



Faculty of Resource Science and Technology

**Microwave-assisted Synthesis of Molecularly Imprinted Polymer for
Sustained Drug Release: A Chemometric Approach on Polymer
Synthesis and Rebinding Study**

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Microwave-assisted Synthesis of Molecularly Imprinted Polymer for
Sustained Drug Release: A Chemometric Approach on Polymer Synthesis and
Rebinding Study

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DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Malaysia Sarawak. Except where due acknowledgements have been made, the work is that of the author alone. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.



.....
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UNIMAS GEMILANG.

ABSTRACT

Molecularly imprinted polymers (MIP) have garnered significant interest as drug delivery system (DDS) in recent years. The conventional thermal heating and one-variable-at-a-time method for synthesising MIP and rebinding capacity evaluation is resource- and time-consuming. Thus, this study focused on the response surface methodology (RSM) optimisation of the polymer synthesis using microwave (MW)-assisted and comparing with conventional thermal synthesis. The influence of two factors (methacrylic acid and divinylbenzene concentration) was studied on the response of the MIP rebinding capacity. Further optimisation on rebinding capacity were employed three factors including MIP dosage, initial drug concentration and contact time using Central Composite Design (CCD). *In vitro* drug release profiles were evaluated in pH 7.4 and pH 2.2 of dissolution media. The aspirin MIP showed spherical particles with smaller particle sizes (500 nm– 1.45 μ m) and yields (0.5–1 g) via MW-assisted synthesis (sample id: ASDW) as compared to the conventional synthesis (sample id: ASD) (size 660 nm –3.65 μ m, yield 0.5–1.15 g). The MW-MIP of paracetamol (sample id: PCMW) also showed similar findings with ASDW (size 1.07 – 1.64 μ m, yield 0.5 - 0.9 g). The optimised formulation of MIP showed polymer P1 with T:FM:CL of 1:4:16 have shown high rebinding capacity in both polymerisation methods of conventional thermal heating (ASD-P1, 3.00 $\pm$ 0.31mg/g)and MW-assisted synthesis (ASDW-P1, 7.05 $\pm$ 0.08 mg/g; PCMW, 6.17 $\pm$ 0.03 mg/g). The optimal rebinding conditions for ASD-P1, ASDW-P1 and PCMW-P1 to achieve high rebinding capacity are as follows: 240 min contact time, 300 mg/L initial concentration and 20 mg MIP dosage. Method validations were performed under optimised conditions and showed only 2-3% error compared with the predicted rebinding capacity. The drug release

profile for ASD-P1, ASDW-P1 and PCMW-P1, has shown a sustained drug release with the highest cumulative release observed at pH 7.4 (64 – 78% for 24 h) than at pH 2.2 (44 – 76%, for 24 h). The release of aspirin and paracetamol at pH 2.2 was fitted in first-order kinetics, followed by the Higuchi model. Meanwhile, all MIP (ASD-P1, ASDW-P1 and PCMW-P1) at pH 7.4 were well-fitted with the Higuchi model. These results collectively demonstrated that the MIP performances are impacted by the choice of the MIP composition (i.e., functional monomer and crosslinker) and the polymerisation method (i.e., MW-assisted synthesis, conventional thermal heating). The optimised formulation ratio of MIP at ratio 1:4:16 can significantly enhance the efficacy, precision, and safety of drug delivery systems, making MIP a valuable component in modern pharmaceuticals. Furthermore, the optimised MIP can potentially reduce cost associated with drug formulation.

Keywords: Molecularly imprinted polymer, response surface methodology, drugs release, microwave-assisted polymerisation

**Sintesis Berbantu Gelombang Mikro Polimer Cetakan Molekul untuk Pelepasan Ubat
Tertahan: Pendekatan Kemometrik pada Kajian Sintesis dan Pengikat Semula Polimer**

ABSTRAK

Polimer cetakan molekul (PCM) telah mendapat kepentingan yang ketara sebagai sistem penghantaran ubat dalam beberapa tahun kebelakangan ini. Pemanasan terma konvensional dan kaedah satu pembolehubah-pada-satu-masa untuk mensintesis PCM dan penilaian kapasiti pengikatan semula adalah memakan sumber dan masa. Oleh itu, kajian ini menumpukan pada pengoptimuman kaedah gerak balas permukaan bagi sintesis polimer menggunakan kaedah bantuan gelombang mikro dan membandingkannya dengan sintesis terma konvensional. Pengaruh dua faktor (kepekatan asid metakrilik dan divinilbenzena) telah dikaji pada tindak balas kapasiti pengikatan semula PCM. Pengoptimuman lanjut pada pembolehubah eksperimen yang mempengaruhi kapasiti pengikatan semula PCM, termasuk jumlah dos, kepekatan ubat semula dan masa interaksi, terus dioptimumkan oleh Reka Bentuk Komposit Memusat. Profil pelepasan ubat in vitro dinilai dalam media larutan pH 7.4 dan pH 2.2. PCM aspirin menunjukkan zarah sfera dengan saiz yang lebih kecil (500 nm– 1.45 μ m) dan hasil yang tinggi (0.5–1 g) melalui sintesis berbantu MW (id sampel: ASDW) berbanding sintesis konvensional (id sampel: ASD) (saiz 660 nm –3.65 μ m, berat 0.5–1.15 g). PCM berbantu gelombang mikro bagi ubat parasetamol (id sampel: PCMW) juga menunjukkan penemuan yang hampir sama dengan ASDW (saiz 1.07 – 1.64 μ m, hasil 0.5 - 0.9 g). Formulasi PCM yang dioptimumkan menunjukkan polimer P1 dengan T:FM:CL sebanyak 1:4:16 mempunyai kapasiti pengikatan semula yang tinggi dalam kedua-dua kaedah pempolimeran (iaitu, pemanasan haba (ASD, 3.00 ± 0.31 mg/g) dan sintesis berbantu gelombang mikro (ASDW-P1, 7.05 ± 0.08 mg/g; PCMW,

6.17±0.03 mg/g). Keadaan pengikatan semula yang optimum untuk ASD-P1, ASDW-P1 dan PCMW-P1 bagi mencapai kapasiti pengikatan semula yang tinggi adalah seperti berikut: masa interaksi 240 minit, kepekatan semula 300 mg/L dan dos 20 mg. Pengesahan kaedah dilakukan di bawah keadaan yang dioptimumkan dan menunjukkan hanya mempunyai ralat 2-3% berbanding dengan kapasiti pengikatan semula yang diramalkan. Profil pelepasan ubat untuk ASD-P1, ASDW-P1 dan PCMW-P1, telah menunjukkan pelepasan ubat yang berterusan dengan pelepasan kumulatif tertinggi diperhatikan pada pH 7.4 (64–78% selama 24 jam), berbanding pelepasan pada pH 2.2 (44–76%, selama 24 jam). Pelepasan aspirin dan parasetamol pada pH 2.2 adalah sesuai di dalam kinetik tertib pertama, diikuti oleh model Higuchi. Sementara itu, semua PCM pada pH 7.4 (ASD-P1, ASDW-P1 dan PCMW-P1) adalah sesuai dengan model Higuchi. Keputusan secara kolektif menunjukkan bahawa prestasi PCM dipengaruhi oleh pilihan komposisi PCM (iaitu, monomer berfungsi dan penghubung silang) dan kaedah pempolimeran (iaitu, sintesis berbantu gelombang mikro (MW), pemanasan haba konvensional). Nisbah optimum PCM pada 1:4:16 menunjukkan peningkatan keberkesanan, ketepatan dan tahap keselamatan sistem penghantaran ubat yang ketara, menjadikan PCM sebagai komponen berharga dalam farmasi moden. Tambahan pula, PCM yang dioptimumkan berpotensi dalam pengurangan kos pembuatan ubat.

.Kata kunci: Polimer cetakan molekul, kaedah gerak balas permukaan, pelepasan ubatan, pempolimeran berbantu gelombang mikro

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LIST OF ABBREVIATIONS

AIBN	Azobisisobutyronitrile
CL	Crosslinker
DVB	Divinylbenzene
DDS	Drug delivery system
EGDMA	Ethylene glycol dimethacrylate
FM	Functional monomer
MAA	Methacrylic acid
MIP	Molecularly imprinted polymer
MW	Microwave
NIP	Non-imprinted polymer
NSAIDs	Non-steroidal anti-inflammatory drugs
NWP	Non-imprinted polymer for microwave
RSM	response surface methodology
T	Template
TRIM	Trimethylolpropane trimethacrylate

INTRODUCTION

1.1 Background of Study

Drug poisoning remains a significant worldwide problem. The availability of drugs over the counter has been a choice for intentional or accidental drug poisoning, including medications like aspirin and paracetamol (Figure 1.1). These are two commonly used drugs that fall under non-steroidal anti-inflammatory drugs (NSAIDs) and antipyretic analgesic drugs (Ibrahim et al., 2023), respectively. Both drugs are readily available in the market and have become the common cause of self-poisoning (Hawton et al., 2019; Kulkarni et al., 2023). Many deaths have been reported in the past due to aspirin and paracetamol overdose, where 15% cause severe aspirin toxicity (Kulkarni et al., 2023) and 12% of paracetamol poisoning cases were reported (Bintalib, 2022). The drug delivery system (DDS) for both drugs is usually designed in a conventional way that employs drug doses in the form of capsules and tablets. These drugs are formed through the compression, coating, and encapsulation of bioactive drug molecules (Srivastava et al., 2016). These systems are designed to promptly release the active drug upon administration, consequently requiring multiple dosing throughout the day and causing poor patient compliance (Ouazib et al., 2019). Frequent burst release phenomena are also observed in the consumption of conventional DDS, which lead to fluctuation of drug plasma level and cause undesirable toxicity effects (Liu et al., 2016; Ouazib et al., 2019).

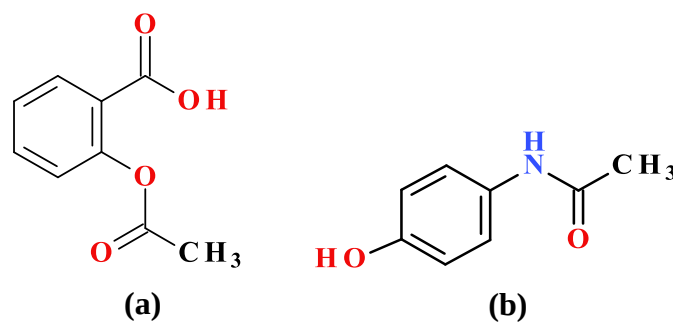


Figure 1.1: Structure of (a) aspirin and (b) paracetamol.

To overcome the poorly features of conventional DDS, an ideal delivery vehicle that can release the drug at the appropriate location, utilising optimal dosage and controlled released mechanism while minimizing or eliminating any adverse side effects and improving patient compliance has actively been researched (Luliński, 2017; Öncel et al., 2017; Liu & Poma, 2021; Lawai et al., 2024). There has been growing interest in polymeric DDS due to their flexibility and potential for further modification. The polymer can be natural (e.g., proteins, polysaccharides, hyaluronic acid, chitosan) and synthetic (e.g., polyester, polylactic acid, polyglycolic acid, polyalcohols) (Agrawal, 2015; Calori et al., 2020). The polymeric DDS can also be categorized as colloidal carriers (e.g., micro/nanoparticles, micelles, micro/nano gels), hydrogels and polymer-drug conjugates (Iheaturu et al., 2019; Trucillo, 2021). In polymeric DDSs, the drug molecules are distributed throughout a polymeric matrix or beneath a polymer membrane, which aims to provide controlled release at a predetermined rate or following physiological parameters (Liu & Poma, 2021).

Molecularly imprinted polymer (MIP) is a new synthetic polymeric material vastly investigated for DDS. MIP is a three-dimensional, microporous cross-linked polymer matrix

capable of selective molecular recognition with the memory of the analyte's shape, size and functional groups (Dong et al., 2021). It is prepared with template, functional monomer, crosslinker, initiator and progenic solvent. Upon polymerization, the template will be removed from the polymer matrix leaving a cavity that has been imprinted with the template. This intrinsic molecular recognition capability imparts MIP with the ability to selectively encapsulate and release drugs, presenting a promising avenue for achieving precision in drug delivery (Liu & Poma, 2021). The polymeric, artificial macromolecular receptors with predetermined selectivity and specificity of a given analyte are designed to mimic the biological receptors with the analogy of "lock and key" such as substrates-enzyme and antigen-antibody (Madikizela et al., 2018; Herrera-Chacón et al., 2021; Liu & Poma, 2021).

Generally, MIP are known to have advantages such as high stability and durability in harsh conditions (high temperature, highly acidic and basic conditions), high selectivity towards extensive template molecules, high drug loading capacity, low cost, and ease in preparation (Asadi et al., 2016; Zaidi, 2016; Li et al., 2019; Malik et al., 2019). Due to the inherent resistance of MIPs to harsh pH, temperature and pressure, MIP can safeguard drugs against enzymatic degradation before they reach its intended site (El-Schich et al., 2020). Another advantage of MIP is high stability for long-term storage while maintaining their recognition properties an easy and inexpensive synthesis method (Ansari & Masoum, 2019). Thus, MIP are considered suitable for DDS due to their specific recognition capabilities, high stability, and potential for targeted and sustained drug release, which potentially reduces off-target effects and improves therapeutic outcomes (Zaidi, 2016; Sanadgol & Wackerlig, 2020). Figure 1.2 illustrates the diagrammatic description of MIP and its functioning as DDS (El-Schich et al., 2020).

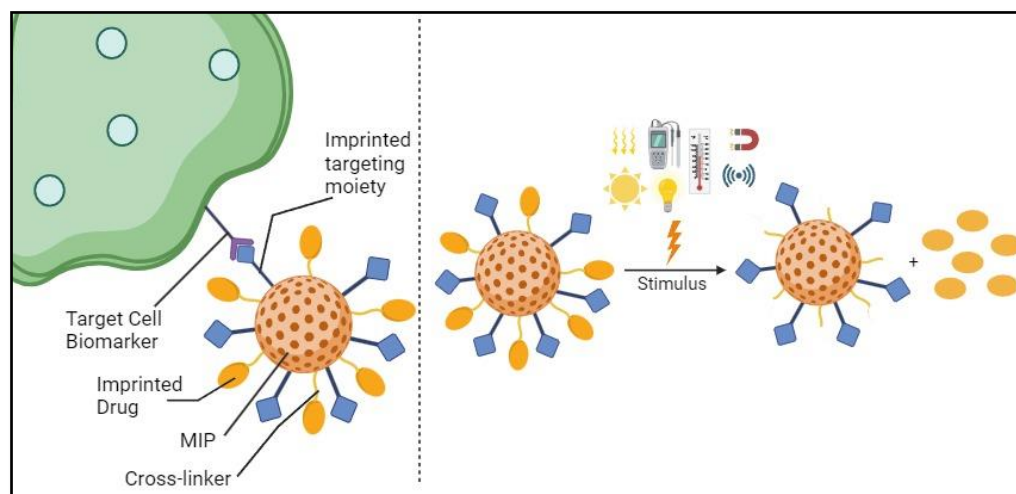


Figure 1.2: General illustration of MIP-based drug delivery (El-Schich et al., 2020).

1.2 Problem Statement

Aspirin and paracetamol which are the most commonly used drugs in intentional poisoning and easily acquired through the market are often produced in a conventional way. However, the weakness of conventional DDS often caused burst release and require frequent dosing that can affect the efficacy, safety, and patient compliance. Aspirin is also well-known to cause gastrointestinal irritation (GI) that can cause ulcers and GI bleeding. To address these issues, a sustained or controlled drug release was designed using MIP to improve the bioavailability, reduce adverse effects, enhance patient compliance and provide more consistent therapeutic effects.

Designing and synthesizing MIP that exhibit high specificity and binding affinity to a wide range of drug molecules and the ability to demonstrate controlled release or targeted delivery has gained wide interest in DDS (Băraian et al., 2022). The process of designing a new MIP with a new polymer composition for DDS also frequently demands an extensive trial-and-

error approach. A simplified and scalable synthesis method is essential for implementing MIP in large-scale drug production. Traditional optimisation methods, such as one-variable-at-a-time, are time-consuming and too often fail to fully extrapolate interactions involving more than two response factors (Heravizadeh et al., 2018). Hence, multivariate analysis, such as Response Surface Methodology (RSM), is opted. RSM is a technique for constructing and investigating the relationship between the response and independent factors to ascertain the most favourable conditions for developing MIP as DDS while minimizing the experimental procedures (Ghanavati et al., 2019; Hashemi & Keykha, 2019).

On the other hand, the conventional thermal heating used in precipitation polymerisation to produce spherical particles is also a limitation due to the prolonged reaction times and uneven heat distribution (Kueseng et al., 2018). It has prompted the exploration of alternative approaches. Therefore, rapid and facile methods such as microwave (MW) assisted synthesis have emerged as an alternative way to synthesize MIP by promoting homogenous heat transfer, leading to remarkably short polymerisation time and high yield (Viveiros et al., 2018; Farooq et al., 2020). However, the existing literature fails to acknowledge the relative influence of the polymerisation method (i.e., conventional thermal heating and MW-assisted synthesis) on the resulting MIP's properties, such as the yield, particle size, rebinding efficiency and drug release application. Therefore, substantial developments have been conducted to optimise the polymer composition and imprinting process, aiming to increase MIP's binding capacity and development for controlled drug release application. The resultant developments can also be applied in other applications such as separation and purification processes, catalysis, chemical sensors and biosensors.

1.3 Objectives

Based on earlier stated problems, the overall aim of the present study was to evaluate the performance of MIP prepared via microwave-assisted synthesis capable as drug delivery system. Therefore, the research embarked on the following objectives:

1. To synthesise MIP and non-imprinted polymer (NIP) using aspirin and paracetamol as templates *via* conventional thermal polymerisation (sample id: ASD and NIP) and microwave-assisted synthesis (sample id: ASDW, PCMW and NWP).
2. To optimise the synthesis of MIP and NIP using Response Surface Methodology (RSM) by evaluating the two factors (i.e. amount of functional monomer and crosslinker).
3. To characterise the physical and chemical properties of MIP and NIP using Fourier Transform Infrared Spectroscopy (FTIR), Field Emission Scanning Electron Microscopy (FESEM) and UV-Visible spectroscopy.
4. To determine the rebinding capacity of the prepared MIP and NIP.
5. To optimise the rebinding capacity of the selected MIP using RSM study by evaluating the three factors (i.e. MIP dosage, initial concentration and contact time).
6. To evaluate the efficiency of *in vitro* drug release of the selected MIP.
7. To determine the release kinetic model of the selected MIP

CHAPTER 2

LITERATURE REVIEW

2.1 Overview of Molecularly Imprinted Polymer

A molecularly imprinted polymer (MIP) is a highly selective polymer with recognition sites created artificially using molecular templates capable of mimicking antibodies and biological receptors (Vasapollo et al., 2011; Yanti et al., 2016). The theory of molecular imprinting was first proposed in the early 1940s by Pauling whom suggesting that antibodies could be form synthetically (Xiao et al., 2018). Later in early 1950s, the imprinting concept was observed in a study that revealed the affinity of the dye molecules towards the silica gel was observed higher after the removal of the templates, thus creating a similarity concept with the antibodies and its antigens in living systems (Lowdon et al., 2020). The first molecular imprinting in organic polymer was revealed in 1970s with the covalent approach in the preparation of MIP capable of discriminating between enantiomers of glyceric acid (Sanadgol & Wackerlig, 2020). Then, the next breakthrough in organic polymer imprinting occurred in 1981 when Mosbach and Ashady reported the preparation of MIP *via* non-covalent interactions which has been widely adapted in nowadays pre-polymerization process due to its fast binding and release rate of the template, easier and simplicity of production, and a variety of available functional monomers (Dong et al., 2021).

In general, an imprinting procedure necessitates using a template, functional monomer, cross-linker, initiator, and porogenic solvent. The preparative steps of MIP comprise (i) the pre-polymerisation of the template and functional monomers complex, (ii) the co-polymerisation in

the presence of excess crosslinker and then (ii) removal of the template by extraction procedure leaving a cavity similar to the template (Samarth et al., 2015; Xiao et al., 2020) (Figure 2.1).

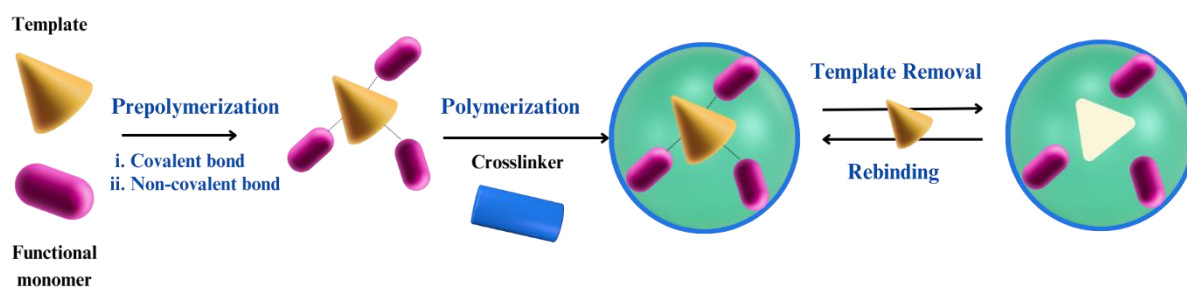


Figure 2.1: Schematic representation of MIP preparation.

MIPs have been synthesised *via* several polymerisation methods, commonly initiated by heat or UV light, to induce free radical polymerisation (Parisi et al., 2022). Free radical polymerisation occurs in the presence of an initiator, consisting of three stages: initiation, propagation, and termination (Hashemi & Keykha, 2019). This process is commonly used in the preparation of MIP, such as in bulk polymerisation, precipitation polymerisation, pickering emulsion polymerisation, multi-step swelling polymerisation, and surface imprinting (Ertürk & Mattiasson, 2017; Fresco-Cala et al., 2020). The high affinity and selectivity of MIP towards the target analyte have made it applicable to many fields of chemistry and biology, particularly in separation and purification processes, catalysis, chemical sensors and biosensors, food technology, and drug delivery systems (DDS) (Malik et al., 2019; Herrera-Chacón et al., 2021; Liu & Poma, 2021).

2.2 MIPs in the Drug Delivery System

MIPs are potentially advantageous polymeric carriers that have the capacity to enhance drug delivery by virtue of their distinctive characteristics. The high loading capacity, prolonged release time of the analytes (e.g. drugs), and versatility in the administration routes give the advantages of MIPs developed for DDS (Vasapollo et al., 2011; Băraian et al., 2022). Furthermore, MIPs have been considered promising DDS due to their ability to provide controlled drug release, high chemical and physical stability, and low cost (Hasanah et al., 2021). The utilisation of functional materials such as bio-macromolecule-sensitive, pH-sensitive, temperature-sensitive and, photosensitive and magnetic materials produce intelligent MIP that is pivotal for targeted delivery (He et al., 2021).

2.2.1 Controlled Release of MIP-DDS

The most important criterion in designing MIPs for DDS is to achieve a controlled released property that can sustain the therapeutic systemic level of the medication for an extended duration (El-Schich et al., 2020). MIPs are smart polymers due to their flexibility in the preparative part and surface modification, which can be designed with stimuli responsiveness and controlled-release properties (Rahim et al., 2021). This helps to mitigate substantial changes in drug concentration and reduce the frequency of administration, which is a hassle in conventional drug delivery (Kazemi et al., 2016; Calori et al., 2020). Figure 2.2 illustrates the pharmacokinetics curves of the drug's plasma concentration *via* a conventional delivery system and a controlled delivery system (Adepu & Ramakrishna, 2021).

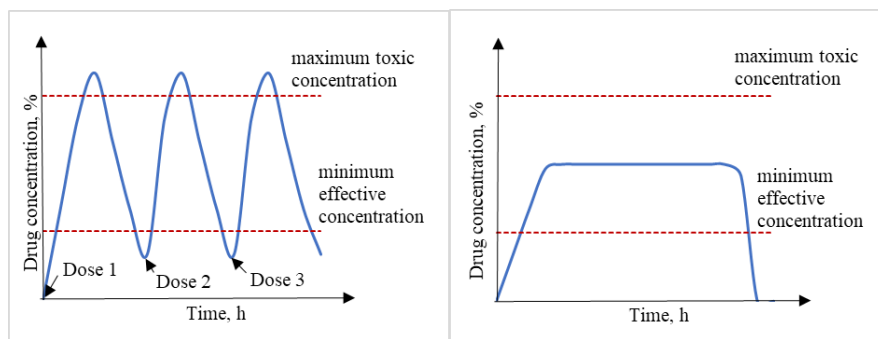


Figure 2.2: Typical drug concentration in (a) conventional delivery system (b) controlled release (Adepu & Ramakrishna, 2021).

An initial study on the controlled release behaviour of MIP was reported in 1998 by Norell and co-workers *via* imprinting theophylline into poly(MAA-EGDMA). The MIP and controlled release were observed in phosphate buffer pH 7.0 media for several hours. Since then, many studies reported on the controlled release of various racemic drugs (S-ibuprofen, S-ketoprofen and R-propranolol) (Suedee et al., 2002). The success of MIP in controlled release behaviour has been explored in a wide range of drugs for cancer treatment such as 5-fluorouracil (An et al., 2020; Asadi et al., 2016a; Hashemi-Moghaddam et al., 2016; Shadabfar et al., 2020), doxorubicin (Hashemi-Moghaddam et al., 2017; Luo et al., 2020; Naqvi et al., 2020), docetaxel (Ali et al., 2022b) and paclitaxel (Bai et al., 2018; Cegłowski et al., 2019). Other types of drugs such as olanzapine for Schizophrenia (Asadi et al., 2016b), amitriptyline hydrochloride for depression (Mohebbi et al., 2021), NSAIDs such as fenbufen (Liu et al., 2018), diclofenac (Zheng et al., 2018), aspirin (Duarte et al., 2006; Kan et al., 2009) and theophylline for pulmonary disease (Parisi et al., 2018) have been studied as MIP-DDS. A controlled release behaviour (Table 2.1) with the ability of targeted delivery (Table 2.2) and stimuli-responsive

(Table 2.3) has been successfully developed using MIP-DDS. Figure 2.3 showed examples of drugs use as template for MIP.

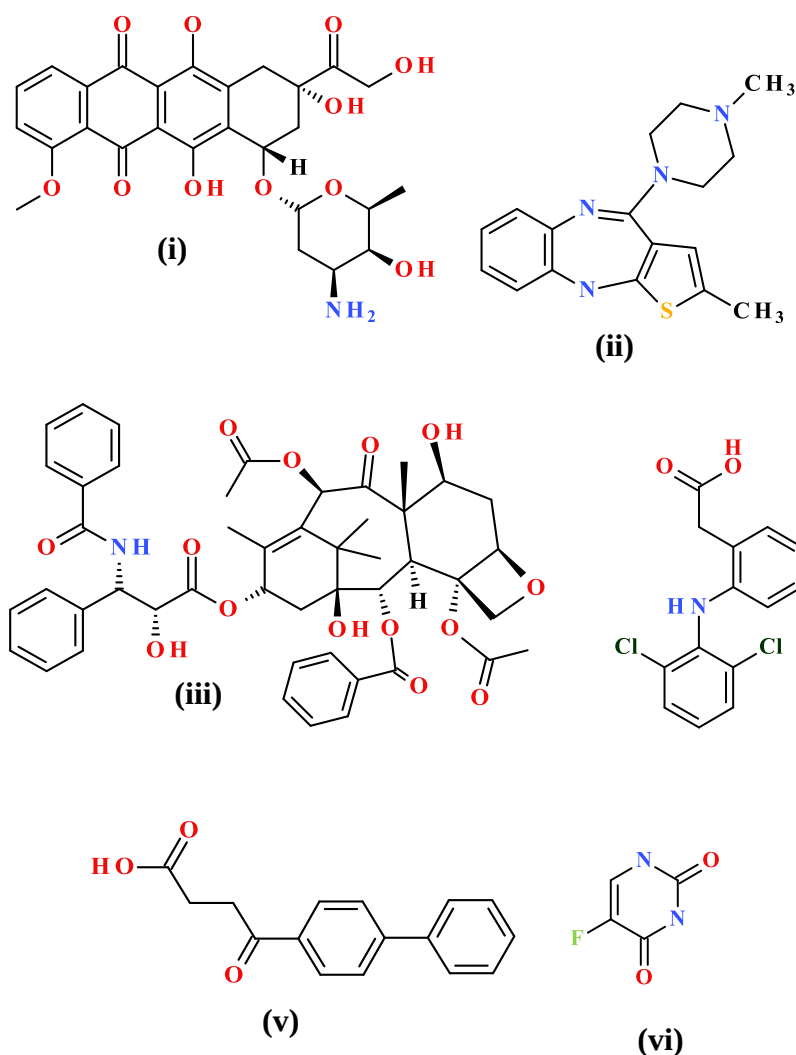


Figure 2.3: Drugs template used in the preparation of MIP for DDS. (i) doxorubicin (Naqvi et al., 2020) (ii) olanzepine (Asadi et al., 2016b) (iii) paclitaxel (Cegłowski et al., 2019) (iv) diclofenac (Zheng et al., 2018) (v) fenbufen (Liu et al., 2018) (vi) 5-fluorouracil (Shadabfar et al., 2020).

Table 2.1: Controlled release MIP-DDS.

Preparation method	Template (T), Monomer (FM) Crosslinker (CL) Initiator (I) Solvent (S)	Components ratio (mmol) (T:FM:CL)	Particle morphology & size	Binding capacity/ Adsorption capacity	<i>In vitro</i> release profiles	<i>In vivo</i> study / cell viability assay	References
Bulk polymerisation (90°C x 24 h)	(T): Glycyrrhizic acid (FM): MAA (CL): EGDMA (I): AIBN (0.1g) (S): Anhydrous DMF (5 mL)	*1 :32 :50	Data not available	40% ± 1.3% (in EtOH) 38% ± 0.9 (in EtOH-H ₂ O)	Medium: pH 2 19% release within 2 h 100% release over 25 h	Not available	(Cirillo et al., 2010)
Bulk polymerisation (40°C x 18 h)	(T): Vancomycin (FM): MAA (CL): PEGDMA (I): KPS (0.018 mmol) (S): Water (5 mL)	*1:8.29:23.79	Hydrogel	Data not available	Medium: pH 7.4. 36% after 12 h. Cumulative release within 24 h is <50%.	Not available	(Kurczewsk a et al., 2017)
Bulk polymerisation (80°C x 7 h)	(T): Tramadol HCl (FM): MAA (CL): EGDMA (I) : Benzoyl peroxide (0.41 mmol) (S): Chloroform (8 mL)	*1 :2.3 :30	Data not available	3.608 mg/g	Medium: pH 7: 96.4% tramadol released over 12 h Release after 3h at: pH 3.6: 76.7% pH 7.0: 68.5% pH 9.8: 60.3%	Not available	(Morsi et al., 2021)
Precipitation polymerisation (90°C x 24 h)	(T): Naltrexone (FM): Acrylic acid (CL): EGDMA (I): AIBN (0.041 g) (S): THF & Water (3:25 mL)	*1: 0.3: 1.4	Hydrodyna mic diameter = 358 nm	75 mg/g	Medium: pH 7.4. 37 mg/g (49%) in about 85 h	Not available	(Rostamiza deh et al., 2012)

Table 2.1: Continued.

Precipitation polymerisation (60°C x 24 h)	(T): Vinblastine (FM): MAA (CL): TRIM (I) : AIBN (15 mg) (S): Acetonitrile (25 mL)	*1:5:2	300-500 nm	76.6% ± 2.06%	Medium: pH 7.4. 70% release at 216 h	Biodistribution study <i>via</i> intravenous injection in rats	(Zhu et al., 2017)
Precipitation polymerisation (50°C x 18 h)	(T): Furosemide (FM): Aam (CL): Cellulose acrylate (I): Ammonium peroxydisulfate (11.04 mg) (S): Water (14 mL)	*1:4:8	Data not available	216.18 umol/g	Medium: pH 7.4. Maximum release is 89.72% (3.23 mg) at 72h	Not available	(Fareghi et al., 2017a)
Precipitation polymerisation (UV light for 12 h)	(T): Humulin (FM): MAA, HEAA (CL): MBAA (I): AIBN (0.04 mmol) (S): Phosphate buffer saline (10 mL) Other : polycaprolactone triol	*1: 8.33 : 3.33: 5.33	198.73 ± 3.05 nm	3.26 mg/g & 3.34 mg/g	Medium: pH 7.4 :30–40 % in the first 30 min and almost 90% at 700 minutes pH 1.2 : less 30%	Yes. Insulin level monitoring in diabetic induced rats	(Paul et al., 2017)
Precipitation polymerisation (60°C x 24 h)	(T): Diclofenac (FM): MAA (CL): EGDMA (I) : AIBN (0.1 mmol) (S) : Acetonitrile (50 mL)	*1:4:20	Spherical uniform, 300 nm	65.18%	Medium: pH 7.4. 19%-25% burst release in first 6h, 65% - 90% release in 108h	Not available	(Zheng et al., 2018)

Table 2.1: Continued.

Precipitation polymerisation (60°C x 18 h)	(T): Paclitaxel (FM): MAA & HEMA (CL): EGDMA (I): AIBN (0.5 mL) (S): Acetonitrile (40 mL)	*1: 6: 17.78	Spherical 0.4 - 1.3 µm	9.86 ± 0.93 mg/g	pH 7.0: ca.40% pH 5.0: ca.22% pH 2.2: 17%	<i>In vitro</i> cytotoxicity against U87 MG, HeLa, KB, A-549, MCF-7	(Ceglowski et al., 2019)
	(T): Paclitaxel (FM): MAA & HEMA (CL): TRIM (I) : AIBN (1 mL) (S): Acetonitrile (40 mL)	*1:6:4	Spherical. 400 - 800 nm	13.32 ± 0.11 mg/g	pH 7.0: ca.85% pH 5.0: ca.65% pH 2.2: 50%	cancer cell lines and HDF normal cell lines.	
Precipitation polymerisation (60°C x 18 h)	(T): 5-Fluorouracil (FM): MAA, HEMA (CL): EGDMA (I): AIBN (1 mL) (S): Methanol (90 mL)	*1:2:1:8.8	Spherical. 300-600nm	22.57mg/g	Highest cumulative release within 160 h: pH 7.4: 12% pH 5.0: 20% pH 2.2: ca.40%	<i>In vitro</i> cytotoxicity against U87 MG, HeLa, KB, A-549, MCF-7	(Ceglowski et al., 2022)
	(T): 5-Fluorouracil (FM): MAA, HEMA (CL): TRIM (I): AIBN (1.4 mL) (S): Methanol (90 mL)	*1:2:1:2	Spherical, 400-800nm	37.30 mg/g	Highest cumulative release within 160 h: pH 7.4: 86% pH 5: 70% pH 2.2: 53%	cancer cell lines and HDF normal cell lines.	
Precipitation polymerisation (60°C x 24 h)	(T): Sunitinib (FM): MAA (CL): EGDMA (I): AIBN (100 mg) (S): Methanol/ acetonitrile (1:1, v/v, 60 mL)	*1:32:40	Hydrodynamic mean diameter is 714.6 ± 9.7 nm	Adsorption kinetics at time interval: 1h - 12.5%, 4h - 50.1%, 24h - 80.3%	Media pH 7.4. Release at time interval: 2h -26%, 6h - 67%, 24h - 79%	Not available	(Parisi et al., 2020)

Table 2.1: Continued.

RAFT precipitation polymerisation (60°C x 22 h)	(T): Phenytoin Base (FM): Aam (CL): EGDMA (I): AIBN (0.02 g) (S): Ethanol: acetonitrile (30:70, v/v, 40 mL) Chloroform (3 mL) <i>Other</i> : Chain transfer agent (CTA)	*1:14.12:14.1 2	Aggregated spherical, 100-600 nm	11200 ug/g	Medium: pH 7.4: 29% drug release during 40 h. 31% after 63 h.	Not available	(Abdollahi et al., 2018)
Mesophase polymerisation (60°C x 24 h)	(T): Theophylline (FM): MAA (C): EGDMA (I): AIBN (100 mg) (S): Chloroform (60 mL)	*1:8.05:20.35	Rod-like geometry	60.7% ±0.8% binding at 24 h incubation time	Medium: pH7.4: 37% released in the first 2 h. Cumulative release at 70%-90% for 24 h.	Not available	(Parisi et al., 2018)
RAFT precipitation polymerisation (60°C x 24 h)	(T): Paclitaxel (FM): MAA (CL): EGDMA (I): AIBN (Nil.) (S): Acetonitrile (40 mL) <i>Other</i> : POSS & Chain transfer agent (CTA)	*1:10:50 (10 mmol POSS)	Monodispersed spherical particles, 187 nm	Drug loading efficiency = 17.1% Entrapment efficiency = 85.5%	At first 50 h: pH 7.0: 3.1% pH 6.0: ca. 8.5% pH 5.0: 10.7%	<i>In vitro</i> cytotoxicity assay on HepG2 cell lines	(Bai et al., 2018)
Surface imprinting (60°C x 14 h)	<i>Surface</i> : Single wall carbon nanotubes (SWCNT) (T): Fenbufen (FM): 4-VP (CL): EGDMA (I): AIBN (20 mg) (S): Chlorine chloride/ ethylene glycol & [BMIM]BF ₄	*1:9:36	External graphitic cylinders between 20 and 50 nm	0.0423 µmol/g	Medium: acetonitrile. 50.1% release over 6 h	Yes. Intragastrically administration.	(Liu et al., 2018)

Table 2.1: Continued.

Surface imprinting (80°C x 24 h)	<i>Surface:</i> β -cyclodextrin-chitosan microsphere (T): Sinomenine hydrochloride (FM): MAA (CL): EGDMA (I): AIBN (0.05 g) (S): DMF (1 L)	*1:6:20	smooth & round, well dispersibility, average 15 μ m	55.9 mg/g	Medium: pH 7.4 17% within 2h without burst release. after 4h, sustained release observed with accumulative of 78% for 24h	Not available	(Chen et al., 2018)
Surface imprinting (1 h)	<i>Surface:</i> Graphene oxide (T): CA125 (FM): Dopamine (CL): Nil. (I): APS (10 mg) (S): Phosphate buffer 250 mmol (pH 8.5, 30 mL))	Unidentified. (T): 10 μ L (FM): 10 mg	GO was ribbon-like with wrinkles at the edge, Nanosheets, Pore size: 16.579 nm	~3.2 nmol/g	Cumulative release of DOX within 50 h: pH 7.4: 2.9% pH 6.0: 28% pH 4.5: 54%	<i>In vitro</i> cytotoxicity against SMMC-7721 and HEK293 cells	(Han et al., 2020)

Note: *Component ratio calculated by author with the given value in mg or mmol and not in ratio formula.

Table 2.2: Magnetic MIP for DDS.

Components : Core Surface (CS), Template (T), Functional Monomer (FM), Crosslinker (CL), Solvent (S), Ratio T:FM:CL (mmol)	Application/ Treatment of Disease	Particle morphology & size	Adsorption capacity (Q) / Extraction efficiency, (E)	Biological Application: Release Profile (<i>In vitro</i> / <i>In vivo</i> / Cytotoxicity study)	References
(CS): Fe ₃ O ₄ -NP (T): Acetylsalicylic acid (FM): MAA (CL): TRIM (S): Chloroform T:FM:CL = 1:4:2	Prevent platelet aggregation & blood clotting	Regular spherical. Average: 500 nm	Q= 99.9 umol/g	<i>In vitro</i> : pH6.8: 50% at first 2 h. Ca. 85% at 10 h <i>In vivo</i> : Nil.	(Kan et al., 2009)
(CS) : Fe ₃ O ₄ -NP (T): 5-FU (FM): Dopamine (CL): Nil. (S): Tris-buffer (10mM, pH 8.5) T:FM = 1:1.7	Ovarian cancer	Spherical. 80 nm	Nil.	<i>In vitro</i> : pH 7.4: Burst release within 4h, release pattern reach maximum within 24h, then sustained release up to 48h. <i>In vivo</i> : Higher concentration of 5-FU in tumor tissue treated with magnetic field. <i>Cytotoxicity test</i> : Nil.	(Hashemi- Moghaddam et al., 2016)
(CS) : Fe ₃ O ₄ @SiO ₂ -FITC (Fluorescein isothiocyanate) (T): Olanzapine (FM): Fructose (CL): Nil. (S): Acetonitrile/DMSO T:CL =1:4	Schizophrenia treatment	Uniform spherical. Z-average = 58 nm	Q= 91 mg/g	<i>In vitro</i> : Nil. <i>In vivo</i> : release in rats targeting at brain site. Drugs injected intravenously and after 24h, drug were observed presence at the rat's brain.	(Asadi et al., 2016b)

Table 2.2: Continued.

(CS): Fe ₃ O ₄ @SiO ₂ -FITC (T): 5-FU (FM): Nil. (CL): Tannic acid (S): Deionized water with SDS Other: Hexadecane (hydrophobic agent) <i>T:CL = 1:1</i>	Cancer treatment	Homogenously & spherical. z-average = 53nm	E= 83.5± 1.7%	<i>In vitro</i> : pH 2: 40% release after 2h. pH 7.4: Sustained release <i>In vivo</i> : release in rats.	(Asadi et al., 2016a)
(CS): Fe ₃ O ₄ -NP (T): Letrozole (FM): MAA (CL): TRIM (S): Acetonitrile <i>T:FM:CL = 1 :1.5 :1</i>	Breast cancer	Agglomerate spherical. Average size 100 nm	Q= 12 mg/g E = 60%	<i>In vitro</i> : pH 7.4: 40% release during 10h. Complete release within 70 h. <i>In vivo</i> : Nil. <i>Cytotoxicity test</i> : Nil.	(Kazemi et al., 2016)
(CS): SiO ₂ - Epitope (T): HER2 Epitope & Doxorubicin (DOX) (FM): Dopamine (CL): Nil. (S): Tris-buffer (10mM, pH 8.5) <i>T:FM = 1: 7.1</i> (DOX:Dopamine)	Ovarian cancer	Uniform spherical. Ca. 80 nm	Nil.	<i>In vitro</i> : pH 7.4: Rapid release within 4 h, and maximum release after 24 h, constant release until 48 h. <i>In vivo</i> :DOX-EPI-IP in distilled water injected intravenously <i>via</i> tail vein of tumor-bearing mice which hindered the tumor growth	(Hashemi- Moghaddam et al., 2017)
(CS): Fe ₃ O ₄ -Graphene oxide (T): CEA (carcino-embryonic antigen) (FM): Dopamine (CL): Nil. (S): Phosphate-buffer (pH 8.5, 450mM)	Cancer	Agglomerate irregular spherical. Pore size: 15.692 nm	MGO-MIP- DOX: Q = Ca. 2.5 nmol/g MGO-MIP: Q=2.61 nmol/g	<i>In vitro</i> : pH 7.0: 3.5% after 10 h. pH5.0: 40% after 10 h pH 6.0: 22% after 10 h <i>In vivo</i> : Nil. <i>Cytotoxicity test</i> : MTT assay against HepG2 and L02 cells.	(Han et al., 2020)

Table 2.2: Continued.

(CS) : Fe ₃ O ₄ @SiO ₂ -FITC-APS (T): 5-FU (FM): MAA (CL): TMPTA (S): DMSO T:FM:CL = 1:4:20	Breast cancer	Uniform spherical. Ca. 200 nm	E= 68%	<i>In vitro</i> : pH 3: Quick release with medium slope within 150 h, then sustained at >80% within 300 h. pH 7.4: Slow release with ca. 20% release within 300 h <i>In vivo</i> : Nil. <i>Cytotoxicity test</i> : MTT assay against MCF-7 cell.	(Shadabfar et al., 2020)
(CS) : Fe ₃ O ₄ @SiO ₂ -FITC-APS (T): Docetaxel (FM): Glucose (modified) (CL): Nil. (S): Acetonitrile/DMSO, 5:1, v/v	Cancer	Spherical. Diameter = 250 nm	Q= 72 mg/g	<i>In vitro</i> : Nil. <i>In vivo</i> : MIPs were orally administered into mice. Drugs were release at targeted area with the guide of external magnetic field. <i>Cytotoxicity test</i> : MTT assay.	(Ali et al., 2022b)

Table 2.3: Stimuli-responsive MIP-DDS.

Type of Stimuli responsive	Components : Core surface (CS) / Polymer backbone (PB), Template (T), Functional Monomer (FM), Crosslinker (CL), Solvent (S), Preparation Method (PM)	Particle morphology & size	Application (<i>In vitro</i> Release / <i>In vivo</i> study)	References
pH responsive	(CS/PB) : Nil. (T): Amitriptyline hydrochloride (FM) : MAA (CS) : TRIM (S) : Chloroform: Amyl acetate, (20:30, v/v) Polymer ID: MIP2 (PM): Precipitation polymerisation	Spherical, 103 ± 10.1 nm	<i>In vitro</i> : Simulated intestinal fluid: 30% release within 15 h More than 80 % release within 120 h. Simulated gastric fluid: 20% release within 15 h Ca. 25% release within 120 h.	(Mohebbi et al., 2021)
pH responsive	(CS/PB) : Graphene oxide (GO) (T): Acrylated β -CD/CUR (FM) : Acrylic acid (CS) : Four functionalized crosslinker modified from HEMA (S): Acetonitrile Polymer ID : GO@CUR-MIP (PM): Surface imprinting	Nil.	<i>In vitro</i> : pH 7.4 : 10% release within 120 h pH 5.5 : 30% release within 120 h	(Sedghi et al., 2021)
Thermo-responsive	(CS/PM): Fe ₃ O ₄ @SiO ₂ (T): Acrylated β -CD/CUR (FM) : NIPAAm (CS) : MBAm (S) : Acetonitrile (PM): Surface imprinting	Spherical. Diameter: 130-150 nm	<i>In vitro</i> : 25°C: 45% released within 7 h. 62% released within 80 h. 38°C: Ca. 65 released within 10 h. 86% released within 80 h.	(Sedghi et al., 2018)

Table 2.3: Continued.

Thermo-responsive	(CS/PB) : Konjac Glucomannan (T): 5-FU (FM) : NIPAAm & AM (CS) : MBAm (S): Water (PM): Graft copolymerisation	Spherical shape on the macrolevel. Approximately nanosized pore.	<i>In vitro</i> : 25°C, pH 7.4: Ca. 75% within 20 h. 38°C, pH 7.4: Ca. 55% within 20 h.	(An et al., 2020)
Redox responsive	(CS/PB) : (T): S-Propранolol (FM) : MAA (CS) : DSDMA / BAC (S): i. Acetonitile ii. DMSO (PM): i. Bulk polymerisation i. High dilution	Nanogels: Ca. 30 nm	<i>In vitro</i> : pH 5.0, with GSH (10 mM): 33% after 1 h and reached equilibrium. Release profile was similar to NIP. pH 5.0, without GSH: 22% after 2 h and reached equilibrium.	(Zhao et al., 2020b)
Redox responsive	(CS/PB) : fluorescent zeolitic imidazolate framework-8 with doxorubicin (FZIF-8/DOX) (T): Epitope of CD59 glycoproteins (FM) : TFMA, DMAEMA, NIPA, TBAm (CS) : BAC (S): Aqueous solution and DMF (PM): Surface imprinting	Spherical. FZIF-8/DOX-MIPs = 88 nm - 130 nm	<i>In vitro</i> : pH 5.0, with GSH (10 mM): 95% release within 15 days. pH 5.0, no GSH: almost no DOX released. pH 6.0, with GSH (10 mM): Ca. 80% release within 15 days. pH 7.4, no GSH: no DOX released.	(Qin et al., 2020)

Table 2.3: Continued.

Redox responsive	(CS/PB) : Nil. (T): Cisplatin (FM) : HEMA (CS) : Disulfide crosslinker, BAC (S): Deionized water and water-ethanol mixture (PM): Precipitation polymerisation	Nanosized hydrogel	<i>In vitro</i> : pH 5.3 and GSH 10mM: Ca. 70% release within 24h pH 5.3 and no GSH: Ca. 60% release within 24 h. pH 7.4 and GSH 2mM: Ca. 20% release within 24 h. pH 7.4 and GSH 10mM: Ca. 40% release within 24 h.	(Loghmani et al., 2021)
Photo responsive	(CS/PB) : Multi wall carbon nanotubes (MWCNT-CH=CH ₂) (T): L-phenylalanine benzyl ester (L-PABE) (FM) : MPABA (CS) : EBMAA (S): Methanol/DMSO (PM): Surface imprinting & Bulk polymerisation	Nanosized tubular moieties with spherical polymer counterpart. Average size: 30 nm.	<i>In vitro</i> : UV light at 365 nm: in the range of 4.0 – 4.5 µmol/g released within 16 h. Visible light: In the range of 1.0 – 1.5 µmol/g released within 16 h.	(Sajini et al., 2019)
Photo responsive	(CS/PB) : UCNPs (NaYF ₄ :Yb, Er@SiO ₂ -MPS) (T): Paracetamol (FM) : MADPADSA (CS) : TEAMA (S): Water: DMF (PM): Surface imprinting	hexagonal prism materials with regular morphology Average diameter: 875 nm	<i>In vitro</i> : pH 7.4, near infrared light (NIR) (980nm): 5.45 µmol/g (91.5%) within 5 h. pH 7.4, dark environment: 0.86 µmol/g (13.7%) within 5 h.	(Liu et al., 2020)

Table 2.3: Continued.

Dual responsive:	(PB) : pH sensitive linker and enzyme sensitive peptide GFLG	Spherical, diameter size: 67.30 ± 7.00 nm	<i>In vitro:</i> DOX release: pH 5.4: complete release within 36 h pH 7.4: less than 10% release after 48 h	(Luo et al., 2020)
pH and enzyme responsive	(T): DOX / NFX (FM) : HPMA (CS) : OEGMA (S) : NH ₄ OAc Polymer ID: CLM (PM): Graft copolymerisation		NFX release: pH 5.4: complete release within 25 h, pH 7.4: 20% release after 48 h. <i>In vivo:</i> anti-cancer and anti-metastasis efficacy of CLM against breast cancer was evaluated on two murine models: orthotropic and lung metastasis breast cancer model	

5-FU, 5-fluorouracil ; β -CD/CUR, β -Cyclodextrin/ Cucurmin; AM, Acrylamide; BAC, N,N'-diacrylylcystamine; DMF, dimethylformamide; DMSO, dimethylsulfoxide; DMAEMA, dimethylaminoethyl methacrylate; DOX, doxorubicin; DSDMA, bis(2-methacryloyloxyethyl)disulfide; EBMAA, N,N-ethylene bismethacrylamide; Fe₃O₄@SiO₂, Iron oxide @ silicon dioxide; GFLG, glycylphenylalanyl- leucyl-glycine; GSH, Gluthathione; HEMA, hydroxyethyl methacrylate; HPMA, hydroxypropyl methacrylate; MAA, methacrylic acid; MADPADSA, 4-[(4-methacryloyloxy)-2,6-dimethyl phenylazo]-3,5-di- methyl benzene-sulfonic acid; MBAm, N, N methylene bisacrylamide; MPABA, 4-[(4-methacryloyloxy)phenylazo]benzoic acid; NIPAAm, N-isopropyl acrylamide; NIPAM, N-isopropylacrylamide; NH₄OAc, Ammonium acetate; NIPA, N-isopropylacrylamide; NFX, nifuroxazide; OEGMA, oligo-(ethylene glycol) methacrylate; TBAm, N-tert-butyl acrylamide; TEAMA, triethanolamine trimethacrylate; TFMA, trifluoromethylacrylic; TRIM, trimethylolpropane trimethacrylate; UCNPs, up conversion nanoparticles.

2.2.2 Drug Release Mechanism from MIP

In general, the controlled release of a drug is governed by chemical or biological mechanisms such as dissolution, diffusion, swelling, erosion, partitioning, osmosis and targeting (Figure 2.4) (Tallapaka et al., 2015; Calori et al., 2020). The three mechanisms controlling the drug release are diffusion, erosion and desorption (Paarakh et al., 2019; Băraian et al., 2022). The medication diffuses past the barrier separating the carrier's interior core from the surrounding bulk, cell, or tissues. The erosion is brought on by an external environment that gradually deteriorates the carrier barrier, allowing the remaining medication to seep and discharge (Trucillo, 2021).

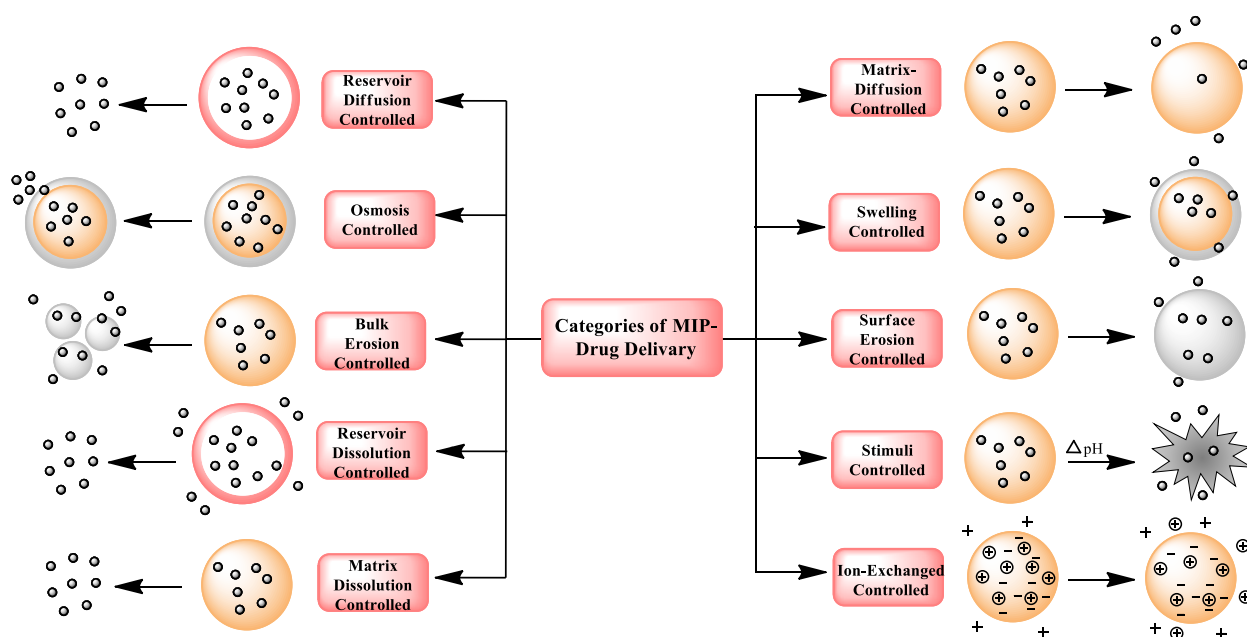


Figure 2.4: Types of MIP-drug-releasing ways for drug delivery (Wang and Recum., 2011; Jameledin et al., 2020).

For MIP, the drug release mechanism is divided into two stages: binding-desorption and diffusion. During the first phase, the therapeutic agent diffuses after desorbing from the MIP's reservoir-like selective binding chambers (Parisi et al., 2020). The sustained release mechanism is determined by the template's and functional monomer's interaction strength during the pre-polymerisation stage. This can be achieved by both non-covalent and covalent approaches (Zaidi, 2016b; He et al., 2021). The release mechanisms of MIP also rely on the polymeric structure of being hydrophobic or hydrophilic, as well as the factor of the dissolution media (acidic, neutral, or basic medium), which eventually resulted in the swelling behaviour of MIP (Fareghi et al., 2017b). The drug release can be optimised by using compatible hydrophilic-hydrophobic polymer blends and improving the drug-matrix compatibility (Calori et al., 2020).

Generally, polymers are hydrophobic beads with high protein binding abilities (Verma et al., 2015). The inclusion of 2-hydroxyethyl methacrylate (HEMA) (Ceglowski et al., 2019) and 2-hydroxyethyl acrylamide (HEAA) (Paul et al., 2017) has increased the hydrophilicity of the polymer and thus induces deprotonation of carboxylic group at high pH value (pH 7) which then causes the polymer to swell by the occurrence of repulsive forces within the polymer matrix and disrupts the structure of the cavities, facilitating the drug release. Meanwhile, the ionisation of the carboxylic groups was minimal in an acidic environment thus resulting in stronger hydrogen bond interactions with the template, which slowed the release process (Ji et al., 2020). The inclusion of hydrophobic molecules such as polyhedral oligomeric silsesquioxanes (POSS) into poly(MAA-EGDMA) polymer network creating a hydrophobic environment was to achieve higher selectivity of drugs has shown lower paclitaxel release in pH 7 compared to pH 5 (Bai et al., 2018).

Simultaneously, the dissolution media of acidic, basic, and neutral medium also plays a major role in the drug release mechanism. The destabilization of polymers such as collapsing or swelling, drug dissociation or changes in the drug partition coefficient are due to the pH-sensitive conformational changes (Rizwan et al., 2017). Components such as amino, imidazolyl and carboxyl groups can easily protonate or deprotonate, which destabilises the polymer network and causes swelling and collapsing (Rizwan et al., 2017; Torchilin, 2018). The solubility of drugs in the dissolution media also affects the release mechanism. This phenomenon is described by Ceglowski et al. (2022) on the release of sparingly soluble properties of 5-fluorouracil in water makes its affinity toward the hydrophilic environment higher. Thus, the hydrogen bond interaction between polymer networks and 5-FU is weak and easily breaks in the hydrophilic environment. Additionally, smaller nanoparticles demonstrate a higher release rate due to their increased surface area-to-volume ratio. This augmented surface area allows for enhanced accessibility, resulting in a greater degree of wettability (Loghmani et al., 2021). This concludes that the release mechanism was affected by the properties of the drug (template), the MIP composition, which reflects on the hydrophilicity and hydrophobicity properties of the MIP, and the interaction of drug molecules with the polymer cavity.

2.3 Other Ideal Parameters for DDS

In designing a controlled-release DDS, various factors must be considered, such as the drug loading, release rate, biocompatibility, ease of preparation and cost. MIP is proven to have a higher loading capacity than its non-imprinted polymer (Bodoki et al., 2019) and controlled release rate (Liu & Poma, 2021; Băraian et al., 2022) as discussed earlier (Section 2.3.2). MIP

also have the advantages of low cost and ease of preparation (Li et al., 2019; Malik et al., 2019), increasing its suitability as DDS. Other factors to consider are biocompatibility and particle size and shape, which are further discussed.

2.3.1 Biocompatibility

MIP as a polymeric DDS, has a biomimetic behaviour that makes them biocompatible with biological systems (Iheaturu et al., 2019). The development of DDS has extensively used acrylic and methacrylic (monomers) and EGDMA (crosslinker) to ensure biocompatibility qualities (Liu & Poma, 2021) (Table 2.1). MAA and EGDMA are biocompatible in their polymerised forms, and substantial research has been done on their extensive use in creating polymers for pharmaceutical and biological applications (Parisi et al., 2020). The usage of different functional monomers and crosslinkers was observed. For example, furosemide-MIP (Fareghi et al., 2017a) and phenytoin base-MIP (Abdollahi et al., 2018) were prepared using acrylamide as functional monomers, and both demonstrated high loading capacity and sustained drug release characteristics. The usage of other crosslinkers, such as TRIM with monomer MAA, was reported in the preparation of MIP-DDS for paclitaxel and 5-fluorouracil, respectively. An *in vitro* cytotoxicity study was performed against several cancer cell lines, which displayed biocompatibility properties (Cegłowski et al., 2019; Ceglowski et al., 2022). Other studies also presented *in vitro* cytotoxicity results (Bai et al., 2018; Han et al., 2020) and *in vivo* studies (Paul et al., 2017; Zhu et al., 2017; Liu et al., 2018; Luo et al., 2019) confirming the biocompatibility of the prepared MIP-DDS.

For DDS application, the crosslinker with the least propensity towards the drug is the best option to reduce non-specific binding affinity and low selectivity (Shadabfar et al., 2020). For example, divinylbenzene (DVB) has the hydrophobic criteria, which is that it has two allyl terminals capable of crosslinking and is mechanically strong and resistant to heat and solvent attacks (Prasetya et al., 2020). It was demonstrated that the DVB was the best crosslinker in the formation of zidovudine MIP compared to EGDMA and TRIM (Muhammad et al., 2012).

The usage of DVB in the preparation of MIP-DDS has not been commonly reported. However, its biocompatibility in other biomedical fields is well-published, such as hydrogel (Hrib et al., 2019), protein immobilization (Rodrigues et al., 2015) and lipase immobilization (Kao et al., 2003). DVB, when combined with biocompatible coatings or in specific forms such as hypercrosslinked polystyrene-poly-DVB, triblock copolymers, and hydrogels, exhibits favourable biocompatibility features, although some forms may have poor mechanical properties (Wendler et al., 2003; Akkaramongkolporn et al., 2009; Davankov & Tsyurupa, 2011; Gonte et al., 2012; Rodrigues et al., 2015; Hrib et al., 2019). Polymers generally exhibit favourable biological behaviour such as bio-adhesion, permeability-enhancing properties and attractive physico-chemical characteristics (Verma et al., 2015). MIP has demonstrated its low toxicity, biodegradability, and biocompatibility as DDS (He et al., 2021; Lusina & Ceglowski, 2022).

2.3.2 Particle Size and Shape

In DDS, the size and shape are important for cell internalization, and particles above 5 μ m are not favourable (Liu et al., 2012). Some studies suggest that spherical particles are

favourable for drug delivery systems due to their consistent drug loading and release, high swelling capability, and good biocompatibility (Kwak et al., 2021; Dhamapurkar & Desai, 2022), tissue penetration, and vascular transport (Cooley et al., 2018; Yang et al., 2019)

Several factors, including the polymerisation method, choice of solvent, monomer concentration and temperature influence the morphology of polymers produced by precipitation polymerisation. The precipitation polymerisation or atom transfer radical precipitation polymerisation, can affect the particle morphology and size distribution (Lu et al., 2020). Spherical particles were most likely to be obtained *via* precipitation polymerisation with particle size of microspheres to nanospheres (Paul et al., 2017; Zheng et al., 2018). As the precipitation polymerisation reaction progresses, monomers first create oligomer radicals, which undergo cross-linking to create cross-linked nuclei. While the oligomers remain soluble in the reaction solvent, the monomers and oligomers are engulfed by the growing nuclei as the reaction proceeds, reaching a critical mass at which point they precipitate to form polymeric beads (Parisi et al., 2020). The usage of the β -cyclodextrin-chitosan microsphere, as reported by Chen and co-workers (2018) produces a well dispersed round particles with an average size of 15 μm . As for the surface imprinting method, the particle morphology will be dependent on the type of surface used, such as single-wall carbon nanotubes producing a graphite cylinder with a size ranging from 20 nm to 50 nm (Liu et al., 2018) and spherical polymer presence on multi-wall carbon nanotubes prepared *via* both surface imprinting and bulk polymerisation (Sajini et al., 2019). Other spherical MIP-DDS was reported by (Abdollahi et al., 2018; Bai et al., 2018; Cegłowski et al., 2019; Cegłowski et al., 2022) prepared *via* precipitation polymerisation.

Another aspect that impacts the polymer morphology is the type of solvent and its interaction with the monomer, which influences the nucleation and growth of polymer particles, ultimately affecting their size and shape (Zhang et al., 2022). The monomer concentration and temperature can also influence the particle size and distribution, with higher monomer concentrations and lower temperatures generally leading to larger particles (Lu et al., 2020), while the use of crosslinkers in precipitation polymerisation can lead to the formation of uniform polymer particles with narrow size distributions (Sinjari et al., 2022). In DDS, smaller particle size has shown burst release compared to larger size due to the diffusion rate, following Fick's law of diffusion. This is due to the larger particles have a lower surface area to volume ratio (Jun et al., 2021). Hence, the development of MIP for DDS is best prepared via precipitation polymerisation to ensure spherical particles. Moreover, these factors must be carefully considered and controlled to achieve the desired polymer morphology in precipitation polymerisation.

2.4 Factors Affecting the Performance of MIPs

The MIP performance (i.e., rebinding or adsorption capacity, drug release) is determined by its binding affinity and selectivity towards the template molecule, which is governed by the MIP composition (i.e., template, functional monomer, crosslinker, porogenic solvent and initiator) (Figure 2.5) (Pratama et al., 2020). Therefore, the design and synthesis of MIP of high selectivity require several considerations in choosing the variables, such as the type of functional monomers, crosslinking agents, initiators, solvents, and the polymer composition ratios (Parisi et al., 2022; Yuksel & Tektas, 2022).

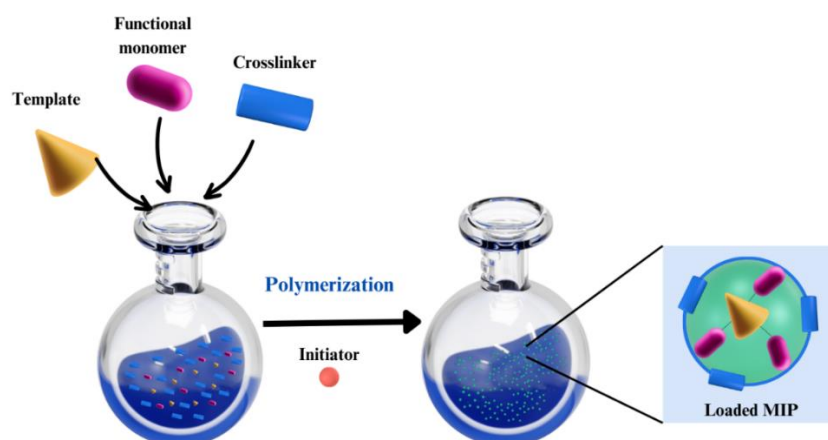


Figure 2.5: Basic components of MIP.

2.4.1 MIP Composition

The main components in the preparation of MIP are the target molecule (template), functional monomer and crosslinker. The template drives the imprinting process and is responsible for the interactions with the functional monomers. The affinity of the template towards the functional monomers is generated by the hydrogen bond or active substituents provided by the functional monomers (Gao et al., 2020a). The structure of the recognition sites of MIP is affected by the choice of functional monomer, while the number of binding sites is influenced by the concentration of the functional monomers (Wloch & Datta, 2019). Thus, the molar ratios between template and functional monomer in the synthesis process can affect the affinity and binding capacity of the MIP (Ariani et al., 2022). An equilibrium process governs the association between the template and functional monomer. Functional monomers are typically used in excess relative to the number of moles of a template (Cormack & Elorza, 2004; Vasapollo et al., 2011).

Many studies reported on the optimum ratios of a template to the functional monomers in designing MIP. The typical molar ratios of template to functional monomers (T:FM) used in non-covalent imprinting have been widely reported as 1:4 (Cormack & Elorza, 2004; Vasapollo et al., 2011; Wloch & Datta, 2019). A computational study performed by Ektirici et al. (2022) using Gaussview 5.0 program suggested that the most stable interaction between sulfamethoxazole and methacrylic acid (MAA) was 1:3 (T:FM) in acetonitrile as a solvent medium (Ektirici et al., 2022). A comparison of other different T:FM ratios in the adsorption of ofloxacin was performed using MAA as a functional monomer at T:FM ratios 1:4, 1:6, 1:8 and 1:10 which suggested that the adsorption properties increased as the T:FM ratio increased to the ratio of 1:8, which then the adsorption properties was decreased as the ratio increased to 1:10 (Wang et al., 2019). On the contrary, a ratio of 1:10 (T:FM) has been reported as optimum in preparing isosorbide dinitrate using MAA as the functional monomer (Mohebbali et al., 2018). The variety of T:FM suggested that the ratio is depending on the type of template and functional monomers where lower molar ratios of T:FM induced binding sites and over-high molar ratios produce higher non-specific binding capacity (Pandey et al., 2020). Figure 2.6 showed the of functional monomers used in the preparation of MIP.

Besides template and functional monomers, the role of crosslinkers (Figure 2.7) in the polymeric network is also vital to provide mechanical stability to the polymer structure. The crosslinker has a significant impact on the morphology characteristic, optimal stiffness, three-dimensional structure, recognition ability, and selectivity of the MIP (Pratama et al., 2020; Hasanah et al., 2021; Ariani et al., 2022). It also plays a significant role in fixing and holding the functional monomers around the template (Amorim et al., 2022). The amount of crosslinker

used can affect the structural stability of MIP. A low amount of crosslinker will reduce the structural stability. In contrast, an excess amount can increase the pore diameter size of the MIP, thus reducing the number of recognition sites and binding capacity of the MIP (Malik et al., 2019). Therefore, uniform functionalized sites throughout the polymeric network can be attained *via* the proper ratio of monomer to crosslinker and reactivity of the crosslinker, which usually uses an excess of 80% crosslink ratios (Pandey et al., 2020; Amorim et al., 2022).

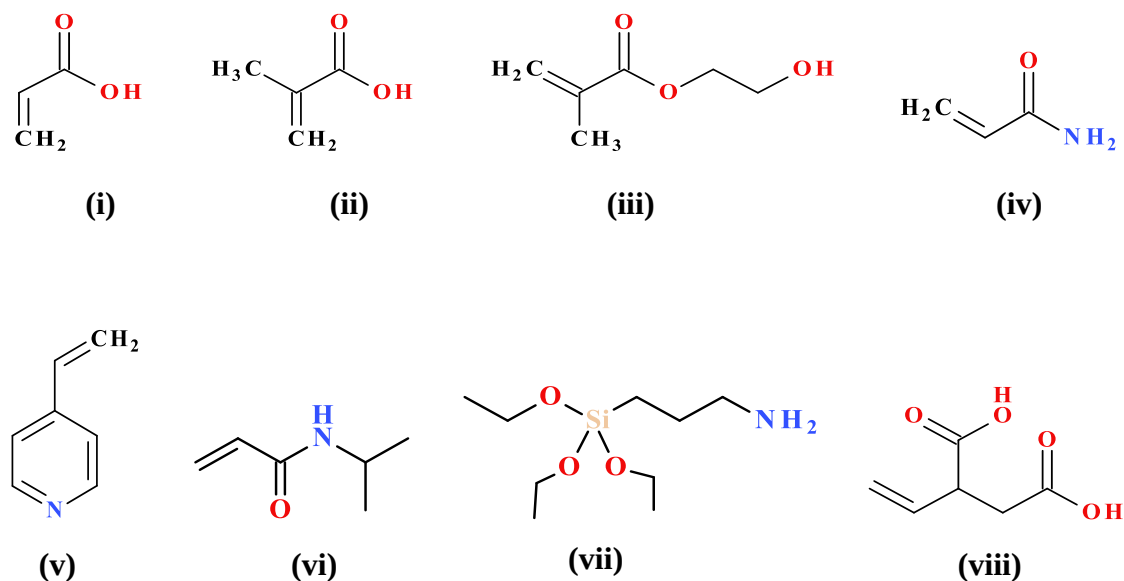


Figure 2.6: Common functional monomers used in the preparation of MIP. (i) acrylic acid (Rostamizadeh et al., 2012), (ii) methacrylic acid (Morsi et al., 2021), (iii) 2-hydroxyethyl methacrylate (Ceglowski et al., 2019), (iv) acrylamide (Fareghi et al., 2017a) (v) 4-vinylpyridine (Yoon & Byun, 2013), (vi) N-Isopropylacrylamide (Sedghi et al., 2018) (vii) 3-aminopropyltriethoxysilane (Wei et al., 2015) (viii) itaconic acid (Alanazi et al., 2021).

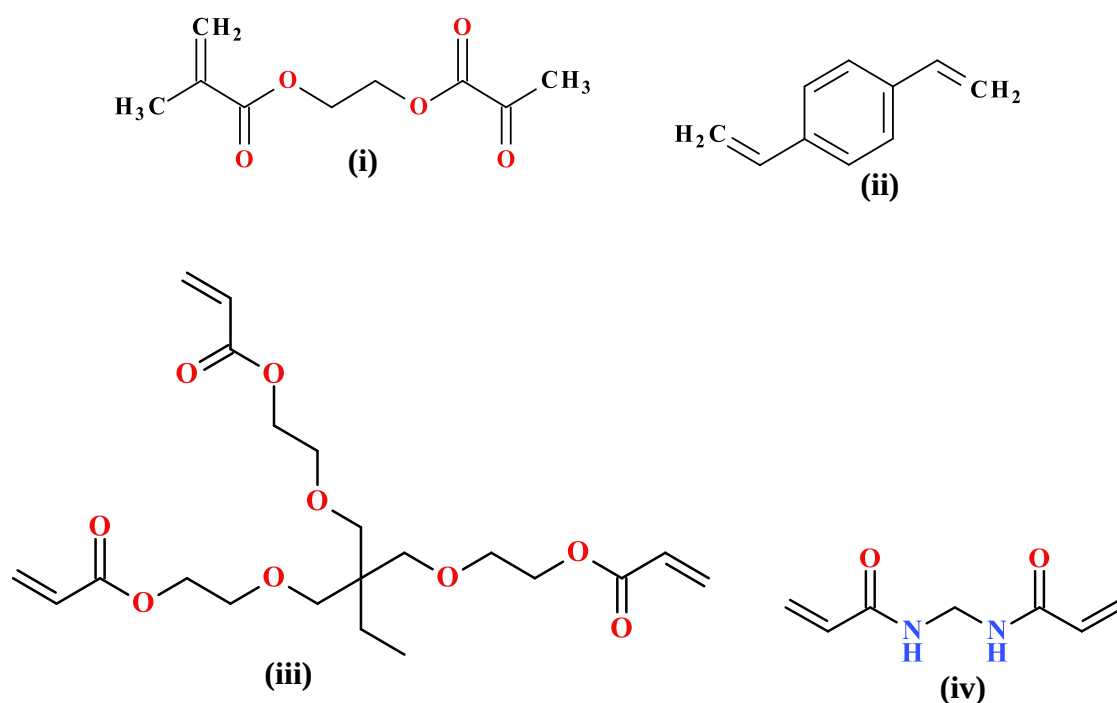


Figure 2.7: Crosslinkers used in the preparation of MIP. (i) Ethylene glycol dimethacrylate (Morsi et al., 2021), (ii) divinylbenzene (Muhammad et al., 2012), (iii) Trimethylolpropane trimethacrylate (Zhu et al., 2017) (iv) N,N'-Methylenebisacrylamide (Paul et al., 2017).

Many reports suggest the standard formulation ratio for the development of MIP as 1:4:20 (template: functional monomer: crosslinker, T:FM:CL) (Kueseng et al., 2018). This is supported by Bhawani et al. (2018) and Til et al. (2020) in their preparation of gallic acid (acrylic acid and ethylene glycol dimethacrylate (EGDMA) as FM and CL, respectively) and sinapic acid (4-vinylpyridine and EGDMA as FM and CL, respectively). However, the ratio of 1:6:20 (T:FM:CL) was found to be the optimum ratio for preparing sulfadimethoxine by using MAA and EGDMA as functional monomers and crosslinkers, respectively. With EGDMA acting as a crosslinker, the synthesis of ciprofloxacin using dual functional monomers (MAA and 2-vinylpyridine) show that 1:10:50 (T:FM:CL) yields the maximum extraction recovery of

ciprofloxacin at $104.6 \pm 7.9\%$ (Thach et al., 2021). Another dual functional monomer, MAA and hydroxyethylmethacrylate, was also reported for preparing paclitaxel using various crosslinkers. The study demonstrated that varied optimum formulation ratio was achieved using different crosslinkers, which were 1:6:17.78 (T:FM:CL) for EGDMA and 1:6:4 (T:FM:CL) for trimethylolpropane trimethacrylate (TRIM) (Ceglowski et al., 2019). Another crosslinker has also been reported for the preparation of MIP for amoxicillin with the formulation ratio of 1:4:8 (T:FM:CL) and showed good binding affinity by MAA-EGDMA compared to MAA-TRIM (Lowdon et al., 2021). This suggested that the optimum formulation ratio will differ for each template and other composition used in the preparation of MIP and require thorough investigation when developing a new MIP composition.

2.4.2 Effect of Solvent in MIP

Porogenic solvents play a vital part in the preparation of MIPs. The solvent acts as a dispersion medium, bringing all the components (template, monomers, crosslinkers and initiator) into one phase (Malik et al., 2019). Porogenic solvents also increase the surface area, ensuring the capacity of the imprinted polymers (Mueller, 2021). The synthesis of MIP usually takes place in an organic solvent for better electrostatic interactions and hydrogen bonding between templates and functional monomers (Ariani et al., 2022). The polarity of organic solvents significantly affects the template-monomer interactions where the less polar solvents will promote the formation of the template-monomer functional complex, whereas more polar solvents interfere with the interactions in the template-monomer functional complex (Bondi et al., 2013; Hasanah et al., 2021).

An aprotic solvent such as toluene (Heravizadeh et al., 2018; Elfadil et al., 2021), dichloromethane (Puzio et al., 2013; Zheng et al., 2018), acetonitrile (Roland & Bhawani, 2016; Cantarella et al., 2019; Hou et al., 2020), dimethylsulfoxide (Kunsa-Ngiem et al., 2018; Lu et al., 2018) and chloroform (Heravizadeh et al., 2020) are often used in non-covalent imprinting method to eliminate the nonspecific hydrophobic interactions, while promoting the hydrogen bonding and electrostatic interactions between the template and monomer complex to obtain good imprinting efficiency (Pichon et al., 2020; Pratama et al., 2020). Meanwhile, a highly polar protic solvent such as ethanol, methanol or water often interferes with hydrogen bonding between the template and functional monomer (Włoch & Datta, 2019). However, when applied to the actual imprinting process, ethanol (Rahimi et al., 2019) and methanol (Ali et al., 2022) showed good template extraction and adsorption capacity, respectively.

The vast usage of acetonitrile in molecular imprinting *via* various methods (i.e. bulk polymerisation, precipitation polymerisation) is due to its capability of producing MIP with high binding capacity and spherically shaped particles (Esfandyari-Manesh et al., 2011; Xavier et al., 2019). Supported by computer simulations, acetonitrile has a great capacity to solvate template functionalities through electrostatic interactions compared to dimethylsulfoxide and ethanol (Olsson et al., 2012; Ektirici et al., 2022) where the high dielectric constant of acetonitrile enhanced the $\text{OH}\cdots\text{O}$ and $\text{C}=\text{O}\cdots\text{H}$ interactions between template and functional monomers (Xavier et al., 2019). Suitable porogen are carefully selected to control and obtain the porous properties of the MIPs, which influences the removal and rebinding process of template molecules from the polymers by facilitating mass transfer and allowing easy access of template to the binding sites (Bondi et al., 2013; Yusof et al., 2020).

2.4.3 Effect of Initiator

An initiator is an agent responsible for forming free radical polymerisation. Peroxy and azo compounds are commonly used as initiators in the synthesis of MIP (Wloch & Datta, 2019). Azobisisobutyronitrile (AIBN) is the most common initiator used for the imprinting process, followed by benzoyl peroxide (Royani et al., 2014; Triadhi et al., 2018), potassium persulfate and ammonium persulfate (Aghoutane et al., 2020; Zeng et al., 2020) (Table 2.1).

The initiator's influence extends to the overall structure of the polymer chains, affecting their length, branching, and, consequently, the formation of binding sites (Chaudhary & Sharma, 2019; Zhang et al., 2021). Lim & Holdsworth (2018) described that a higher amount of AIBN initiators (0.1 mmol) could affect template stability and functional monomer interactions due to heat release during polymerisation. Meanwhile, Mohebbi et al. (2018) used AIBN in the 0.5 - 3 mmol range and reported that the particle size increased as the initiator concentration increased. A similar finding was reported by Zhao et al. (2020a), employing potassium persulfate (KPS) in 8 – 16 mg as an initiator. Higher initiator concentrations impact the polymerisation rate, which in turn affects the physical characteristics of the MIP, such as pore volume and pore structure.

This suggested that larger particle size was produced at a higher amount of initiator. In contrast, a low amount of initiator induces low polymer conversion (Xiao et al., 2016; Lim & Holdsworth, 2018; Mohebbi et al., 2018). Additionally, initiators contribute to the MIP's porosity and surface area, potentially enhancing mass transfer kinetics (Mohebbi et al., 2018).

Table 2.4: General preparation of MIP *via* various polymerisation methods.

Template (T), (Category), *Type of MIP	Functional monomers (FM), Crosslinker (CL), Solvent (S)	Initiator (Amount)	Polymerisation Method and Temperature	Findings	References
Quercetin hydrate (Flavonoids) *MIP monolith powder	(FM): AA (CL): TRIM (S): Tetrahydrofuran (5 mL) <i>T:FM:CL 1:8:40</i>	AIBN (Nil)	Bulk polymerisation i. 60°C x 16 h ii. 70°C x 3 h	Particle: MIP with amorphous surface. Adsorption capacity: 169 mg/g IF: 2.41	(Amiri et al., 2014)
Quercetin (Flavonoids) *MIP-coated fiber	(FM): APTES (CL): TEOS (S): Ethanol (9 mL) <i>T:FM:CL Nil.</i>	Nil.	Sol-gel method Room temperature, 12 h	Particle: uniform structure with many cavities. Extraction capacity: MIP: 19.5 ng NIP: 9.97 ng	(Rahimi et al., 2019)
2,3, 7,8- tetrachlorodibenzo- p-dioxin (Organic pollutant) *MIP monolith powder	(FM): MAA (CL): EGDMA (S): Chloroform (6 mL) <i>T:FM:CL 1:6:32</i>	1,1'- azobis(cyclohexa- necarbonitrile) ABCHC (0.103 g)	Bulk polymerisation 60°C x 24 h	Particle: irregular particles. Size range 56 – 74 µm Binding capacity: MIP: 10 mg/g NIP: 3.5 mg/g	(Sikiti et al., 2014)
Diclofenac (NSAIDs) *MIP monolith powder	(FM): MAA (CL): EGDMA (S): Chloroform: toluene (8 mL, 5:2, v/v) <i>T:FM:CL 1:4:24</i>	AIBN (0.06 mmol)	Bulk polymerisation 60°C x 20 h	Particle: Less than 75 µm Binding affinity in acetonitrile: MIP: 649±10.04 ug, IF: 1.35 Binding capacity in water: MIP: 1140±10.02 ug, IF: 1.32 Release profile at 20 h: pH 1.5: 14%, pH 6.0: 65% pH 8.0: 90%	(Mohajeri et al., 2012)

Table 2.4: Continued.

Diclofenac sodium (NSAIDs)	(FM): MAA (CL): TRIM (S): Tetrahydrofuran/ Acetonitrile (33 mL, 1:10, v/v) <i>T:FM:CL</i> <i>1:5:15</i>	AIBN (0.01 mmol)	Precipitation polymerisation	Particle: Uniform size and shape. 145.7 nm	(Noee et al., 2013)
*MIP powder			60°C x 17 h	Binding capacity: MIP: 150.4 mg/g IF: 125.4 mg/g	
Diclofenac sodium (NASIDs)	(FM): MAA (CL): EGDMA (S): Acetonitrile (8 mL) <i>T:FM:CL</i> <i>1:10:50</i>	AIBN (5.1 mmol)	Bulk polymerisation	Particle: Nil. Adsorption capacity: MIP: 110 umol/g NIP: 13 umol/g	(Cantarella et al., 2019)
*MIP monolith powder			70°C x 4 h		
Erythromycin (Antibiotic)	(FM): AA (CL): MBA (S): Acetonitrile: Deionized Water (16 mL: 20 mL) <i>T:FM:CL</i> <i>1:72:3</i>	AIBN (0.45 g, 3 mmol)	Bulk polymerisation	SEM: Rough surface of MIP than NIP.	(Hou et al., 2020)
*MIP monolith powder			60°C x 6 h	Binding capacity: MIP: 1048 mg/g	
Ciprofloxacin (Antibiotic)	(FM): MAA and 2-VP (CL): EGDMA (S): Chloroform: Methanol (12 mL, 0:1, v/v) <i>T:FM:CL</i> <i>1:6.6:3.3:50,</i> <i>(Cip: MAA:2- VP:EGDMA)</i>	AIBN (20 mg)	Bulk polymerisation	SEM: non-porous and equant polymer particles	(Thach et al., 2021)
*MIP monolith powder			60°C x 24 h	Binding capacity: MIP: 2.4 mg/g NIP: 1.45 mg/g	

Table 2.4: Continued.

Ziram (Fungicide)	(FM): MAA (CL): EGDMA (S): Chloroform (20 mL) Other: PVA solution <i>T:FM:CL</i> <i>1 :8 :40</i>	AIBN (20 mg)	Suspension polymerisation 60°C x 24 h	SEM: irregular spherical shapes with heterogeneous sizes. MIP = 30 µm Adsorption coefficient, Qmax MIP: 56.98 mg/g, NIP: 17.75 mg/g	(Öter & Zorer, 2021)
Piperine (Alkaloid) *MIP powder	(FM): AA (CL): EGDMA (S): Acetonitrile (75 mL) <i>T:FM:CL</i> <i>1 :4:24</i>	AIBN (0.011 g)	Precipitation polymerisation. 60°C x 6 h	Morphology: Spherical particles Binding capacity: 84.94 %	(Roland & Bhawani, 2016)
Ofloxacin (Antibacterial agent) *MIP powder	(FM) : MAA and GMA (CL) : EDMA (S): MeOH/H ₂ O (50 mL) <i>T:FM:CL 1 :8:8:30</i>	AIBN (25 mg)	Precipitation polymerisation. 60°C x 24 h	Morphology: anomalistically spherical in shape that the surface was coarse Maximum adsorption capacity : 17.49 mg/g	(Wang et al., 2019)
Tartrazine (Water soluble acid dyes) *Magnetic-MIP	(FM): 1-(α-methyl acrylate)-3- methylimidazolium bromide (1-MA-3MI-Br) (CL): TRIM (S): Toluene:Water (2 mL: 130 mL) Other: Span 80 Magnetic: Fe ₃ O ₄ <i>T:FM:CL 1 :4:31</i>	AIBN (0.1 g)	Emulsion polymerisation 70°C x 12 h	Morphology: spherical shaped particles with diameter distribution from 20 - 100 µm. Number of binding sites: MIP: 25.43±0.60 mg/g NIP: 8.32 ±0.69 mg/g	(Luo et al., 2011)

Table 2.4: Continued.

Tartrazine (Water soluble acid dyes) *Magnetic-MIP	(FM): Acrylamide (CL): N,N'-methylene- bis- acrylamide (S): Distiled water (40 mL) Magnetic: Fe ₃ O ₄ <i>T:FM:CL</i> <i>1 :2:10</i>	KPS (0.025 g, 0.185 mmol)	Precipitation polymerisation. 70°C x 22 h	Morphology: Agglomerated spherical particles under SEM. TEM images showed Cubic shaped magnetic particles surrounded by the polymeric network. Tartrazine degradation efficiency: 98.7% within 180 min.	(Arabzadeh et al., 2019)
Fenthion (Organo- phosphorous pesticide) *MIP powder	(FM) : MAA (CL) : EGDMA (S): Chloroform (70 mL) <i>T:FM:CL</i> <i>1 :4:20</i>	AIBN (40 mg)	Precipitation polymerisation. 55°C x 18 h	Morphology: Spherical porous, rough surfaces with diameter between 31–42nm. Adsorption efficiency of MIP is 97.87 ± 1.13% compared to NIP of 34.91 ± 2.48%.	(Heravizadeh et al., 2020)
Tylosin tartrate (Microlide antibiotic) *MIP powder	(FM) : MAA (CL) : EGDMA (S) : DMSO:MeOH (70:3, v/v, mL) <i>T:FM:CL</i> <i>1:10:20</i>	Ammoniu m persulfate (APS) (0.1g)	Seeded precipitation polymerisation. 55°C x 12 h	Morphology: Rough surface with porous structure. Statistic average diameter of MIP is about 50 µm and that of NIP is 35 µm. Adsorption capacity: MIP: 106.5 mg/g IF: 3.6	(Zeng et al., 2020)
Malathion (Herbicide) *MIP sensor	FM) : Acrylamide (CL) : Bisacrylamide (S) : PBS solution (5 mL, pH 7.45) <i>T:FM:CL</i> <i>1:4:2</i>	Ammoniu m persulfate (APS) (0.1g)	Surface imprinting 74°C x overnight	Morphology : rough and uniform surface	(Aghoutane et al., 2020)

Table 2.4: Continued.

Atrazine (Herbicide)	(FM) : MAA (CL) : EGDMA (S): Acetonitrile (30 mL) <i>T:FM:CL</i> <i>1 :14.3:57.1</i>	AIBN (280 mg)	Bulk polymerisation 65°C x 14 h	Morphology: Nil. Extraction efficiency: Optimum extraction at pH 4 – 9, temperature 45°C. High selectivity for atrazine compared to other analogue.	(Djozan & Ebrahimi, 2008)
*MIP monolith fibers					
Atrazine (Herbicide)	(FM) : MAA (CL) : EGDMA (S): Chloroform (2.01 mL) <i>T:FM:CL</i> <i>1 :4:20</i>	BPO (0.07 g)	Bulk polymerisation 70°C x 150 min	Morphology: Rough surface with cavities size range 80 -230 nm Binding capacity: MIP: 14.9 mmol/g NIP: 1.99 mmol/g	(Royani et al., 2014)
*MIP monolith					
Solanesol (Intermediate Drugs)	(FM): MMA (CL): EGDMA (S) : SDS:H2O (40:15, mg:mL) <i>T:FM:CL</i> <i>1 :8:30</i>	KPS (10 mg)	Emulsion polymerisation 70°C	Morphology: Spherical particles. Size distribution at 50 – 500 µm, with 76% at 125 – 300 µm. Adsorption capacity: MIP: 67.55 mg/g NIP: 26.87 mg/g IF: 2.51	(Zhao et al., 2020a)
*MIP					

AA, Acrylic acid; TRIM, trimethylolpropane trimethacrylate, AIBN, Azobisisobutyronitrile ; APTES, 3-Aminopropyltriethoxysilane ; TEOS, Tetraethyl orthosilicate ; MAA, methacrylic acid ; EGDMA, ethylene glycol dimethacrylate ; ABCHC, 1,1'-azobis(cyclohexanecarbonitrile); MeOH, methanol; H₂O, water; MBA, N,N'-Methylenebisacrylamide; GMA, glycidyl methacrylate; PVA, polyvinyl alcohol; 2-VP, 2-vinylpyridine; KPS, potassium persulfate; BPO, benzoyl peroxide, PBS, phosphate buffer solution; DMSO, dimethylsulfoxide;

2.4.4 Polymerisation Temperature

The polymerisation temperature can affect the selectivity of MIP and is often associated with the use of an initiator. The performance of MIP might be impacted by the decomposition temperature of the initiator used in the synthesis process (Sadia et al., 2023). Research has shown that polymerisation at lower temperatures (60 °C) can lead to MIP with higher selectivity and improved enantioselectivity in some cases (Sajini & Mathew, 2021). MIP are also known for their resistance to elevated temperatures and ability to maintain recognition capacity for several years at room temperature, highlighting their stability over a wide temperature range (Vasapollo et al., 2011). Elevated temperatures can reduce the uncertainty of the thermal resistance in MIP-based biomimetic sensors, resulting in a more stable signal (Geerets et al., 2013). MIP have been shown to withstand exposure to temperatures of up to 150 °C for 24 h without losing affinity for the template, indicating a degree of thermal stability (Svenson & Nicholls, 2001).

Additionally, some MIP have been reported to exhibit thermal stability up to 300 °C (Elugoke et al., 2021) and 320 °C (Jian et al., 2020), with specific monomers contributing to their stability. Polymerisation at 125 °C for 48 h was reported, resulting in the formation of solids during polymerisation. Amorphous solids were observed after 20 min of reaction when benzoyl peroxide was used as the initiator, while no solids were formed after 16 h when nitroxide-mediated polymerisation was used (Boonpangrak et al., 2006). The polymerisation temperature can vary from room temperature (Mazzotta et al., 2023) to an elevated temperature (Jian et al., 2020), as shown in Table 2.4.

Overall, the effect of temperature on MIP is a critical factor in their synthesis and performance, influencing their selectivity, sensitivity, and potential applications. It is important

to note that the selectivity of MIP is not solely dependent on temperature but also on other factors, such as polymer components and the application for which the MIP is employed (Beckskereki et al., 2021). This is due to the MIP properties, such as particle morphology, size, and binding affinity, affected by the choice of the components and polymerisation method (Pratama et al., 2020).

2.5 MIP for Analgesic Drugs

The development of MIP for many types of drugs has received broad interest among researchers. MIP for analgesic drugs, including NSAIDs such as diclofenac (Mohajeri et al., 2012; Noee et al., 2013; Cantarella et al., 2019), naproxen (Dehghani et al., 2021; Husin et al., 2021), ibuprofen (Yildirim & Caykara, 2018) and aspirin (Abd et al., 2019) have been reported for various applications (e.g., SPE, DDS). The daily usage and availability of these drugs in the market have prompted an exploration of the impact of these drugs on patient compliance as well as their discharge as environmental waste (Huang et al., 2014).

Utilising aspirin in DDS was only reported by Duarte et al. (2006) and Kan et al. (2009) using two different polymerisation methods, namely supercritical fluid-assisted polymerisation and precipitation polymerisation, respectively (Table 2.5). The magnetic MIP for aspirin has shown sustained release up to 10 h with 50% drug release at 2 h (Kan et al., 2009). Meanwhile, up to 90% of aspirin release in the first 10 h was observed by MIP prepared *via* supercritical fluid polymerisation (Duarte et al., 2006). No current research was observed in the preparation of aspirin MIP for DDS except for its usage in other applications such as separation (Sooraj &

Mathew, 2015), solid-phase extraction (Meischl et al., 2016; Abd et al., 2019), and sensor (Pawley et al., 2017; Mansoor & Al-Bayati, 2021).

Paracetamol is another analgesic drug; however, less reported for DDS (refer to Section 2.2) (Liu et al., 2020). The MIP for paracetamol was widely applied as a sensor (Huang et al., 2018; Decorbie et al., 2020; Alanazi et al., 2021; Yang et al., 2021; Zheng et al., 2022) and solid phase extraction (Abbasi et al., 2019) as shown in Table 2.6. It was observed that various polymer composition ratios were reported for the preparation of aspirin and paracetamol MIP, indicating that any new development for aspirin and paracetamol MIP should consider a thorough investigation. Also, considering the lack of data in the preparation of MIP-DDS for both aspirin and paracetamol, the urge to develop a new formulation of MIP-DDS for both aspirin and paracetamol has emerged, and an optimisation and cost-effective approach is required.

Table 2.5: The preparation of MIP for aspirin in various applications.

Types of MIP/ Application	Preparation method	Template (T), Monomer (FM) Crosslinker (CL) Solvent (S) Initiator (I)	Components ratio (mmol) (T:FM:CL)	Particle morphology & size	Findings	Reference
Controlled drug release	Supercritical fluid assisted polymerisation. (65°C x 180 min, 19 MPa pressure)	T: Aspirin FM: DEDGMA Stabiliser : Krytox S: Methanol (5 mL) I : AIBN	*Not applicable to be calculated due to lack of information	Discrete spherical particle with 1.7 µm diameter.	Supercritical fluid polymerisation successfully prepares molecularly imprinted polymers for controlled drug delivery, with salicylic acid and acetylsalicylic acid templates showing promising results.	(Duarte et al., 2006)
Magnetic MIP / controlled drug release	Precipitation polymerisation (70°C x 6 h)	Magnetic functionalized : Fe ₃ O ₄ (0.2g) T: Aspirin FM: MAA CL: TRIM S: Chloroform (40mL) I :AIBN (50mg)	1:4:2	Regular spherical shape Average size 500 nm	Adsorption capacity: 99.9 umol/g, IF 2.39 Release profile: 50% release within 2 h, and slower and achieved controlled manner at 10 h.	(Kan et al., 2009)
Separation	Supercritical fluid assisted polymerisation. (65°C x 20 h, 300 bar pressure)	T: Aspirin/ paracetamol FM1: MMA FM2 : MAA / 4-VP CL: EGDMA S: THF (1mL) I :AIBN (0.3g) Dispersion agent: PHDFDMA (0.65g)	1:10:2.5:1.5 (T:FM1:FM2 :CL)	Irregular shape. Average size: 250 – 300 nm.	MIPs synthesised using supercritical fluid technology efficiently separate and detect acetaminophen (paracetamol) and aspirin, with 4-VP as a more efficient binding monomer	(Yoon & Byun, 2013)

Table 2.5: Continued.

Separation	Surface imprinting (70°C x 24 h)	Surface : MWCNT T: Aspirin FM: 1-vinylimidazole CL: EGDMA S: Chloroform (25mL) I :AIBN (50mg)	1:5:10	Turbular morphology due to the presence of MWCNT	Adsorption capacity: 0.6442 mmol/g at 4 mmol/L 16.14% higher than MIP prepared via bulk polymerisation	(Sooraj & Mathew, 2015)
MISPE	Sol-gel imprinting (50°C x 3 h)	T: Aspirin and metabolites (2mL) FM: APTES (200µL) CL: TEOS (800µL) S: acetonitrile (2mL) I :Ammonium hydroxide (400µL)	*Not applicable to be calculated due to the mixture of aspirin metabolites	Uniform irregular particle size with average of 150 nm.	Binding efficiency: 70-95%	(Bhatia et al., 2016)
SPE	Bulk polymerisation (60°C x 24 h)	T: Aspirin FM: AA CL: EGDMA S: Acetonitrile (10 mL) I : AIBN (24.6 mg)	1:6:30	Non-spherical particles. Surface area 334.5 m ² /g	Maximum loading capacity at pH 3 is 328.6 mg/g	(Meischl et al., 2016)
CdTe-QDs-MIP / sensor	Surface imprinting / RT for 12 h	Surface : CdTe-QDs T: Aspirin FM: APTES CL: Tetraethoxysilane S: Water (10mL) I : Nil. Catalyst: NH ₃ .H ₂ O (0.1 mL)	1:4:20	imprinted QDs exhibited a well-defined core-shell structure and had shell thicknesses of about 8–15 nm	Core-shell CdTe quantum dot-imprinted polymers effectively recognize and determine aspirin in human urine and saliva, with optimised optical stability, detection time, and detection limits.	(Wei et al., 2015)

Table 2.5: Continued.

Monolithic solid phase micro-extraction (SPME) / Extraction	Bulk polymerisation (60°C x 24 h)	T: Aspirin FM: MAA CL: TRIM S: Ethanol (7 mL) I : benzoyl peroxide (0.5g)	1:2.33: 20.33 1:4.94:53.92 1:3.02:29.79 (<i>optimum</i>)	Spherically form particles. 2.941-11.64 µm.	98.88% recovery (0.5g MIP, 60ppm)	(Abd et al., 2019)
		T: Aspirin FM: Styrene CL: TRIM S: Ethanol (7 mL) I : benzoyl peroxide (0.5g)	1:3.22:46.11 1:3.88:42.77 1:4.09:41.63 (<i>optimum</i>)	Spherically form particles. 2.50-4.16 µm.	97.63% recovery (0.5g MIP, 60ppm)	
MIP-CS-g-PMMA / Sensors	Surface grafting	Surface: Chitosan T: Aspirin FM: MMA CL: MBAA S: Acetic acid aqueous solution (60mL) I : ammonium persulfate (8.5mL)	*Not applicable to be calculated due to lack of data information	unimodal size distribution within 150– 200 nm	MIP-CS-g-PMMA nanoparticles show high efficiency selective recognition and temperature memory, offering a new functional polymer material for biosensors	(Zheng et al., 2016)
Ag-nanoporous imprinted matrix / Sensor	Bulk polymerisation (60°C x 24 h)	T: Aspirin FM: Acrylamide (AA) CL: EGDMA S: acetonitrile (5mL) I : AIBN (0.025g)	1:6:30	Nanosponge structure with large set of nano-sized cavities	Rebinding study: concentration-dependent increase in the phase shift of a thermal wave.	(Pawley et al., 2017)

Table 2.5: Continued.

Chemo-sensors	Electro-polymerisation	MIP-ano-film: T: Aspirin FM: 3-TCA and 3-TBA CL: Thiopene S: acetonitrile I :benzoyl peroxide Other: sacrificial alumina plates MIP-mic-film T: Aspirin FM: 3-TCA and 3-TBA CL: Thiopene S: SDS-water I :benzoyl peroxide	1 :5 :20	• MIP-ano-film: densely packed nanowires or brush like structures with long-range uniformity thicknesses in the range 46 ± 5.2 nm MIP-mic films: uniform coatings in the micron size range (1.1 ± 0.3 μ m) that coalesced to a particulate film	Sensor sensitivity towards aspirin: (i) MIP-ano-based chemosensor: 20.62 Hz/mM (ii) MIP-mic film: 2.80 hz/mM MIP-ano-film is eight times sensitive towards aspirin than MIP-mic film.	(Suriyanar ayanan et al., 2017)
Electrode MIP / sensor	Bulk polymerisation (60°C until hardened)	T: Aspirin FM: vinyl acetate CL: MBAA S: Methanol (5 mL) I : Benzoyl peroxide (0.32g)	1:6:30	Irregular agglomerated particles	The MIP electrode have high sensitivity towards aspirin	(Mansoor & Al-Bayati, 2021)

*3-TCA, 3-thiopheneacetic acid; 3-TBA, 3-thienylboronic acid; 4-VP, 4-vinylpyridine; DEDGMA, Di-ethylene glycol dimethacrylate; APTES, 3-aminopropyltriethoxysilane; TEOS, tetraethyl orthosilicate; MBAA, N,N'-Methylenebisacrylamide; MMA, methyl methacrylate; THF, tetrahydrofuran; SDS, sodium dodecyl sulfate;

Table 2.6: The preparation of MIP for paracetamol in various applications.

Types of MIP/ Application	Preparation method	Template (T), Monomer (FM) Crosslinker (CL) Solvent (S) Initiator (I)	Components ratio (mmol) (T:FM:CL)	Particle morphology & size	Findings	References
Sensor	Surface Imprinting. (65°C x 24 h)	Surface: Bulk acoustic wave (BAW) sensor T: Paracetamol FM: 4-VP and MAA CL:EGDMA S: Acetonitrile (6 mL) I : AIBN (20mg)	1:2:2:20	Nil.	MIP sensors prepared with both FM (4-VP & MAA) showed high selectivity towards paracetamol.	(Tan et al., 2001)
Sensors	Electropolymerisation (cyclic voltammetry in the potential range between -0.1 and 1.25 V during 20 cycles at a scan rate of 50 mV/s)	Surface: Carbon fiber microcylinder electrodes T: Paracetamol FM: o-PD and aniline S: H2SO4 (0.1 M) I : Nil.	*Not applicable to be calculated due to lack of data information	Nil.	Paracetamol voltammetric microsensors based on electrocopolymerised–molecularly imprinted film modified carbon fiber microelectrodes	(Gómez-Caballero et al., 2005)
Electrochemical sensor	Electropolymerisation (cycling potential for 8 times between –0.6 V and 1.8 V)	Surface: PCz-co-PPy)/AuPd/GN- CNTs-IL/GCE T: Paracetamol FM: Carbazole and Pyrrole S: Acetonitrile	Not applicable for calculation due to lack of data information	MIP(PCz-co-PPy)/ AuPd/GN- CNTs-IL composite is more porous and has larger surface area than MIP film	detection of PCM in biological samples, with acceptable recoveries of 84.5%-102% and with a low limit of detection of 50 nM	(Yang et al., 2021)

Table 2.6: Continued.

Electrochemical Sensor/ drug detection	Surface imprinting (UV exposure for 1.5 min)	Surface: screen-printed carbon electrodes T: Carboxyl-paracetamol (4-(4-hydroxyanilino)-4-oxobutanoic acid FM: Itaconic acid CL: EGDMA and TRIM S: DMF (22 mL) I: N,N-diethyldithiocarbamic acid benzyl ester (0.3g) Redox label: FcMMA (0.17g) CTA: PETMP (0.1g)	T:FM = 1:5	NanoMIPs obtained were spherical with average size of 186 ± 38 nm	Sensor shows satisfactory performance in human plasma with recoveries of 94-108 %	(Alanazi et al., 2021)
Electrochemical Sensor	Surface imprinting (50°C x 24 h)	Surface: Glassy carbon electrode T: Paracetamol FM: α -methylphenenic acid CL:EGDMA S: DMSO (400 mL) I : AIBN (20mg)	T:FM = 1:5	Nil.	MIP-based electrochemical sensor effectively detects paracetamol with a detection limit of 3.6 pM and excellent anti-interference performance.	(Zheng et al., 2022)
Magnetic-MIP / SPE	Precipitation polymerisation (60°C x 20 h)	T: Paracetamol FM: MAA CL: 2-(methacrylamido) ethyl methacrylate S: acetonitrile/ chloroform (5:1, v/v, 30 mL) I: AIBN (0.1 mmol) Magnetic: Fe ₃ O ₄	1:4:10	Spherical magnetic particles.	Maximum adsorption capacity: 84 mg/g at pH 9.0.	(Azodi-Deilami et al., 2014)

Table 2.6: Continued.

MI-SPE	Precipitation polymerisation (80°C x 24 h	T: Paracetamol FM: MAA CL: EGDMA S: Acetonitrile (50mL) I: AIBN (10 mg)	1:15:10	Nil.	Extraction recovery of paracetamol: 96 – 103.8%	(Abbasi et al., 2019)
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2.6 Optimisation and Cost-Effective Approach in MIP Preparation

2.6.1 Multivariate Analysis using Response Surface Methodology (RSM)

A laborious experimental setup was required to develop a new formulation of MIP for DDS, especially when utilizing a one-variable-at-a-time approach (Renkecz et al., 2014; Lim & Holdsworth, 2018). The process is time-consuming, and the interaction that involves more than two response factors is not fully extrapolated (Heravizadeh et al., 2018). Multivariate techniques are cost-effective, robust and efficient statistical approaches widely applied for optimising analytical procedures (Nezhadali & Mojarab, 2014). The response surface methodology (RSM) is a statistical tool used to optimise large numbers of parameters and illustrates the interaction between variables while minimizing experimental procedures (Vera et al., 2014; Mourabet et al., 2017; Xu et al., 2021). This method has been widely used in various analytical fields and MIP.

Application of RSM in the preparation of MIP is often observed in the optimisation of the extraction procedure (Nasab et al., 2019; Hashemi et al., 2020; Heravizadeh et al., 2020; Kalogiouri et al., 2020; Sadegh et al., 2021; Tamandani et al., 2022), adsorption study (Xu et al., 2021), photocatalytic degradation process (Arabzadeh et al., 2019), and separation (Ferey et al., 2013). Only a few studies reported on utilizing RSM to prepare MIP, such as diazinon (Rahiminezhad et al., 2014) and fenoxycarb (Atayat et al., 2019). Successful interactional behaviour was observed *via* response surface graph between five critical factors (i.e. the amounts of template, porogenic solvent, initiator, functional monomer to cross-linker ratio, and polymerisation temperature) (Rahiminezhad et al., 2014) and two critical factors (i.e. volume of

acetonitrile and amount of crosslinker) (Atayat et al., 2019) while minimizing the experimental procedures indicating that RSM is suitable for optimisation approach in the preparation of MIP.

2.6.2 Conventional and Microwave-assisted Synthesis of MIP

MIP can be synthesised *via* several methods developed over time to obtain a better MIP result (Yemis et al., 2013). Typically, bulk polymerisation, precipitation polymerisation, pickering emulsion polymerisation, multi-step swelling polymerisation, and surface imprinting processes are used in the conventional heating process of synthesising MIP (Ertürk & Mattiasson, 2017; Fresco-Cala et al., 2020). Nevertheless, the conventional method offers a longer reaction time (6-24 h), and uneven heat distribution prompts the exploration of alternative approaches (Kueseng et al., 2018).

In recent years, microwave (MW)-assisted synthesis has emerged as an alternative and efficient technique for polymerisation (Lamaoui et al., 2021). A cost-effective approach also can be achieved *via* MW-assisted synthesis by reducing the polymerisation time (Kunsa-Ngiem et al., 2018). MW-assisted synthesis is a fast and well-controlled reaction used extensively in organic synthesis and recently explored in polymerisation reactions (Brahmbhatt et al., 2020). The MW irradiation interact with polar molecules and ions, causing them to oscillate and rotate. This molecular movement generates heat through dielectric heating and dipolar polarization (Grewal et al., 2013). The direct interaction with the reactants leads to rapid and uniform heating, reducing reaction times and improving reaction efficiency. This can also lead to higher yields and selectivity in the products (Kumar et al., 2020). There are various types of MW and

irradiation settings used in the polymerisation of MIP, such as at MW power 80 W (Liang et al., 2020; Zhu et al., 2021), 700W (Lu et al., 2018; Zhang et al., 2021) and 800 W (Awokoya et al., 2022). The rapid polymerisation *via* MW observed in various research is shown in Table 2.7.

The utilization of MW has also been reported in various steps in MIP preparation, such as in the synthesis of aldehyde functionalized magnetic nanoparticles prior to the imprinting process (Sullivan et al., 2021), MW-synthesis of MBA-CdTe QDs as an epitope for imprinted polymer (Amiri & Hosseini, 2020), and in extraction process from MIP (Bi et al., 2019; Maragou et al., 2020). Triadhi et al. (2018) synthesised a non-imprinted polymer (NIP) for the retention of catechin *via* the conventional thermal method (60-65 °C x 60 min) and MW-assisted synthesis (60-65 °C x 4 min), indicating that no physical differences between the NIP produced *via* the two polymerisation methods. Brahmabhatt et al. (2020) reported similar average pore radii observed on MIP prepared at a range of microwave power from 5W to 300W, indicating the particle size was not affected by the change of irradiation power. However, specific details on morphological changes induced by MW-assisted synthesis are not sufficiently explored compared to the conventional polymerisation method. MIP prepared using MW-assisted synthesis have demonstrated enhanced molecular selectivity. In general, the optimum MW irradiation setting on the synthesis of polymer can vary depending on the polymer composition.

Resveratrol-imprinted polymers synthesised with MW assistance showed similar binding capacities but superior binding site selectivity compared to conventional thermal synthesised polymers (Schwarz et al., 2015). Lamaoui et al. (2021) also reported efficient adsorption properties for the selective determination of sulfamethoxazole using magnetic MIP synthesised using MW assistance. Therefore, utilizing MW in MIP synthesis reduces

polymerisation time, improves MIP's adsorption properties and energy efficiency (Kunsa-Ngiem et al., 2018).

Table 2.7: Application of MW-assisted synthesis in the preparation of MIP.

Type of Microwave and Power Setting	Template (T), Functional monomers (FM), Crosslinker (CL), Initiator (I), Solvent (S)	T:FM:CL	Findings / Application	References
Mono-made Microwave synthesizer, MAS-ii, Shanghai, Power: 800W, 60°C x 1h	(T) : Malachite Green (FM) : Styrene (CL) : DVB (S) : Distilled water (5mL) (I) : Benzoyl peroxide (20mg) Other: Watermelon rind	1: 5.07 : 25.62	• Binding capacity: 92.5% • Morphology: Porous with irregular pores • Application: Dye removal from wastewater	(Awokoya et al., 2022)
ETHOS 1 Milestone Advanced Microwave Digestion System. 70°C x 20 min	(T) : Sulfamethoxazole (FM) : Acrylamide or MAA (CL) : EGDMA (S) : DMSO (8.5mL) (I) : APS (12mg) Magnetic: MNPs, PEG	1: 19.7 : 14.1	• Acrylamide monomer gives high binding capacity than MAA with 79.07 $\mu\text{mol/g}$ and 58.34 $\mu\text{mol/g}$, respectively. • Morphology: Nil. • Application: Selective determination of sulfamethoxazole	(Lamaoui et al., 2021)
A microwave oven (max power = 700W, Midea Inc., Guangdong, China. Power: 700W 15 min	(T) : Biacalin (FM) : Imidazolium Ionic Liquid (IL-1C) (CL) : EGDMA (S) : DMSO/1,4-butanediol/1-dodecanol (100 μL /100 μL /600 μL) (I):Nil. Other: POSS	Nil	• Using 1-allyl-3-methylimidazolium tetrafluoroborate (IL-1C) as monomer gave highest imprinting factor of 5.4, compared to IL-2C, IL-4C and IL-8C. • Morphology: Irregular spherical • Application: Column in on-line SPME-HPLC and off-line LC-MS/MS	(Zhang et al., 2021)

Table 2.7: Continued.

BFY-205 micro- computer microwave chemical reactor (Gongyi Yuhua Instrument Co. LTD, China). Power : 80W x 3 min	(T) : CdCl ₂ .2.5H ₂ O (FM) : Salicylic aldehyde ethylene diamine Schiff Base (CL) : EGDMA (S) : Water (135mL) (I) : AIBN (0.15g)	Nil.	• Binding capacity: 179.04 mg/g • Morphology: Globular shape • Application : Extraction	(Zhu et al., 2021)
Single mode CEM Discover SP ® microwave synthesis. Power: 80W	(T) : 4-nitrophenol (FM) : γ -ureidopropyltrimethoxysilane (CL) : TEOS (S) : Methanol (100mL) (I) : AIBN (0.7 mmol) Magnetic: Fe ₃ O ₄ @SiO ₂	1:4:9	• Binding capacity:134.23 mg/g • Morphology:MIP has rough surface compared to NIP • Application: SPE	(Liang et al., 2020)
Scientific microwave oven equipped with IR sensor. Power: 400W 80°C x 30 min	(T) : Perphenazine (PPZ) (FM) : MAA (CL) : EGDMA (S) : Acetonitrile (25mL) (I) : AIBN (0.05g) Magnetic: Fe ₃ O ₄ @MAA	1:10:90	• Binding capacity: 86.48 mg/g • Morphology: Uniform nanoparticles with diameter of 7.79 $\pm$ 0.53 nm • Application: pre-concentrate and separate PPZ in human plasma and urine	(Safdarian & Ramezani, 2019)
Microwave synthesizer 80°C x 2 h	(T) : Chloramphenicol (FM) : 4-VP (CL) : TRIM (S) : DMSO (16 mL) (I) : ABCN (0.02g) Magnetic: vinyl-modified Fe ₃ O ₄	*1:14.98:8.35	• Binding capacity: 172.54$\pm$9.99μmol/g • Morphology: irregular spherical with size ranges 2-5 μm • Application: preconcentration and clean up chicken feed extract	(Kunsa-Ngiem et al., 2018)

Table 2.7: Continued.

Microwave synthesizer (XH-300UL, Beijing XiangHu Science and Technology Development Lo., Ltd) Power: 700W 60°C x 1 h	(T) : Tetracycline (FM) : Ag/PEDOT (CL) : TRIM (S) : DMSO (20mL) (I) : AIBN (0.01g) Other: ZnFe ₂ O ₄	*1:31.32 T:CL	• Binding capacity: 1.47mg/g. 2.04 times higher than ZnFe₂O₄ • Morphology: Partial hollow spherical. Average diameter 480nm. • Application : photocatalytic activity and selectivity for tetracycline degradation	(Lu et al., 2018)
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2.7 Summary

In summary, MIP offers advantages and current advances as a controlled release DDS by discussing the release mechanism and factors to consider in developing a DDS, as well as factors affecting the MIP performance (i.e., template, functional monomer, crosslinker, porogenic solvent, and initiator). As a highly crosslinked polymer with interconnected structures that can effectively store any active target, MIP can promote sustained drug delivery, allowing for a longer administration period (Gagliardi, 2023). It was envisaged that the development of MIP for aspirin and paracetamol as DDS would promote sustained drug delivery and improve patient compliance.

Based on the literature precedent and preliminary findings, the utilization of MW technology in the preparation of MIP for DDS has not been explored recently. MW not only can significantly reduce the polymerisation time, but it also can improve the MIP yield, binding selectivity, and particle morphology compared to conventional thermal polymerisation. The polymerisation time can be reduced from hours to minutes due to the reduction in activation energy barrier (Kumar et al., 2020). Upon utilising the MW in the synthesis process, the optimum irradiation time envisaged are within minutes. Moreover, exploring the integration of RSM and MW-assisted methods for MIP synthesis offers an opportunity to improve both efficiency and cost-effectiveness in polymerisation processes, ultimately contributing a valuable insight into industrial scalability and reproducibility.

CHAPTER 3

SYNTHESIS OF MIP VIA CONVENTIONAL THERMAL HEATING AND MICROWAVE-ASSISTED SYNTHESIS: OPTIMISATION OF POLYMER SYNTHESIS USING RSM

3.1 Introduction

The conventional method of MIP synthesis typically involves thermal heating, a well-established process in bulk polymerisation, precipitation polymerisation, pickering emulsion polymerisation, multi-step swelling polymerisation, and surface imprinting techniques (Ertürk & Mattiasson, 2017; Fresco-Cala et al., 2020). However, the prolonged reaction times and uneven heat distribution prompt the exploration of alternative approaches (Kueseng et al., 2018). In recent years, MW-assisted synthesis has emerged as an alternative and efficient technique for polymerisation (Lamaoui et al., 2021). MW-assisted synthesis is a fast and well-controlled reaction used extensively in organic synthesis and recently explored in polymerisation reactions (Brahmbhatt et al., 2020). Despite the substantial progress in both conventional thermal heating and MW-assisted synthesis methods for MIP preparation, a critical knowledge gap exists regarding the comparative impact of these techniques on the resulting MIP. The choice between thermal and MW-assisted methods may significantly influence the overall performance, structural characteristics, and binding capabilities of MIP.

Therefore, a comprehensive understanding of each synthesis approach's distinct effects and potential advantages is essential for advancing the field and optimising MIP design for DDS. This research aims to address this critical knowledge gap by systematically comparing MIPs prepared through conventional thermal heating and MW-assisted synthesis. The study will

explore the impact of the synthesis method on crucial parameters, including polymer morphology, binding affinity, and selectivity. Additionally, the investigation will consider each method's scalability and reproducibility, providing insights that can inform the selection of the most suitable polymer compositions and synthesis approach for specific MIP applications.

3.2 Materials and Methods

3.2.1 Materials and Reagents

All chemicals and solvents were commercially available and used as received. Methacrylic acid (MAA, 99% purity; contain 250 ppm MEHQ as inhibitor), divinylbenzene (DVB, technical grade; 80% purity), 2,2'-azobisisobutyronitrile (AIBN; 0.2M in toluene) were all purchased from Sigma Aldrich. Acetylsalicylic acid (aspirin, 99% purity) was purchased from Acros Organic. Paracetamol (4-acetamidophenol, 98%) was purchased from Fisher Scientific. Acetonitrile technical grade was purchased from Merck. Methanol, AR grade, was purchased from HmBG. Acetic acid was purchased from Fisher Scientific. Potassium bromide (KBr) for FTIR was purchased from Merck.

3.2.2 Instrumentations

Anton Paar Monowave 400 microwave synthesizer was purchased from Anton Paar Malaysia Sd. Bhd. A JSM-IT500HR Field Emission Scanning Electron Microscope (FE-SEM) was purchased from Japan Electron Optics Laboratory Co., Ltd, Japan. The Fourier Transform Infrared (FTIR) spectra were obtained from Thermo Scientific Nicolet Model iS10 with Smart

Omni-Transmission attachment and was purchased from ThermoFisher Scientific, USA. The UV-Vis analysis was performed using a UV-1280 UV-VIS spectrophotometer from Shimadzu Corporation. All the instruments were located in the Faculty of Resource Science and Technology, Universiti Malaysia Sarawak.

3.2.3 Synthesis of MIP and NIP

3.2.3.1 *via* Conventional Thermal Heating

The MIP for aspirin (sample id.: ASD) was synthesised by precipitation polymerisation. Aspirin (1 mmol, 0.18 g) and MAA (4 mmol, 0.34 mL) were dissolved in acetonitrile (50 mL) in a round bottom flask. The mixture was stirred at room temperature for 1 h followed by the addition of DVB (16 mmol, 2.28 mL) and AIBN (3 mL). The reaction mixture was purged with nitrogen gas for 15 min and constantly stirred at 80 °C for 6 h. The synthesised MIP was filtered, rinsed with acetonitrile, and dried in the oven at 50 °C for 18 h. The dried polymers were washed with methanol and acetic acid (9:1, v/v, 7 x 25 mL) followed by acetonitrile (4 x 25 mL). The template removal was monitored using UV-Vis. The non-imprinted polymer (NIP) was synthesised by following the same procedure without the template during polymerisation.

3.2.3.2 *via* Microwave-assisted Synthesis

i. Determination of MW irradiation setting

The polymerisation of MIP and NIP was performed in an Anton Paar Monowave 400 microwave synthesiser with a vial capacity of 30 mL using aspirin as template. The MW

irradiation setting was determined in a preliminary study using aspirin as a template. Aspirin (1 mmol) was dissolved in acetonitrile (15 mL), followed by the addition of MAA (4 mmol, 0.34 mL), and stirred at room temperature for 1 h. DVB (16 mmol, 2.28 mL) and AIBN (3 mL) were added to the mixture and purged with nitrogen gas for 15 min. After this, the reaction mixture was heated *via* MW (800 W) at the polymerisation temperature stated in Table 3.1. The synthesised MIP was filtered, rinsed with acetonitrile, and dried in the oven at 50°C for 18 h. The washing process followed the procedure mentioned previously.

Table 3.1: MW irradiation setting for the preliminary study.

Polymer ID	Temperature	Time
MW80	80°C	60 min
MW100	100°C	15 min
MW110	110°C	8 min
MW120	120°C	4 min

ii. MW Polymerisation of MIP using Aspirin and Paracetamol as Template

The polymerisation of MIP for aspirin (sample id: ASDW) and paracetamol (sample id: PCMW) as templates was performed by dissolving aspirin or paracetamol (1 mmol) in acetonitrile (15 mL). MAA (4 mmol) was added to the mixture and stirred for 1 h. DVB (16 mmol) and AIBN (3 mL) were added into the mixture and nitrogen-degassed (15 min) prior to MW heating (800W). The synthesised MIP was filtered, rinsed with acetonitrile, and dried in

the oven at 50°C for 18 h. The washing process followed the previously described protocol. The NIP was synthesised by following the same procedure without a template during polymerisation.

3.2.3.3 Optimisation of the Polymer Synthesis: Experimental Design using Central Composite Design (CCD)

A CCD was used to determine the optimal condition for the two critical factors in preparing MIP and NIP using polymerisation methods *via* MW-assisted and conventional thermal heating, as stated in Section 3.2.3.1 and Section 3.2.3.2(ii). An experimental rotatable, full factorial (2^n) central composite design (CCD) of orthogonal type with 5 central and 1 axial points ($\alpha = 1.41421$) was established *via* Design Expert software version 10 (Table 3.2). The Design-Expert is a statistical software package from Stat-Ease Inc. that is specifically dedicated to performing design of experiments (DOE) with designing and analysing experiments. It provides tools to analyse the experimental data, including statistical tests and graphical representations, as well as to identify the important trends and relationships (Lamidi et al., 2016; Stat-Ease, 2024). Significant variables such as the amount of functional monomer (FM) and cross-linker (CL) (mmol) were chosen as the independent variables affecting the rebinding capacity as a response. A total of 13 experimental runs (Table 3.3) were derived from Equation 3.1 where k is number of factors; C_p is number of central points. Whilst preparing all 13 polymers as described in Table 3.2, the amount of other components was kept constant, including initiator and porogenic solvent, following the procedures described in Section 3.2.3.1 and 3.2.3.2.

$$\text{Number of experiments} = 2^k + 2k + C_p$$

Equation 3.1

Table 3.2: The range of independent variables for the experimental design.

Factors	Levels				
	Low (-1)	Central (0)	High (+1)	- α	+ α
Functional Monomer	4	7	10	2.76	11.24
Crosslinker	16	18	20	15.17	20.83

Table 3.3: Experimental factors for the optimisation of the MIP and NIP synthesis using template of aspirin or paracetamol.

Standard Run No.	Run Order	Polymer ID	Template (mmol)	Factors	
				MAA (mmol)	DVB (mmol)
1	6	P1	1.00	4.00	16.00
2	3	P2	1.00	10.00	16.00
3	8	P3	1.00	4.00	20.00
4	4	P4	1.00	10.00	20.00
5	13	P5	1.00	2.76	18.00
6	12	P6	1.00	11.24	18.00
7	9	P7	1.00	7.00	15.17
8	11	P8	1.00	7.00	20.83
9	5	P9	1.00	7.00	18.00
10	10	P10	1.00	7.00	18.00
11	1	P11	1.00	7.00	18.00
12	2	P12	1.00	7.00	18.00
13	7	P13	1.00	7.00	18.00

3.2.4 Characterisation of MIP and NIP

Fourier Transform Infrared (FTIR) analysis was conducted on the MIP and NIP in KBr pellet. The polymer sample was mixed with KBr powder to form a pellet, and infrared spectra of unwashed and washed MIP and NIP were recorded over a frequency range of 4000 to 900 cm^{-1} . Field Emission Scanning Electron Microscope (FE-SEM) analysis was conducted to compare the surface morphology of all polymers prepared *via* conventional thermal heating and MW-assisted synthesis. The particle size was measured using ImageJ software version 1.53t with 35 sample numbers.

3.2.5 Batch Rebinding Study

A preliminary rebinding study was conducted to obtain the rebinding capacity as a response to the optimisation in the synthesis of MIP and NIP. MIP and NIP (80 mg) were placed in a flask containing drug solution (20 mL, 100 mg/L) and shaken at room temperature for 2 h at 150 rpm. The solution was filtered and measured using a UV-vis-spectrophotometer at 276 nm and 289 nm for aspirin and paracetamol. Each sample was performed in triplicate. A calibration curve was generated using 7 standard drug solutions (10 mg/L to 110 mg/L). The rebinding capacity of MIP and NIP was calculated from the following Equation 3.2, where C_i was the initial concentration (mg/L), C_f was the final concentration (mg/L), V was the volume of the solution in litre, and m was the weight of adsorbent (g). The selectivity of the prepared MIP was evaluated with the different behaviours of MIPs and NIPs using Equation 3.3, where good selectivity was equal to high IFs ($\text{IF} > 1$).

$$\text{Adsorption capacity, } Q = \frac{(C_i - C_f)V}{m} \quad \text{Equation 3.2}$$

$$IF_{\text{template}} = \frac{Q_{\text{MIP}}}{Q_{\text{NIP}}} \quad \text{Equation 3.3}$$

3.3 Results and Discussion

3.3.1 Synthesis of MIP and NIP for Aspirin and Paracetamol

The polymer was prepared following precipitation polymerisation using conventional synthesis (ASD and NIP) and MW-assisted synthesis (ASDW and NWP) (Figure 3.1). The polymerisation mixture contains MAA and DVB as functional monomers and crosslinkers at various formulation ratios (P1-P13) (Table 3.3). The selection of MAA as functional monomer was based on its ability to serve as both a hydrogen-bond donor and acceptor, resulting in it being well-suited for ionic interactions (Chen et al., 2016). Meanwhile, DVB was used as a crosslinker and known to be a relatively hydrophobic compound with minimal interaction with the template, resulting in a decrease in non-specific binding site phenomena (Muhammad et al., 2012; Meischl et al., 2016).

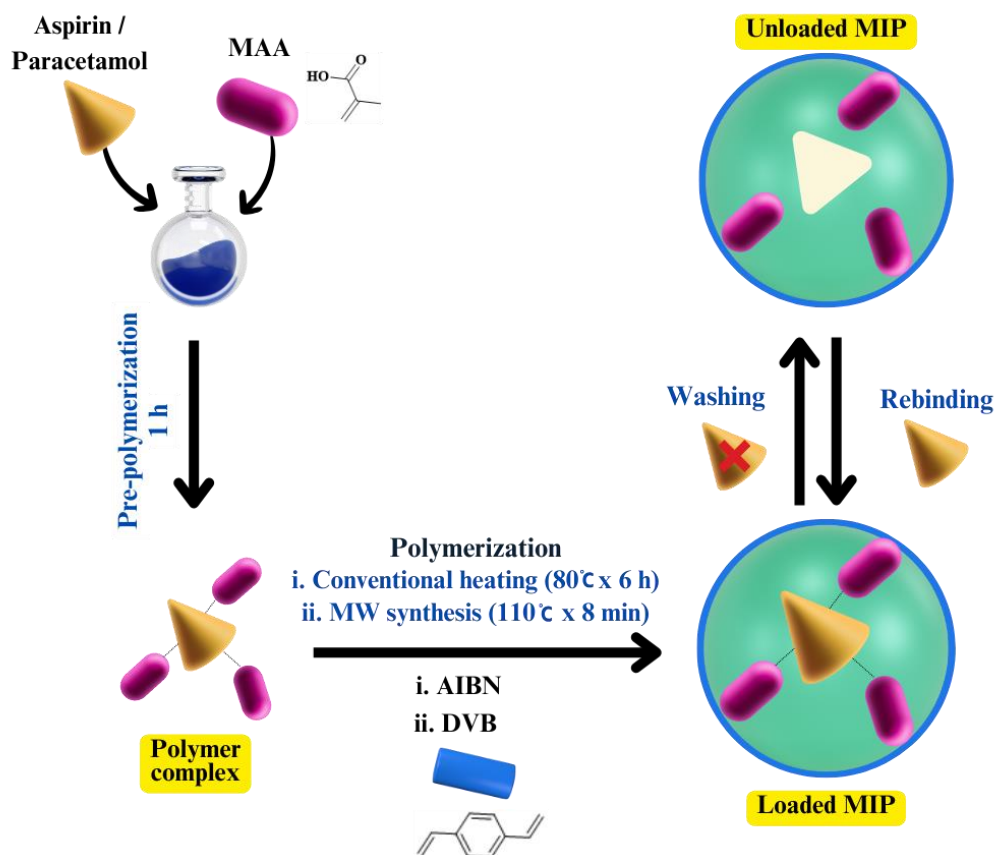


Figure 3.1: Schematic diagram of the synthesis of MIP employing aspirin and paracetamol as template.

In this study, the interaction between MAA and aspirin was the hydrogen bonding between the carboxyl group (C=O) and hydroxyl group (-OH) present in both molecules (Figure 3.2a). Similarly, utilizing paracetamol as a template has formed hydrogen bonding interaction with MAA *via* carboxyl (C=O), hydroxyl (-OH) and an amino group (-NH) as shown in Figure 3.2b (Özcan & Şahin, 2007; Zheng et al., 2022). It is known that the interaction of template molecules and functional monomers drove the molecular recognition of MIP.

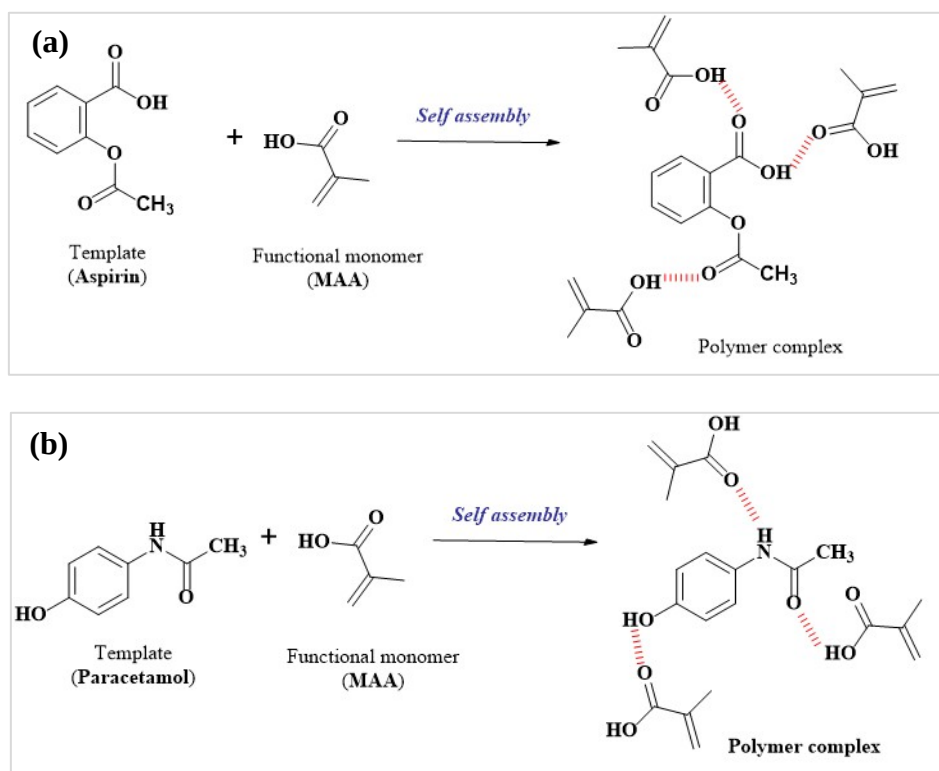


Figure 3.2: Molecular recognition of the polymer complex in the preparation of MIP for (a) aspirin and (b) paracetamol (Özcan & Şahin, 2007; Zheng et al., 2022).

3.3.2 Synthesis of Polymer via Conventional Thermal Heating

3.3.2.1 Polymer Yield

The influence of the amount of functional monomer and crosslinker on ASD and NIP yield was observed in this study. As seen in Figure 3.3, the yield of MIP and NIP was obtained at 80 °C for 6 h. Higher ASD yield was observed when the amount of functional monomer and crosslinker was higher. Similar findings were found in the preparation of NIP. This indicates that the total amount of functional monomers and crosslinkers affects the yield (Gao et al., 2020b). The yield of polymer in free radical polymerisation is governed by several factors,

including the monomers and their concentration (Tanaka et al., 2022), initiator and solvent system (Meena & Singh, 2023), external factors (i.e., high pressure, sonication) and reaction conditions (i.e., agitation speed, temperature, time) (Rodríguez-Pizano et al., 2019).

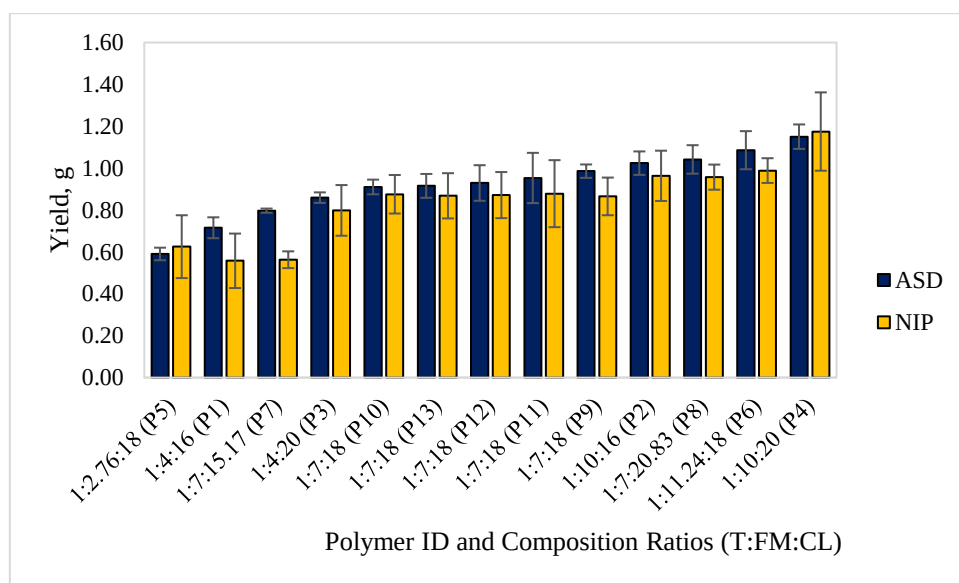


Figure 3.3: Yield of ASD and NIP on the factor of the polymer composition (template: functional monomer: crosslinker, T:FM:CL).

3.3.2.2 FTIR Spectra of ASD and NIP

The functional groups of the synthesised ASD and its NIP were analysed using FTIR. Predominantly, the FTIR spectra of MIP showed no significant difference from the NIP peaks as the polymers consist of similar molecular structure of MAA and DVB. This finding has also been reported by Shahar and his team (2016) on the FTIR analysis of NIP and MIP using MAA and EGDMA as functional monomer and crosslinker, respectively. In the NIP spectra (Figure 3.4a), broad -OH absorption band at 3472 cm^{-1} was observed which attributed to the stretching

vibration of O-H in the functional monomer MAA. The medium peak observed at 1699 cm^{-1} was correspond to the carboxyl group (C=O) in MAA (Ali et al., 2022). The C=C double bond attributed to DVB was observed at 1603 cm^{-1} (Li & Larock, 2003). The appearance of peak at 2920 cm^{-1} corresponded to C-H and the disappearance of the peak at $1620\text{ -}1680\text{ cm}^{-1}$ attributed to C=C in MAA and DVB, and the appearance of a medium peak indicates the occurrence of polymerisation in the MIP (Mustafai et al., 2018).

Meanwhile, the spectra of MIP having aspirin molecule as the template in the polymer network is shown in Figure 3.4b and Figure 3.4c. The peak corresponded to O-H group at 3472 cm^{-1} in NIP was slightly shifted upfield to 3450 cm^{-1} in MIP (Figure 3.4b). While a strong C=O absorption was slightly shifted downfield from 1699 cm^{-1} in NIP to 1700 cm^{-1} in MIP. This phenomenon is due to the formation of hydrogen bonds between the functional groups of aspirin and MAA (Ektirici et al., 2022). After template removal, the C=O peak was shifted to 1701 cm^{-1} and the O-H stretching shifted to 3449 cm^{-1} (Figure 3.4c) which indicates the change in the polymer environment. The C-H bending of the benzene ring in DVB was observed at the $900\text{ to }600\text{ cm}^{-1}$ regions.

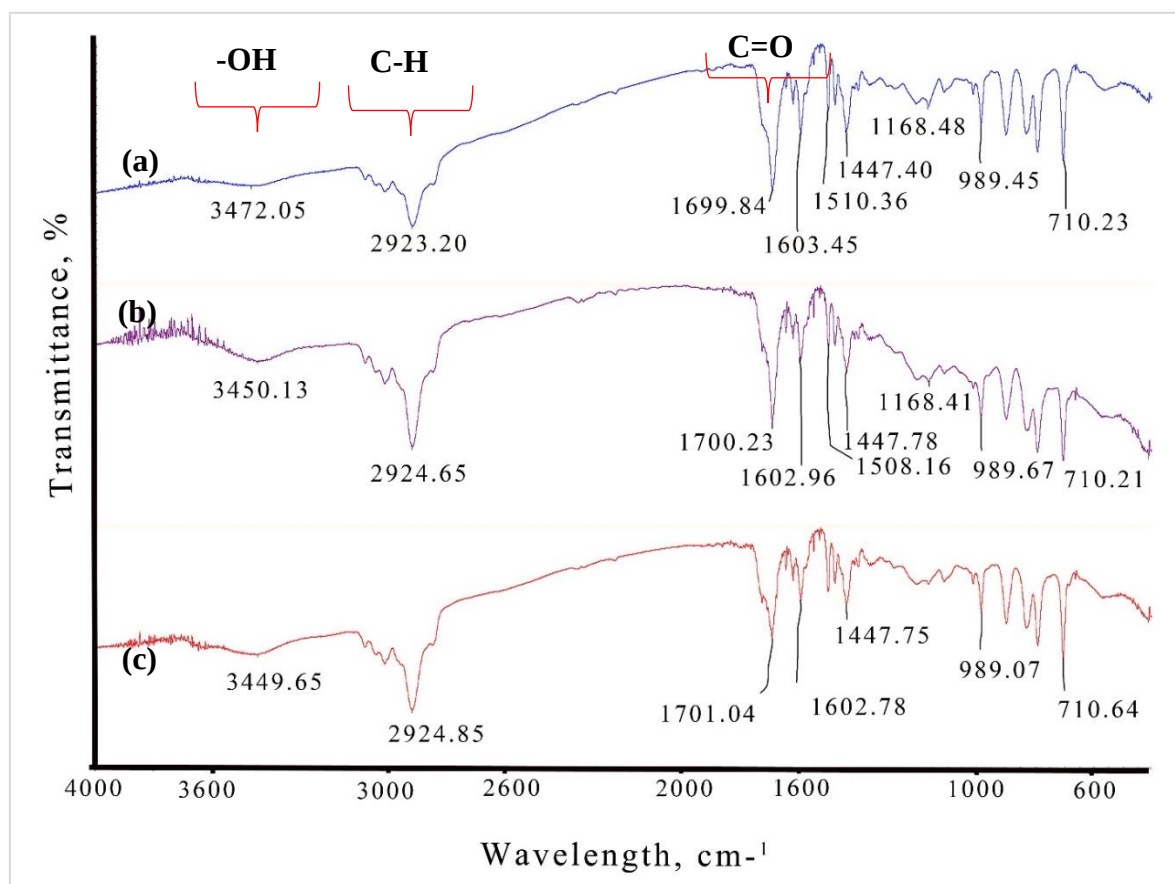


Figure 3.4: FTIR spectra of (a) NIP (b) MIP before washing (c) MIP after washing of ASD.

3.3.2.3 Surface Morphology and Particle Size

An assessment of the physical properties of the polymer was undertaken to elucidate the effect of formulation ratio on the surface morphology using FE-SEM. Table 3.4 shows the morphology of all polymers produced *via* precipitation polymerisation of conventional thermal heating. The use of acetonitrile in this study has produced MIP with spherical particles. ASD (P1, P3, P4, and P7) showed an irregular uniform spherical particles while ASD-P2, P5, P6, P8, P9, P10, P11, P12, and P13 appeared as agglomerate particles. A study conducted by Zheng and team (2018) revealed that acetonitrile produced a more spherical particles compared to other

solvents such as chloroform, toluene, and ethanol. Similar findings was reported by Moheballi and team (2021) that the used of acetonitrile as a porogenic solvent in precipitation polymerisation often produced spherical polymer particles (Moheballi et al., 2021). This indicated that solvents play a significant role in the synthesis of MIP. Besides toluene, chloroform, and dichloromethane, the usage of acetonitrile has been widely reported (Roland & Bhawani, 2016; Cantarella et al., 2019; Hou et al., 2020).

The shape of NIP was observed as more monodisperse than ASD. This is due to the absence of a template in the NIP preparation that has some effect to the shape of MIP (Hasanah et al., 2021). In developing drug carriers, the shape of MIP is important as it contributes to the therapeutic delivery processes such as particle adhesion, distribution, and cell internalization in the *in vivo* application (Burton & Goodwin, 2010; Liu et al., 2012; Danaei et al., 2018). Microspheres or nanospheres are well-established drug carriers for many pharmaceutical industries (Das & Kumar, 2020), and numerous spherical MIPs have been prepared for a sustained release application that showed promising results (Abdollahi et al., 2018; Zheng et al., 2018; Ceglowski et al., 2022).

Table 3.4: Surface morphology of ASD and NIP at 5000x magnification.

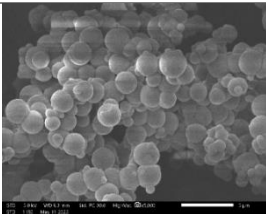
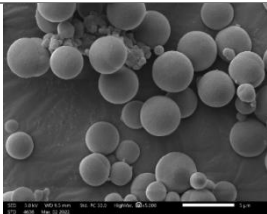
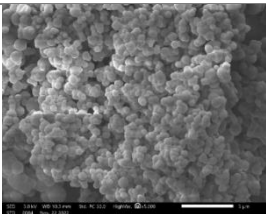
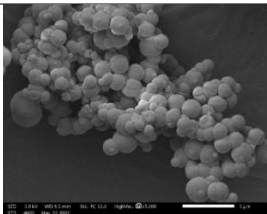
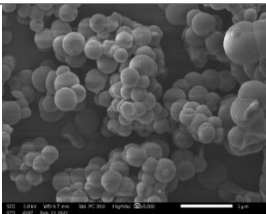
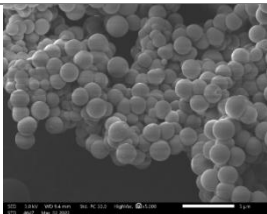
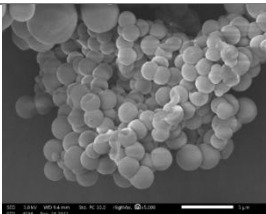
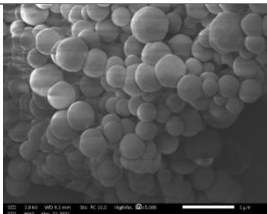
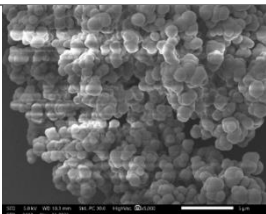
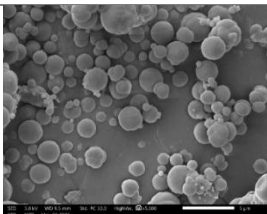
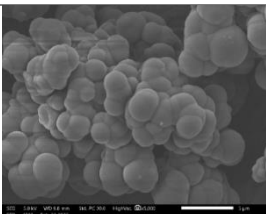
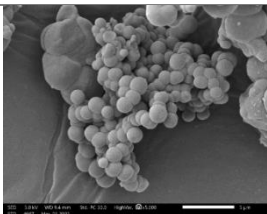
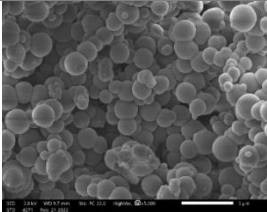
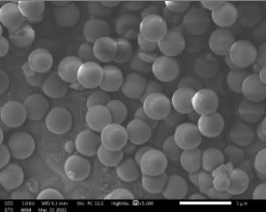
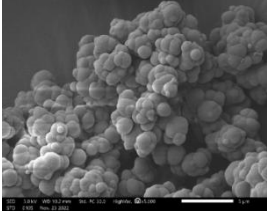
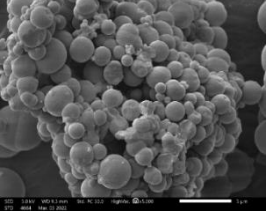
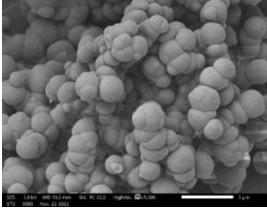
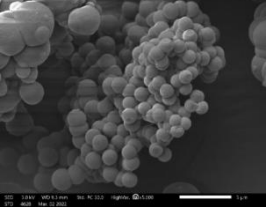
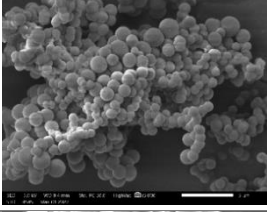
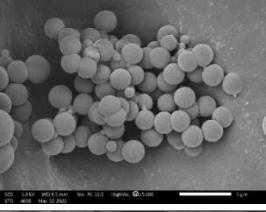
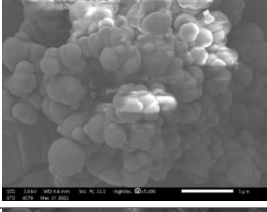
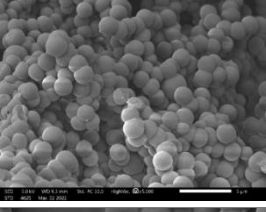
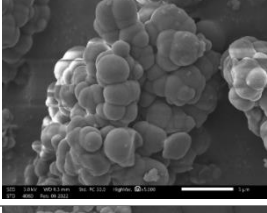
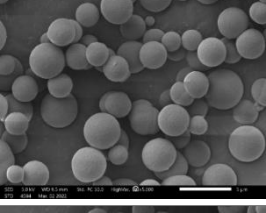
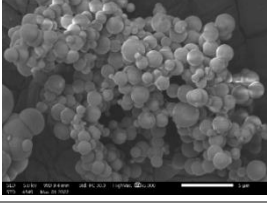
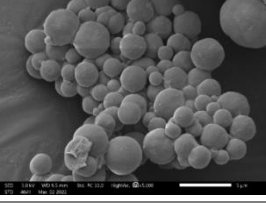
Polymer ID	Formulation ratio (T:FM:CL)	ASD	NIP
P1	1:4:16		
P2	1:10:16		
P3	1:4:20		
P4	1:10:20		
P5	1:2.76:18		
P6	1:11.24:18		

Table 3.4: Continued.

P7	1:7:15.17		
P8	1:7:20.83		
P9	1:7:18		
P10	1:7:18		
P11	1:7:18		
P12	1:7:18		
P13	1:7:18		

The polymer particles were measured using ImageJ and presented in Figure 3.5. In the preparation of both MIP and NIP, several polymer compositions (P1 – P13) based on the amount of FM and CL were employed in acetonitrile solvent (50 mL). ASD-P2 showed a smaller particle size at $0.66 \pm 0.13 \mu\text{m}$ and its corresponding NIP at $1.75 \pm 0.38 \mu\text{m}$. The next smaller particles were observed in ASD-P5 at $0.88 \pm 0.23 \mu\text{m}$ and its corresponding NIP at $1.76 \pm 0.67 \mu\text{m}$. Both polymers were prepared at different formulation ratios at T:FM:CL of 1:10:16 and 1:2.76:18, respectively. A microsphere can be produced using precipitation polymerization. In precipitation polymerization, the initiation of polymer formation involves the generation of nuclei and oligomers where crosslinking occurs as the oligomers increase in size. Then, the polymer chains extend individually by incorporating newly formed oligomers and monomers present in the solvent instead of overlapping (Orowitz et al., 2020).

In the case of polymer composition, the particle size is influenced by the presence of crosslinker where the amount of crosslinker can affect the particle size and crosslinking density (Du et al., 2020; Wang et al., 2021). A high concentration of crosslinkers can lead to surface shrinkage and smaller particle sizes (Chaudhary & Sharma, 2019). However, the outcome of this study is contrary to the previous research. The particle size of P8 (CL=20.83 mmol) and P5 (CL=18 mmol) was larger than P2 (CL=16 mmol).

This finding also suggested that other factors may come in play in affecting the formation of polymer particle size, such as the size of the template, and polymerisation temperature, which induces the growing nuclei, monomer and initiator concentration, and type of solvent (Parisi et al., 2020; Pratama et al., 2020). In this case, conventional thermal heating often causes inconsistency in the polymerisation temperature which affecting the chain-growth

polymerization and thus can affect the size of MIPs (Turner et al., 2010; Brahmabhatt et al., 2020). The temperature affects the polymer nucleation rate which at lower temperature larger particles were obtained due to more time for the monomers to grow (Teixeira et al., 2021). The monomer concentration and temperature can also influence the particle size and distribution, with higher monomer concentrations and lower temperatures generally leading to larger particles (Lu et al., 2020). This is also in corresponds to the number of initiation sites present in the mixture where ASD-P2 has higher alkyl group with a total of 26 mmol (MAA and DVB) compared to ASD-P5 with a total of 20.76 mmol (MAA and DVB). Higher initiation sites resulting in smaller particles size (Lok & Ober, 2011; Liu et al., 2015). Increasing the initiator concentration also causing an increased in the particle size which was similar to what was reported by Moheballi and team (2018).

On other hand, solvents with different dielectric constants can affect the rates of polymerization reactions where the high dielectric constant solvents can stabilize transition states and ionic intermediates, potentially increasing the rate of propagation and decreasing the rate of termination (Liu et al., 2015; Pryamitsyn & Ganesan, 2015). In this study, acetonitrile ($\epsilon = 35.09$) has high dielectric constant which often lead to narrower molecular weight distributions due to better solvation and stabilization of growing polymer chains (Bornardd et al., 2021). The absence of template in the polymerization process of NIPs revealed that the particle size was mainly larger than their corresponding MIPs, indicating that the presence of a template can affect the particle size (Yoshimastu et al., 2007; Hasanah et al., 2021). The average particle size obtained for MIP using aspirin via conventional thermal heating in this study was in the range of 0.66 – 3.65 μm . Similar particle size range were observed in the

preparation of MIP for aspirin via conventional thermal heating using bulk polymerization (Abd et al., 2019). Moreover, the smaller particle size observed by ASD-P2 ($0.66 \pm 0.13 \mu\text{m}$) was in comparable with the MIP prepared with magnetic nanoparticles which was reported at 500 nm (Kan et al., 2009).

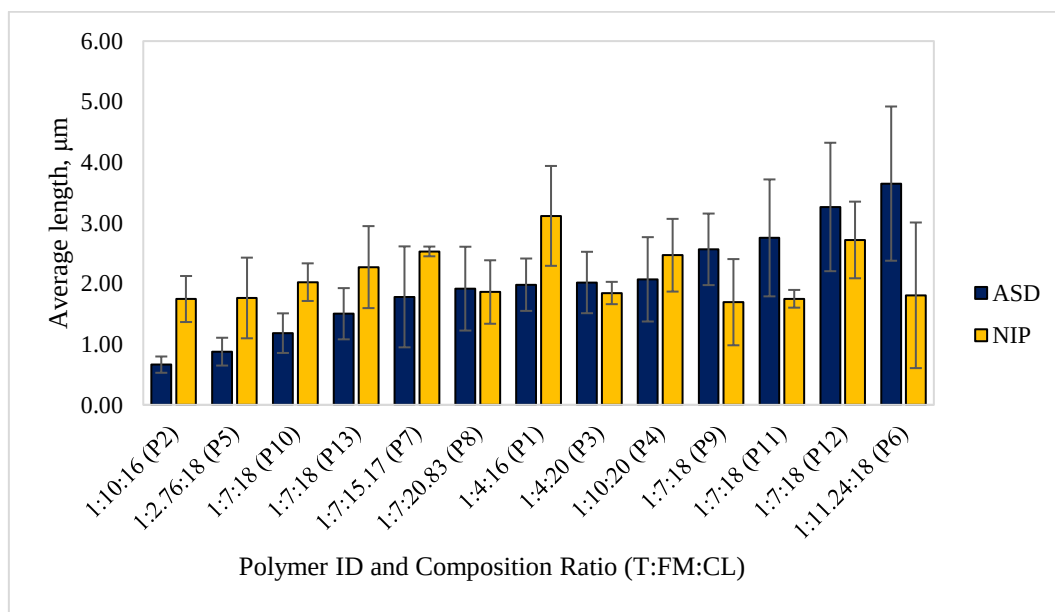


Figure 3.5: Average particle size of ASD and NIP in corresponds of ASD average diameter from smallest to larger particle size. N=35.

3.3.2.4 Template Removal Monitoring

Having the MIP prepared, the template was removed by washing with methanol:acetic acid (9:1, v/v) several times prior to determining the rebinding properties of MIP. Figure 3.6 shows the template removal observed by the UV-Spectrophotometer. In this study, a complete template removal from the MIP requires two washing steps. The first step was the removal with

10% acetic acid in methanol, while the second step involved washing with acetonitrile to remove the remaining acetic acid. All the synthesised MIP showed a similar washing pattern (Appendix A2), which requires about 6-7 times of washing with methanol:acetic acid (9:1, v/v). Acetic acid was added to the washing solution to promote acidic conditions that break the hydrogen bonding between the template-monomer complex (Wei et al., 2017). To ensure the entirety of green procedures, the used solvents were recycled by performing solvent distillation.

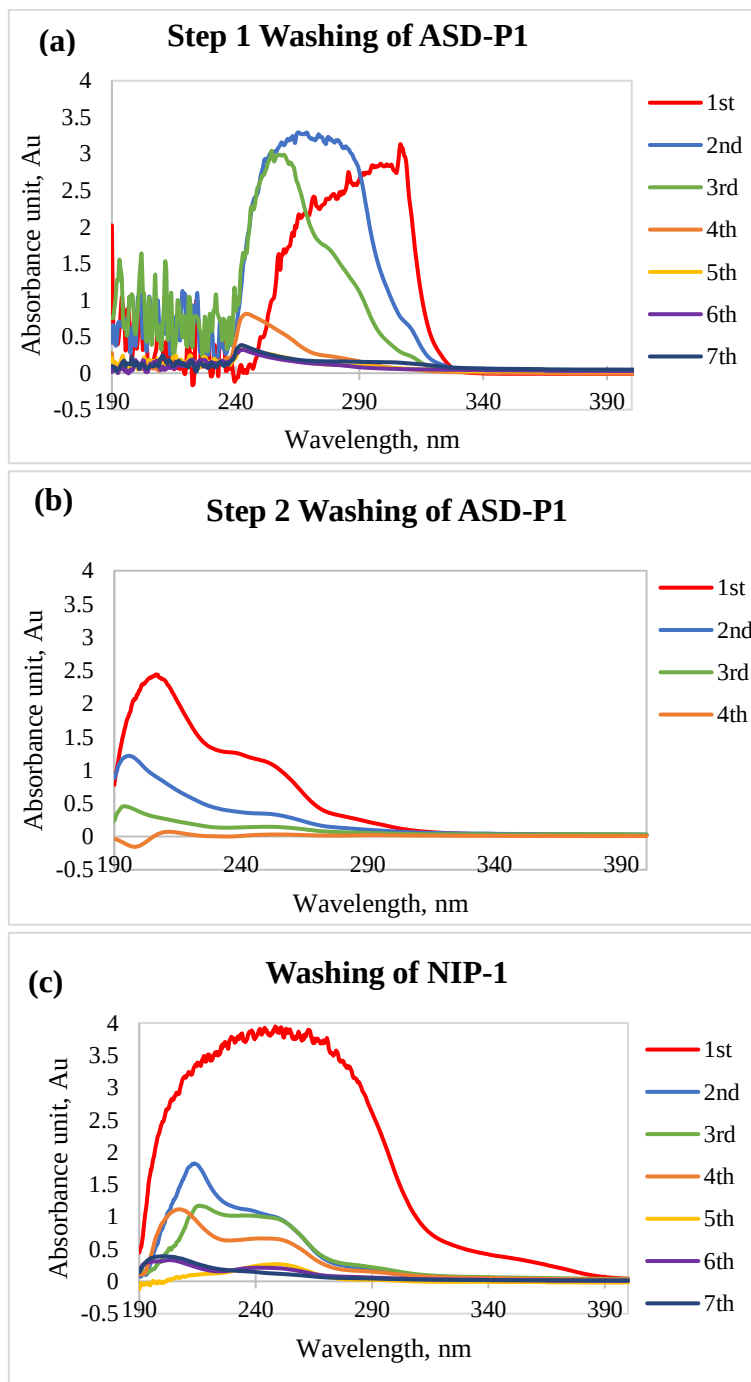


Figure 3.6: UV-spectrophotometry monitoring on the removal of the template using (a) methanol:acetic acid (9:1, v/v) and (b) acetonitrile (c) NIP using only acetonitrile.

3.3.2.5 Batch Rebinding Study.

The batch rebinding study employed a CCD with 13 experimental runs (all in triplicates) to synthesise ASD and NIP. The design response was obtained from the preliminary rebinding study, and the average responses are presented in Table 3.5. ASD-P1 obtained the highest rebinding capacity (T:FM:CL, 1:4:16) at 3.00 ± 0.31 mg/g, followed by ASD-P6 (T:FM:CL, 1:11.24:18) at 2.74 ± 0.14 mg/g. Polymers achieved the lowest rebinding capacity with a T:FM:CL ratio of 1:7:18. It was supported by the imprinting factor (IF) value obtained from the study. IF indicates the strength of the polymer interactions against the template molecule (Pratama et al., 2020). In this study, all polymers produced presented an IF value greater than 1, indicating that the MIP produced has high-specific interactions with the template aspirin (Zhang et al., 2021). A high IF value can be obtained when the NIP shows a lower rebinding capacity than its MIP. The low rebinding capacity of NIP was due to the absence of template molecules in the polymerisation process, thus lowering the recognition affinity (Mohebbi et al., 2021). The highest IF value in this study was obtained by ASD-P1 (1:4, aspirin:MAA) with 2.71. In comparison with other study, Wang and his team reported the IF value obtained with ratio 1:4 of the template ofloxacin with functional monomer MAA was 2.1 which is slightly lower than this study. Higher IF was obtained by Wei and team (2015) for template aspirin when it was imprinted onto surface of CdTe quantum dots with value of 3.31. Therefore, the IF value obtained from this method has showed a high-specific interactions with the template aspirin.

Table 3.5: Preliminary rebinding capacity and imprinting factor of MIP and NIP prepared using conventional thermal heating.

Polymer ID	T:FM:CL	Rebinding Capacity, mg/g		Imprinting factor, IF
		ASD	NIP	
P1	1:4:16	3.00±0.31	1.11±0.02	2.71
P2	1:10:16	2.59±0.50	1.60±0.03	1.62
P3	1:4:20	2.52±0.35	1.52±0.05	1.65
P4	1:10:20	2.28±0.16	1.12±0.06	2.04
P5	1:2.76:18	2.61±0.33	1.20±0.08	2.18
P6	1:11.24:18	2.74±0.15	1.60±0.08	1.71
P7	1:7:15.17	2.56±0.09	1.63±0.06	1.58
P8	1:7:20.83	2.38±0.10	1.07±0.09	2.23
P9	1:7:18	1.13±0.16	0.96±0.06	1.18
P10	1:7:18	1.32±0.10	0.92±0.01	1.43
P11	1:7:18	1.18±0.16	0.99±0.04	1.18
P12	1:7:18	1.04±0.10	0.98±0.02	1.05
P13	1:7:18	1.24±0.17	0.94±0.06	1.33

*T, template; FM, functional monomer ; CL, crosslinker

3.3.3 Synthesis of Polymer via MW-assisted Synthesis

MIP was successfully prepared using conventional thermal heating. However, the longer reaction time (6 h) and uneven heat distribution prompt the exploration of alternative approaches. The polymerisation of MIP *via* MW has been reported as a rapid synthesis with lower energy consumption than conventional heating (Chen et al., 2015; Liang et al., 2018; Lamaoui et al., 2021; Zhang et al., 2021). The green synthesis of MIP *via* MW irradiation has significantly influenced the overall performance, structural characteristics, morphology, shape

and binding capabilities of MIPs (Brahmbhatt et al., 2020). In this study, two different templates were used in the MIP synthesised, namely aspirin (sample id: ASDW) and paracetamol (sample id: PCMW).

3.3.3.1 Determination of MW-irradiation Setting

An evaluation of the microwave setting was performed using aspirin as the model template. Recent developments of MIP using MW technology employing irradiation settings at 60 °C to 80 °C from 15 mins to 1 h (Chen et al., 2015; Liang et al., 2018; Lamaoui et al., 2021; Zhang et al., 2021). Herein, MIP was polymerised at several MW temperature settings (80, 100, 110 and 120 °C) and evaluated the prepared MIP's yield, particle morphology and rebinding capacity.

i. The Effect of MW Temperature on Polymer Yield

The yield of MIP obtained *via* MW-assisted synthesis is shown in Figure 3.7. The yield of polymers obtained using MW is comparable to that of MIP produced *via* traditional thermal heating (ASD-P1) in a shorter reaction time. MW80 produced a higher yield (0.87 g), followed by MW110 (0.62 g), MW120 (0.58 g) and MW100 (0.53 g). Nevertheless, the low polymerisation temperature of MW80 at 1 h produced a higher yield than ASD-P1 (6 h). In a shorter polymerisation time, MW110 (8 min) showed higher yield than MW100 (15 min) and MW120 (4 min). The increase of the reaction rate *via* internal energy transfer by MW led to an

increase in particle formation, enhancing both the yield and polymerisation time (Turner et al., 2010; Leadbeater, 2014; Shahar et al., 2016; Viveiros et al., 2018; Farooq et al., 2020).

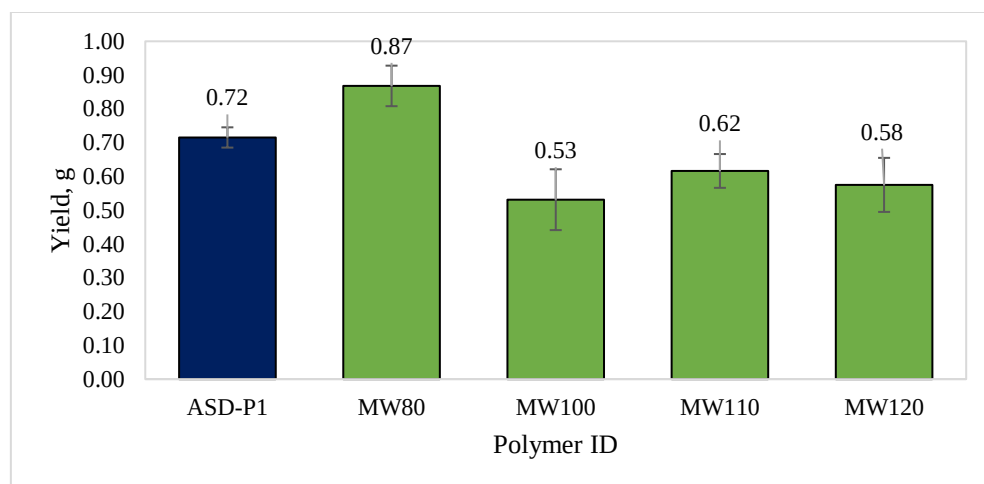


Figure 3.7: Yield of MIP produced by MW-assisted synthesis (MW80, MW100, MW110 and MW120) and conventional thermal heating (ASD-P1) using aspirin as template.

ii. Functional Group Characterisation of MIP

The spectra of prepared MIP via MW-assisted synthesis at various polymerization temperature and time is presented in Table 3.6 and in comparison, to the MIP prepared by conventional thermal heating (ASD-MIP). Similar to the ASD-MIP, the O-H stretching vibration in the functional monomer MAA appeared at 3440 to 3455 cm^{-1} regions. The disappearance of the peak at 1620 -1680 cm^{-1} corresponded to C=C in MAA and DVB, and the appearance of a strong peak at 2920 cm^{-1} for C-H stretching indicates the occurrence of polymerisation in MIP (Mustafai et al., 2018). The peak corresponding to C=O was observed at 1700 - 1705 cm^{-1} . The C=O was slightly shifted upfield which indicated that as the temperature increased, the formation of rigid networks and crosslinked polymer increased. Therefore, the

restricted movement of C=O group leading to higher vibrational frequencies and causes blue shifting (Larkin, 2011).

Table 3.6: FTIR spectra of MW-assisted synthesis of MIP aspirin at different polymerisation setting and incomparison with conventional thermal heatig aspirin-MIP (ASD-MIP)

Functional group	Wavelength (cm⁻¹)				
	ASD-MIP	MW80 (MIP)	MW100 (MIP)	MW110 (MIP)	MW120 (MIP)
O-H stretching	3449	3453	3450	3436	3442
C-H stretching	2924	2924	2924	2924	2928
C=O stretching	1701	1701	1703	1703	1708
C=C stretching (aromatic)	1602	1602	1600	1604	1611

iii. Particle Morphology and Size of MIP

Figure 3.8 shows the morphology of MIP using FESEM spectroscopy. All MIP prepared via MW irradiation showed smaller agglomerate spherical particles compared to ASD-P1. The spherical particles were obtained using precipitation polymerisation in acetonitrile which was due to the growing of chain lengths that exceed its solubility and precipitates from the solution (Orowitz et al., 2020; Mohebalı et al., 2021). Introducing MW in the precipitation polymerization has increased the polymerization rate which increased the particle collision and

causes aggregation which was also observed by Sullivan and team (2023). The morphological characterisation of all MIP synthesised *via* MW showed distinct particle size reduction compared to the conventionally prepared MIP (Fig 4a). The average particle size of all MIP was measured using ImageJ with 35 sample sizes (Figure 3.9). MW120 produced the smallest particle sizes of $1.00 \pm 0.24 \text{ }\mu\text{m}$, while MW100, MW110 and MW80 gave decreasing polydisperse particle sizes of $1.25 \pm 0.22 \text{ }\mu\text{m}$, $1.24 \pm 0.17 \text{ }\mu\text{m}$ and $1.14 \pm 0.25 \text{ }\mu\text{m}$, respectively (Shahar et al., 2016). Rapid polymerisation *via* MW contributed to the particle nucleation and growth during polymerisation, resulting in smaller particle sizes of the polymers (Burton & Goodwin, 2010). Although MW120 has a smaller particle size than MW110, the morphology of MW120 showed high polydispersity and agglomerated particles compared to MW110. The inhomogeneous distribution of binding sites in the agglomerated polymer particles resulted in reduced binding affinity (Zhao, 2022) and smaller surface area, thus inducing fewer binding sites available to the template (Xia et al., 2017).

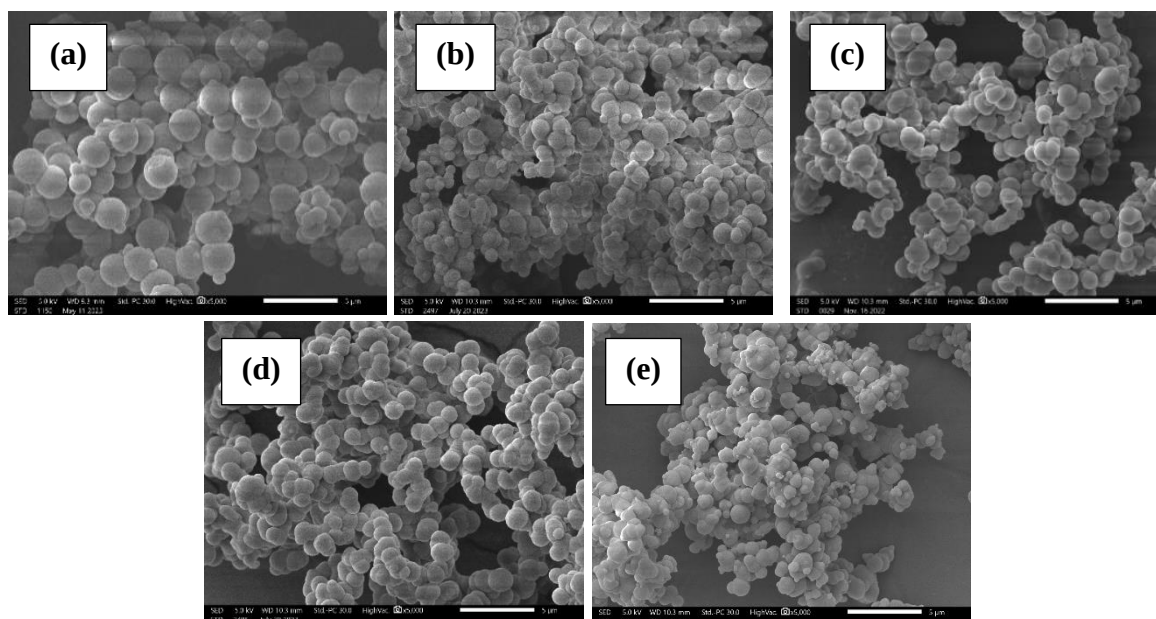


Figure 3.8: SEM images of MIP at 5,000 magnifications of different microwave heating settings (a) ASD-P1 (b) MW80 (c) MW100 (d) MW110 (e) MW120.

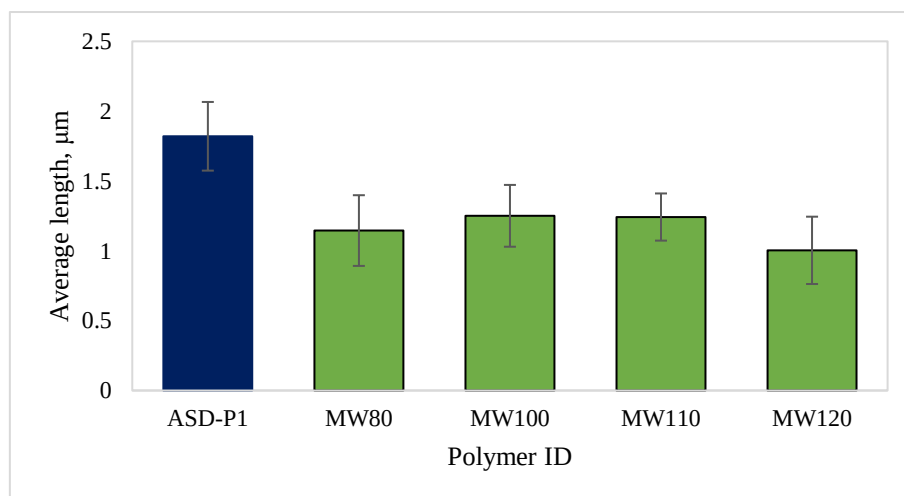


Figure 3.9: Average diameter of MIP. n=35.

iv. The Effect of Particle Size on the Rebinding Capacity

The performance of the prepared MIP was evaluated for their rebinding efficiency. The binding mechanism involved in molecular imprinting was driven by the interactions between the template molecule and the functional monomers during the imprinting (Cowen et al., 2020). Figure 3.10 shows the rebinding capacity of the prepared MIP *via* MW compared to ASD-P1. MW110 showed the highest rebinding capacity at 7.27 ± 0.20 mg/g compared to MW80 (5.33 ± 0.21 mg/g), MW100 (6.14 ± 0.27 mg/g) and MW120 (5.78 ± 0.38 mg/g). The size of MIP particles significantly influences the rebinding efficiency of MIP. Greater surface area-to-volume ratios frequently associated with smaller particle sizes can result in more binding sites and better access to the binding locations, affecting rebinding capacity (Gkika et al., 2024). The low particle polydispersity of MW110 compared to other MIPs has allowed more template access into the binding sites, resulting in higher rebinding capacity (Xia et al., 2017). From this preliminary study, the MW temperature setting at 110 °C for 8 min was chosen as the polymerisation setting in the subsequent study.

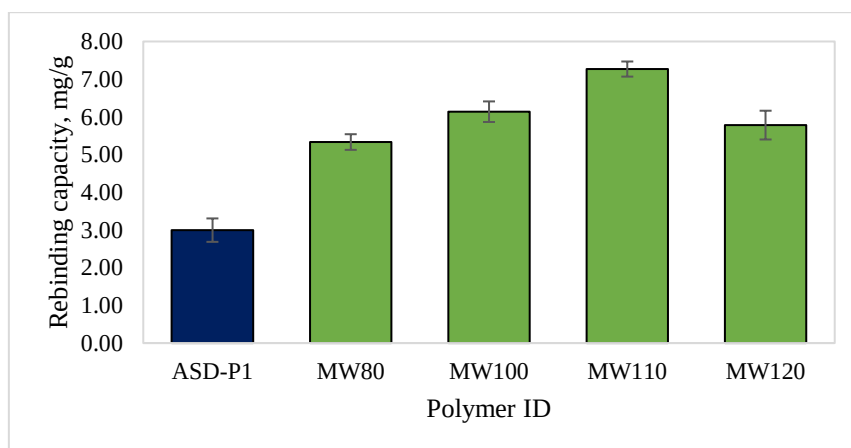


Figure 3.10: Rebinding capacity of MIP for aspirin prepared at various MW settings.

3.3.3.2 Polymer Yield of ASDW, PCMW and NWP.

The MIP for aspirin (ASDW), paracetamol (PCMW), and NIP (NWP) were synthesised *via* MW irradiation at 110 °C for 8 mins. The polymer obtained from ASDW was comparable to the MIP obtained *via* conventional thermal heating (ASD) with the range of 0.5 - 1.0 g (Figure 3.11). The polymerisation in the MW synthesizer was in controlled compared to conventional thermal heating. Similar findings were observed in PCMW, where about 0.5 - 0.9 g of polymer was obtained. In this study, MW-assisted synthesis showed uniform heating through the stirring process and produced by the internal energy transfer. The heating process occurred internally as the MW interacts with polar molecules and ions in the material, causing the molecule to rotate and move. The molecular movement generates heat through friction and dielectric heating, leading to rapid and efficient energy transfer throughout the substance, resulting in better yield in a shorter time (Kappe, 2004; Gaba & Dhingra, 2011; Shahar et al., 2016; Viveiros et al., 2018; Farooq et al., 2020).

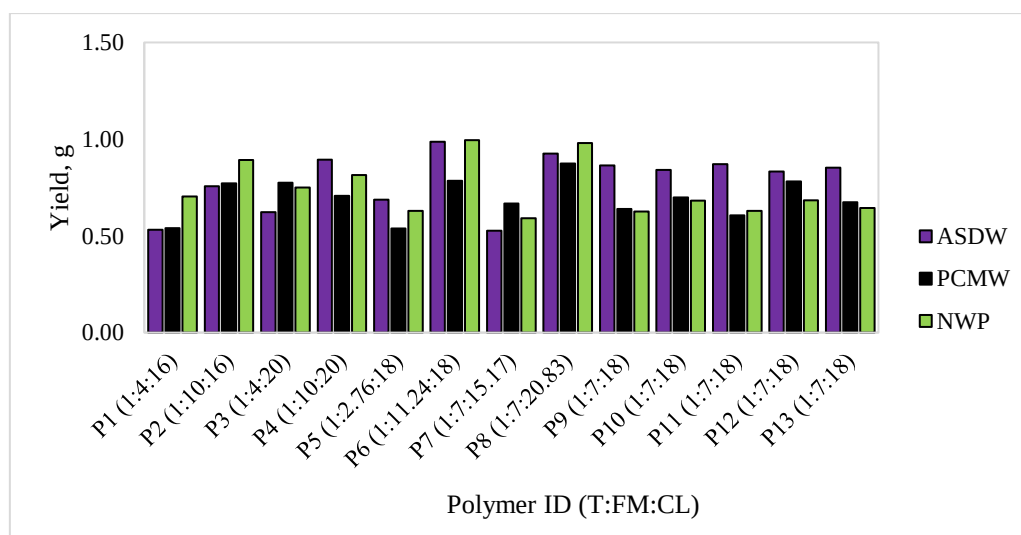


Figure 3.11: Polymer yield of (a) ASDW, (b) PCMW and (c) NWP.

3.3.3.3 Template Removal Monitoring

The template removal was performed following two washing steps and monitored by UV-Vis. The washing patterns observed in ASDW, PCMW and NWP also required about seven times of washing with methanol:acetic acid (9:1, v/v, 25 mL) and four times with acetonitrile for a complete removal of drugs. The overall washing process of ASDW, PCMW and NWP were presented in Appendix B2 and Appendix C2.

3.3.3.4 FTIR Spectra of MIP and NWP

The functional group confirmation was performed using FTIR. Table 3.7 showed the spectra of NWP, unwashed ASDW and PCMW, and washed ASDW and PCMW. The polymer backbone for both ASDW and PCMW were consists of MAA and DVB as functional monomers and crosslinkers, similar to the previous ASD structure. The O-H stretching from carboxylic acid was observed at 3430 to 3443 cm^{-1} regions. The C=C stretching of vinyl groups present in MAA and DVB supposedly showed a peak at 1620 to 1680 cm^{-1} regions. However, the peak disappeared due to the polymerisation of crosslinked polymer and a strong peak at 2920 cm^{-1} regions attributed to the C-H stretching formed from the crosslinked polymer (Prasetya et al., 2020). The carbonyl stretching (C=O) of MAA was observed in 1705 to 1712 cm^{-1} region for all spectra. A slight shift was observed in the C=O stretching to downfield region after template removal indicating a change in the polymer environment (Ektirici et al., 2022). The C-H bending of the benzene ring in DVB was observed at the 900 to 600 cm^{-1} regions. In the PCMW-MIP before washing, a peak at 1206 cm^{-1} was observed and attributed to the present of C-N stretching

of amide presence in paracetamol (Roland & Bhawani, 2014). The peak disappeared after washing showing that the removal of paracetamol from the MIP matrix.

Table 3.7: FTIR spectra of NWP, unwashed ASDW, washed ASDW, unwashed PCMW and washed PCMW.

Functional group	Wavelength (cm⁻¹)				
	NWP	ASDW-MIP (before washing)	ASDW-MIP (after washing)	PCMW-MIP (before washing)	PCMW-MIP (after washing)
O-H stretching	3443	3434	3435	3436	3434
C-H stretching	2925	2927	2928	2931	2927
C=O stretching	1705	1708	1711	1708	1712
C=C stretching (aromatic)	1597	1615	1603	1614	1602
C-N stretching	-	-	-	1206	-

3.3.3.5 Surface Morphology and Particle Size

The morphology of ASDW, PCMW and NWP are presented in Table 3.8. The MW-assisted synthesis produced microspheres as polydisperse agglomerated particles. In polymers, P1 and P2, the NWP showed a more regular spherical polymer compared to its corresponding MIP (ASDW and PCMW). This suggests that the presence of a template is affecting the shape of MIPs by creating specific recognition sites that complement the template's size, shape, and functional group arrangement (Cegłowski et al., 2019; Hasanah et al., 2021). Meanwhile, other

polymer compositions (P3-P13) of NWP and its corresponding MIP (ASDW and PCMW) showed agglomerated polydisperse spherical particles compared to conventional thermal heating. The morphology of polymers produced by precipitation polymerisation is influenced by several factors, including the polymerisation method (Zheng et al., 2018), choice of solvent (Esfandyari-Manesh et al., 2011; Xavier et al., 2019), monomer concentration, and temperature (Lu et al., 2020). In comparison to conventional thermal heating, the differences with MW synthesis are in the polymerisation temperature setting, and the volume of solvent. Due to the limitations of vial capacity, the maximum volume used to synthesise MIP *via* MW was 15 mL. Thus, the agglomerated polydisperse spherical particles obtained in this study are caused by the solvent volume (Lu et al., 2020).

Table 3.8: SEM micrographs of ASDW, PCMW and NWP synthesised at 110°C for 8 min.

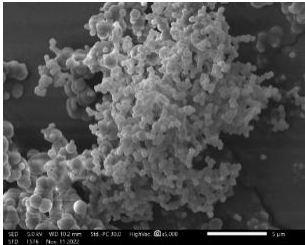
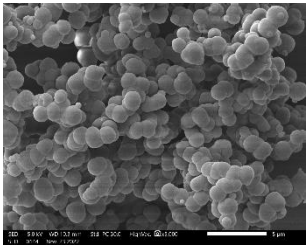
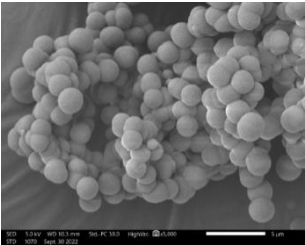
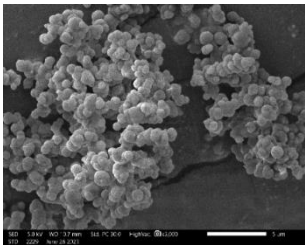
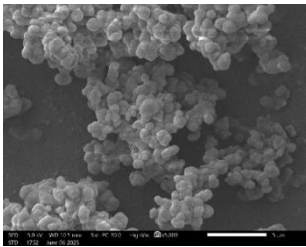
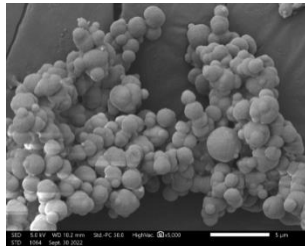
Polymer ID	ASDW	PCMW	NWP
P1			
P2			

Table 3.8: Continued.

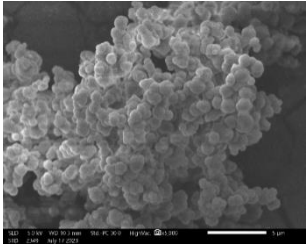
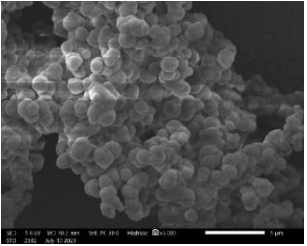
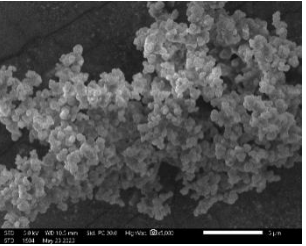
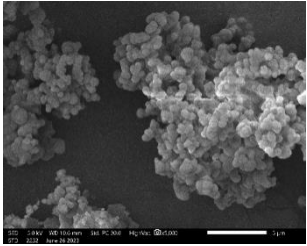
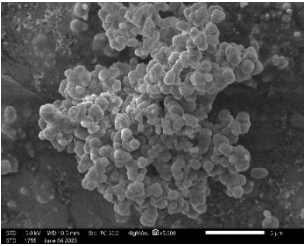
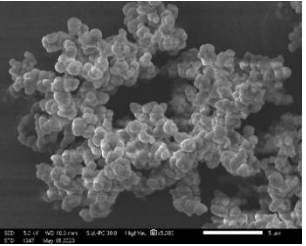
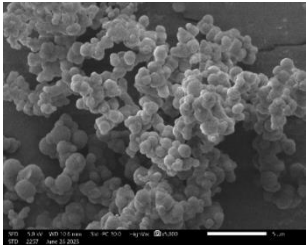
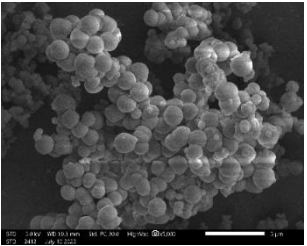
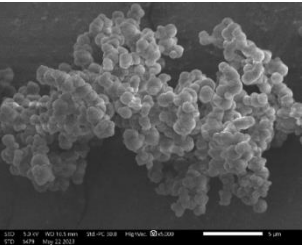
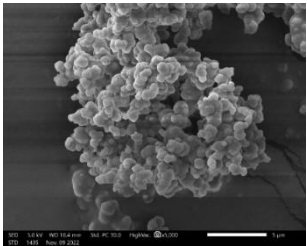
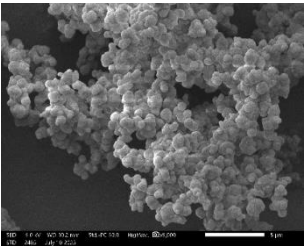
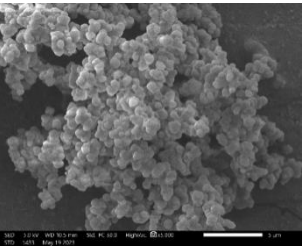
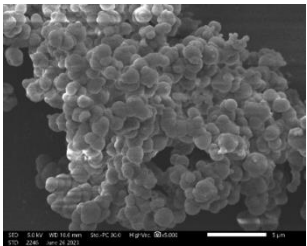
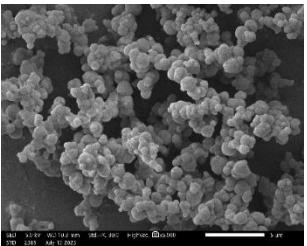
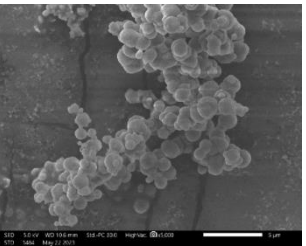
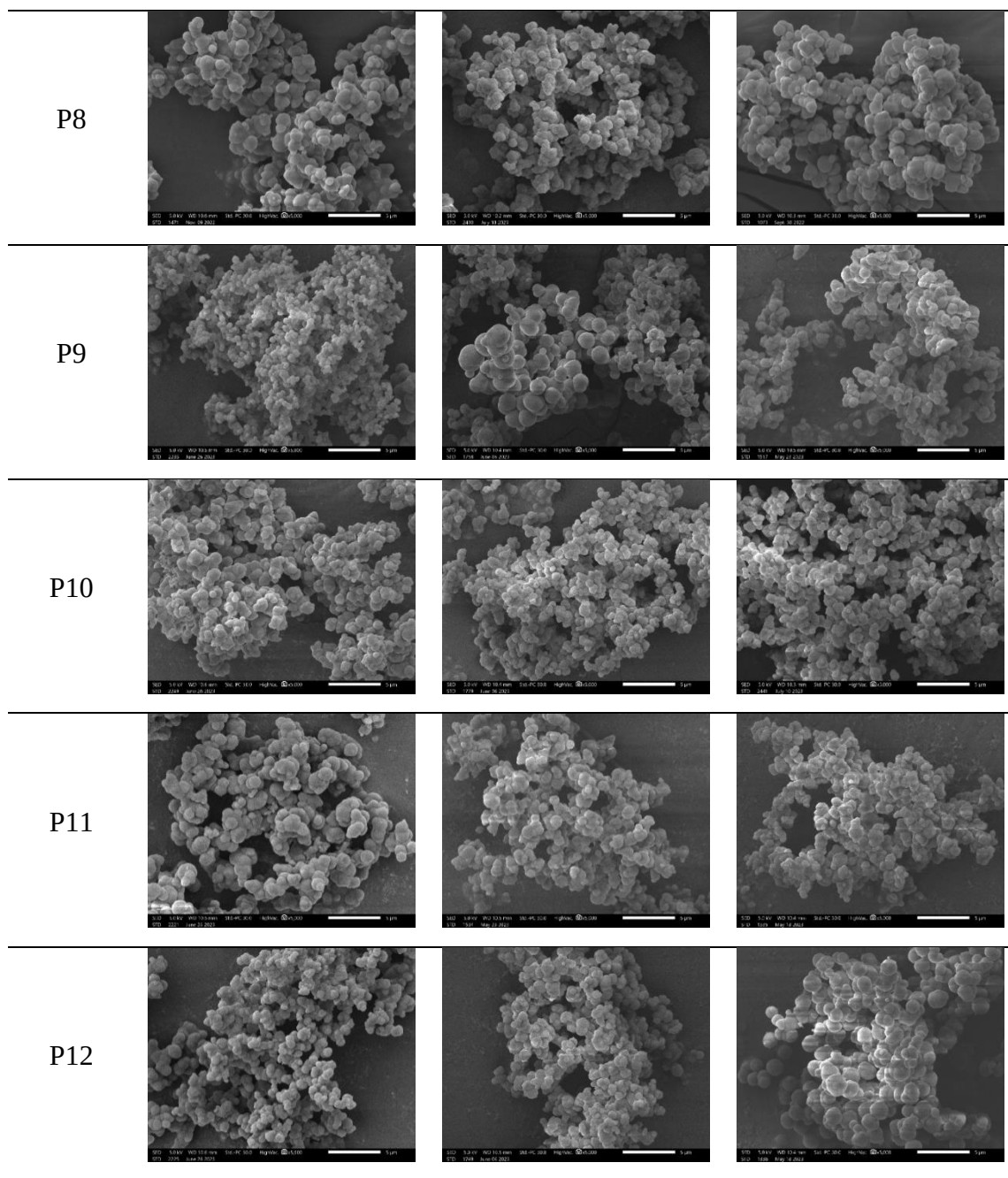
P3			
P4			
P5			
P6			
P7			

Table 3.8: Continued.



P13

Three SEM images of P13 at different magnifications. The left image is at 1000x magnification, the middle at 2000x, and the right at 5000x. All images show a dense, granular morphology of the P13 material. Each image includes technical data at the bottom and a 1 μm scale bar.

96

slightly high standard deviation. In comparison to other study, the particle size obtained in this study showed smaller particle size compared to what was reported by Khunsa Ngiem and team in 2018, who obtained particle size ranging from 2 to 5 μm using MW.

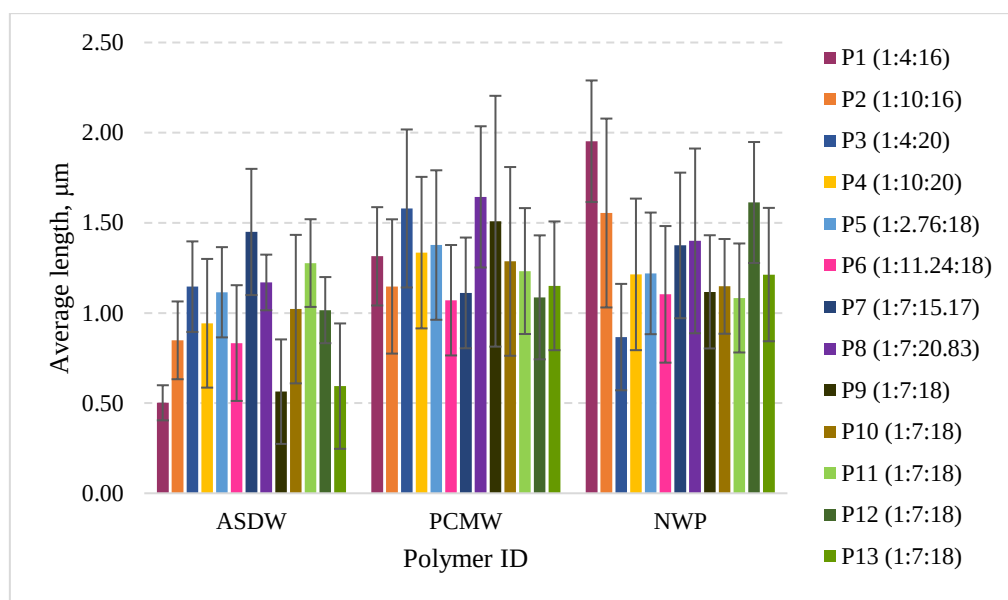


Figure 3.12: Average particle size of (a) ASDW, (b) PCMW and (c) NWP measured by ImageJ. N=35.

3.3.3.6 Rebinding Study of ASDW, PCMW and its NWP.

In the MW-assisted synthesis of aspirin MIP (ASDW), the rebinding capacity observed from all the polymers was higher than conventional precipitation polymerisation (Figure 3.13). The rebinding capacity was 7.15 ± 0.08 mg/g from ASDW-P1 compared to 3.00 ± 0.31 mg/g from the conventional thermal heating (ASD-P1). On the other hand, PCMW_P1 also showed a higher rebinding capacity at 6.17 ± 0.03 mg/g. This outcome contradicts that of Rahiminezhad et al. (2014), who reported that high temperature has a negative impact on the complex formation

between the template and functional monomer, which is associated with the binding affinity of the prepared MIP (Wloch & Datta, 2019; Pratama et al., 2020). These results can be explained by the fact that MIP can withstand high temperatures without losing its binding affinity up to 150°C for 24 h (Svenson & Nicholls, 2001). This finding can be supported by the IF value, which higher IF value indicates the specific recognition of the prepared MIP towards the drug compared to its NWP (Pratama et al., 2020). ASDW-P5 (T:FM:CL, 1:2.76:18) showed a high IF value and, followed by ASDW-P1 (T:FM:CL, 1:4:16). Meanwhile, PCMW-P7 (T:FM:CL, 1:7:15.17) showed higher IF value. Therefore, The MW polymerisation setting at 110°C for 8 min used in this study does not negatively affect the binding affinity of the prepared MIP.

The higher rebinding capacity observed in both ASDW and PCMW is also in accordance with the particle size obtained *via* MW synthesis. The particle size of MIP and NIP prepared *via* MW-assisted synthesis appeared slightly smaller than conventional thermal heating. Smaller particle size often offers a larger surface area-to-volume ratio, leading to more binding sites and improved access to the binding locations that influence the rebinding capacity (Gkika et al., 2024).

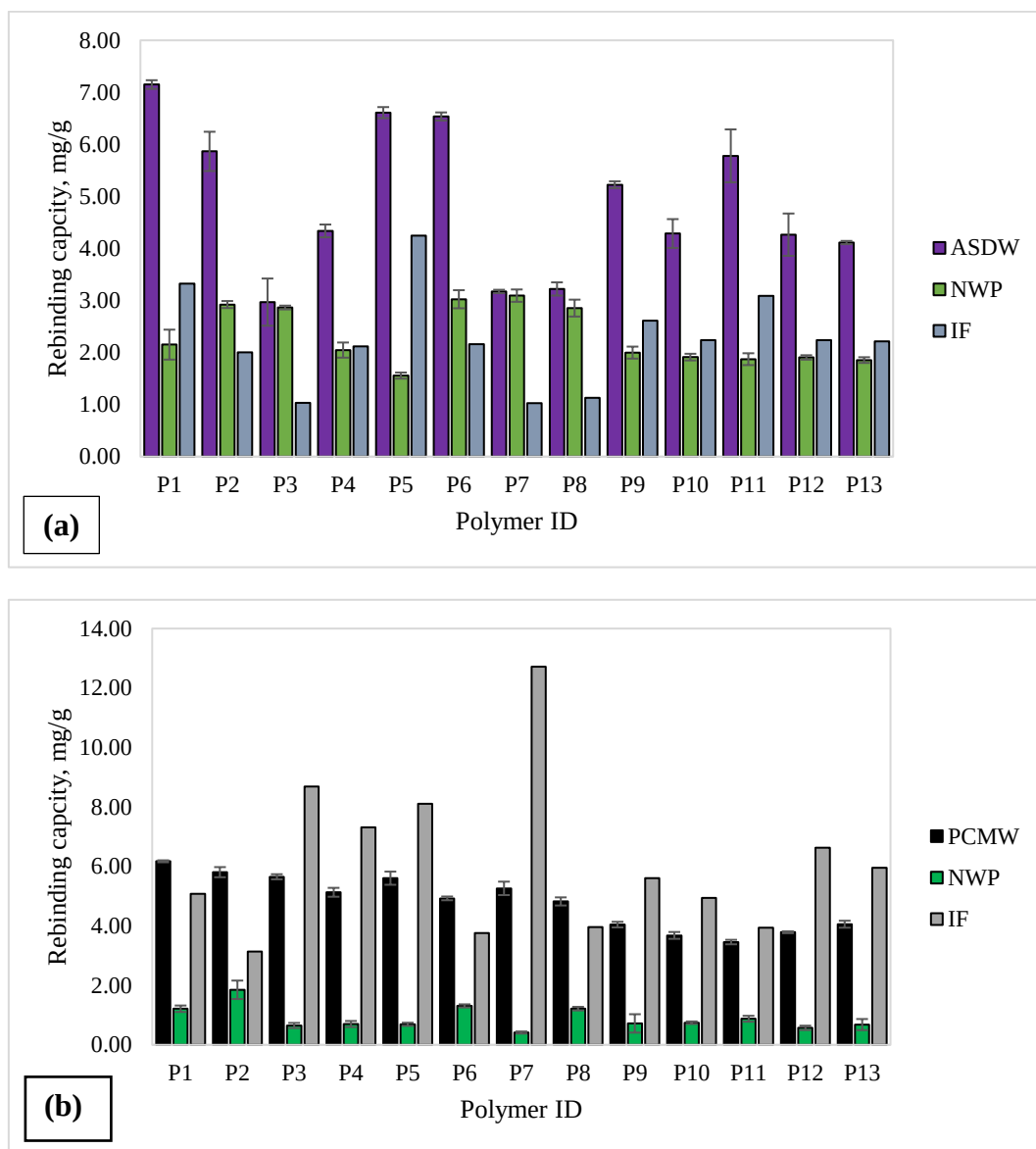


Figure 3.13: Rebinding capacity of (a) ASDW and (b) PCMW.

3.3.4 Optimisation of the Polymer Synthesis

3.3.4.1 Fitting the Design Model

The synthesis of MIP was optimised employing CCD of RSM. The collected rebinding study data was analysed using Design Expert software (version 10) and applied the second-order

polynomial model. The fit summary on the response model revealed that the quadratic model was statistically significant for the rebinding capacity of ASD, ASDW and PCMW. Therefore, it will be used in further analysis.

i. Conventional Thermal Heating of Aspirin MIP (ASD)

The analysis of variance (ANOVA) for the rebinding capacity of ASD is shown in Table 3.9. The model F-value of 51.85 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise which refers to the variability or random error present in the experimental data. The value of “Prob.>F” for the model is less than 0.05, which indicates that the model is significant, which is desirable as it indicates that the terms in the model have significantly affected the response. In this case B, A^2 , B^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. The lack-of-fit F-value of 3.65 implies the lack-of-fit is insignificant relative to pure error. There is a 12.15% chance that a lack-of-fit this large could occur due to noise. The quality of fit for the polynomial model equation is expressed by the ANOVA coefficient of determination (R^2 and adjusted- R^2). R^2 is a measure of variation in the response that can be explained by predictors in the model and was used to determine the relationship between the actual and predicted rebinding capacity, as shown in Figure 3.14. Based on the results, a desirable R^2 and adjusted- R^2 value, were close to 1, representing a high correlation between the experimental and the fitted data. A zero R^2 value indicated no relationship between the actual and predicted rebinding capacity. Furthermore, the predicted- R^2 value of 0.8542 was in agreement with the adjusted- R^2 of 0.9550, which indicates that factors affecting the efficiency of the rebinding capacity of ASD have been selected

correctly. The adequate precision measures the signal to noise ratio with a ratio greater than 4 is desirable. In this study, a ratio of 15.470 indicates an adequate signal, thus, establishing this model to be used to navigate the design space.

Table 3.9: ANOVA for the response surface quadratic models for rebinding capacity of ASD.

Source	Sum of Squares	df	Mean Square	F-Value	p-value Prob > F
Model	6.27	5	1.25	51.85	< 0.0001
A-Monomer	0.027	1	0.027	1.12	0.3244
B-Crosslinker	0.14	1	0.14	5.64	0.0493
AB	7.225E-003	1	7.225E-003	0.30	0.6016
A²	3.94	1	3.94	163.00	< 0.0001
B²	2.94	1	2.94	121.63	< 0.0001
Residual	0.17	7	0.024		
Lack Of Fit	0.12	3	0.041	3.65	0.1215
Pure Error	0.045	4	0.011		
Cor Total	6.44	12			

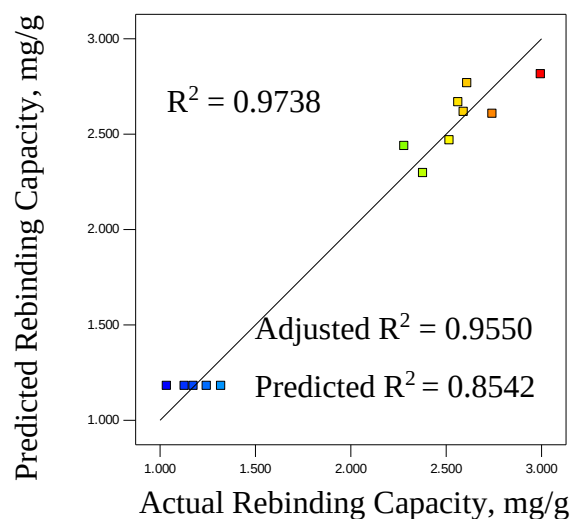


Figure 3.14: Predicted vs actual data for rebinding capacity (mg/g) of ASD.

The quadratic models for the rebinding capacity with coded and actual factors are shown in Equation 3.4 and Equation 3.5, respectively. In both equations, A and B correspond to independent variables of the amount of functional monomer and amount of crosslinker, respectively. The negative coefficients for model components A and B indicate the undesirable effects on the rebinding capacity of ASD. While the positive coefficients for AB, A^2 and B^2 indicate desirable effects on the rebinding capacity of ASD. The equation in terms of coded factors can be used to predict the response for given levels of each factor. By default, the high levels of the factors are coded as +1, and the low levels are coded as -1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients. However, the levels should be specified in the original units of each factor in the actual factors. This equation should not be used to determine the relative impact of each factor because the

coefficients are scaled to accommodate the units of each factor, and the intercept is not at the centre of the design space.

ASD Rebinding capacity (mg/g) Equation 3.4

$$= +1.18 - 0.058A - 0.13B + 0.043AB \\ + 0.75A^2 + 0.65B^2$$

ASD Rebinding capacity (mg/g) Equation 3.5

$$= +60.15414 - 1.31787A - 5.9712B \\ + 7.08333E - 0.03AB + 0.083639A^2 \\ + 0.16256B^2$$

ii. MW-assisted Synthesis of Aspirin MIP (ASDW)

The ANOVA of the response surface quadratic model for ASDW is shown in Table 3.10. The model F-value and p-value are significant and desirable. The coefficient of multiple determinations R^2 of 0.7304 is relatively close to 1. However, the negative predicted- R^2 implies that overall means were a better predictor for the response than the current model. Further analyses were conducted as described in Table 3.11, where the standard deviation for the model was 0.96. The standard deviation value indicates a good model that gives a near value between predicted and actual values for the response of rebinding capacity. The adequate precision measurement obtained further confirmation of whether this design can be used. The signal to noise ratio with a ratio greater than 4 is desirable. This study's adequacy precision ratio of 6.740 indicates an adequate signal; thus, this model can be used to navigate the design space.

Regardless of the prior establishment, an improvement in the coefficient of determination for ASDW was achieved *via* model modification by adding one more term (A^2B) to the model (Rheem et al., 2017) (Table 3.12). The R^2 has improved to 0.9063, and the predicted- R^2 was in reasonable fit with the adjusted- R^2 . The overall analysis of the modified model term is reported in Appendix B6.2.

Table 3.10: ANOVA for the response surface quadratic models for rebinding capacity of ASDW.

Source	Sum of Squares	df	Mean Square	F- Value	p-value Prob > F
Model	17.34	5	3.47	3.79	0.0555
A-Monomer	1.011E-005	1	1.011E-005	1.106E-005	0.9974
B-Crosslinker	3.98	1	3.98	4.35	0.0755
AB	1.76	1	1.76	1.92	0.2084
A^2	6.53	1	6.53	7.14	0.0319
B^2	3.62	1	3.62	3.96	0.0870
Residual	6.40	7	0.91		
Lack Of Fit	4.26	3	1.42	2.65	0.1848
Pure Error	2.14	4	0.54		
Cor Total	23.75	12	3.47		
R^2	0.7304				
Adjusted R^2	0.5379				
Predicted R^2	-0.4166				

Table 3.11: Additional statistical data for ASDW.

Significant factors	ASDW
Standard. Deviation (Std. Dev).	0.96
Mean	4.89
Coefficient of Variance (CV) %	19.56
Adequate Precision	6.740
PRESS	33.64

Table 3.12: ANOVA for response surface reduced cubic model for ASDW.

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F
Model	21.52	6	3.59	9.68	0.0071
A-Monomer	1.011E-005	1	1.011E-005	2.728E-005	0.9960
B-Cross-linker	1.250E-003	1	1.250E-003	3.372E-003	0.9556
AB	1.76	1	1.76	4.74	0.0724
A ²	6.53	1	6.53	17.61	0.0057
B ²	3.62	1	3.62	9.76	0.0205
A ² B	4.18	1	4.18	11.27	0.0153
Residual	2.22	6	0.37		
Lack of Fit	0.082	2	0.041	0.077	0.9272
Pure Error	2.14	4	0.54		
Cor Total	23.75	12			
Model	21.52	6	3.59	9.68	0.0071
R ²	0.9063				
Adjusted R ²	0.8126				
Predicted R ²	0.7722				

The predicted vs. actual plot for the rebinding capacity of ASDW is shown in Figure 3.15. Multiple regression analysis was used to create empirical models based on coded and actual factors to predict the rebinding capacity of ASDW using the experimental data. The rebinding capacity of ASDW was expressed in Equation 3.6. and Equation 3.7, for both coded and actual factors, respectively. In all equations, A and B correspond to independent variables of the amount of functional monomer and amount of crosslinker, respectively. The equation, expressed in terms of coded factors, allows for making predictions about the reaction based on specific quantities of each factor. The default coding for high levels of the factors is +1, while the default coding for low levels is -1. The coded equation is beneficial for determining the relative influence of the components by comparing the coefficients of the factors.

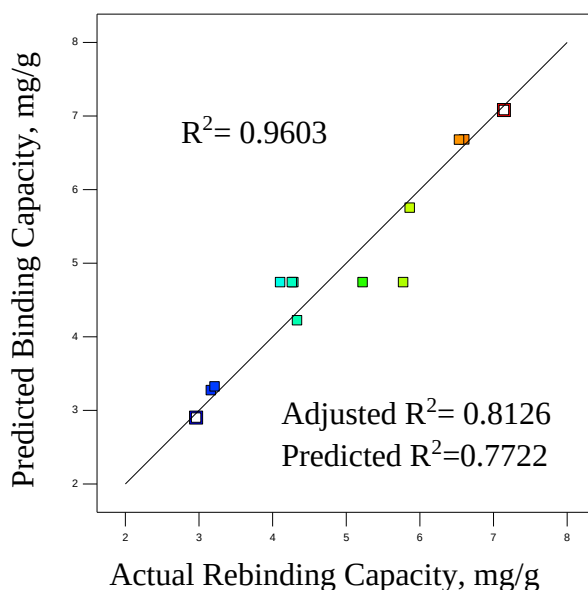


Figure 3.15: Predicted vs actual data for rebinding capacity (mg/g) of ASDW.

ASDW Rebinding capacity (mg/g)

Equation 3.6

$$\begin{aligned} &= +4.74 - 1.124E - 003A + 0.018B \\ &+ 0.66AB + 0.97A^2 - 0.72B^2 - 1.45 A2B \end{aligned}$$

ASDW Rebinding capacity (mg/g)

Equation 3.7

$$\begin{aligned} &= +36.16959 - 23.72750A + 1.79195B \\ &+ 1.23444AB \\ &+ 1.55283A^2 - 0.18028B^2 - 0.080288A2B \end{aligned}$$

iii. MW-assisted Synthesis of Paracetamol MIP (PCMW)

As for PCMW, the ANOVA for the quadratic response surface is shown in Table 3.13. The Model F-value of 14.26 implies the model is significant. There is only a 0.15% chance that an F-value this large could occur due to noise. The Lack of Fit F-value of 3.24 implies that it is non-significant, which is a good fitting to the model with only a 14.29% chance that it occurs due to noise. The predicted vs. actual plot for rebinding properties of PCMW is shown in Figure 3.16. The rebinding capacity of PCMW was expressed in Equation 3.8. and Equation 3.9, for both coded and actual factors, respectively. In all equations, A and B correspond to independent variables of the amount of functional monomer and amount of crosslinker, respectively.

Table 3.13: ANOVA for the response surface quadratic models for rebinding capacity of PCMW.

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F
Model	8.92	5	1.78	14.26	0.0015
A-Monomer	0.42	1	0.42	3.34	0.1104
B-Cross-linker	0.42	1	0.42	3.32	0.1112
AB	6.400E-003	1	6.400E-003	0.051	0.8275
A ²	5.19	1	5.19	41.52	0.0004
B ²	3.93	1	3.93	31.41	0.0008
Residual	0.88	7	0.13		
Lack of Fit	0.62	3	0.21	3.24	0.1429
Pure Error	0.26	4	0.064		
Cor Total	9.79	12			

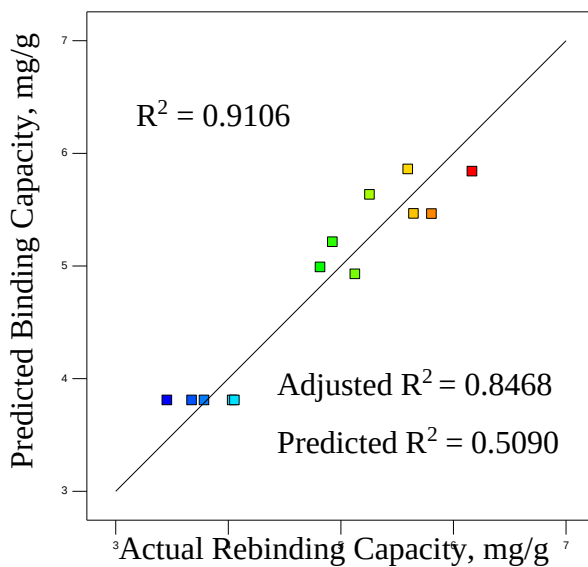


Figure 3.16: Predicted vs actual data for rebinding capacity (mg/g) of PCMW.

Equation 3.8

$$\begin{aligned} \text{PCMW Rebinding capacity (mg/g)} \\ = +3.81 - 0.23A - 0.23B - 0.040AB \\ + 0.86A^2 + 0.75B^2 \end{aligned}$$

Equation 3.9

$$\begin{aligned} \text{PCMW Rebinding capacity (mg/g)} \\ = +71.11376 - 1.29995A - 6.82960B \\ - 6.66667E - 003AB + 0.095986A^2 \\ + 0.18784B^2 \end{aligned}$$

3.3.4.2 Effect of Functional Monomer and Crosslinker (FM:CL) Ratio on Rebinding Capacity

The interactive relationship of independent variables (FM and CL) upon the achieved responses of rebinding capacity was represented graphically by a 3D response surface graph. In this study, the formulation ratio 1:4:16 (template: functional monomer: crosslinker, T:FM:CL) gave the highest rebinding capacity for ASD, ASDW and PCMW (Figure 3.17). This shows that T:FM of 1:4 have better binding affinity for aspirin and paracetamol. The molar ratios of T:FM in the synthesis process can affect the affinity and binding capacity of the MIP (Ariani et al., 2022). It was reported that lower T:FM molar ratios induced binding sites, and over-high T:FM molar ratios produced higher non-specific binding capacity (Pandey et al., 2020).

The recognition sites of MIP are affected by the choice of FM, while the number of binding sites is influenced by the concentration of the FM (Wloch & Datta, 2019). MAA, as functional monomer, can act as both hydrogen-bond donor and acceptor, as well as showing

good suitability for ionic interactions (Chen et al., 2016). At a low FM value of 2.8 mmol, high rebinding capacity was predicted at 15 mmol CL for ASD, ASDW and PCMW, respectively (Figure 3.18). Increasing the CL value to 20 mmol also showed high rebinding capacity for ASD and PCMW but low rebinding capacity for ASDW. At a high FM value of 11 mmol, high rebinding capacity can be achieved for ASD and PCMW (Figure 3.19).

Meanwhile, a contradicting trend was observed for ASDW, where the predicted rebinding capacity was decreased as the amount of CL increased. This indicates that the concentration of CL has a significant impact on the rebinding capacity of ASDW. A high CL concentration produces a larger pore diameter size and reduces the number of recognition sites (Malik et al., 2019). Although high rebinding capacity was predicted for all polymers at several FM:CL ratios, it is noteworthy to mention the effect of such ratios on the polymer morphology and particle size. In DDS, spherical particles with less polydispersity and smaller size opted for better cell internalization (Di et al., 2021; Kwak et al., 2021).

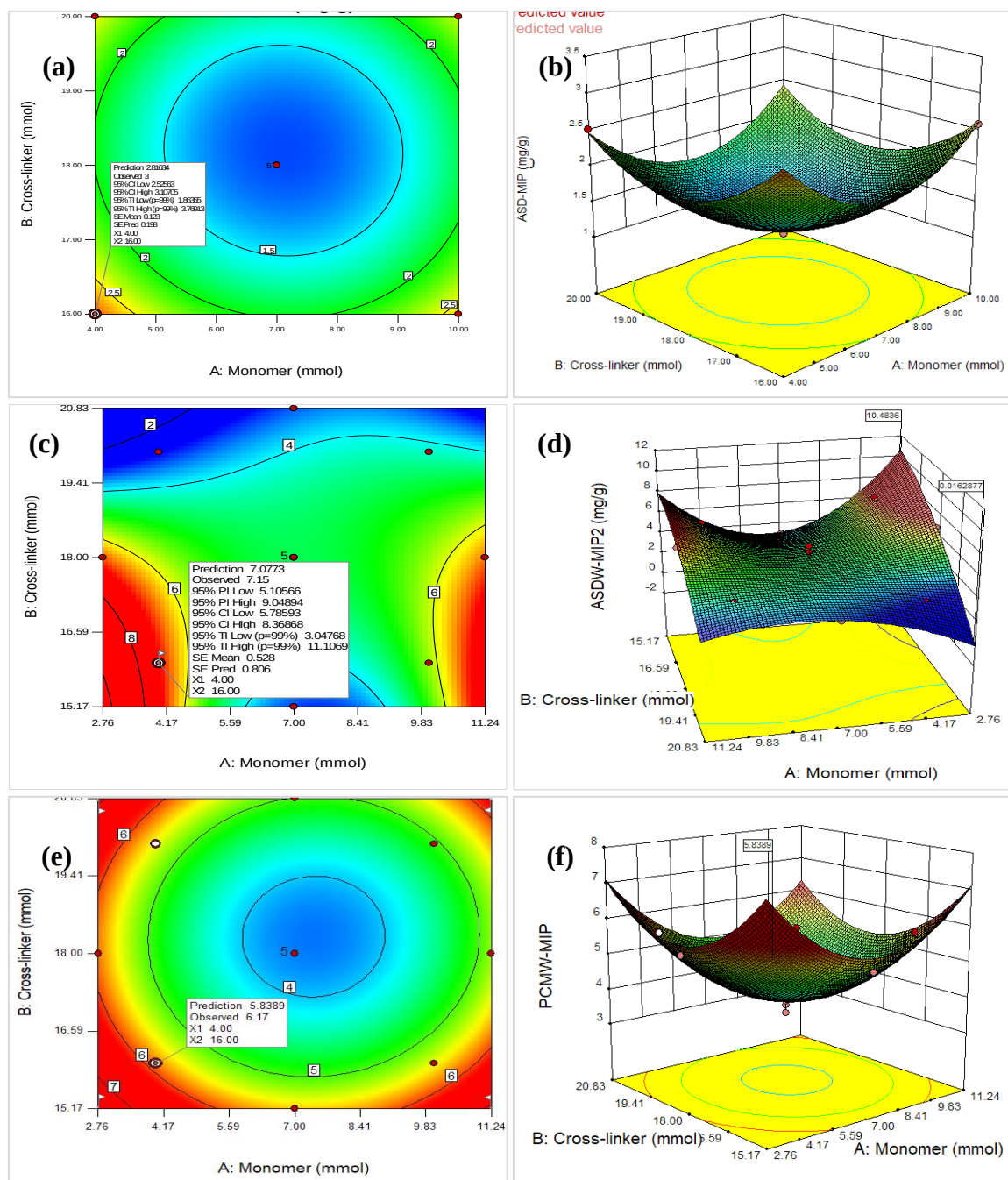


Figure 3.17: Contour plot and response surface plot illustrating the overall interaction of FM and CL on rebinding capacity at ratio 1:4:16 (T:FM:CL) (a-b) ASD, (c-d) ASDW and (e-f) PCMW.

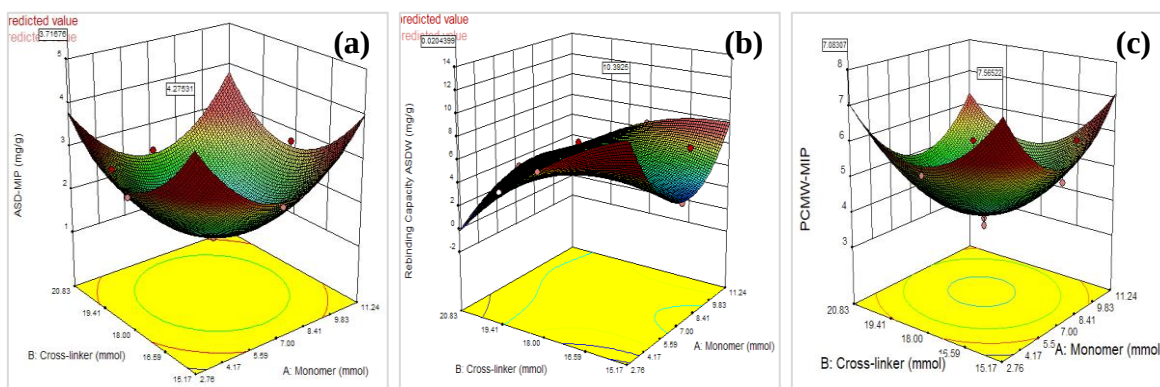


Figure 3.18: 3D surface graph showing the effect of low FM value (2.76 mmol) on the rebinding capacity of (a) ASD, (b) ASDW and (c) PCMW.

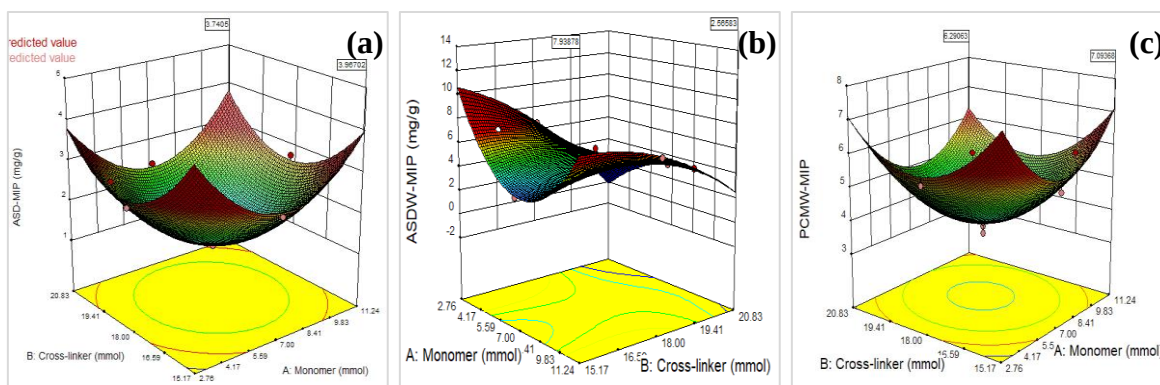


Figure 3.19: 3D surface graph showing the effect of high FM value (11.23 mmol) on the rebinding capacity of (a) ASD, (b) ASDW and (c) PCMW.

3.3.4.3 Method Validation

The method validation was performed under optimised conditions to validate that the predicted results were not biased toward practical value (Figure 3.20). The actual experimental data of the rebinding capacity were collected by performing duplicate experimental runs, as shown in Table 3.14. As can be seen, the actual values are proportionate with the predicted value.

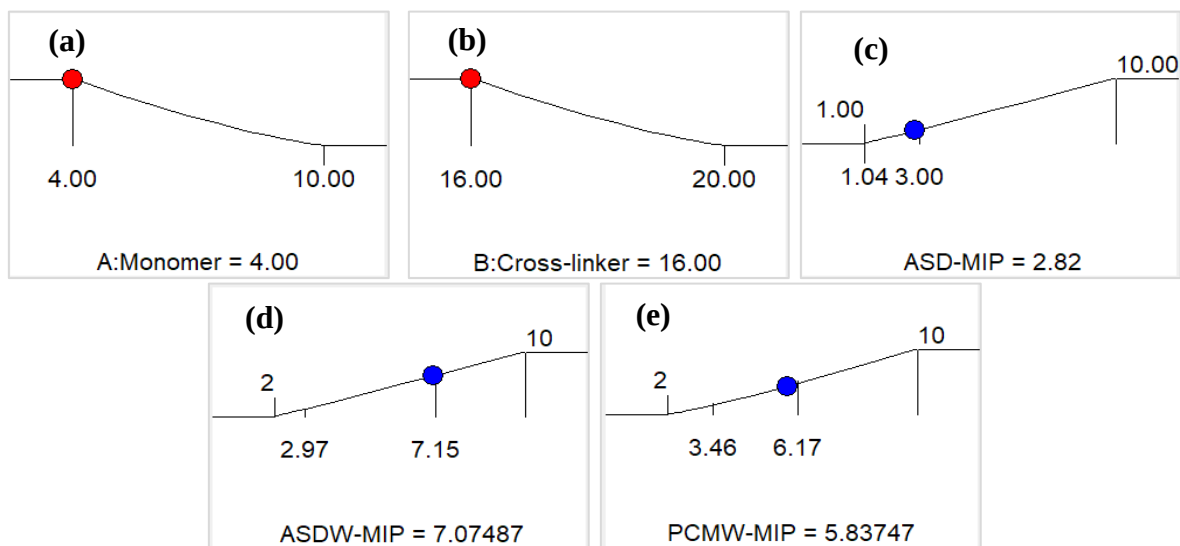


Figure 3.20: Optimised formulation ratio for the preparation of MIP (a) amount of monomer (mmol), (b) amount of crosslinker (mmol), and predicted rebinding capacity of (c) ASD, (d) ASDW (e) PCMW.

Table 3.14: Validation of rebinding capacity for ASD, ASDW and PCMW.

Polymer	Rebinding capacity, mg/g				
	R1	R2	Actual value (Average)	s.d.	Predicted value
ASD-P1	2.85	2.92	2.88	0.05	2.82
ASDW-P1	6.85	7.15	7.00	0.21	7.07
PCMW-P1	5.87	6.08	5.97	0.10	5.84

*R1, replicate 1; R2, replicate 2; s.d, standard deviation

3.4 Conclusion

Through systematic experimentation, the findings in this study reveal a significant advancement in the MIP properties achieved through MW-assisted synthesis, surpassing the

capabilities of conventionally synthesised MIPs. The polymer yield obtained *via* MW-assisted synthesis was comparable with conventional thermal heating and polymerised in a shorter time (110°C for 8 mins). Although the conventional thermal heating precipitation polymerisation produced more homogenous spherical particles than MW-assisted synthesis, the average particle size of MW-assisted synthesised MIP was smaller than conventional thermal heating.

The optimisation of polymer synthesis using CCD of RSM showed polymer P1 with T:FM:CL of 1:4:16 have high rebinding capacity in both polymerisation methods (i.e., thermal heating (ASD) and MW-assisted synthesis (ASDW, PCMW). Higher rebinding capacity achieved by ASDW-P1 (7.15 ± 0.08 mg/g) compared to ASD-P1 (3.00 ± 0.31 mg/g). High rebinding capacity was also observed by PCMW-P1 (6.17 ± 0.03 mg/g). It is consistent with the findings of the particle size of the MIP obtained by ASDW-P1 (0.50 ± 0.09 μm), PCMW-P1 (1.31 ± 0.2 μm) and ASD-P1 (1.98 ± 0.43 μm), respectively.

The utilization of RSM for polymer synthesis optimisation enabled simultaneous evaluation of the factors of the amount of functional monomer and crosslinker for the response of MIP rebinding capacity. A quadratic model was obtained to navigate the design space for both ASD, ASDW and PCMW, with some model modification performed for ASDW. The interactional behaviour of the two factors studied was successfully displayed in the three-dimensional response surface graph, generating substantial information from 13 polymer formulation ratios. In conclusion, it was demonstrated that different polymerisation processes (i.e., thermal heating and MW-assisted synthesis), including the type and amount of FM and CL in the formulation, can affect the final morphology of the resultant MIP or NIP, and the rebinding properties of MIP.

CHAPTER 4

APPLICATION OF RESPONSE SURFACE METHODOLOGY IN THE OPTIMISATION OF REBINDING CAPACITY OF MOLECULARLY IMPRINTED POLYMER AND EVALUATION OF SUSTAINED RELEASE OF ASPIRIN AND PARACETAMOL

4.1 Introduction

The response surface methodology (RSM) is a statistical tool that optimises large parameters and illustrates the interaction between variables while minimizing experimental procedures (Vera et al., 2014; Mourabet et al., 2017; Xu et al., 2021). By systematically manipulating and fine-tuning the variables, RSM enables a thorough understanding of the relationships between parameters and the performance of the resulting MIPs (Breig & Luti, 2021). The significance of optimisation using RSM lies in its potential to revolutionize DDS. Through a meticulous and statistical approach, this research aims to enhance the efficiency, specificity, and reproducibility of MIPs, advancing the prospect of precise drug delivery and also tailored to the unique characteristics of each therapeutic agent.

4.2 Materials and Methods

4.2.1 Materials, Reagents, and Instrumentation

Selected MIPs (ASD-P1, ASDW-P1 and PCMW-P1) were obtained and prepared according to the method described in Chapter 3. Acetylsalicylic acid (aspirin, 99% purity) was purchased from Acros Organic. Paracetamol (4-acetamidophenol, 98%) and hydrochloric acid (HCl, 37%) were purchased from Fisher Scientific. Acetonitrile technical grade and sodium

hydroxide (NaOH) were purchased from Merck and used as received. Potassium chloride (KCl), potassium dihydrogen phosphate (KH_2PO_4), and disodium hydrogen phosphate (Na_2HPO_4) were purchased from R&M and used as received. Sodium chloride (NaCl) was purchased from Bendosen. The UV-Vis analysis was performed using a UV-1280 UV-VIS spectrophotometer from Shimadzu Corporation.

4.2.2 Optimisation of Rebinding Study using CCD (RSM)

The selected MIP (ASD-P1, ASDW-P1, PCMW-P1) was optimised using CCD to obtain an exceptional binding property. Three factors influencing the rebinding capacity of MIP were critically studied which were the adsorbent dosage (20 – 40 mg), initial concentration (50 – 300 mg/L) and contact time (30 – 240 min) (Table 4.1). A total of 20 experimental runs were generated by Design Expert version 10, as shown in Table 4.2.

Generally, the selected MIP (20 mg) was added into a 50 mL conical flask containing the drug solution (20 mL, 50 mg/L). The solution was shaken at room temperature at 150 rpm for 30 mins. The solution was filtered and measured using a UV-vis-spectrophotometer at 276 nm and 289 nm for aspirin and paracetamol. A calibration curve was generated using 7 standard drug solutions (10 mg/L to 400 mg/L). The adsorbed amount of template was calculated from the same Equation 3.2 in Chapter 3.

Table 4.1: The range of independent variables for the experimental design.

Factors	Levels			-α	+α
	Low (-1)	Central (0)	High (+1)		
MIP dosage	20.00	30.00	40.00	13.18	46.82
Initial concentration	50.00	175.00	300.00	10.00	385.22
Contact time	30.00	135.00	240.00	10.00	312.00

Table 4.2: Experimental runs for optimisation of rebinding study of ASD-P1, ASDW-P1 and PCMW-P1.

Std. Run No.	Run Order	MIP dosage (mg)	Initial concentration (mg/L)	Contact Time (mins)
1	B14	20.00	50.00	30.00
2	B3	40.00	50.00	30.00
3	B12	20.00	300.00	30.00
4	B7	40.00	300.00	30.00
5	B4	20.00	50.00	240.00
6	B1	40.00	50.00	240.00
7	B17	20.00	300.00	240.00
8	B19	40.00	300.00	240.00
9	B13	13.18	175.00	135.00
10	B20	46.82	175.00	135.00
11	B9	30.00	10.00	135.00
12	B10	30.00	385.22	135.00
13	B5	30.00	175.00	10.00
14	B2	30.00	175.00	312.00

Table 4.2: Continued.

15	B15	30.00	175.00	135.00
16	B16	30.00	175.00	135.00
17	B8	30.00	175.00	135.00
18	B6	30.00	175.00	135.00
19	B18	30.00	175.00	135.00
20	B11	30.00	175.00	135.00

4.2.2.1 Preparation of Drug Solution

The stock solution (500 mg/L) for both aspirin (1 g) and paracetamol (1 g) was prepared in acetonitrile (500 mL) and kept at 4 °C until further use. The working solutions, as stated in Table 4.1, were obtained by diluting the stock solution with acetonitrile following Equation 4.1, where V_1 is the volume of stock solution needed to make a new solution, C_1 is the concentration of the stock solution, V_2 is the final volume of new solution, and C_2 is the concentration of the new solution.

$$C_1 v_1 = C_2 v_2 \quad \text{Equation 4.1}$$

4.2.3 *In vitro* Drug Release of Aspirin and Paracetamol

4.2.3.1 Drug Loading

Prior to the drug release study, drug loading was performed by incubating selected MIP (80 mg) in the drug solution (20 mL, 100 mg/L) for 18 h (overnight). The MIP loaded with drug (aspirin or paracetamol) was filtered and dried at room temperature for 24 h. The supernatant was measured using UV-vis spectrophotometer, and the adsorbed drug was calculated using Equation 3.2.

4.2.3.2 Preparation of Dissolution Medium

The hydrochloric acid-potassium chloride buffer solution at pH 2.2 was prepared by dissolving KCl (3.725 g, 0.1M) and HCl (0.386 g, 0.02M) in a flask of distilled water (500 mL). The pH value was adjusted using HCl and NaOH. Meanwhile, the buffer solution of pH 7.4 was prepared by dissolving NaCl (8 g), KCl (0.2 g), Na₂HPO₄ (1.44 g) and KH₂PO₄ (0.245 g) in distilled water (1 L). The solutions were kept at 4°C until further use.

4.2.3.3 Aspirin or Paracetamol Drug Release

The drug release was performed by placing loaded MIP (20 mg) into a Visking tube and immersed in the dissolution media of pH 2.2 (20 mL) and pH 7.4 (20 mL). The solution was placed in an incubator at 37±1 °C with agitation at 150 rpm. At specified time intervals within

2 to 24 h, samples (3 mL) were withdrawn from the medium and replaced with an equivalent volume of fresh dissolution media. The withdrawn samples were analysed using UV-vis spectrophotometer at 296 nm for aspirin and 289 nm for paracetamol. The cumulative percentage of drug release was calculated following the steps presented in Equation 4.2, Equation 4.3, Equation 4.4, and Equation 4.5, respectively. P_t is the percentage release at time t and $P(t-1)$ is the cumulative percentage release previous to ' t '. V_1 is the bath volume, and V_2 is the volume removed from the solution. The experimental drug release data were analysed using zero-order, first-order, simplified Higuchi models, the Korsmeyer-Peppas, and the Hixson-Crowell Cube Root Law kinetic models.

$$\text{Concentration of drug } \left(\frac{\text{mg}}{\text{L}}\right) = (\text{slope} \times \text{absorbance}) \pm \text{intercept} \quad \text{Equation 4.2}$$

$$\text{Amount of drug release (mg)} \quad \text{Equation 4.3}$$

$$= \text{Concentration } \left(\frac{\text{mg}}{\text{L}}\right) \times \text{Dissolution bath volume (L)} \times \text{dilution factor}$$

$$\text{Cumulative drug release (\%)} \quad \text{Equation 4.4}$$

$$= \left(\frac{\text{volume of sample withdrawn}}{\text{Bath volume}} \right) \times P(t-1) + P_t$$

$$\text{Dilution factor} = \frac{V_1 - V_2}{V_1} \quad \text{Equation 4.5}$$

4.3 Results and Discussion

The rebinding efficiency of MIPs can also be influenced by factors such as the MIP dosage, initial concentration, and contact time. Optimizing the rebinding capacity is essential for applying MIPs in various fields, including molecular recognition and the detection of target molecules. In a static rebinding assay at 100 mg/L initial concentration, 80 mg MIP dosage, and 120 min contact time, as discussed in Chapter 3, ASD-P1, ASDW-P1 and PCMW-P1 showed the highest rebinding capacity at 3.00 ± 0.31 mg/g, 7.15 ± 0.08 mg/g and 6.17 ± 0.03 mg/g, respectively. Based on the highest rebinding capacity of MIP P1 for all polymerisation methods (ASD, ASDW and PCMW) with the formulation ratio 1:4:16 (T:FM:CL) from the previous Chapter 3, the following strategy was to optimise the rebinding capacity of the prepared MIP using RSM.

4.3.1 Rebinding Study and Optimisation using Central Composite Design (CCD)

In order to achieve the highest rebinding capacity of MIPs, namely ASD-P1, ASDW-P1 and PCMW-P1, several factors such as the MIP dosage, initial concentration, and contact time were evaluated and optimised using CCD. A total of 20 experimental runs were employed, and actual experimental factors with the response of rebinding capacity are presented in Table 4.3. The data obtained were analysed using Design Expert software.

Table 4.3: Central composite design (CCD) for rebinding study of ASD-P1, ASDW-P1, and PCMW-P1.

Std. Order	Run Order	MIP dosage (g)	Contact time, (min)	Initial concentration (mg/L)	Rebinding capacity (mg/g)		
					ASD-P1	ASDW-P1	PCMW-P1
1	R14	0.02	30.00	50.00	1.84	3.14	1.84
2	R3	0.04	30.00	50.00	2.07	3.52	2.08
3	R12	0.02	30.00	300.00	1.86	3.88	1.83
4	R7	0.04	30.00	300.00	3.46	4.89	3.45
5	R4	0.02	240.00	50.00	2.23	5.51	2.17
6	R1	0.04	240.00	50.00	2.42	6.92	2.39
7	R17	0.02	240.00	300.00	5.00	8.47	4.96
8	R19	0.04	240.00	300.00	5.65	9.59	5.64
9	R13	0.01	135.00	175.00	2.63	4.46	2.56
10	R20	0.05	135.00	175.00	3.00	8.15	2.99
11	R9	0.03	135.00	10.00	1.96	2.88	1.91
12	R10	0.03	135.00	385.22	6.27	9.28	6.25
13	R5	0.03	10.00	175.00	1.46	2.57	1.45
14	R2	0.03	312.00	175.00	3.30	8.13	3.31
15	R15	0.03	135.00	175.00	2.64	4.51	2.60
16	R16	0.03	135.00	175.00	2.18	3.69	2.15
17	R8	0.03	135.00	175.00	2.69	4.56	2.67
18	R6	0.03	135.00	175.00	2.13	3.64	2.09
19	R18	0.03	135.00	175.00	2.23	3.79	2.19
20	R11	0.03	135.00	175.00	2.69	4.81	2.38

*std, standard;

ANOVA summary generated by the Design Expert software for the response surface quadratic model of ASD-P1, ASDW-P1 and PCMW-P1 is presented in Appendix A6.2, Appendix B6.2, and Appendix C6.2, respectively. In all models, the F-value were significant, implying that there was only a 0.01% chance that the F-value that large could be due to the noise. All models that were presented as non-significant based on their lack-of-fit F-values show that the quadratic model is statistically significant for the response (Xu et al., 2021).

Figure 4.1 shows the correlation between the predicted and actual value of rebinding capacity for all MIPs (a) ASD-P1, (b) ASDW-P1 and (c) PCMW-P1, which confirms the accuracy of the model (Arabzadeh et al., 2019). The goodness of fit of the models was further checked with the coefficient of determination (R^2) where the values are closer to 1, indicating a good fit. In addition, all predicted- R^2 values were in agreement with the adjusted- R^2 , which means that the significant terms have been included in the proposed empirical models and a good correlation between experimental results and the predicted data (Heravizadeh et al., 2020; Sadegh et al., 2021).

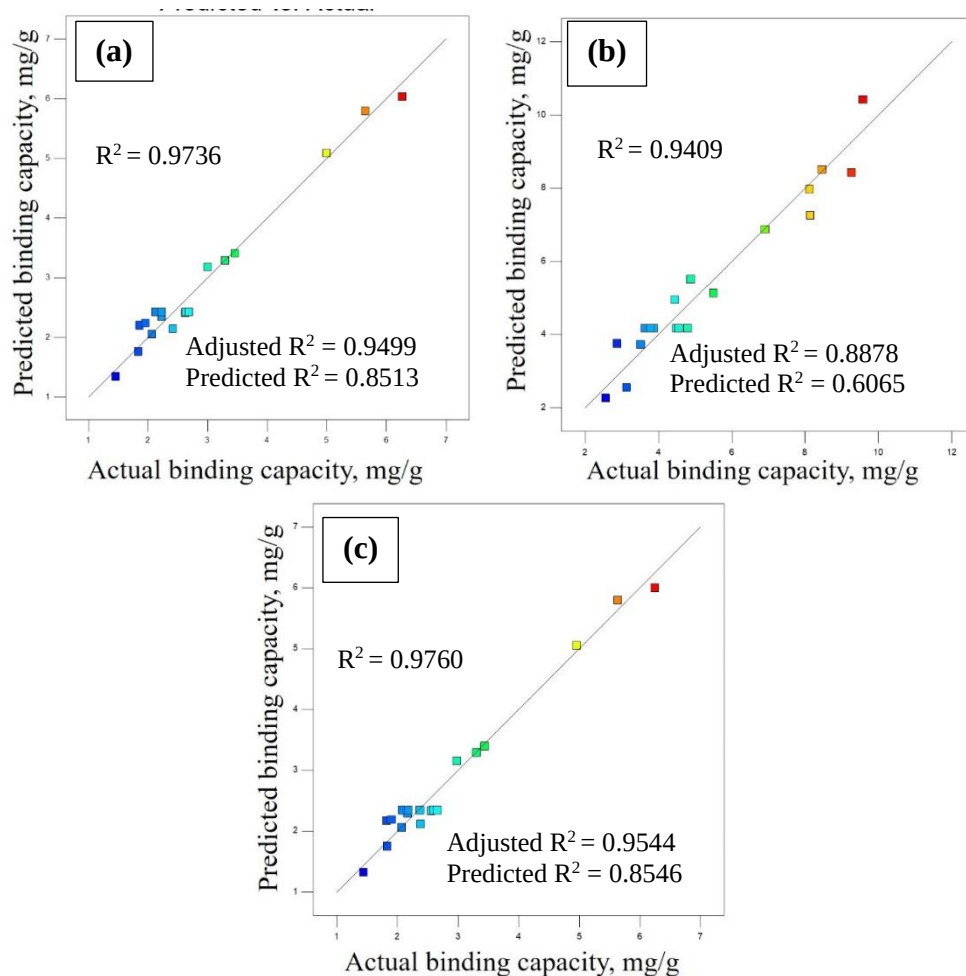


Figure 4.1: Predicted vs actual rebinding capacity of (a) ASD-P1 (b) ASDW-P1 and (c) PCMW-P1.

The equations below show the empirical regression model between the chosen variables and the rebinding capacity response. The coded and actual factors for ASD-P1 are shown in Equation 4.6 and Equation 4.7, respectively. Equation 4.8 and Equation 4.9 represent the coded and actual factors for ASDW-P1. Meanwhile, Equation 4.10 and Equation 4.11 represent the coded and actual factors for PCMW-P1. In all equations, A, B, and C correspond to independent variables of the adsorbent dosage, initial concentration and contact time, respectively.

Rebinding capacity of ASD – P1

Equation 4.6

$$\begin{aligned} &= +2.42 + 0.25A + 1.02B + 0.74C + 0.23AB \\ &- 0.12AC + 0.57BC + 0.14 A^2 \\ &+ 0.67 B^2 - 0.14 C^2 \end{aligned}$$

Rebinding capacity of ASD – P1

Equation 4.7

$$\begin{aligned} &= +3.15214 - 0.075601A - 0.018210B \\ &+ 6.28821E - 003C + 1.83000E - 004AB \\ &- 1.17857E - 004AC + 4.37143E - 005BC \\ &+ 1.40728E - 003A^2 + 4.28580E - 005B^2 \\ &- 1.22871E - 005C^2 \end{aligned}$$

Rebinding capacity of ASDW – P1

Equation 4.8

$$\begin{aligned} &= +4.16 + 0.77A + 1.29B + 1.87C + 0.042AB \\ &+ 0.14 AC + 0.44BC + 0.72A^2 + 0.74B^2 \\ &+ 0.23C^2 \end{aligned}$$

Rebinding capacity of ASDW – P1

Equation 4.9

$$\begin{aligned} &= +7.48902 - 0.38093A - 0.011781B \\ &+ 2.26527E - 003C + 3.40000E - 005 AB \\ &+ 1.35714E - 004AC + 3.35238E - 005BC \\ &+ 7.23118E - 003 A^2 + 4.73299E - 005 B^2 \\ &+ 2.08323E - 005C^2 \end{aligned}$$

Rebinding capacity of PCMW – P1

Equation 4.10

$$\begin{aligned} &= +2.34 + 0.26A + 1.02B + 0.73C \\ &+ 0.23AB - 0.12AC + 0.58BC + 0.15A^2 \\ &+ 0.69B^2 - 0.10C^2 \end{aligned}$$

Rebinding capacity of PCMW – P1

Equation 4.11

$$\begin{aligned} &= +3.27048 - 0.081593A - 0.018707B \\ &+ 5.12829E - 003C + 1.84096E - 004AB \\ &- 1.14830E - 004AC + 4.45539E - 005BC \\ &+ 1.51935E - 003A^2 + 4.38942E - 005B^2 \\ &- 9.21675E - 006C^2 \end{aligned}$$

4.3.1.1 Response Surface Plot

The three-dimensional response surface plots and contour plots were developed to visualize and understand the interaction effect between all the factors by evaluating two variables at a time on the rebinding capacity of all MIPs while maintaining another variable at a fixed level (Asfaram et al., 2015; Nazmi & Sarbon, 2019; Xu et al., 2021).

i. Effect of Initial Concentration and Contact Time on the Rebinding Capacity

Figure 4.2a-c shows the combined effect of initial concentration and contact time on the rebinding capacity of ASD-P1, ASDW-P1 and PCMW-P1 at a 20 mg dosage of MIP. As the concentration increased to 300 mg/L and extended contact time was applied until 240 min, high rebinding capacity was observed in all MIPs at 20 mg dosage. ASD-P1 showed a rebinding capacity of 5.07 mg/g, and ASDW-P1 showed a rebinding capacity of 8.50 mg/g. In comparison, PCMW-P1 showed a rebinding capacity of 5.04 mg/g. Higher rebinding capacity can be achieved at low MIP dosage due to the high number of binding sites fabricated in the prepared

MIP (Heravizadeh et al., 2020). As the MIP dosage was increased to 40 mg (Figure 4.2d-f), the rebinding capacity of ASD-P1, ASDW-P1 and PCMW-P1 increased to 5.78 mg/g, 10.41 mg/g and 5.79 mg/g, respectively. Introducing more MIP dosage creates more binding sites and increases the chances of successful rebinding (Tamandani et al., 2022).

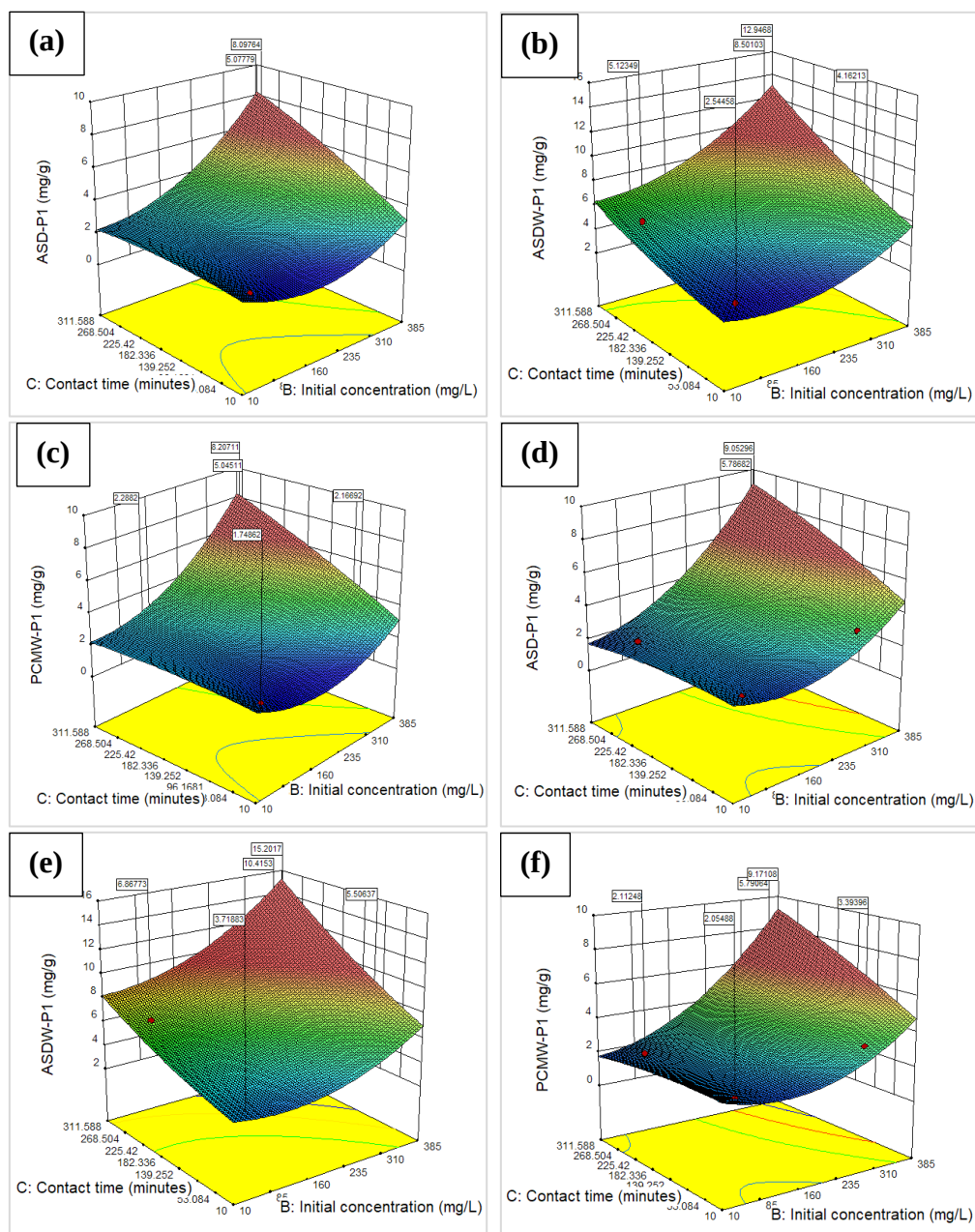


Figure 4.2: Effect of initial concentration and contact times on the rebinding capacity of (a) ASD-P1 (b) ASDW-P1 (c) PCMW-P1 at 20 mg MIP dosage and (d) ASD-P1 (e) ASDW-P1 (f) PCMW-P1 at 40 mg MIP dosage.

i. Effect of MIP Dosage and Contact Time on the Rebinding Capacity

Figure 4.3 shows the effect of MIP dosage and contact time on the rebinding capacity at low initial concentrations of 50 mg/L. At low initial concentration, no significant changes were observed in the rebinding capacity of ASD-P1 and PCMW-P1, although prolonged contact time (310 min) and increased adsorbent dosage (45 mg) were employed. This phenomenon is attributed to low solute concentrations, which allows for the occupation of the active sites on MIPs (Asfaram et al., 2015). Meanwhile, ASDW-P1 showed high rebinding capacity at 10.05 mg/g at the low initial concentration (50 mg/L), 45 mg MIP dosage and 310 min contact time.

It was noted that ASDW-P1 has the smallest particle size, as discussed in Chapter 3, Section 3.3.3.5, and often has higher surface area-to-volume ratios, which produced more binding sites and improved access to the binding locations (Gkika et al., 2024). Thus, high rebinding capacity was observed by ASDW-P1. High rebinding capacity was achieved when the initial concentration was increased to 300 mg/L and agitated at a longer contact time (300 min) and a high MIP dosage (40 mg) (Figure 4.4). This behaviour is ascribed to the formation of specific cavities within the MIP, which allow more solute molecules to rebind within the cavities (Ghani et al., 2020). Additionally, many active sites on the MIP can adsorb the target molecules (Asfaram et al., 2015).

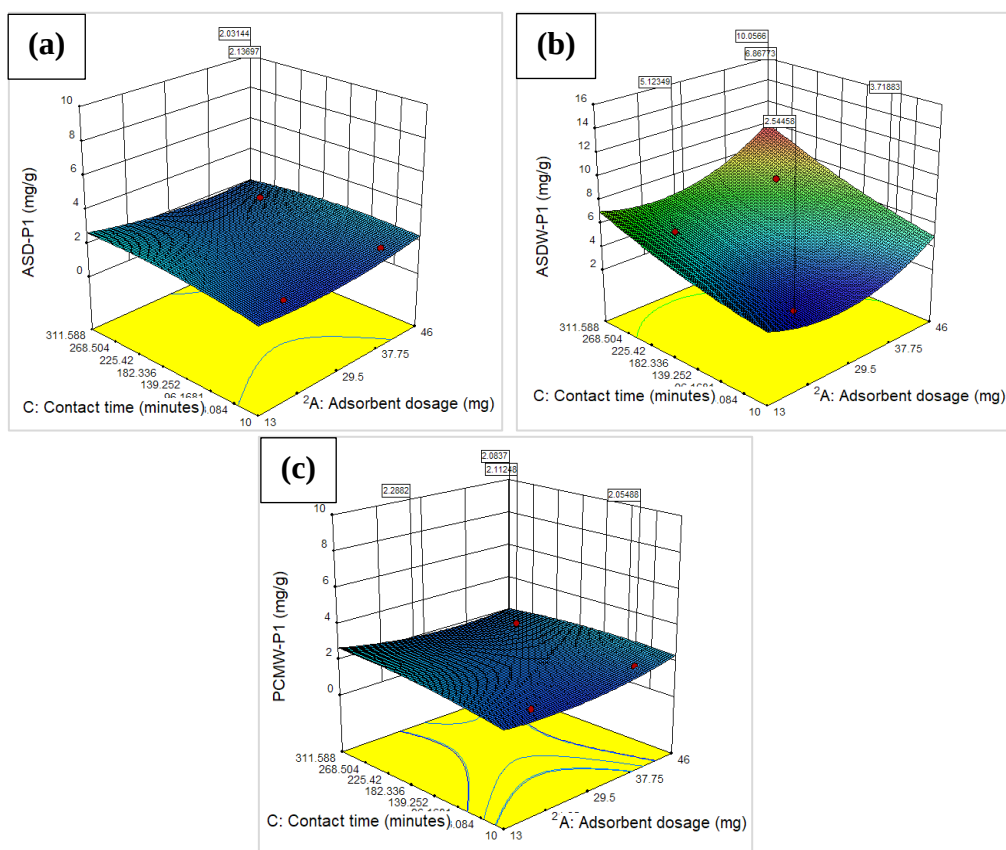


Figure 4.3: Effect of MIP dosage and contact time on the rebinding capacity of (a) ASD-P1, (b) ASDW-P1 and (c) PCMW-P1 at initial concentration of 50 mg/L.

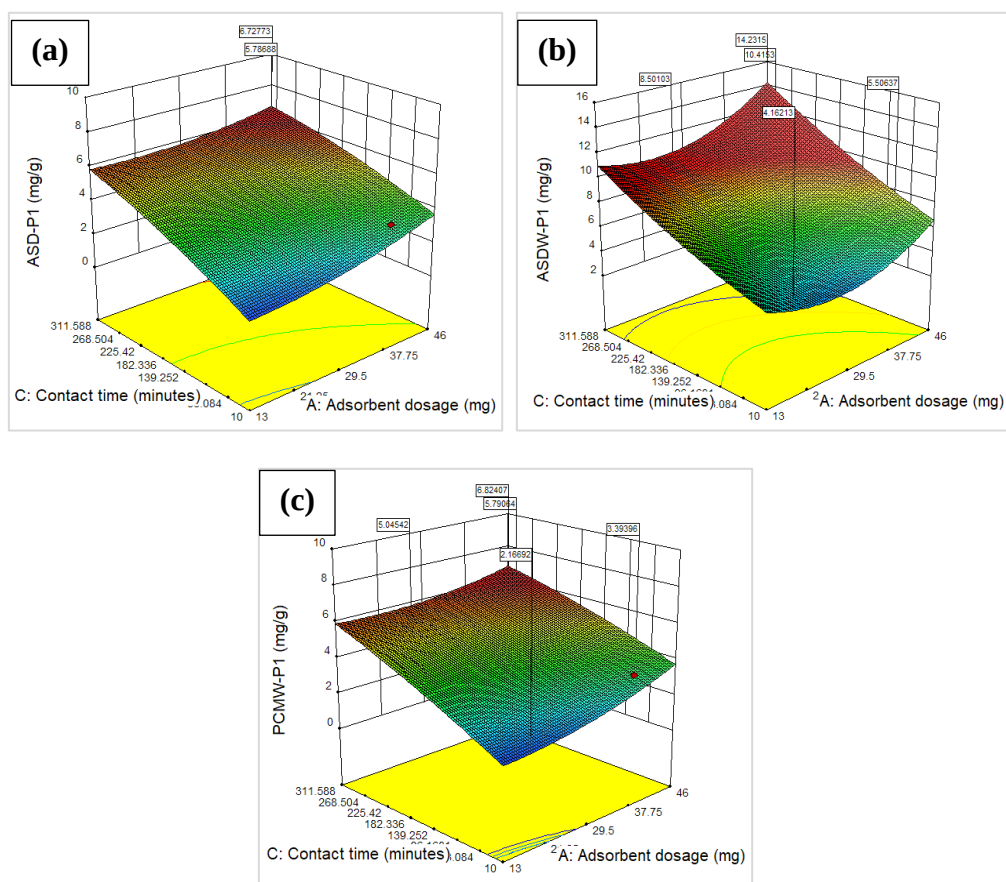


Figure 4.4: Effect of MIP dosage and contact time on rebinding capacity of (a) ASD-P1, (b) ASDW-P1 and (c) PCMW-P1 at initial concentration at 300 mg/L.

ii. Effect of Initial Concentration and MIP Dosage on the Rebinding Capacity

Other factors impacting the rebinding capacity were the initial concentration and MIP dosage. Figure 4.5 shows the effect of initial concentration and MIP dosage on the rebinding capacity of MIPs at different contact times. At 30 min contact time, 300 mg/L initial concentration and 40 mg MIP dosage, low rebinding capacity were observed for ASD-P1 (3.46 mg/g), ASDW-P1 (5.51 mg/g) and PCMW-P1 (3.39 mg/g) (Figure 4.5a-c). The rebinding

capacity was increased to 5.74 mg/g for ASD-P1, 8.96 mg/g for ASDW-P1 and 5.76 mg/g for PCMW-P1 as the amount of MIP was increased to 45 mg and 384 mg/L high initial concentration at 30 min contact time. Moreover, increasing the contact time to 240 min with similar MIP dosage (40 mg) and initial concentration (300 mg/L) has improved the rebinding capacity to 5.78 mg/g for ASDW-P1, 10.41 mg/g for ASDW-P1, and 5.79 mg/g for PCMW-P1 (Figure 4.5d-f). It was observed that high rebinding capacity was achieved at a shorter contact time when high initial concentration and MIP dosage were employed. Meanwhile, prolonged contact time allowed more adsorption into specific binding sites located in the deeply crosslinked polymer network (Xu et al., 2021).

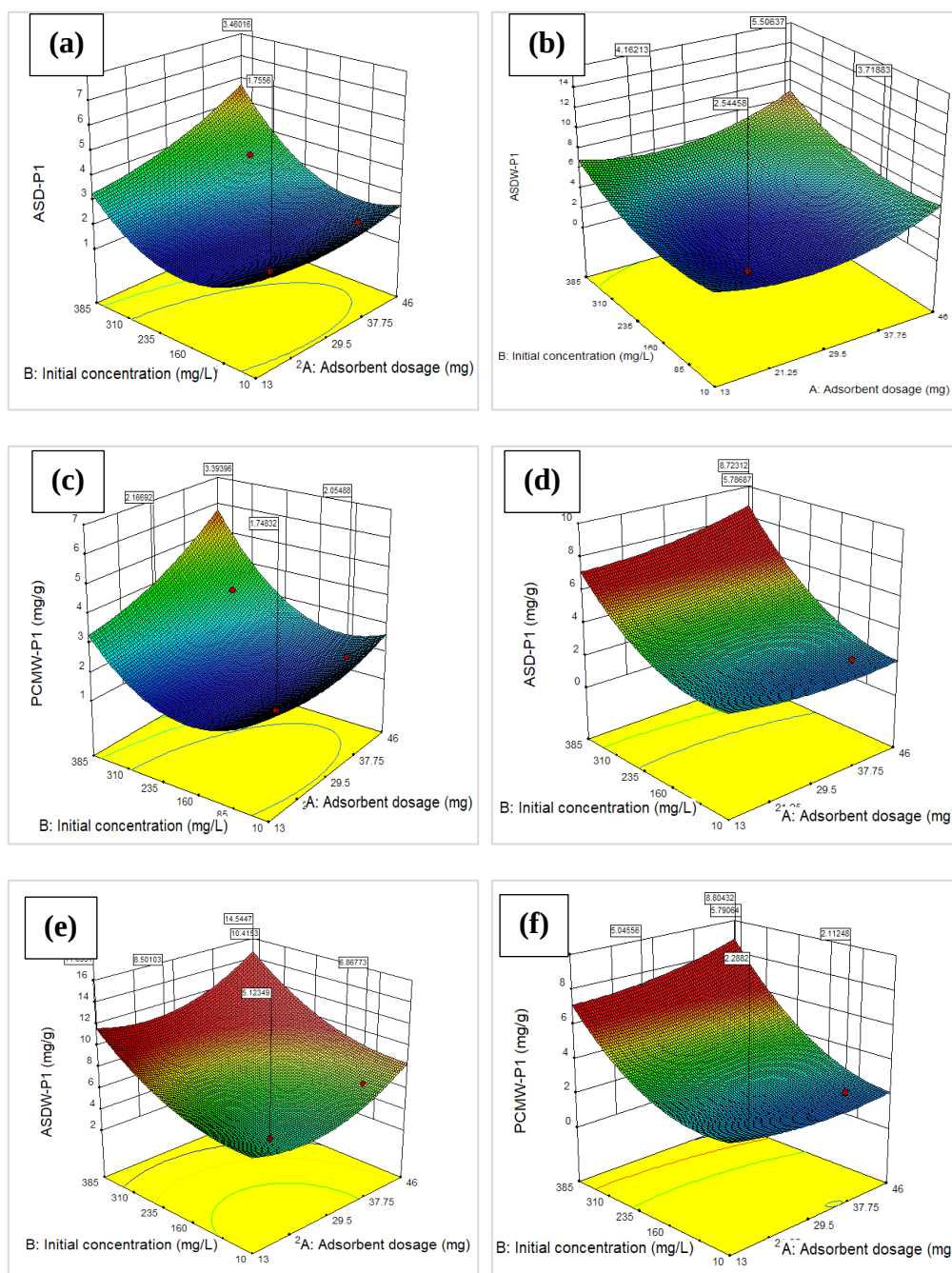


Figure 4.5: Effect of initial concentration and MIP dosage on the rebinding capacity of (a) ASD-P1, (b) ASDW-P1 and (c) PCMW-P1 at 30 min and on (d) ASD-P1, (e) ASDW-P1 and (f) PCMW-P1 at 240 min.

Based on the designed empirical models discussed above, the overall optimum operating conditions for the rebinding capacity of all MIPs are shown in Figure 4.6. The optimised design is illustrated based on the minimum MIP dosage in producing high rebinding performance. All three factors are the most prominent affecting the rebinding capacity of the prepared MIP. It can be seen even at low MIP dosage (20 mg); high rebinding capacity can be achieved at high initial concentration (300 mg/L) and longer contact time (240 min). The predicted rebinding capacity at optimised conditions for ASD-P1, ASDW-P1 and PCMW-P1 was 5.08 mg/g, 8.50 mg/g and 5.05 mg/g, respectively. The validation of the optimised condition is reported in Table 4.3. A 2 to 3 % error was observed from the actual validation with the predicted results.

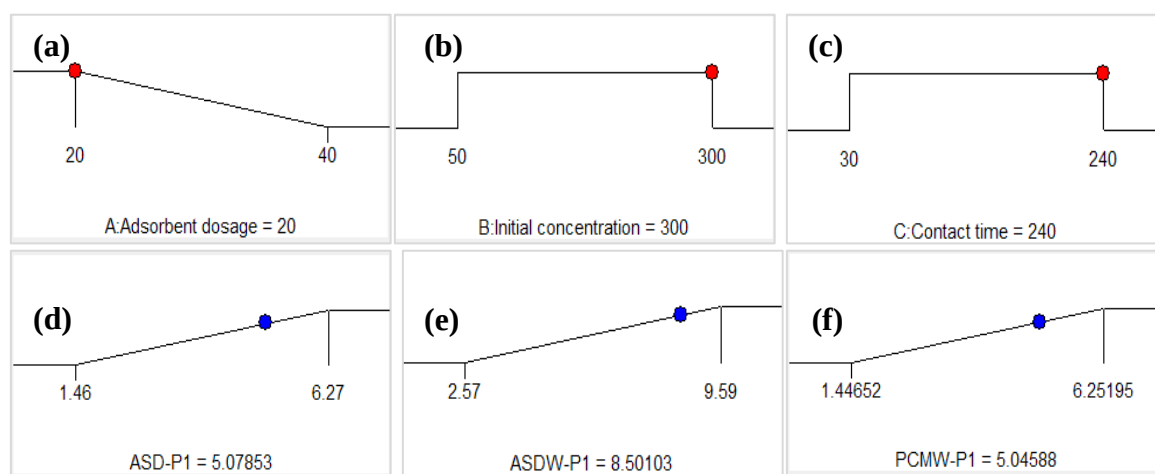


Figure 4.6: Optimised condition on the rebinding capacity of the selected MIP based on the (a) adsorbent dosage (b) initial concentration (c) contact time and the predicted rebinding capacity of (d) ASD-P1, (e) ASDW-P1 and (f) PCMW-P1.

Table 4.4: Validation of rebinding capacity for ASD-P1, ASDW-P1 and PCMW-P1 based on the optimised condition.

MIP	Rebinding capacity, mg/g					Predicted value	Error
	Actual value						
	R1	R2	Average	s.d.			
ASD-P1	5.31	5.16	5.24	0.10	5.08	3.05%	
ASDW-P1	8.61	8.73	8.67	0.08	8.50	2.00%	
PCMW-P1	5.11	5.21	5.16	0.07	5.05	2.17%	

4.3.2 *In vitro* Drug Release Profile

Having all the MIPs rebinding performance and characterisation analysed, the final stage of this study is to evaluate the in-vitro drug release of aspirin and paracetamol. The release profile of MIPs was evaluated by performing dialysis technique using a Visking tube, a semipermeable membrane, for a diffusion-controlled release experiment (Figure 4.7). The dissolution media employed was phosphate-buffered saline (PBS, pH 7.4) solution and hydrochloric acid-potassium chloride buffer solution (pH 2.2), which simulated the condition of intestinal fluid and human stomach, respectively (Sheybani et al., 2015).

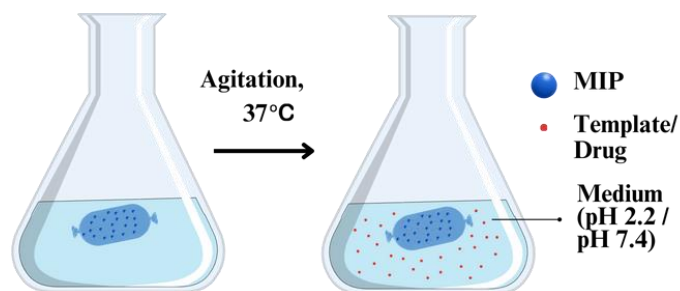


Figure 4.7: Experimental setup for *in vitro* drug release.

4.3.2.1 Cumulative Drug Release of Aspirin and Paracetamol

Cumulative drug release was also conducted on both aspirin (ASD-P1 and ASDW-P1) and paracetamol (PCMW-P1) at pH 2.2 and pH 7.4 (Figure 4.8). The release pattern of ASD-P1 particles in pH 2.2 and 7.4 is shown in Figure 4.8a. The cumulative amount of aspirin released from ASD-P1 particles in pH 7.4 increases gradually with time until it reaches equilibrium at 78%. Meanwhile, the drug release in pH 2.2 showed an inconsistent released profile where the release achieved 51% in the first 2 h, then decreased to 22% at 4 h, before it increased to 56% at 8 h and finally reached equilibrium at 24 h.

Similar release pattern was observed by ASDW-P1 at pH 2.2 (Figure 4.8b). Burst release was observed until 33.05% in the first 2 h. The release profile then decreased to 6.53% after 4 h before escalating to 47.91% after 6 h. The burst release observed was due to drug molecules present at the surface or outer layer of MIP (Sheybani et al., 2015). At 12 h, the drug release increased to 80.38% before reaching equilibrium. At pH 7.4, ASDW-P1 showed higher aspirin released at 2 h with 43.23% release. This could be due to the swelling of the polymer network facilitating the drug release (Kurczewska et al., 2017).

As for PCMW-P1 (Figure 4.8c), 33.03% paracetamol was released within 2 h under acidic conditions. It was then decreased to 29.67% at 8 h before it increased to 56.42% at 12 h before reaching equilibrium. While at pH 7.4, a higher release profile was observed at 44.73% at 2 h and increased to 59.82% at 8 h, window before a sustained release until 24 h. Low drug release in acidic conditions was due to the hardly ionized carboxylic groups in the polymer network, causing less repulsion with the polymer chain (Ji et al., 2020).

The release profile shown by ASD-P1, ASDW-P1 and PCMW-P1 indicated that all MIPs are pH sensitive. The design of this MIP, which consists of MAA and DVB as FM and CL, respectively, created both hydrophilic and hydrophobic polymer matrix system which plays an essential role in determining drug distribution profiles and release characteristics (Huang & Brazel, 2001). At a higher pH value (pH 7.4), the carboxylic groups present in the MAA structure were deprotonated, which induces a distinct repulsive force within the polymer matrix and causes swelling, then facilitating the release of aspirin (Figure 4.9) (Cegłowski et al., 2019). At a lower pH value (pH 2.2), the protonation of the carboxylic groups causes less swelling effect therefore, fewer drug (aspirin or paracetamol) was released (Rizwan et al., 2017).

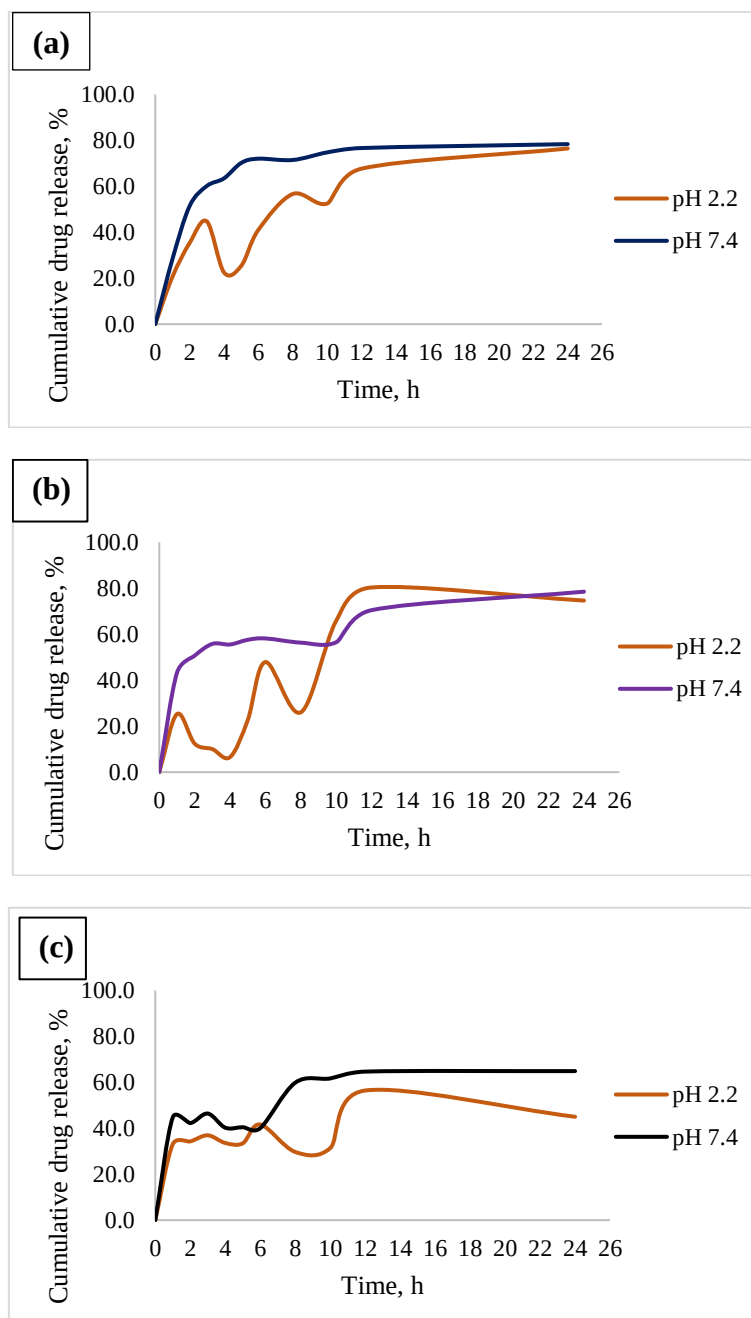


Figure 4.8: Cumulative drug release of (a) ASD-P1, (b) ASDW-P1 and (c) PCMW-P1 in pH 2.2 and pH 7.4.

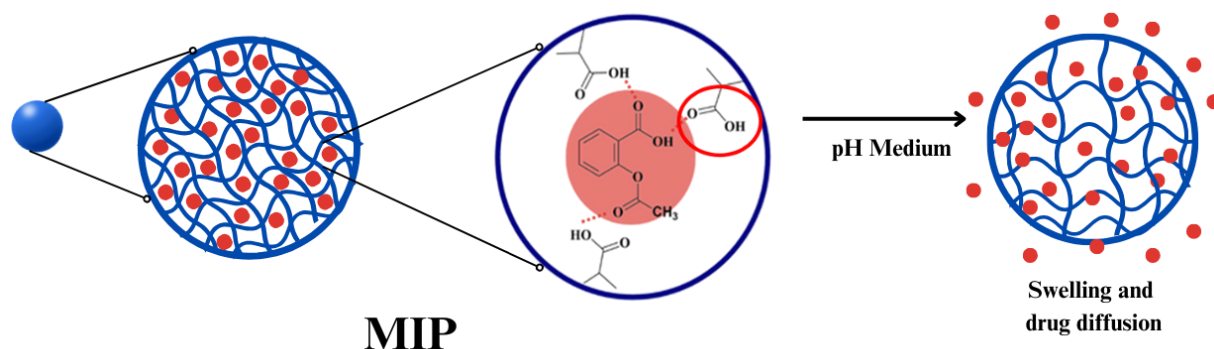


Figure 4.9: The swelling effect of MIP induced by the pH medium.

4.3.2.2 Drug Release Kinetic Modelling

The experimental data obtained from the release study were fitted to various kinetic models to explain the release kinetics of aspirin from the polymeric matrix in pH 2.2 and pH 7.4 medium (Table 4.5 and Table 4.6). Under acidic conditions (pH 2.2), the first-order kinetics produces the best fit for ASD-P1 with a higher coefficient of determination closer to 1 ($R^2=0.8246$), followed by the Higuchi model ($R^2=0.8171$). Similar release kinetic were observed by PCMW-P1. The first-order model is commonly used to describe the release of drugs from a system where the release rate is concentration-dependent, such as matrix-based drug delivery systems (Bruschi, 2015; Paarakh et al., 2019). Meanwhile, ASDW-P1 shows the best fit into the Higuchi model under acidic conditions, which is also observed in all MIPs performed at pH 7.4. This established the release mechanism of MIP as a three-dimensional polymeric matrix system as a diffusion-controlled type of release (Frenning, 2011). The Higuchi release kinetics model describes the release of drugs from an insoluble matrix based on a simple law of

diffusion from the spherical MIP (Singhvi & Singh, 2011; Reddy et al., 2018). It also addresses the release rate of a solute, typically a drug from a polymer matrix, where the loading of the solute exceeds its solubility (Paul, 2011).

Table 4.5: Linear fitting of cumulative release kinetics in pH 2.2.

Kinetic models	ASD-P1	ASDW-P1	PCMW-P1
Zero-order	$y = 2.7938x + 21.678$ $R^2 = 0.6863$	$y = 3.4417x + 10.34$ $R^2=0.6835$	$y = 1.9659x + 18.145$ $R^2=0.6358$
First -Order	$y = -0.0247x + 1.9068$ $R^2=0.8246$	$y = -0.0297x + 1.9693$ $R^2=0.6895$	$y = -0.0144x + 1.9203$ $R^2=0.7316$
Higuchi model	$y = 15.47x + 5.8318$ $R^2=0.8171$	$y = 17.713x - 6.2252$ $R^2=0.7105$	$y = 10.74x + 6.9583$ $R^2=0.7095$
Korsmeyer-peppas model	$y = 0.6695x + 1.0842$ $R^2=0.4523$	$y = 0.7453x + 0.8573$ $R^2=0.4737$	$y = 0.6855x + 0.9236$ $R^2=0.3877$
Hixson-Crowell Cube Root Law	$y = -0.9313x + 26.107$ $R^2= 0.6863$	$y = -1.1472x + 29.887$ $R^2=0.6835$	$y = -0.6553x + 27.285$ $R^2=0.6358$

Table 4.6: Linear fitting of cumulative release kinetics in pH 7.4.

Kinetic models	ASD-P1	ASDW-P1	PCMW-P1
Zero-order	$y = 2.553x + 39.89$ $R^2 = 0.4419$	$y = 2.0835x + 38.475$ $R^2=0.5144$	$y = 1.846x + 33.33$ $R^2=0.4686$
First -Order	$y = -0.0257x + 1.7533$ $R^2=0.5924$	$y = -0.021x + 1.7884$ $R^2=0.7498$	$y = -0.0152x + 1.8166$ $R^2=0.588$
Higuchi model	$y = 16.737x + 20.078$ $R^2=0.7376$	$y = 12.79x + 23.987$ $R^2=0.7606$	$y = 11.727x + 19.065$ $R^2=0.7071$
Korsmeyer-peppas model	$y = 0.6774x + 1.2443$ $R^2=0.4032$	$y = 0.5176x + 1.2982$ $R^2=0.2583$	$y = 0.7014x + 1.0802$ $R^2=0.352$
Hixson-Crowell Cube Root Law	$y = -0.851x + 20.037$ $R^2= 0.4419$	$y = -0.6945x + 20.508$ $R^2=0.5144$	$y = -0.6153x + 22.223$ $R^2=0.4686$

4.4 Conclusion

RSM is a highly beneficial tool for determining optimum conditions to achieve high rebinding capacity. The present study showed that the optimised parameters to achieve high rebinding capacity for ASD-P1, ASDW-P1, and PCMW-P1 were at 20 mg MIP, 300 mg/L initial concentration and 240 min contact time. While minimizing the number of experiments, RSM can visualize the interactional behaviour between each parameter. Under optimised conditions, the rebinding capacity of ASD-P1, ASDW-P1 and PCMW-P1 was at 5.24 mg/g, 8.67 mg/g and 5.16 mg/g, respectively. There was only a 2 - 3 % error in validation results compared to the predicted rebinding capacity.

The prepared MIP also showed sustained drug delivery under pH 7.4 due to the inclusion of DVB into the MAA polymeric network (poly(MAA-DVB)), which has created a slightly hydrophobic environment. For all examined MIP (ASD-P1, ASDW-P1 and PCMW-P1), the highest cumulative release was observed at pH 7.4 compared to pH 2.2. The release of aspirin and paracetamol at pH 2.2 was fitted in first-order kinetics, followed by the Higuchi model. Meanwhile, all MIP (ASD-P1, ASDW-P1 and PCMW-P1), were well-fitted with the Higuchi model at pH 7.4. It was demonstrated that the ultimate properties of MIP, particularly concerning drug release, are significantly impacted by the type of functional monomer and crosslinker.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The molecular imprinting of aspirin and paracetamol has been successfully carried out *via* conventional thermal heating (sample id: ASD) and MW-assisted synthesis (sample id: ASDW and PCMW). This study has revealed a significant advancement in the MIP properties achieved through MW-assisted synthesis (ASDW), surpassing the capabilities of conventionally synthesised aspirin MIP (ASD). The polymerization time has shortened to 8 mins (110 °C) of MW-irradiation setting from 6 h (80 °C) of the conventional thermal heating. The polymer yield obtained *via* MW-assisted synthesis (ASDW; 0.5 – 1 g) was comparable with conventional thermal heating (ASD; 0.5 – 1.2 g) promoting the rapid polymerization time using MW enhances the yield. The polymer morphology obtained *via* the two methods revealed an irregular uniform spherical particles and agglomerated particles, suggesting that further optimisation in the polymer synthesis (i.e. amount of solvent) should be conducted to obtain a smaller and regularly uniform polymer particles that could benefits in the DDS. Furthermore, the average particle size of MW-assisted synthesised (ASDW) was smaller (500 nm – 1.95 µm) than conventional thermal heating (ASD; 660 nm – 3.65 µm). This is beneficial in the DDS as the size should be large enough to prevent the rapid leakage into the blood capillaries but small enough to escape capture by fixed macrophages that are present in the reticuloendothelial system such as liver and spleen and this should be further explored in future study. Similar findings were also observed in the preparation of MIP using paracetamol *via* MW-assisted synthesis with comparable polymer yield (0.5 – 0.9 g) and smaller particle size distribution (1.07 – 1.64 µm).

It also evident from the study that the utilisation of RSM for polymer synthesis optimisation has allowed for the simultaneous assessment of the impact of the functional monomer and crosslinker concentration on the rebinding ability of MIPs. The optimisation of MIP synthesis using CCD of RSM showed polymer P1 with T:FM:CL of 1:4:16 have shown high rebinding capacity in both polymerisation methods (i.e., thermal heating (ASD) and MW-assisted synthesis (ASDW, PCMW). Rapid polymerisation of MW can produce smaller particle size with greater surface-to-volume ratio that allow more access to binding sites and eventually increased the rebinding capacity. Ratio 1:4 of template to MAA was optimum for the interaction of template and MAA to take place. It was demonstrated that different polymerisation processes (i.e., thermal heating and MW-assisted synthesis), including the type and amount of FM and CL in the formulation, can affect the final morphology of the resultant MIP or NIP and the rebinding properties of MIP. Following the optimised MIP synthesis, ASD-P1, ASDW-P1 and PCMW-P1 have shown high rebinding capacity at optimised rebinding parameters of 240 min contact time, 300 mg/L initial concentration and 20 mg MIP dosage. The validation method has been performed and shows only a 2 - 3 % error compared with the predicted rebinding capacity.

Subject to drug release application, the prepared MIP (ASD-P1, ASDW-P1 and PCMW-P1) has shown a sustained drug release under pH 7.4 and pH 2.2. The highest cumulative release was observed at pH 7.4 than at pH 2.2 suggesting that the drug release is higher in intestinal fluid than gastric fluid and it may reduce the gastrointestinal ulcer caused by aspirin. The release of aspirin and paracetamol at pH 2.2 was fitted in first-order kinetics, followed by the Higuchi model. Meanwhile, all MIPs (ASD-P1, ASDW-P1 and PCMW-P1) were well-fitted with the Higuchi model at pH 7.4 indicating that the prepared MIP is spherically formed polymer

although it was observed that the morphology are irregularly uniform. It was demonstrated that the ultimate properties of MIPs, particularly concerning drug release are significantly impacted by the choice of the MIP composition (i.e., functional monomer and crosslinker) and the polymerisation method (i.e., MW-assisted synthesis). The successful development of MIP *via* MW with spherical morphology, smaller size and high rebinding capacity for both aspirin and paracetamol as template was evidence that optimum formulation ratio for polymer synthesis was at 1:4:16. This can be a new guidance in the selection of polymer composition ratio.

5.2 Recommendations

In order to advance the utilization of MW-assisted synthesis for the production of MIP-DDS, several recommendations emerge from this study. Firstly, further exploration of additional reaction parameters used in RSM study for the synthesis of MIP, such as amount of solvent, another type of functional monomer (two types of monomers) and crosslinker (EGDMA or TRIM), could provide valuable insights into enhancing the efficiency and selectivity of the prepared MIP for DDS. Evaluating other polymer composition could provide a better understanding of the formation of morphology, particle size and rebinding efficiency of the MIP produced *via* MW. Preliminary screening of the template-functional monomer interactions could also be performed using Gaussian software in order to confirm the interaction between template and functional monomer.

Additionally, scale-up studies are warranted to assess the feasibility of transitioning from laboratory-scale to industrial-scale production, evaluating factors such as equipment and infrastructure cost, energy consumption, waste management, regulatory compliance, and

production throughput. The development of the infrastructure could be costly if it was built from scratch. However, a possible collaboration with existing pharmaceutical industry could reduce the cost and time with explicit studies on the safety and risk issues. Obtaining regulatory approvals require several steps and time consuming as the development of this DDS are for clinical implementation. Firstly, the *in vivo* evaluation study and cytotoxicity assay are crucial in confirming the biocompatibility of the prepared MIP in the preclinical research. Then, clinical trial is expected for Phase I till III before it can be approved by the regulatory bodies. Moreover, compliance with environmental regulations and occupational health and safety regulations are essentials as the MW-assisted synthesis involves high frequency electromagnetic fields that could pose a safety risk. A structured action plan and research strategy in implementing the above recommendations are firstly to ensure the equipment safety by selecting a certified MW. Then, an operational safety protocol including safety training for personnel, radiation monitoring, and shielding and containment activity should be performed. Thirdly, an environmental safety assessment should be conducted to monitor the emissions control, energy efficiency and waste management. Lastly, a regulatory compliance and risk management should be performed.

Assessing MW-assisted synthesis's environmental and safety implications compared to conventional methods is crucial to ensure its efficiency, sustainability, and safety in pharmaceutical manufacturing. This includes a statistical process control study during the pilot plant to ensure that the process operates efficiently, producing more specification-conforming products with less waste and risk. Translating the process into larger scale will require a huge cost which can be mitigated by collaboration or partnerships with pharmaceutical industry and

other academia institutions, ensuring additional expertise. These recommendations collectively aim to propel the utilization of MW-assisted synthesis as a superior method for the synthesis of MIP, offering improved efficiency, sustainability, and cost-effectiveness to the industry.

REFERENCES

- Abbasi, S., Haeri, S. A., & Sajjadifar, S. (2019). Bio-dispersive liquid liquid microextraction based on nano rhamnolipid aggregates combined with molecularly imprinted-solid phase extraction for selective determination of paracetamol in human urine samples followed by HPLC. *Microchemical Journal*, 146, 106–114. <https://doi.org/10.1016/j.microc.2018.12.065>
- Abd, F. N., Al-Bayati, Y. K., & Joda, B. A. (2019). Synthesis of molecularly imprinted polymers for estimation of aspirin by using different functional monomers. *International Journal of Drug Delivery Technology*, 9(04), 660–665. <https://doi.org/10.25258/ijddt.9.4.24>
- Abdollahi, E., Khalafi-Nezhad, A., Mohammadi, A., Abdouss, M., & Salami-Kalajahi, M. (2018). Synthesis of new molecularly imprinted polymer via reversible addition fragmentation transfer polymerisation as a drug delivery system. *Polymer*, 143, 245–257. <https://doi.org/10.1016/j.polymer.2018.03.058>
- Adepu, S., & Ramakrishna, S. (2021). Controlled drug delivery systems: Current status and future directions. *Molecules*, 26(19). <https://doi.org/10.3390/molecules26195905>
- Aghoutane, Y., Diouf, A., Österlund, L., Bouchikhi, B., & El Bari, N. (2020). Development of a molecularly imprinted polymer electrochemical sensor and its application for sensitive detection and determination of malathion in olive fruits and oils. *Bioelectrochemistry*, 132, 107404. <https://doi.org/10.1016/j.bioelechem.2019.107404>
- Agrawal, P. (2015). Significance of Polymers in Drug Delivery System. *Journal of*

Pharmacovigilance, 03(01), 1–2. <https://doi.org/10.4172/2329-6887.1000e127>

- Alanazi, K., Garcia Cruz, A., Di Masi, S., Voorhaar, A., Ahmad, O. S., Cowen, T., Piletska, E., Langford, N., Coats, T., Sims, M. R., & Piletsky, S. A. (2021). Disposable paracetamol sensor based on electroactive molecularly imprinted polymer nanoparticles for plasma monitoring. *Sensors and Actuators, B: Chemical*, 329, 129128. <https://doi.org/10.1016/j.snb.2020.129128>
- Ali, F., Shah, Z., Khan, A., Saadia, M., Alothman, Z. A., & Cheong, W. J. (2022a). Synthesis, column packing and liquid chromatography of molecularly imprinted polymers for the acid black 1, acid black 210 and acid brown 703 dyes. *RSC Advances*, 12, 19611–19623. <https://doi.org/10.1039/d2ra02357a>
- Ali, Z., Sajid, M., Manzoor, S., Ahmad, M. M., Khan, M. I., Elboughdiri, N., Kashif, M., Shanableh, A., Rajhi, W., Mersni, W., Bayraktar, E., & Salem, S. Ben. (2022b). Biodegradable magnetic molecularly imprinted anticancer drug carrier for the targeted delivery of docetaxel. *ACS Omega*, 7(32), 28516–28524. <https://doi.org/10.1021/acsomega.2c03299>
- Akkaramongkolporn, P., Ngawhirunpat, T., & Opanasopit, P. (2009). Preparation and evaluation of differently sulfonated styrene-divinylbenzene cross-linked copolymer cationic exchange resins as novel carriers for drug delivery. *AAPS PharmSciTech*, 10(2), 641–648. <https://doi.org/10.1208/s12249-009-9259-5>
- Amiri, A., Ramazani, A., Jahanshahi, M., & Moghadam Nia, A. A. (2014). Synthesis of nanostructured molecularly imprinted acrylic acid-based network copolymer as a solid

- sorbent for the quercetin extraction. *Journal of Nanostructures*, 4, 277–283.
<https://doi.org/10.7508/jns.2014.03.004>
- Amiri, N. S., & Hosseini, M.-R. (2020). Application of ratiometric fluorescence sensor-based microwave-assisted synthesized CdTe quantum dots and mesoporous structured epitope-imprinted polymers for highly efficient determination of tyrosine phosphopeptide. *Analytical Methods*, 12(1), 63–72. <https://doi.org/10.1039/c9ay00276f>
- Amorim, M. S., Sales, M. G. F., & Frasco, M. F. (2022). Recent advances in virus imprinted polymers. *Biosensors and Bioelectronics*: X, 10, 100131
<https://doi.org/10.1016/j.biosx.2022.100131>
- An, K., Kang, H., Zhang, L., Guan, L., & Tian, D. (2020). Preparation and properties of thermosensitive molecularly imprinted polymer based on konjac glucomannan and its controlled recognition and delivery of 5-fluorouracil. *Journal of Drug Delivery Science and Technology*, 60, 101977. <https://doi.org/10.1016/j.jddst.2020.101977>
- Ansari, S., & Masoum, S. (2019). Molecularly imprinted polymers for capturing and sensing proteins: Current progress and future implications. *TrAC - Trends in Analytical Chemistry*, 114, 29–47. <https://doi.org/10.1016/j.trac.2019.02.008>
- Arabzadeh, N., Mohammadi, A., Darwish, M., & Abuzerr, S. (2019). Construction of a TiO₂ – Fe₃O₄ -decorated molecularly imprinted polymer nanocomposite for tartrazine degradation: Response surface methodology modeling and optimisation. *Journal of the Chinese Chemical Society*, 66(5), 474–483. <https://doi.org/10.1002/jccs.201800302>
- Ariani, M. D., Zuhrotun, A., Manesiotis, P., & Hasanah, A. N. (2022). Magnetic molecularly

- imprinted polymers : An update on their use in the separation of active compounds from natural products. *Polymers*, 14, 1389. <https://doi.org/10.3390/polym14071389>
- Arshady, R., & Mosbach, K. (1981). Synthesis of Substrate-selective Polymers by Host-Guest Polymerization. *Die Makromolekulare Chemie*, 182, 687–692. Retrieved from <http://onlinelibrary.wiley.com/doi/10.1002/macp.1981.021820240/abstract>
- Asadi, E., Abdouss, M., Leblanc, R. M., Ezzati, N., Wilson, J. N., & Azodi-Deilami, S. (2016a). *In vitro / in vivo* study of novel anti-cancer, biodegradable cross-linked tannic acid for fabrication of 5-fluorouracil-targeting drug delivery nano-device based on a molecular imprinted polymer. *RSC Advances*, 6(43), 37308–37318. <https://doi.org/10.1039/c6ra03704f>
- Asadi, E., Abdouss, M., Leblanc, R. M., Ezzati, N., Wilson, J. N., & Kordestani, D. (2016b). Synthesis, characterization and *in vivo* drug delivery study of a biodegradable nano-structured molecularly imprinted polymer based on cross-linker of fructose. *Polymer*, 97, 226–237. <https://doi.org/10.1016/j.polymer.2016.05.031>
- Asfaram, A., Ghaedi, M., Agarwal, S., Tyagi, I., & Gupta, V. K. (2015). Removal of basic dye Auramine-O by ZnS: Cu nanoparticles loaded on activated carbon. Optimisation of parameters using response surface methodology with central composite design. *RSC Advances*, 5(24), 18438–18450. <https://doi.org/10.1039/C4RA15637D>
- Atayat, A., Mergola, L., Mzoughi, N., & Del Sole, R. (2019). Response surface methodology approach for the preparation of a molecularly imprinted polymer for solid-phase extraction of fenoxycarb pesticide in mussels. *Journal of Separation Science*, 42(18), 3023–3032.

<https://doi.org/10.1002/jssc.201900344>

Awokoya, K. N., Oninla, V. O., Adeyinka, G. C., Ajadi, M. O., Chidimma, O. T., Fakola, E. G., & Akinyele, O. F. (2022). Experimental and computational studies of microwave-assisted watermelon rind – styrene based molecular imprinted polymer for the removal of malachite green from aqueous solution. *Scientific African*, 16, e01194.

<https://doi.org/10.1016/j.sciaf.2022.e01194>

Azodi-Deilami, S., Najafabadi, A. H., Asadi, E., Abdouss, M., & Kordestani, D. (2014). Magnetic molecularly imprinted polymer nanoparticles for the solid-phase extraction of paracetamol from plasma samples, followed its determination by HPLC. *Microchimica Acta*, 181(15–16), 1823–1832. <https://doi.org/10.1007/s00604-014-1230-9>

Bai, J., Zhang, Y., Chen, L., Yan, H., Zhang, C., Liu, L., & Xu, X. (2018). Synthesis and characterization of paclitaxel-imprinted microparticles for controlled release of an anticancer drug. *Materials Science and Engineering C*, 92, 338–348.

<https://doi.org/10.1016/j.msec.2018.06.062>

Bai, Y., Luo, X., Han, Y., Liu, B., Zhang, J., & Zhang, M. (2020). Facile synthesis of narrow particle size distribution, high solid content, cationic polymer latexes by macroemulsion polymerization-based particle coagulation mechanism. *Journal of Macromolecular Science, Part A*, 57, 116 - 122. <https://doi.org/10.1080/10601325.2019.1673175>.

Băraian, A. I., Iacob, B. C., Bodoki, A. E., & Bodoki, E. (2022). In vivo applications of molecularly imprinted polymers for drug delivery: A pharmaceutical perspective. *International Journal of Molecular Sciences*, 23(22), 14071.

<https://doi.org/10.3390/ijms232214071>

Beckskereki, G., Horvai, G., & Tóth, B. (2021). The selectivity of molecularly imprinted polymers. *Polymers*, 13(11). <https://doi.org/10.3390/polym13111781>

Bhatia, T., Gupta, M. K., Singh, P., Chauhan, A., Saxena, P. N., & Mudiam, M. K. R. (2016). Sol-gel approach for extracting highly versatile aspirin and its metabolites using MISPE followed by GC-MS/MS analysis. *Bioanalysis*, 8(8), 795–805. <https://doi.org/10.4155/bio.16.28>

Bhawani, S. A., Sen, T. S., & Mohammad Ibrahim, M. N. (2018). Synthesis of molecular imprinting polymers for extraction of gallic acid from urine. *Chemistry Central Journal*, 12(1), 1–7. <https://doi.org/10.1186/s13065-018-0392-7>

Bi, X., Tian, W., Wang, X., Cao, W., Gao, L., Fan, S., Wang, Y., Wang, M., & Niu, L. (2019). Preparation of a POSS-hybridized molecularly imprinted monolith for the analysis of baicalin and its analogues in a microwave-assisted extract from: *Scutellaria baicalensis* by means of on-line SPME-HPLC and off-line LC-MS/MS. *Analytical Methods*, 11(17), 2351–2361. <https://doi.org/10.1039/c9ay00568d>

Bintalib, M. (2022). Clinical Profile and Outcomes of Poisonings and Drug Overdose at King Saud Medical City. *International Journal of Pharmaceutical and Bio-Medical Science*, 02(07), 206–210. <https://doi.org/10.47191/ijpbms/v2-i7-03>

Bondi, M.C.M., Urraca, J.L., Carrasco, S., & Navarro-Villoslada, F. (2013). Preparation of molecularly imprinted polymers. In *Handbook of molecularly Imprinted Polymers* (C.

Alvarez-Lorenzo, & A.Concheiro), pp. 23-86. United Kingdom: Smithers Rapra.
https://books.google.com.my/books?id=dQImDwAAQBAJ&printsec=copyright&redir_esc=y#v=onepage&q&f=false

Bodoki, A. E., Iacob, B. C., & Bodoki, E. (2019). Perspectives of molecularly imprinted polymer-based drug delivery systems in cancer therapy. *Polymers*, 11(12).
<https://doi.org/10.3390/polym11122085>

Bonardd, S., Saldías, C., Leiva, Á., Díaz Díaz, D., & Kortaberria, G. (2021). Molecular Weight Enables Fine-Tuning the Thermal and Dielectric Properties of Polymethacrylates Bearing Sulfonyl and Nitrile Groups as Dipolar Entities. *Polymers*, 13(3), 317–336.
<https://doi.org/10.3390/polym13030317>

Bossi, A., Bonini, F., Turner, A. P. F., & Piletsky, S. A. (2007). Molecularly imprinted polymers for the recognition of proteins: The state of the art. *Biosensors and Bioelectronics*, 22(6), 1131–1137. <https://doi.org/10.1016/j.bios.2006.06.023>

Boonpangrak, S., Whitcombe, M. J., Prachayasittikul, V., Mosbach, K., & Ye, L. (2006). Preparation of molecularly imprinted polymers using nitroxide-mediated living radical polymerisation. *Biosensors and Bioelectronics*, 22(3), 349–354.
<https://doi.org/10.1016/j.bios.2006.04.014>

Brahmbhatt, H. A., Surtees, A., Tierney, C., Ige, O. A., Piletska, E. V., Swift, T., & Turner, N. W. (2020). Effect of polymerisation by microwave on the physical properties of molecularly imprinted polymers (mips) specific for caffeine. *Polymer Chemistry*, 11(36), 5778–5789. <https://doi.org/10.1039/d0py00921k>

- Breig, S. J. M., & Luti, K. J. K. (2021). Response surface methodology: A review on its applications and challenges in microbial cultures. *Materials Today: Proceedings*, 42(5), 2277–2284. <https://doi.org/10.1016/j.matpr.2020.12.316>
- Bruschi, M. L. (Ed.). (2015). Mathematical models of drug release. In *Strategies to Modify the Drug Release from Pharmaceutical Systems* (pp. 63–86). <https://doi.org/10.1016/b978-0-08-100092-2.00005-9>
- Burton, P., & Goodwin, J. (2010). Solubility and permeability measurement and applications in drug discovery. *Combinatorial Chemistry & High Throughput Screening*, 13(2), 101–111. <https://doi.org/10.2174/138620710790596763>
- Calori, I. R., Braga, G., de Jesus, P. D. C. C., Bi, H., & Tedesco, A. C. (2020). Polymer scaffolds as drug delivery systems. *European Polymer Journal*, 129, 109621. <https://doi.org/10.1016/j.eurpolymj.2020.109621>
- Cantarella, M., Carroccio, S. C., Dattilo, S., Avolio, R., Castaldo, R., Puglisi, C., & Privitera, V. (2019). Molecularly imprinted polymer for selective adsorption of diclofenac from contaminated water. *Chemical Engineering Journal*, 367(February), 180–188. <https://doi.org/10.1016/j.cej.2019.02.146>
- Ceglowski, M., Kurczewska, J., Ruszkowski, P., & Schroeder, G. (2019). Application of paclitaxel-imprinted microparticles obtained using two different cross-linkers for prolonged drug delivery. *European Polymer Journal*, 118, 328–336. <https://doi.org/10.1016/j.eurpolymj.2019.06.010>
- Ceglowski, M., Kurczewska, J., Lusina, A., & Nazim, T. (2022). EGDMA- and TRIM-based

- microparticles imprinted with 5-Fluorouracil for prolonged drug delivery. *Polymers*, 14(5), 1207. <https://doi.org/10.3390/polym14051027>
- Chaudhary, V., & Sharma, S. (2019). Effect of various synthesis parameters on styrene–divinylbenzene copolymer properties. *Journal of Porous Materials*, 26(6), 1559–1571. <https://doi.org/10.1007/s10934-019-00753-7>
- Chen, H., Son, S., Zhang, F., Yan, J., Li, Y., Ding, H., & Ding, L. (2015). Rapid preparation of molecularly imprinted polymers by microwave-assisted emulsion polymerisation for the extraction of florfenicol in milk. *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences*, 983–984, 32–38. <https://doi.org/10.1016/j.jchromb.2015.01.003>
- Chen, L., Wang, X., Lu, W., Wu, X., & Li, J. (2016). Molecular imprinting: Perspectives and applications. *Chemical Society Reviews*, 45(8), 2137–2211. <https://doi.org/10.1039/c6cs00061d>
- Chen, H., Zhang, W., Yang, N., Chen, C., & Zhang, M. (2018). Chitosan-Based Surface Molecularly Imprinted Polymer Microspheres for Sustained Release of Sinomenine Hydrochloride in Aqueous Media. *Applied Biochemistry and Biotechnology*, 185(2), 370–384. <https://doi.org/10.1007/s12010-017-2658-2>
- Cirillo, G., Parisi, O. I., Curcio, M., Puoci, F., Iemma, F., Spizzirri, U. G., & Picci, N. (2010). Molecularly imprinted polymers as drug delivery systems for the sustained release of glycyrrhizic acid. *Journal of Pharmacy and Pharmacology*, 62(5), 577–582. <https://doi.org/10.1211/jpp.62.05.0003>

- Cooley, M., Sarode, A., Hoore, M., Fedosov, D. A., Mitragotri, S., & Sen Gupta, A. (2018). Influence of particle size and shape on their margination and wall-adhesion: implications in drug delivery vehicle design across nano-to-micro scale. *Nanoscale*, 10(32), 15350–15364. <https://doi.org/10.1039/c8nr04042g>
- Cormack, P. A. G., & Elorza, A. Z. (2004). Molecularly imprinted polymers: Synthesis and characterisation. *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences*, 804(1), 173–182. <https://doi.org/10.1016/j.jchromb.2004.02.013>
- Danaei, M., Dehghankhold, M., Ataei, S., Hasanzadeh Davarani, F., Javanmard, R., Dokhani, A., ... Mozafari, M. R. (2018). Impact of particle size and polydispersity index on the clinical applications of lipidic nanocarrier systems. *Pharmaceutics*, 10(2), 1–17. <https://doi.org/10.3390/pharmaceutics10020057>
- Das, S., & Kumar, N. (2020). Microsphere: A unique and useful drug delivery system. *Journal of Gastroenterology & Digestive Systems*, 4(1), 16–18. <https://doi.org/10.33140/jgds.04.01.05>
- Davankov, V., & Tsyurupa, M. (2011). Hypercrosslinked polystyrene as hemosorbents. *Comprehensive Analytical Chemistry*, 56(C), 567–583. [https://doi.org/10.1016/S0166-526X\(11\)56015-5](https://doi.org/10.1016/S0166-526X(11)56015-5)
- Decorbie, N., Tijunelyte, I., Gam-Derouich, S., Solard, J., Lamouri, A., Decorse, P., Felidj, N., Gauchotte-Lindsay, C., Emmanuel, R., Mangeney, C., & Lidgi-Guigui, N. (2020). Sensing polymer/paracetamol interaction with an independent component analysis-based SERS-MIP nanosensor. *Plasmonics*, 15(5), 1533–1539. <https://doi.org/10.1007/s11468-020->

- Dehghani, Z., Akhond, M., & Absalan, G. (2021). Carbon quantum dots embedded silica molecular imprinted polymer as a novel and sensitive fluorescent nanoprobe for reproducible enantioselective quantification of naproxen enantiomers. *Microchemical Journal*, 160, 105723. <https://doi.org/10.1016/j.microc.2020.105723>
- Dhamapurkar, S. P., & Desai, D. M. (2022). A review on microsphere for novel drug delivery system. *World Journal of Advanced Research and Reviews*, 16(3), 529–538. <https://doi.org/10.30574/wjarr.2022.16.3.1368>
- Di, J., Gao, X., Du, Y., Zhang, H., Gao, J., & Zheng, A. (2021). Size, shape, charge and “stealthy” surface: Carrier properties affect the drug circulation time in vivo. *Asian Journal of Pharmaceutical Sciences*, 16(4), 444–458. <https://doi.org/10.1016/j.ajps.2020.07.005>
- Dickey, F. H. (1949). The Preparation of Specific Adsorbents. *Proceedings of the National Academy of Sciences of the United States of America*, 35(5), 227–229. <https://doi.org/10.1073/pnas.35.5.227>
- Djozan, D., & Ebrahimi, B. (2008). Preparation of new solid phase micro extraction fiber on the basis of atrazine-molecular imprinted polymer: Application for GC and GC/MS screening of triazine herbicides in water, rice and onion. *Analytica Chimica Acta*, 616(2), 152–159. <https://doi.org/10.1016/j.aca.2008.04.037>
- Dong, C., Shi, H., Han, Y., Yang, Y., Wang, R., & Men, J. (2021). Molecularly imprinted polymers by the surface imprinting technique. *European Polymer Journal*, 145, 110231. <https://doi.org/10.1016/j.eurpolymj.2020.110231>

- Du, W., Wu, P., Zhao, Z., & Zhang, X. (2020). Facile preparation and characterization of temperature-responsive hydrophilic crosslinked polymer microspheres by aqueous dispersion polymerisation. *European Polymer Journal*, 128, 109610. <https://doi.org/10.1016/j.eurpolymj.2020.109610>
- Duarte, A. R. C., Casimiro, T., Aguiar-Ricardo, A., Simplício, A. L., & Duarte, C. M. M. (2006). Supercritical fluid polymerisation and impregnation of molecularly imprinted polymers for drug delivery. *Journal of Supercritical Fluids*, 39(1), 102–106. <https://doi.org/10.1016/j.supflu.2006.01.013>
- Ektirici, S., Kurç, Ö., Jalilzadeh, M., Aşlr, S., & Türkmen, D. (2022). Computational investigation of the monomer ratio and solvent environment for the complex formed between sulfamethoxazole and functional monomer methacrylic acid. *ACS Omega*, 7, 17175–17184. <https://doi.org/10.1021/acsomega.2c00862>
- El-Schich, Z., Zhang, Y., Feith, M., Beyer, S., Sternbæk, L., Ohlsson, L., Stollenwer, M., & Wingren, A. G. (2020). Molecularly imprinted polymers in biological applications. *BioTechniques*, 69(6), 407–420. <https://doi.org/10.2144/btn-2020-0091>
- Elfadil, D., Lamaoui, A., Pelle, F. Della, Amine, A., & Compagnone, D. (2021). Molecularly imprinted polymers combined with electrochemical sensors for food contaminants analysis. *Molecules*, 26(15), 1–40. <https://doi.org/10.3390/molecules26154607>
- Elugoke, S. E., Adekunle, A. S., Fayemi, O. E., Akpan, E. D., Mamba, B. B., Sherif, E. S. M., & Ebenso, E. E. (2021). Molecularly imprinted polymers (MIPs) based electrochemical sensors for the determination of catecholamine neurotransmitters – Review.

- Electrochemical Science Advances*, 1(2), 1–43. <https://doi.org/10.1002/elsa.202000026>
- Ertürk, G., & Mattiasson, B. (2017). Molecular imprinting techniques used for the preparation of biosensors. *Sensors*, 17(2), 1–17. <https://doi.org/10.3390/s17020288>
- Esfandiyari-Manesh, M., Javanbakht, M., Atyabi, F., Badiei, A., & Dinarvand, R. (2011). Effect of porogenic solvent on the morphology, recognition and release properties of carbamazepine-molecularly imprinted polymer nanospheres. *Journal of Applied Polymer Science*, 121(2), 1118–1126. <https://doi.org/10.1002/app.33812>
- Fareghi, A. R., Moghadam, P. N., & Khalafy, J. (2017a). Synthesis and characterization of a cellulose-based molecularly imprinted polymer in aqueous solution: The study of furosemide slow release. *Starch*, 69(11–12), 1–43. <https://doi.org/10.1002/star.201700002>
- Fareghi, A. R., Moghadam, P. N., Khalafy, J., Bahram, M., & Moghtader, M. (2017b). Preparation of a new molecularly imprinted polymer based on self-crosslinkable cellulose acrylate in aqueous solution: A drug delivery system for furosemide. *Journal of Applied Polymer Science*, 134(48), 1–11. <https://doi.org/10.1002/app.45581>
- Farooq, S., Ngaini, Z., & Mortadza, N. A. (2020). Microwave-assisted synthesis and molecular docking study of heteroaromatic chalcone derivatives as potential antibacterial agents. *Bulletin of the Korean Chemical Society*, 41(9), 918–924. <https://doi.org/10.1002/bkcs.12088>
- Ferey, L., Delaunay, N., Rutledge, D. N., Huertas, A., Raoul, Y., Gareil, P., & Vial, J. (2013). Use of response surface methodology to optimize the simultaneous separation of eight polycyclic aromatic hydrocarbons by capillary zone electrophoresis with laser-induced

- fluorescence detection. *Journal of Chromatography A*, 1302, 181–190.
<https://doi.org/10.1016/j.chroma.2013.06.027>
- Fresco-Cala, B., Batista, A. D., & Cárdenas, S. (2020). Molecularly imprinted polymer micro- and nano-particles: A review. *Molecules*, 25(20), 4740.
<https://doi.org/10.3390/molecules25204740>
- Frenning, G. (2011). Modelling drug release from inert matrix systems: From moving-boundary to continuous-field descriptions. *International Journal of Pharmaceutics*, 418(1), 88–99.
<https://doi.org/10.1016/j.ijpharm.2010.11.030>
- Gaba, M., & Dhingra, N. (2011). Microwave chemistry: General features and applications. *Indian Journal of Pharmaceutical Education and Research*, 45(2), 175–183.
- Gagliardi, M. (2023). Design and development of molecularly imprinted biodegradable polymers for nanomedicine. *Advanced Industrial and Engineering Polymer Research*, 6(4), 396–406. <https://doi.org/10.1016/j.aiepr.2023.04.002>
- Gao, M., Gao, Y., Chen, G., Huang, X., Xu, X., Lv, J., Wang, J., Xu, D., & Liu, G. (2020a). Recent advances and future trends in the detection of contaminants by molecularly imprinted polymers in food samples. *Frontiers in Chemistry*, 8, 1–20.
<https://doi.org/10.3389/fchem.2020.616326>
- Gao, Y., Zhou, D., Lyu, J., Sigen, A., Xu, Q., Newland, B., Matyjaszewski, K., Tai, H., & Wang, W. (2020b). Complex polymer architectures through free-radical polymerisation of multivinyl monomers. *Nature Reviews Chemistry*, 4(4), 194–212.
<https://doi.org/10.1038/s41570-020-0170-7>
- Garcia, M., Kempe, K., Haddleton, D. M.,

- Khan, A., & Marsh, A. (2015). Templated polymerizations on solid supports mediated by complementary nucleoside interactions. *Polymer Chemistry*, 6(11), 1944–1951. <https://doi.org/10.1039/c4py01783h>
- Geerets, B., Peeters, M., van Grinsven, B., Bers, K., de Ceuninck, W., & Wagner, P. (2013). Optimizing the thermal read-out technique for MIP-based biomimetic sensors: Towards nanomolar detection limits. *Sensors*, 13(7), 9148–9159. <https://doi.org/10.3390/s130709148>
- Ghani, S. M., Rezaei, B., Jamei, H. R., & Ensafi, A. A. (2020). Preparation and comparison of molecularly imprinted polymer fluorimetric nanoprobe based on polymer dots and carbon quantum dots for determination of acetamiprid using response surface method. *Microchimica Acta*, 187(5). <https://doi.org/10.1007/s00604-020-04283-x>
- Gkika, D. A., Tolkou, A. K., Lambropoulou, D. A., Bikiaris, D. N., Kokkinos, P., Kalavrouziotis, I. K., & Kyzas, G. Z. (2024). Application of molecularly imprinted polymers (MIPs) as environmental separation tools. *RSC Applied Polymers*. <https://doi.org/10.1039/d3lp00203a>
- Gómez-Caballero, A., Goicolea, M. A., & Barrio, R. J. (2005). Paracetamol voltammetric microsensors based on electrocopolymerized- molecularly imprinted film modified carbon fiber microelectrodes. *Analyst*, 130(7), 1012–1018. <https://doi.org/10.1039/b502827b>
- Gonte, R., Balasubramanian, K., Deb, P. C., & Singh, P. (2012). Synthesis and characterization of mesoporous hypercrosslinked poly (Styrene Co- Maleic Anhydride) microspheres. *International Journal of Polymeric Materials and Polymeric Biomaterials*, 61(12), 919–

930. <https://doi.org/10.1080/00914037.2011.610057>

Grewal, A. S., Kumar, K., Redhu, S., & Bhardwaj, S. (2013). Microwave assisted synthesis: A green chemistry approach and. *International Research Journal of Pharmaceutical and Applied Sciences*, 3(5), 278–285.

Guo, Z., Xu, W., Xu, G., & Jia, Q. (2023). An updated overview of MOF@ MIP in drug carrier and drug analysis: Construction, application and prospective. *TrAC Trends in Analytical Chemistry*, 167(October 2023), 117275. <https://doi.org/10.1016/j.trac.2023.117275>

Han, S., Teng, F., Wang, Y., Su, L., Leng, Q., & Jiang, H. (2020). Drug-loaded dual targeting graphene oxide-based molecularly imprinted composite and recognition of carcino-embryonic antigen. *RSC Advances*, 10, 10980–10988. <https://doi.org/10.1039/d0ra00574f>

Han, Y., Tao, J., Ali, N., Khan, A., Malik, S., Khan, H., Yu, C., Bilal, M., & Mohamed, A. A. (2022). Molecularly imprinted polymers as the epitome of excellence in multiple fields. *European Polymer Journal*, 179, 111582. <https://doi.org/10.1016/j.eurpolymj.2022.111582>

Hasanah, A. N., Safitri, N., Zulfa, A., Neli, N., & Rahayu, D. (2021). Factors affecting preparation of molecularly imprinted polymer and methods on finding template-monomer interaction as the key of selective properties of the materials. *Molecules*, 26, 5612. <https://doi.org/10.3390/molecules26185612>

Hashemi-Moghaddam, H., Kazemi-Bagsangani, S., Jamili, M., & Zavareh, S. (2016). Evaluation of magnetic nanoparticles coated by 5-fluorouracil imprinted polymer for controlled drug delivery in mouse breast cancer model. *International Journal of*

Pharmaceutics, 497(1–2), 228–238. <https://doi.org/10.1016/j.ijpharm.2015.11.040>

Hashemi-Moghaddam, H., Zavareh, S., Karimpour, S., & Madanchi, H. (2017). Evaluation of molecularly imprinted polymer based on HER2 epitope for targeted drug delivery in ovarian cancer mouse model. *Reactive and Functional Polymers*, 121(July), 82–90. <https://doi.org/10.1016/j.reactfunctpolym.2017.10.025>

Hashemi, S. H., & Keykha, F. (2019). Application of the response surface methodology in the optimisation of modified molecularly imprinted polymer-based pipette-tip micro-solid phase extraction for spectrophotometric determination of nicotine in seawater and human plasma. *Analytical Methods*, 11(42), 5405–5412. <https://doi.org/10.1039/c9ay01496a>

Hashemi, S. H., Ziyaadini, M., Kaykhahi, M., Jamali Keikha, A., & Naruie, N. (2020). Separation and determination of ciprofloxacin in seawater, human blood plasma and tablet samples using molecularly imprinted polymer pipette-tip solid phase extraction and its optimisation by response surface methodology. *Journal of Separation Science*, 43(2), 505–513. <https://doi.org/10.1002/jssc.201900923>

Hawton, K., Ferrey, A., Casey, D., Wells, C., Fuller, A., Bankhead, C., Clements, C., Ness, J., Gunnell, D., Kapur, N., & Geulayov, G. (2019). Relative toxicity of analgesics commonly used for intentional self-poisoning: a study of case fatality based on fatal and non-fatal overdose. *Journal of Affective Disorders*, 246, 814–819.

He, S., Zhang, L., Bai, S., Yang, H., Cui, Z., Zhang, X., & Li, Y. (2021). Advances of molecularly imprinted polymers (MIP) and the application in drug delivery. *European Polymer Journal*, 143, 110179. <https://doi.org/10.1016/j.eurpolymj.2020.110179>

- Heravizadeh, O. R., Khadem, M., Nabizadeh, R., & Shahtaheri, S. J. (2018). Synthesis of molecular imprinted polymer nanoparticles followed by application of response surface methodology for optimisation of metribuzin extraction from urine samples. *Chemical Papers*, 72(12), 3057–3068. <https://doi.org/10.1007/s11696-018-0546-z>
- Heravizadeh, O. R., Khadem, M., Dehghani, F., & Shahtaheri, S. J. (2020). Determination of fenthion in urine samples using molecularly imprinted nanoparticles: Modelling and optimisation by response surface methodology. *International Journal of Environmental Analytical Chemistry*, 102(17), 6105–6119. <https://doi.org/10.1080/03067319.2020.1808630>
- Herrera-Chacón, A., Cetó, X., & del Valle, M. (2021). Molecularly imprinted polymers - towards electrochemical sensors and electronic tongues. *Analytical and Bioanalytical Chemistry*, 413(24), 6117–6140. <https://doi.org/10.1007/s00216-021-03313-8>
- Hou, L., Han, X., & Wang, N. (2020). High performance of molecularly imprinted polymer for the selective adsorption of erythromycin in water. *Colloid and Polymer Science*, 298(8), 1023–1033. <https://doi.org/10.1007/s00396-020-04660-1>
- Hrib, J., Krumbholcova, E. C., Duskova-Smrckova, M., Hobzova, R., Sirc, J., Hruby, M., Michalek, J., Hodan, J., lesny, P., & Smucler, R. (2019). Hydrogel tissue expanders for stomatology. Part II. Poly(styrene-maleic anhydride) hydrogels. *Polymers*, 11(7). <https://doi.org/10.3390/polym11071087>
- Huang, X., & Brazel, C. S. (2001). On the importance and mechanisms of burst release in matrix-controlled drug delivery systems. *Journal of Controlled Release*, 73(2–3), 121–136.

[https://doi.org/10.1016/S0168-3659\(01\)00248-6](https://doi.org/10.1016/S0168-3659(01)00248-6)

Huang, D. L., Wang, R. Z., Liu, Y. G., Zeng, G. M., Lai, C., Xu, P., Lu, B. A., Xu, J. J., Wang, C., & Huang, C. (2014). Application of molecularly imprinted polymers in wastewater treatment: a review. *Environmental Science and Pollution Research*, 22(2), 963–977. <https://doi.org/10.1007/s11356-014-3599-8>

Huang, J., Tong, J., Luo, J., Zhu, Y., Gu, Y., & Liu, X. (2018). Green synthesis of water-compatible fluorescent molecularly imprinted polymeric nanoparticles for efficient detection of paracetamol. *ACS Sustainable Chemistry and Engineering*, 6(8), 9760–9770. <https://doi.org/10.1021/acssuschemeng.8b00823>

Husin, N. A., Muhamad, M., Yahaya, N., Miskam, M., Syazni Nik Mohamed Kamal, N. N., Asman, S., Roov, M., & Zain, N. N. M. (2021). Application of a new choline-imidazole based deep eutectic solvents in hybrid magnetic molecularly imprinted polymer for efficient and selective removal of naproxen from aqueous samples. *Materials Chemistry and Physics*, 261, 124228. <https://doi.org/10.1016/j.matchemphys.2021.124228>

Ibrahim, T., Gebril, A., Nasr, M. K., Samad, A., & Zaki, H. A. (2023). Unlocking the Optimal Analgesic Potential: A Systematic Review and Meta-Analysis Comparing Intravenous, Oral, and Rectal Paracetamol in Equivalent Doses. *Cureus*, 15(7), 1–13. <https://doi.org/10.7759/cureus.41876>

Iheaturu, N. C., Diwe, I. V., Daramola, O. O., & Sadiku, E. R. (2019). Polymeric nanomaterials for drug delivery. In *Green Biopolymers and their Nanocomposites. Materials Horizons: From Nature to Nanomaterials* (Dhorali, G.), pp. 251–266. Springer Singapore.

https://doi.org/10.1007/978-981-13-8063-1_11

- Jamaledin, R., Makvandi, P., Yiu, C. K., Agarwal, T., Vecchione, R., Sun, W., Maiti, T. K., Tay, F. R., & Netti, P. A. (2020). Engineered microneedle patches for controlled release of active compounds: recent advances in release profile tuning. *Advanced Therapeutics*, 3(12), 2000171. <https://doi.org/10.1002/adtp.202000171>
- Janczura, M., Luliński, P., & Sobiech, M. (2021). Imprinting technology for effective sorbent fabrication: Current state-of-art and future prospects. *Materials*, 14(8), 1850. <https://doi.org/10.3390/ma14081850>
- Ji, K., Luo, X., He, L., Liao, S., Hu, L., Han, J., Chen, C., Liu, Y., & Tan, N. (2020). Preparation of hollow magnetic molecularly imprinted polymer and its application in silybin recognition and controlled release. *Journal of Pharmaceutical and Biomedical Analysis*, 180, 113036. <https://doi.org/10.1016/j.jpba.2019.113036>
- Jian, Y., Chen, L., Cheng, J., Huang, X., Yan, L., & Li, H. (2020). Molecularly imprinted polymers immobilized on graphene oxide film for monolithic fiber solid phase microextraction and ultrasensitive determination of triphenyl phosphate. *Analytica Chimica Acta*, 1133, 1–10. <https://doi.org/10.1016/j.aca.2020.08.003>
- Jun, Y., Oh, H., Karpoormath, R., Jha, A., & Patel, R. (2021). Role of microsphere as drug carrier for osteogenic differentiation. *International Journal of Polymeric Materials and Polymeric Biomaterials*, 70(5), 318–327. <https://doi.org/10.1080/00914037.2020.1713783>
- Kalogiouri, N. P., Tsalbouris, A., Kabir, A., Furton, K. G., & Samanidou, V. F. (2020). Synthesis and application of molecularly imprinted polymers using sol – gel matrix

- imprinting technology for the efficient solid-phase extraction of BPA from water. *Microchemical Journal*, 157. <https://doi.org/10.1016/j.microc.2020.104965>
- Kan, X., Geng, Z., Zhao, Y., Wang, Z., & Zhu, J. J. (2009). Magnetic molecularly imprinted polymer for aspirin recognition and controlled release. *Nanotechnology*, 20(16). <https://doi.org/10.1088/0957-4484/20/16/165601>
- Kao, C. Y., Lo, T. C., & Lee, W. C. (2003). Influence of polyvinylpyrrolidone on the hydrophobic properties of partially porous poly(styrene-divinylbenzene) particles for biological applications. *Journal of Applied Polymer Science*, 87(11), 1818–1824. <https://doi.org/10.1002/app.11653>
- Kappe, C. O. (2004). Controlled microwave heating in modern organic synthesis. *Angewandte Chemie International Edition*, 43(46), 6250–6284. <https://doi.org/10.1002/anie.200400655>
- Karasu, T., Özgür, E., & Uzun, L. (2023). MIP-on-a-chip: Artificial receptors on microfluidic platforms for biomedical applications. *Journal of Pharmaceutical and Biomedical Analysis*, 226, 115257. <https://doi.org/10.1016/j.jpba.2023.115257>
- Kazemi, S., Sarabi, A. A., & Abdouss, M. (2016). Synthesis and characterization of magnetic molecularly imprinted polymer nanoparticles for controlled release of letrozole. *Korean Journal of Chemical Engineering*, 33(11), 3289–3297. <https://doi.org/10.1007/s11814-016-0171-x>
- Kim, M., Jiang, Y., & Kim, D. (2013). Zn²⁺-imprinted porous polymer beads: Synthesis, structure, and selective adsorption behavior for template ion. *Reactive & Functional Polymers*, 73, 821–827. <https://doi.org/10.1016/J.REACTFUNCTPOLYM.2013.03.012>

- Kueseng, P., Nisoa, M., & Sontimuang, C. (2018). Rapid preparation of molecularly imprinted polymers by custom-made microwave heating for analysis of atrazine in water. *Journal of Separation Science*, 41(13), 2783–2789. <https://doi.org/10.1002/jssc.201800198>
- Kulkarni, A., Shrivastava, P., & Phulara, R. K. (2023). Timely hemodialysis for successful treatment of acute salicylate overdose in a young adult female – A case report. *Indian Journal of Nephrology*, 1–3. <https://doi.org/10.4103/ijn.ijn>
- Kumar, A., Kuang, Y., Liang, Z., & Sun, X. (2020). Microwave chemistry, recent advancements, and eco-friendly microwave-assisted synthesis of nanoarchitectures and their applications: a review. *Materials Today Nano*, 11, 100076. <https://doi.org/10.1016/j.mtnano.2020.100076>
- Kunsa-Ngiem, S., Sutthivaiyakit, P., Lowmunkhong, P., Harir, M., Schmitt-Kopplin, P., & Sutthivaiyakit, S. (2018). Magnetic molecularly imprinted polymer prepared by microwave heating for confirmatory determination of chloramphenicol in chicken feed using high-performance liquid chromatography-tandem mass spectrometry. *Journal of Environmental Science and Health - Part B Pesticides, Food Contaminants, and Agricultural Wastes*, 53(11), 738–745. <https://doi.org/10.1080/03601234.2018.1480161>
- Kurczewska, J., Cegłowski, M., Pecyna, P., Ratajczak, M., Gajęcka, M., & Schroeder, G. (2017). Molecularly imprinted polymer as drug delivery carrier in alginate dressing. *Materials Letters*, 201, 46-49. <https://doi.org/10.1016/j.matlet.2017.05.008>

- Kwak, T. J., Jung, H., Allen, B. D., Demirel, M. C., & Chang, W. J. (2021). Dielectrophoretic separation of randomly shaped protein particles. *Separation and Purification Technology*, 262. <https://doi.org/10.1016/j.seppur.2020.118280>
- Lamaoui, A., Palacios-Santander, J. M., Amine, A., & Cubillana-Aguilera, L. (2021). Fast microwave-assisted synthesis of magnetic molecularly imprinted polymer for sulfamethoxazole. *Talanta*, 232, 122430. <https://doi.org/10.1016/j.talanta.2021.122430>
- Lamidi, S., Olaleye, N., Bankole, Y., Obalola, A., Aribike, E., & Adigun, I. (2022). Applications of response surface methodology (RSM) in product design, development, and process optimization. *IntechOpen*.
- Larkin, P. (2011). *Infrared and Raman Spectroscopy: Principles and Spectral Interpretation*. Amsterdam, Netherlands: Elsevier Science. Retrieved from <https://books.google.com.my/books?id=KPyV1DRMRbwC>
- Lawai, V., & Ngaini, Z. (2021). Chitosan magnetic nanocomposites for gene delivery. In *Polysaccharide-based Nanocomposites for Gene Delivery and Tissue Engineering* (S., Bhawani, Z., Karim, M., Jawaid), pp. 279-294. United Kingdom: Woodhead Publishing.
- Lawai, V., Ngaini, Z., Bhawani, S. A., Farooq, S., & Wahi, R. (2024). Microwave-assisted molecularly imprinted polymer synthesis for chemometric and rebinding mechanism of drug release applications. *Polymer Advanced Technologies*, 35(1), 1–12. <https://doi.org/10.1002/pat.6291>
- Leadbeater, N.E. (2014). Microwave-Assisted Synthesis: General Concepts. In R., Hoogenboom, U., Schubert, F., Wiesbrock (Eds), *Microwave-assisted Polymer Synthesis*.

Advances in Polymer Science (vol. 274). Springer, Cham.
https://doi.org/10.1007/12_2013_274

Li., F., & Larock, R.C. (2003). Synthesis, Structure and Properties of New Tung Oil–Styrene–Divinylbenzene Copolymers Prepared by Thermal Polymerization. *Biomacromolecules*, 4(4), 1018-1025. <https://doi.org/10.1021/bm034049j>

Li, R., Feng, Y., Pan, G., & Liu, L. (2019). Advances in molecularly imprinting technology for bioanalytical applications. *Sensors*, 19(1), 177. <https://doi.org/10.3390/s19010177>

Liang, W., Lu, Y., Li, N., Li, H., & Zhu, F. (2020). Microwave-assisted synthesis of magnetic surface molecular imprinted polymer for adsorption and solid phase extraction of 4-nitrophenol in wastewater. *Microchemical Journal*, 159, 105316. <https://doi.org/10.1016/j.microc.2020.105316>

Lim, K. F., & Holdsworth, C. I. (2018). Effect of formulation on the binding efficiency and selectivity of precipitation molecularly imprinted polymers. *Molecules*, 23(11), 1–19. <https://doi.org/10.3390/molecules23112996>

Liu, Y., Tan, J., Thomas, A., Ou-Yang, D., & Muzykantov, V. R. (2012). The shape of things to come: Importance of design in nanotechnology for drug delivery. *Therapeutic Delivery*, 3(2), 181–194. <https://doi.org/10.4155/tde.11.156>

Liu, B., Zhang, M., Liu, Y., Tan, Z., Zhou, C., & Zhang, H. (2015). Particle nucleation and growth in the emulsion polymerization of styrene: Effect of monomer/water ratio and electrolyte concentration. *Journal of Macromolecular Science, Part A: Pure and Applied Chemistry*, 52(2), 147–154. <https://doi.org/10.1080/10601325.2015.980765>.

- Liu, D., Yang, F., Xiong, F., & Gu, N. (2016). The smart drug delivery system and its clinical potential. *Theranostics*, 6(9), 1306–1323. <https://doi.org/10.7150/thno.14858>
- Liu, X. L., Yao, H. F., Chai, M. H., He, W., Huang, Y. P., & Liu, Z. S. (2018). Green synthesis of carbon nanotubes-reinforced molecularly imprinted polymer composites for drug delivery of fenbufen. *AAPS PharmSciTech*, 19(8), 3895–3906. <https://doi.org/10.1208/s12249-018-1192-z>
- Liu, L., Chen, M., Yang, H., Huang, Z., Tang, Q., Chow, C., Gong, C., Zu, M., & Xiao, B. (2020). An NIR-light-responsive surface molecularly imprinted polymer for photoregulated drug release in aqueous solution through porcine tissue. *Materials Science & Engineering C*, 106, 110253. <https://doi.org/10.1016/j.msec.2019.110253>
- Liu, R., & Poma, A. (2021). Advances in molecularly imprinted polymers as drug delivery systems. *Molecules*, 26(12), 3589. <https://doi.org/10.3390/molecules26123589>
- Loghmani, M. H., Shojaie, A. F., & Hosseini, S. A. (2021). Glutathione-responsive hydrogel and molecularly imprinted polymer nanospheres: New aspect on cisplatin delivery. *Journal of Industrial and Engineering Chemistry*, 96, 98–108. <https://doi.org/10.1016/j.jiec.2020.12.018>
- Lok, K. P., & Ober, C. K. (2011). Particle size control in dispersion polymerization of polystyrene. *Canadian Journal of Chemistry*, 63(1), 209–216. <https://doi.org/10.1139/v85-033>

- Lowdon, J. W., Diliën, H., van Grinsven, B., Eersels, K., & Cleij, T. J. (2021). Colorimetric sensing of amoxicillin facilitated by molecularly imprinted polymers. *Polymers*, 13, 2221. <https://doi.org/10.3390/polym13132221>
- Lu, Z., Yu, Z., Dong, J., Song, M., Liu, Y., Liu, X., Ma, Z., Su, H., Yan, Y., & Huo, P. (2018). Facile microwave synthesis of a Z-scheme imprinted ZnFe₂O₄/Ag/PEDOT with the specific recognition ability towards improving photocatalytic activity and selectivity for tetracycline. *Chemical Engineering Journal*, 337, 228–241. <https://doi.org/10.1016/j.cej.2017.12.115>
- Lu, H., Tian, H., Wang, C., & Xu, S. (2020). Designing and controlling the morphology of spherical molecularly imprinted polymers. *Materials Advances*, 1(7), 2182–2201. <https://doi.org/10.1039/d0ma00415d>
- Luliński, P. (2017). Molecularly imprinted polymers based drug delivery devices: A way to application in modern pharmacotherapy. A review. *Materials Science and Engineering C*, 76, 1344–1353. <https://doi.org/10.1016/j.msec.2017.02.138>
- Luo, X., Zhan, Y., Huang, Y., Yang, L., Tu, X., & Luo, S. (2011). Removal of water-soluble acid dyes from water environment using a novel magnetic molecularly imprinted polymer. *Journal of Hazardous Materials*, 187(1–3), 274–282. <https://doi.org/10.1016/j.jhazmat.2011.01.009>
- Luo, L., Xu, F., Peng, H., Luo, Y., Tian, X., Battaglia, G., Zhang, H., Gong, Q., Gu, Z., & Luo, K. (2020). Stimuli-responsive polymeric prodrug-based nanomedicine delivering nifuroxazide and doxorubicin against primary breast cancer and pulmonary metastasis.

<https://doi.org/10.1016/j.jconrel.2019.12.017>

Lusina, A., & Ceglowski, M. (2022). Molecularly imprinted polymers as state-of-the-art drug carriers in hydrogel transdermal drug delivery applications. *Polymers*, 14, 640.

Madikizela, L., Tavengwa, N., & Pakade, V. (2018). Molecularly Imprinted Polymers for Pharmaceutical Compounds: Synthetic Procedures and Analytical Applications. In N. Chenkaya (Ed), *Recent Research in Polymerisation* (pp 47-67). <https://doi.org/10.5772/intechopen.71475>

Malik, M. I., Shaikh, H., Mustafa, G., & Bhanger, M. I. (2019). Recent applications of molecularly imprinted polymers in analytical chemistry. *Separation and Purification Reviews*, 48(3), 179–219. <https://doi.org/10.1080/15422119.2018.1457541>

Mansoor, A., & Al-Bayati, Y. K. (2021). Electrochemical preparation of a molecularly imprinted polymer electrode for estimation of aspirin using two different functional monomers. *Iraqi Journal of Market Research and Consumer Protection*, 13(1), 14–25.

Maragou, N. C., Thomaidis, N. S., Theodoridis, G. A., Lampi, E. N., & Koupparis, M. A. (2020). Determination of bisphenol A in canned food by microwave assisted extraction, molecularly imprinted polymer-solid phase extraction and liquid chromatography-mass spectrometry. *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences*, 113, 121938. <https://doi.org/10.1016/j.jchromb.2019.121938>

- Mazzotta, E., Giulio, T. Di, Mariani, S., Corsi, M., Malitesta, C., & Barillaro, G. (2023). Vapor-phase synthesis of molecularly imprinted polymers on nanostructured materials at room temperature. *Small*, 19(38), 2302274. <https://doi.org/10.1002/sml.202302274>
- Meena, S. K., & Singh, S. (2023). A comparative thermal stability study of n-(4-nitrophenyl) maleimide and n-(2, 4-di-nitrophenyl) maleimide with methylacrylate. *International Journal for Research in Applied Science and Engineering Technology*, 11(1), 1574–1585. <https://doi.org/10.22214/ijraset.2023.48874>
- Meischl, F., Schemeth, D., Harder, M., Köpfle, N., Tessadri, R., & Rainer, M. (2016). Synthesis and evaluation of a novel molecularly imprinted polymer for the selective isolation of acetylsalicylic acid from aqueous solutions. *Journal of Environmental Chemical Engineering*, 4(4), 4083–4090. <https://doi.org/10.1016/j.jece.2016.09.013>
- Mohajeri, S. A., Malaekheh-nikouei, B., & Sadegh, H. (2012). Development of a pH-responsive imprinted polymer for diclofenac and study of its binding properties in organic and aqueous media. *Drug Development and Industrial Pharmacy*, 38, 616–622. <https://doi.org/10.3109/03639045.2011.621126>
- Mohebalı, A., Abdouss, M., & Zahedi, P. (2018). Isosorbide dinitrate template-based molecularly imprinted poly(methacrylic acid) nanoparticles: effect of initiator concentration on morphology and physicochemical properties. *Chemical Papers*, 72(12), 3005–3016. <https://doi.org/10.1007/s11696-018-0536-1>
- Mohebalı, A., Abdouss, M., Kazemi, Y., & Daneshnia, S. (2021). Fabrication and characterization of pH-responsive poly (methacrylic acid)-based molecularly imprinted

- polymers nanosphere for controlled release of amitriptyline hydrochloride. *Polymers for Advanced Technologies*, 32(11), 4386–4396. <https://doi.org/10.1002/pat.5440>
- Morsi, S., N.A., A., Abd-elFatah, N., Morsi, M., & Shaker, N. (2021). Molecular imprinted-polymer as a controlled release material for tramadol. *Egyptian Journal of Chemistry*, 64(1), 253–262. <https://doi.org/10.21608/ejchem.2020.35500.2737>
- Mourabet, M., Rhilassi, A. El, Boujaady, H. El, & Taitai, A. (2017). Use of response surface methodology for optimisation of fluoride adsorption in an aqueous solution by Brushite. *Arabian Journal of Chemistry*, 10, 3292–3302.
- Mueller, A. (2021). A note about crosslinking density in imprinting polymerisation. *Molecules*, 26(17). <https://doi.org/10.3390/molecules26175139>
- Muhammad, T., Nur, Z., Piletska, E. V., Yimit, O., & Piletsky, S. A. (2012). Rational design of molecularly imprinted polymer: The choice of cross-linker. *Analyst*, 137(11), 2623–2628. <https://doi.org/10.1039/c2an35228a>
- Mustafai, F. A., Balouch, A., Abdullah, Jalbani, N., Bhanger, M. I., Jagirani, M. S., Kumar, A., & Tunio, A. (2018). Microwave-assisted synthesis of imprinted polymer for selective removal of arsenic from drinking water by applying Taguchi statistical method. *European Polymer Journal*, 109, 133–142. <https://doi.org/10.1016/j.eurpolymj.2018.09.041>
- Naqvi, S. T. R., Rasheed, T., Hussain, D., Majeed, S., Fatima, B., Haq, M. N. ul, Zarin, A., & Nawaz, R. (2020). Development of molecularly imprinted magnetic iron oxide nanoparticles for doxorubicin drug delivery. *Monatshefte Für Chemie - Chemical Monthly*, 151, 1049–1057. <https://doi.org/org/10.1007/s00706-020-02644-z>

- Nasab, S. G., Semnani, A., Karim, M., Yazd, M. J., & Cheshmekhezr, S. (2019). Synthesis of ion-imprinted polymer-decorated SBA-15 as a selective and efficient system for the removal and extraction of Cu(ii) with focus on optimisation by response surface methodology. *Analyst*, 144(15), 4596–4612. <https://doi.org/10.1039/c9an00586b>
- Nazmi, N. N. M., & Sarbon, N. M. (2019). Response surface methodology on development and formulation optimisation of chicken skin gelatine film blended with carboxymethyl cellulose as affected by varying plasticiser concentrations. *International Food Research Journal*, 26(1), 47–57.
- Nezhadali, A., & Mojarab, M. (2014). Computational study and multivariate optimisation of hydrochlorothiazide analysis using molecularly imprinted polymer electrochemical sensor based on carbon nanotube/polypyrrole film. *Sensors and Actuators, B: Chemical*, 190, 829–837. <https://doi.org/10.1016/j.snb.2013.08.086>
- Noee, S., Salimraftar, N., Abdouss, M., & Riazi, G. (2013). Imprinted microspheres and nanoparticles with diclofenac sodium: Effect of solvent on the morphology and recognition properties. *Polymer International*, 62(12), 1711–1716. <https://doi.org/10.1002/pi.4471>
- Norell, M. C., Andersson, H. S., & Nicholls, I. A. (1998). Theophylline molecularly imprinted polymer dissociation kinetics: a novel sustained release drug dosage mechanism. *Journal of Molecular Recognition*, 11(1-6), 98-102. [https://doi.org/10.1002/\(SICI\)1099-1352\(199812\)11:1/6<98::AID-JMR399>3.0.CO;2-Y](https://doi.org/10.1002/(SICI)1099-1352(199812)11:1/6<98::AID-JMR399>3.0.CO;2-Y)
- Olsson, G. D., Karlsson, B. C. G., Shoravi, S., Wiklander, J. G., & Nicholls, I. A. (2012). Mechanisms underlying molecularly imprinted polymer molecular memory and the role of

- crosslinker: Resolving debate on the nature of template recognition in phenylalanine anilide imprinted polymers. *Journal of Molecular Recognition*, 25(2), 69–73. <https://doi.org/10.1002/jmr.2147>
- Öncel, P., Çetin, K., Topçu, A. A., Yavuz, H., & Denizli, A. (2017). Molecularly imprinted cryogel membranes for mitomycin C delivery. *Journal of Biomaterials Science, Polymer Edition*, 28(6), 519–531. <https://doi.org/10.1080/09205063.2017.1282772>
- Öter, Ç., & Zorer, Ö. S. (2021). Molecularly imprinted polymer synthesis and selective solid phase extraction applications for the detection of ziram, a dithiocarbamate fungicide. *Chemical Engineering Journal Advances*, 7(April). <https://doi.org/10.1016/j.ceja.2021.100118>
- Ouazib, F., Bouslah Mokhnachi, N., Haddadine, N., & Barille, R. (2019). Role of polymer/polymer and polymer/drug specific interactions in drug delivery systems. *Journal of Polymer Engineering*, 39(6), 534–544. <https://doi.org/10.1515/polyeng-2018-0403>
- Özcan, L., & Şahin, Y. (2007). Determination of paracetamol based on electropolymerized-molecularly imprinted polypyrrole modified pencil graphite electrode. *Sensors and Actuators, B: Chemical*, 127(2), 362–369. <https://doi.org/10.1016/j.snb.2007.04.034>
- Paarakh, M. P., Jose, P. A. N. I., Setty, C. M., & Peter, G. V. (2019). Release kinetics – Concepts and applications. *International Journal of Pharmacy Research & Technology*, 8(1), 12–20. <https://doi.org/10.31838/ijprt/08.01.02>
- Pandey, H., Khare, P., Singh, S., & Singh, S. P. (2020). Carbon nanomaterials integrated molecularly imprinted polymers for biological sample analysis: A critical review.

Materials Chemistry and Physics, 239, 121966.

<https://doi.org/10.1016/j.matchemphys.2019.121966>

Parisi, O. I., Scrivano, L., Candamano, S., Ruffo, M., Vattimo, A. F., Spanedda, M. V., & Puoci, F. (2018). Molecularly imprinted microrods via mesophase polymerisation. *Molecules*, 23(63), 1–9. <https://doi.org/10.3390/molecules23010063>

Parisi, O. I., Ruffo, M., Malivindi, R., Vattimo, A. F., Pezzi, V., & Puoci, F. (2020). Molecularly imprinted polymers (MIPs) as theranostic systems for sunitinib controlled release and self-monitoring in cancer therapy. *Pharmaceutics*, 12(1). <https://doi.org/10.3390/pharmaceutics12010041>

Parisi, O. I., Francomano, F., Dattilo, M., Patitucci, F., Prete, S., Amone, F., & Puoci, F. (2022). The evolution of molecular recognition: from antibodies to molecularly imprinted polymers (MIPs) as artificial counterpart. *Journal of Functional Biomaterials*, 13(1). <https://doi.org/10.3390/jfb13010012>

Paul, P. K., Treetong, A., & Suedee, R. (2017). Biomimetic insulin-imprinted polymer nanoparticles as a potential oral drug delivery system. *Acta Pharmaceutica*, 67(2), 149–168. <https://doi.org/10.1515/acph-2017-0020>

Pauling, L. (1940). A Theory of the Formation of Antibodies. *Journal of the American Chemical Society*, 372(62), 2643–2657.

Pawley, C. J., Perez-Gavilan, A., Foley, K. S., Lentink, S., Welsh, H. N., Tuijthof, G., Redeker, E. S., Dillen, H., Eersels, K., Grinsven B. V., & Cleij, T. J. (2017). Studying the drug delivery kinetics of a nanoporous matrix using a MIP-based thermal sensing platform.

Polymers, 9(11), 1–10. <https://doi.org/10.3390/polym9110560>

Pichon, V., Delaunay, N., & Combès, A. (2020). Sample preparation using molecularly imprinted polymers. *Analytical Chemistry*, 92(1), 16–33. <https://doi.org/10.1021/acs.analchem.9b04816>

Prasetya, N. B. A., Ngadiwiyana, N., Ismiyarto, I., & Sarjono, P. R. (2020). Effects of percent weight of divinylbenzene as crosslinking agent on the properties of eugenol-divinylbenzene copolymers. *Journal of Physics: Conference Series*, 1524(1). <https://doi.org/10.1088/1742-6596/1524/1/012089>

Pratama, K. F., Manik, M. E. R., Rahayu, D., & Hasanah, A. N. (2020). Effect of the molecularly imprinted polymer component ratio on analytical performance. *Chemical and Pharmaceutical Bulletin*, 68(11), 1013–1024. <https://doi.org/10.1248/cpb.c20-00551>

Pryamitsyn, V., & Ganesan, V. (2015). Pair interactions in polyelectrolyte-nanoparticle systems: Influence of dielectric inhomogeneities and the partial dissociation of polymers and nanoparticles. *The Journal of Chemical Physics*, 143 16, 164904 . <https://doi.org/10.1063/1.4934242>.

Puzio, K., Delépée, R., Vidal, R., & Agrofoglio, L. A. (2013). Combination of computational methods, adsorption isotherms and selectivity tests for the conception of a mixed non-covalent-semi-covalent molecularly imprinted polymer of vanillin. *Analytica Chimica Acta*, 790, 47–55. <https://doi.org/10.1016/j.aca.2013.06.036>

Qin, Y. T., Feng, Y. S., Ma, Y. J., He, X. W., Li, W. Y., Zhang, Y. K., & Zhang, Y. K. (2020).

- Tumor-sensitive biodegradable nanoparticles of molecularly imprinted polymer-stabilized fluorescent zeolitic imidazolate framework-8 for targeted imaging and drug delivery. *ACS Applied Materials and Interfaces*, 12(22), 24585–24598. <https://doi.org/10.1021/acsami.0c05154>
- Rahim, M. A., Jan, N., Khan, S., Shah, H., Madni, A., Khan, A., Jabar, A., Khan, S., Elhissi, A., Hussain, Z., Aziz, H. C., Sohail, M., Khan, M., & Thu, H.E. (2021). Recent advancements in stimuli responsive drug delivery platforms for active and passive cancer targeting. *Cancers*, 13(4), 670. <https://doi.org/10.3390/cancers13040670>
- Rahimi, M., Bahar, S., Heydari, R., & Amininasab, S. M. (2019). Determination of quercetin using a molecularly imprinted polymer as solid-phase microextraction sorbent and high-performance liquid chromatography. *Microchemical Journal*, 148, 433–441. <https://doi.org/10.1016/j.microc.2019.05.032>
- Rahiminezhad, M., Shahtaheri, S. J., Ganjali, M. R., & Forushani, A. R. R. (2014). Application of response surface methodology to synthesize appropriate molecularly imprinted polymer for diazinon. *Key Engineering Materials*, 605(1), 67–70. <https://doi.org/10.4028/www.scientific.net/KEM.605.67>
- Rajpal, S., & Mishra, P. (2022). Next generation biosensors employing molecularly imprinted polymers as sensing elements for *in vitro* diagnostics. *Biosensors and Bioelectronics: X*, 11, 100201. <https://doi.org/10.1016/j.biosx.2022.100201>
- Reddy, B. B. K., Nagoji, K. E. V., & Sahoo, S. (2018). Preparation and *in vivo* evaluation of cephalexin matrix tablest. *Brazilian Journal of Pharmaceutical Sciences*, 54(3), 1–12.

- Renkecz, T., László, K., & Horváth, V. (2014). Molecularly imprinted microspheres prepared by precipitation polymerisation at high monomer concentrations. *Molecular Imprinting*, 2(1), 1–17. <https://doi.org/10.2478/molim-2014-0001>
- Rheem, S., Rheem, I., & Oh, S. (2017). Response surface methodology using a fullest balanced model: A re-analysis of a dataset in the Korean journal for food science of animal resources. *Korean Journal for Food Science of Animal Resources*, 37(1), 139–146. <https://doi.org/10.5851/kosfa.2017.37.1.139>
- Rizwan, M., Yahya, R., Hassan, A., Yar, M., Azzahari, A. D., Selvanathan, V., Sonsudin, F., & Abouloula, C. N. (2017). pH sensitive hydrogels in drug delivery: Brief history, properties, swelling, and release mechanism, material selection and applications. *Polymers*, 9(4). <https://doi.org/10.3390/polym9040137>
- Rodrigues, R. C., Hernandez, K., Barbosa, O., Rueda, N., Garcia-Galan, C., dos Santos, C. S. J., Berenguer-Murcia, A., & Fernandez-Lafuente, R. (2015). Immobilization of proteins in poly-styrene-divinylbenzene matrices: functional properties and applications. *Current Organic Chemistry*, 19(17), 1707–1718. <https://doi.org/10.2174/1385272819666150429231728>
- Rodríguez-Pizano, J. J., Granados-Rivera, L. E., Hernández-Escoto, H., & Contreras-López, D. (2019). Effect of the operating conditions on the particle size distribution by the suspension polymerisation process. *ECORFAN Journal Bolivia*, 6(10), 1–12. <https://doi.org/10.35429/ejb.2019.10.6.1.12>
- Roland, R. M., & Bhawani, S. A. (2014). Synthesis of molecularly imprinted polymer for the

removal of melamine. *Asian Journal of Chemistry*, 26(12), 70–73.

- Roland, R. M., & Bhawani, S. A. (2016). Synthesis and characterization of molecular imprinting polymer microspheres of piperine: extraction of piperine from spiked urine. *Journal of Analytical Methods in Chemistry*, 2016. <https://doi.org/10.1155/2016/5671507>
- Rostamizadeh, K., Vahedpour, M., & Bozorgi, S. (2012). Synthesis, characterization and evaluation of computationally designed nanoparticles of molecular imprinted polymers as drug delivery systems. *International Journal of Pharmaceutics*, 424, 67–75. <https://doi.org/10.1016/j.ijpharm.2011.12.054>
- Royani, I., Widayani, Abdullah, M., & Khairurrijal. (2014). An atrazine molecularly imprinted polymer synthesized using a cooling-heating method with repeated washing: Its physico-chemical characteristics and enhanced cavities. *International Journal of Electrochemical Science*, 9(10), 5651–5662. [https://doi.org/10.1016/S1452-3981\(23\)08195-6](https://doi.org/10.1016/S1452-3981(23)08195-6)
- Sadegh, N., Asfaram, A., Javadian, H., Haddadi, H., & Sharifpour, E. (2021). Ultrasound-assisted solid phase microextraction-HPLC method based on Fe₃O₄ @ SiO₂ -NH₂ - molecularly imprinted polymer magnetic nano-sorbent for rapid and efficient extraction of harmaline from *Peganum harmala* extract. *Journal of Chromatography B*, 117, 122640. <https://doi.org/10.1016/j.jchromb.2021.122640>
- Sadia, M., Ahmad, I., Ul-Saleheen, Z., Zubair, M., Zahoor, M., Ullah, R., Bari, A., & Zekker, I. (2023). Synthesis and characterization of MIPs for selective removal of textile dye acid black-234 from wastewater sample. *Molecules*, 28(4), 1–17. <https://doi.org/10.3390/molecules28041555>

- Safdarian, M., & Ramezani, Z. (2019). Rapid microwave-assisted distillation-precipitation polymerisation for the synthesis of magnetic molecular imprinted polymers coupled to HPTLC determination of perphenazine in human urine. *New Journal of Chemistry*, 43(1), 48–57. <https://doi.org/10.1039/c8nj05062g>
- Sajini, T., Thomas, R., & Mathew, B. (2019). Rational design and synthesis of photo-responsive molecularly imprinted polymers for the enantioselective intake and release of L-phenylalanine benzyl ester on multiwalled carbon nanotubes. *Polymer*, 173(April), 127–140. <https://doi.org/10.1016/j.polymer.2019.04.031>
- Sajini, T., & Mathew, B. (2021). A brief overview of molecularly imprinted polymers: Highlighting computational design, nano and photo-responsive imprinting. *Talanta Open*, 4, 100072. <https://doi.org/10.1016/j.talo.2021.100072>
- Samarth, N. B., Kamble, V., Mahanwar, P. A., Rane, A. V., & K., A. V. (2015). A historical perspective and the development of molecular imprinting polymer-A review. *Chemistry International*, 1(4), 202–210.
- Sanadgol, N., & Wackerlig, J. (2020). Developments of smart drug-delivery systems based on magnetic molecularly imprinted polymers for targeted cancer therapy: A short review. *Pharmaceutics*, 12(9), 1–31. <https://doi.org/10.3390/pharmaceutics12090831>
- Sarhan, A., & Wulff, G. (1972). On the Use of Enzyme-Analogous Polymers for Racemate Separation. *Angew. Chem., Int. Ed.*, 84(669), 364.
- Schwarz, L. J., Potdar, M. K., Danylec, B., Boysen, R. I., & Hearn, M. T. W. (2015). Microwave-assisted synthesis of resveratrol imprinted polymers with enhanced selectivity.

Analytical Methods, 7(1), 150–154. <https://doi.org/10.1039/c4ay02518k>

Sedghi, R., Yassari, M., & Heidari, B. (2018). Thermo-responsive molecularly imprinted polymer containing magnetic nanoparticles: Synthesis, characterization and adsorption properties for curcumin. *Colloids and Surfaces B: Biointerfaces*, 162, 154–162. <https://doi.org/10.1016/j.colsurfb.2017.11.053>

Sedghi, R., Ashrafzadeh, S., & Heidari, B. (2021). pH-sensitive molecularly imprinted polymer based on graphene oxide for stimuli actuated controlled release of curcumin. *Journal of Alloys and Compounds*, 857, 157603. <https://doi.org/10.1016/j.jallcom.2020.157603>

Shadabfar, M., Abdouss, M., & Khonakdar, H. A. (2020). Synthesis, characterization, and evaluation of a magnetic molecular imprinted polymer for 5-fluorouracil as an intelligent drug delivery system for breast cancer treatment. *Journal of Materials Science*, 55(26), 12287–12304. <https://doi.org/10.1007/s10853-020-04887-x>

Shahar, T., Tal, N., & Mandler, D. (2016). Molecularly imprinted polymer particles: Formation, characterization and application. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 495, 11–19. <https://doi.org/10.1016/j.colsurfa.2016.01.027>

Sheybani, S., Hosseinifar, T., Abdouss, M., & Mazinani, S. (2015). Mesoporous molecularly imprinted polymer nanoparticles as a sustained release system of azithromycin. *RSC Advances*, 5(120), 98880–98891. <https://doi.org/10.1039/c5ra11970g>

Sikiti, P., Msagati, T. A. M., Mamba, B. B., & Mishra, A. K. (2014). Synthesis and characterization of molecularly imprinted polymers for the remediation of PCBs and dioxins in aqueous environments. *Journal of Environmental Health Science and*

Engineering, 12(1), 1–8. <https://doi.org/10.1186/2052-336X-12-82>

Singhvi, G., & Singh, M. (2011). *In vitro* drug release characterization models. *International Journal of Pharmaceutical Studies and Research*, 2(1), 77–84. Retrieved from https://www.researchgate.net/publication/285447890_Review_In_vitro_Drug_Release_Characterization_Models

Sinjari, S., Zhao, Y., Li, J., Skreta, M., Sweny, H., Hong, C., & Stöver, H. D. H. (2022). Lightly cross-linked microgels by precipitation polymerisation of maleic anhydride and styrenic monomers. *ACS Applied Polymer Materials*, 4(8), 5680–5687. <https://doi.org/10.1021/acsapm.2c00651>

Sooraj, M. P., & Mathew, B. (2015). Artificial recognition sorbents on multiwalled carbon nanotubes for the separation of aspirin from its structural analogues. *International Journal of Scientific & Engineering Research*, 6(9), 723–733.

Srivastava, A., Yadav, T., Sharma, S., Nayak, A., Akanksha Kumari, A., & Mishra, N. (2016). Polymers in Drug Delivery. *Journal of Biosciences and Medicines*, 04(01), 69–84. <https://doi.org/10.4236/jbm.2016.41009>

Suedee, R., Srichana, T., & Rattananont, T. (2002). Enantioselective release of controlled delivery granules based on molecularly imprinted polymers. *Drug Delivery*, 9(1), 19–30. <https://doi.org/10.1080/107175402753413145>

Sullivan, M. V, Stockburn, W. J., Hawes, P. C., Mercer, T., & Reddy, S. M. (2021). Green synthesis as a simple and rapid route to protein modified magnetic nanoparticles for use in the development of a fluorometric molecularly imprinted polymer-based assay for

- detection of myoglobin. *Nanotechnology*, 32(9), 095502. doi: 10.1088/1361-6528/abce2d
- Suriyanarayanan, S., Mandal, S., Ramanujam, K., & Nicholls, I. A. (2017). Electrochemically synthesized molecularly imprinted polythiophene nanostructures as recognition elements for an aspirin-chemosensor. *Sensors and Actuators, B: Chemical*, 253, 428–436. <https://doi.org/10.1016/j.snb.2017.05.076>
- Svenson, J., & Nicholls, I. A. (2001). On the thermal and chemical stability of molecularly imprinted polymers. *Analytica Chimica Acta*, 435(1), 19–24. [https://doi.org/10.1016/S0003-2670\(00\)01396-9](https://doi.org/10.1016/S0003-2670(00)01396-9)
- Stat-Ease, 2024. Design of Experiment (DOE) Made Easy. Stat-Ease. <https://www.statease.com/software/design-expert/>
- Tajani, A. S., Soheili, V., Moosavi, F., Ghodsi, R., Alizadeh, T., & Bazzaz, B. S. F. (2022). Ultra selective and high-capacity dummy template molecular imprinted polymer to control quorum sensing and biofilm formation of *Pseudomonas aeruginosa*. *Analytica Chimica Acta*, 1199, 339574. <https://doi.org/10.1016/j.aca.2022.339574>
- Tallapaka, S. H., Karuturi, V. K., Sotthivirat, S., & Vetro, J. A. (2015). Controlled Drug Delivery. In A.K., Mitra, D., Kwatra, A.D., Vadlapudi (Eds). *Drug Delivery* (pp.108-128). Burlington: Jones and Bartlet Learning. https://www.google.com.my/books/edition/Drug_Delivery_book/pwdvBAAAQBAJ?hl=en&gbpv=0
- Tamandani, M., Hashemi, S. H., Kaykhahi, M., Jamali Keikha, A., & Nasiryan, A. (2022). Determination of profenofos in seawater and foodstuff samples after its molecularly

- imprinted polymer pipette-tip micro solid phase extraction optimized by response surface methodology. *BMC Chemistry*, 16(1), 1–11. <https://doi.org/10.1186/s13065-022-00807-z>
- Tan, Y., Zhou, Z., Wang, P., Nie, L., & Yao, S. (2001). A study of a bio-mimetic recognition material for the BAW sensor by molecular imprinting and its application for the determination of paracetamol in the human serum and urine. *Talanta*, 55(2), 337–347. [https://doi.org/10.1016/S0039-9140\(01\)00442-8](https://doi.org/10.1016/S0039-9140(01)00442-8)
- Tanaka, H., Kii, M., Yamamoto, Y., & Tsuiki, H. (2022). Dependence of polymer tacticity and polymerisation rate on conversion, solvent, chain length in radical polymerisation of captodative-substituted ethyl acetoxyacrylate. *Polymer Bulletin*, 79(8), 6547–6564. <https://doi.org/10.1007/s00289-021-03821-8>
- Thach, U. D., Thi, H. H. N., Pham, T. D., Mai, H. D., & Nhu-Trang, T. T. (2021). Synergetic effect of dual functional monomers in molecularly imprinted polymer preparation for selective solid phase extraction of ciprofloxacin. *Polymers*, 13(16). <https://doi.org/10.3390/polym13162788>
- Torchilin, V. P. (2018). Fundamentals of stimuli-responsive drug and gene delivery systems. In A., Singh, M. M., Amiji (Eds). *Stimuli-responsive Drug Delivery Systems* (pp. 1-32). United Kingdom: The Royal Society of Chemistry. <https://doi.org/10.1039/9781788013536>
- Triadhi, U., Zulfikar, M. A., Setiyanto, H., & Amran, M. B. (2018). Effects of (monomer - crosslinker - initiator) composition during non imprinted polymers synthesis for catechin retention. *Journal of Physics: Conference Series*, 1013(1). <https://doi.org/10.1088/1742->

- Trucillo, P. (2021). Drug carriers: Classification, administration, release profiles, and industrial approach. *Processes*, 9(470), 1–18. <https://doi.org/10.3390/pr9030470>
- Turner, N. W., Holdsworth, C. I., Donne, S. W., McCluskey, A., & Bowyer, M. C. (2010). Microwave induced MIP synthesis: Comparative analysis of thermal and microwave induced polymerisation of caffeine imprinted polymers. *New Journal of Chemistry*, 34(4), 686–692. <https://doi.org/10.1039/b9nj00538b>
- Vasapollo, G., Sole, R. Del, Mergola, L., Lazzoi, M. R., Scardino, A., Scorrano, S., & Mele, G. (2011). Molecularly imprinted polymers: Present and future prospective. *International Journal of Molecular Sciences*, 12(9), 5908–5945. <https://doi.org/10.3390/ijms12095908>
- Vera, L., Zan, M. M. De, Cámara, M. S., & Goicoechea, C. (2014). Experimental design and multiple response optimisation . Using the desirability function in analytical methods development. *Talanta*, 124, 123–138.
- Verma, N. K., Alam, G., D K, V., Mishra, J. N., Khan, W. U., Singh, A. P., & Roshan, A. (2015). Recent advances in microspheres technology for drug delivery. *International Journal of Pharmaceutical Sciences and Nanotechnology*, 8(2), 2799–2813. <https://doi.org/10.37285/ijpsn.2015.8.2.2>
- Viveiros, R., Rebocho, S., & Casimiro, T. (2018). Green strategies for molecularly imprinted polymer development. *Polymers*, 10(3), 306. <https://doi.org/10.3390/polym10030306>
- Wang, N. X., & von Recum, H. A. (2011). Affinity-based drug delivery. *Macromolecular bioscience*, 11(3), 321–332. <https://doi.org/10.1002/mabi.201000206>

- Wang, X., Zhao, L., Sun, Z., & Gong, B. (2019). Preparation of ofloxacin-restricted access media—molecularly imprinted polymers for its selective recognition of ofloxacin in milk samples. *Chromatographia*, 82(7), 1041–1050. <https://doi.org/10.1007/s10337-019-03738-5>
- Wang, Z., Lin, M., Dong, Z., Zhang, J., & Yang, Z. (2021). Synthesis and radiation grafting modification of hydroxyl controlled AM/HEMA polymer microspheres. *Journal of Dispersion Science and Technology*, 42(7), 1031–1041. <https://doi.org/10.1080/01932691.2020.1730887>
- Wang, S., Zhang, L., Zeng, J., Hu, X., Wang, X., Yu, L., Wang, D., Cheng, L., Ahmed, R., Romanovski, V., Li, P., & Zhang, Z. (2023). Multi-templates molecularly imprinted polymers for simultaneous recognition of multiple targets: From academy to application. *TrAC Trends in Analytical Chemistry*, 166, 117173. <https://doi.org/10.1016/j.trac.2023.117173>
- Wei, X., Zhou, Z., Hao, T., Xu, Y., Li, H., Lu, K., Dai, J., Zheng X., Gao, L., Wang J., Yan, Y., & Zhu, Y. (2015). Specific recognition and fluorescent determination of aspirin by using core-shell CdTe quantum dot-imprinted polymers. *Microchimica Acta*, 182(7–8), 1527–1534. <https://doi.org/10.1007/s00604-015-1463-2>
- Wei, Y., Zeng, Q., Bai, S., Wang, M., & Wang, L. (2017). Nanosized difunctional photo responsive magnetic imprinting polymer for electrochemically monitored light-driven paracetamol extraction. *ACS Applied Materials and Interfaces*, 9(50), 44114–44123. <https://doi.org/10.1021/acsami.7b14772>

- Wendler, T., Duhr, C., & Bosch, T. (2003). Ex vivo biocompatibility of a new β 2-microglobulin hemoperfusion polymer. *International Journal of Artificial Organs*, 26(6), 467–476. <https://doi.org/10.1177/039139880302600604>
- Wloch, M., & Datta, J. (2019). Synthesis and polymerisation techniques of molecularly imprinted polymers. In M. Marc (Ed). *Comprehensive Analytical Chemistry* (vol. 86, pp. 17–40). <https://doi.org/10.1016/bs.coac.2019.05.011>
- Xavier, L. P. dos S., Dias, A. C., Baeta, B. E. L., De Azevedo Santos, L., Ramalho, T. C., De Aquino, S. F., & Da Silva, A. C. (2019). Experimental and theoretical studies of solvent polarity influence on the preparation of molecularly imprinted polymers for the removal of estradiol from water. *New Journal of Chemistry*, 43(4), 1775–1784. <https://doi.org/10.1039/C8NJ03639J>
- Xia, Q., Yun, Y., Li, Q., Huang, Z., & Liang, Z. (2017). Preparation and characterization of monodisperse molecularly imprinted polymer microspheres by precipitation polymerisation for kaempferol. *Designed Monomers and Polymers*, 20(1), 201–209. <https://doi.org/10.1080/15685551.2016.1239174>
- Xiao, D., Su, L., Teng, Y., Hao, J., & Bi, Y. (2020). Fluorescent nanomaterials combined with molecular imprinting polymer: synthesis, analytical applications, and challenges. *Microchimica Acta*, 187(7). <https://doi.org/10.1007/s00604-020-04353-0>
- Xiao, D., Jiang, Y., & Bi, Y. (2018). Molecularly imprinted polymers for the detection of illegal drugs and additives: a review. *Microchimica Acta*, 185(4). <https://doi.org/10.1007/s00604-018-2735-4>

- Xu, H., Gao, Y., Tao, Q., Li, A., Liu, Z., Jiang, Y., Liu, H., Yang, R., & Liu, Y. (2021). Synthesizing a surface-imprinted polymer based on the nanoreactor SBA-15 for optimizing the adsorption of salicylic acid from aqueous solution by response surface methodology. *New Journal of Chemistry*, 45(14), 6192–6205. <https://doi.org/10.1039/d1nj00016k>
- Yang, Y., Nie, D., Liu, Y., Yu, M., & Gan, Y. (2019). Advances in particle shape engineering for improved drug delivery. *Drug Discovery Today*, 24(2), 575–583. <https://doi.org/10.1016/j.drudis.2018.10.006>
- Yang, L., Zhang, B., Xu, B., Zhao, F., & Zeng, B. (2021). Ionic liquid functionalized 3D graphene-carbon nanotubes–AuPd nanoparticles–molecularly imprinted copolymer based paracetamol electrochemical sensor: Preparation, characterization and application. *Talanta*, 22. <https://doi.org/10.1016/j.talanta.2020.121845>
- Yanti, Nurhayati, T., Royani, I., Widayani, & Khairurrijal. (2016). Synthesis and characterization of MAA-based molecularly-imprinted polymer (MIP) with D-glucose template. *Journal of Physics: Conference Series*, 739(1), 0121. <https://doi.org/10.1088/1742-6596/739/1/012143>
- Yemiş F, Alkan P, Yenigül B, Yenigül M. (2013). Molecularly imprinted polymers and their synthesis by different methods. *Polymers and Polymer Composites*, 21(3):145-150. doi:10.1177/096739111302100304
- Yildirim, E., & Caykara, T. (2018). Ibuprofen-imprinted ultrathin poly[N-(2-hydroxypropyl) methacrylamide] films. *Journal of Applied Polymer Science*, 135(4), 1–7. <https://doi.org/10.1002/app.45707>

- Yoon, S. Do, & Byun, H. S. (2013). Molecularly imprinted polymers for selective separation of acetaminophen and aspirin by using supercritical fluid technology. *Chemical Engineering Journal*, 226, 171–180. <https://doi.org/10.1016/j.cej.2013.04.052>
- Yoshimatsu, K., Reimhult, K., Krozer, A., Mosbach, K., Sode, K., & Ye, L. (2007). Uniform molecularly imprinted microspheres and nanoparticles prepared by precipitation polymerisation: The control of particle size suitable for different analytical applications. *Analytica Chimica Acta*, 584(1), 112–121. <https://doi.org/10.1016/j.aca.2006.11.004>
- Yuksel, N., & Tektas, S. (2022). Molecularly imprinted polymers: preparation, characterisation, and application in drug delivery systems. *Journal of Microencapsulation*. <https://doi.org/10.1080/02652048.2022.2055185>
- Yusof, N. A., Khairi, N. A. S., & Rahman, S. K. A. (2020). Molecularly imprinted polymer for water contaminants. In R. Bouhfid, A. el K. Qaiss, & M. Jawaid (Eds.). *Polymer Nanocomposite-Based Smart Materials. From Synthesis to Application* (pp. 211–233). Elsevier Ltd. <https://doi.org/10.1016/b978-0-08-103013-4.00012-1>
- Zaidi, S. A. (2016a). Molecular imprinted polymers as drug delivery vehicles. *Drug Delivery*, 23(7), 2262–2271. <https://doi.org/10.3109/10717544.2014.970297>
- Zaidi, S. A. (2016b). Latest trends in molecular imprinted polymer based drug delivery systems. *RSC Advances*, 6(91), 88807–88819. <https://doi.org/10.1039/c6ra18911c>
- Zeng, H., Yu, X., Wan, J., & Cao, X. (2020). Rational design and synthesis of molecularly imprinted polymers (MIP) for purifying tylosin by seeded precipitation polymerisation. *Process Biochemistry*, 94, 329–339. <https://doi.org/10.1016/j.procbio.2020.03.025>

- Zhang, X., Gao, L., Niu, L., & Bi, X. (2021). Microwave-assisted preparation of a molecularly imprinted monolith combining an imidazolium ionic liquid and POSS for enhanced extraction of baicalin-like compounds in *Scutellaria baicalensis* by means of in-capillary SPME followed by on-line LC and off-line. *New Journal of Chemistry*, 45(11), 5195–5205. <https://doi.org/10.1039/d0nj06254e>
- Zhang, R., Gao, R., Gou, Q., Lai, J., & Li, X. (2022). Precipitation polymerisation: A powerful tool for preparation of uniform polymer particles. *Polymers*, 14(9). <https://doi.org/10.3390/polym14091851>
- Zhao, G., Liu, J., Liu, M., Han, X., Peng, Y., Tian, X., liu, J., & Zhang, S. (2020a). Synthesis of molecularly imprinted polymer via emulsion polymerisation for application in solanesol separation. *Applied Sciences*, 10(8). <https://doi.org/10.3390/APP10082868>
- Zhao, Y., Simon, C., Daoud Attieh, M., Haupt, K., & Falcimaigne-Cordin, A. (2020b). Reduction-responsive molecularly imprinted nanogels for drug delivery applications. *RSC Advances*, 10(10), 5978–5987. <https://doi.org/10.1039/c9ra07512g>
- Zhao, Y. (2022). Molecularly imprinted materials for glycan recognition and processing. *Journal of Materials Chemistry B*, 10(35), 6607–6617. <https://doi.org/10.1039/d2tb00164k>
- Zheng, X. F., Lian, Q., Yang, H., Wu, H. X., Cheng, C., Yin, G., & Zhang, W. (2016). Preparation and characterization of temperature-memory nanoparticles of MIP-CS-: G - PMMA. *RSC Advances*, 6(112), 110722–110732. <https://doi.org/10.1039/c6ra22730a>
- Zheng, L., Wang, H., & Cheng, X. (2018). Molecularly imprinted polymer nanocarriers for recognition and sustained release of diclofenac. *Polymers for Advanced Technologies*,

29(5), 1360–1371. <https://doi.org/10.1002/pat.4247>

Zheng, B., He, X., Zhang, Q., & Duan, M. (2022). A novel molecularly imprinted membrane for highly sensitive electrochemical detection of paracetamol. *International Journal of Electrochemical Science*, 17, 1–11. <https://doi.org/10.20964/2022.07.37>

Zhu, Y., Yang, L., Huang, D., & Zhu, Q. (2017). Molecularly imprinted nanoparticles and their releasing properties, bio-distribution as drug carriers. *Asian Journal of Pharmaceutical Sciences*, 12, 172–178. <https://doi.org/10.1016/j.ajps.2016.08.008>

Zhu, F., Li, L., Li, N., Liu, W., Liu, X., & He, S. (2021). Selective solid phase extraction and preconcentration of Cd(II) in the solution using microwave-assisted inverse emulsion-suspension Cd(II) ion imprinted polymer. *Microchemical Journal*, 164. <https://doi.org/10.1016/j.microc.2021.106060>

APPENDICES

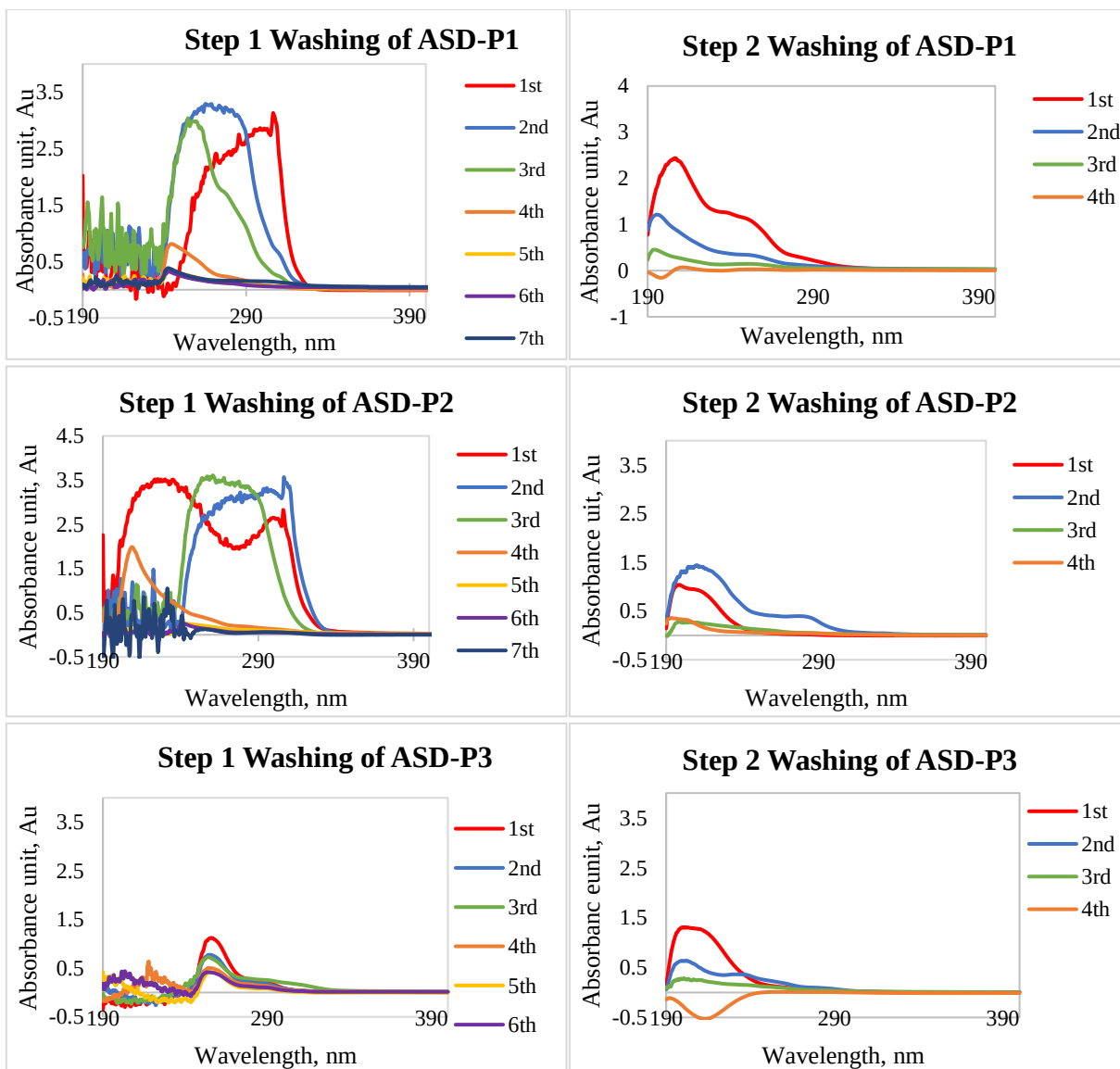
Appendix A: Synthesis of MIP using Aspirin as Template via Conventional Thermal Heating for Drug Delivery Application

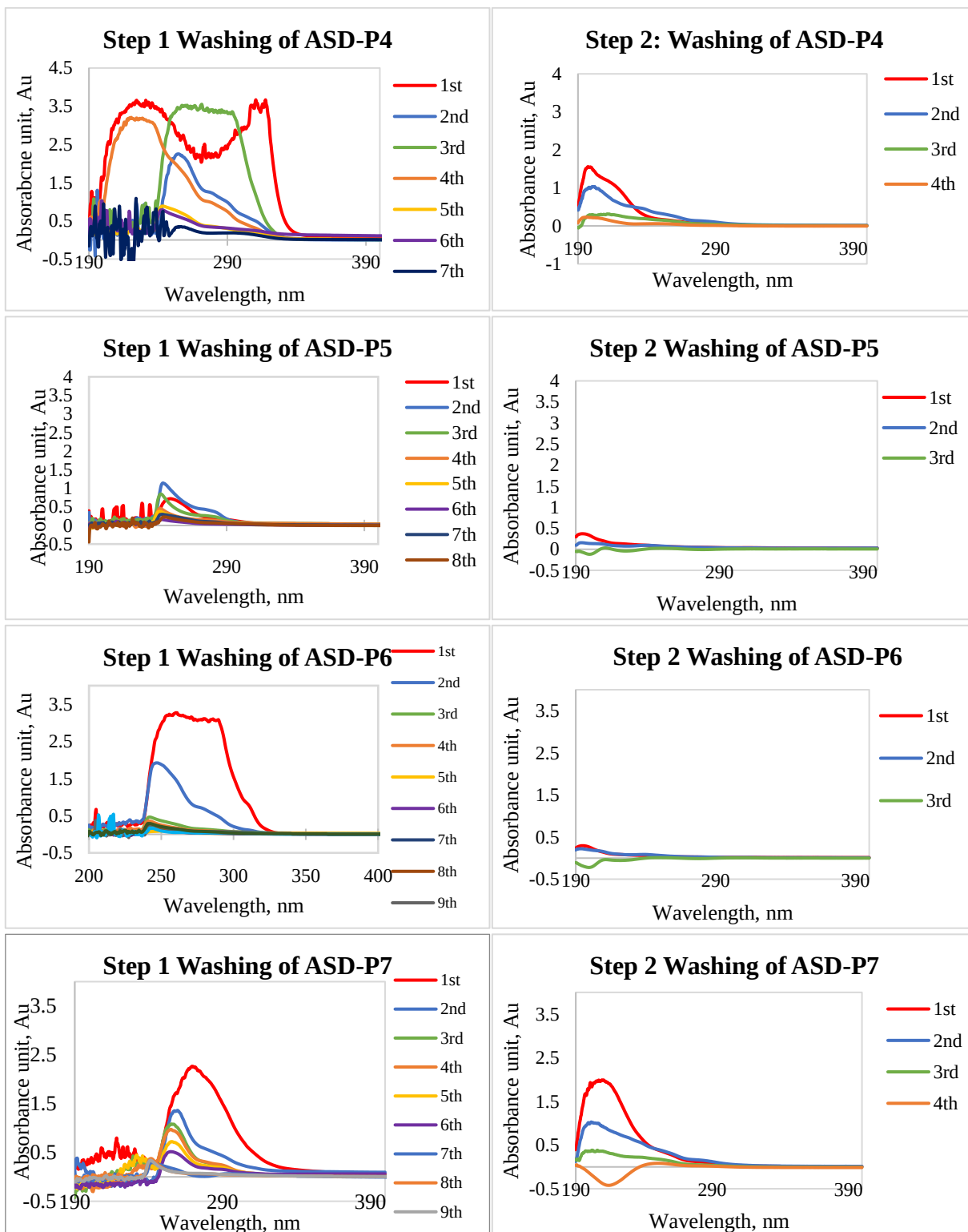
Appendix A1: Polymer Yield

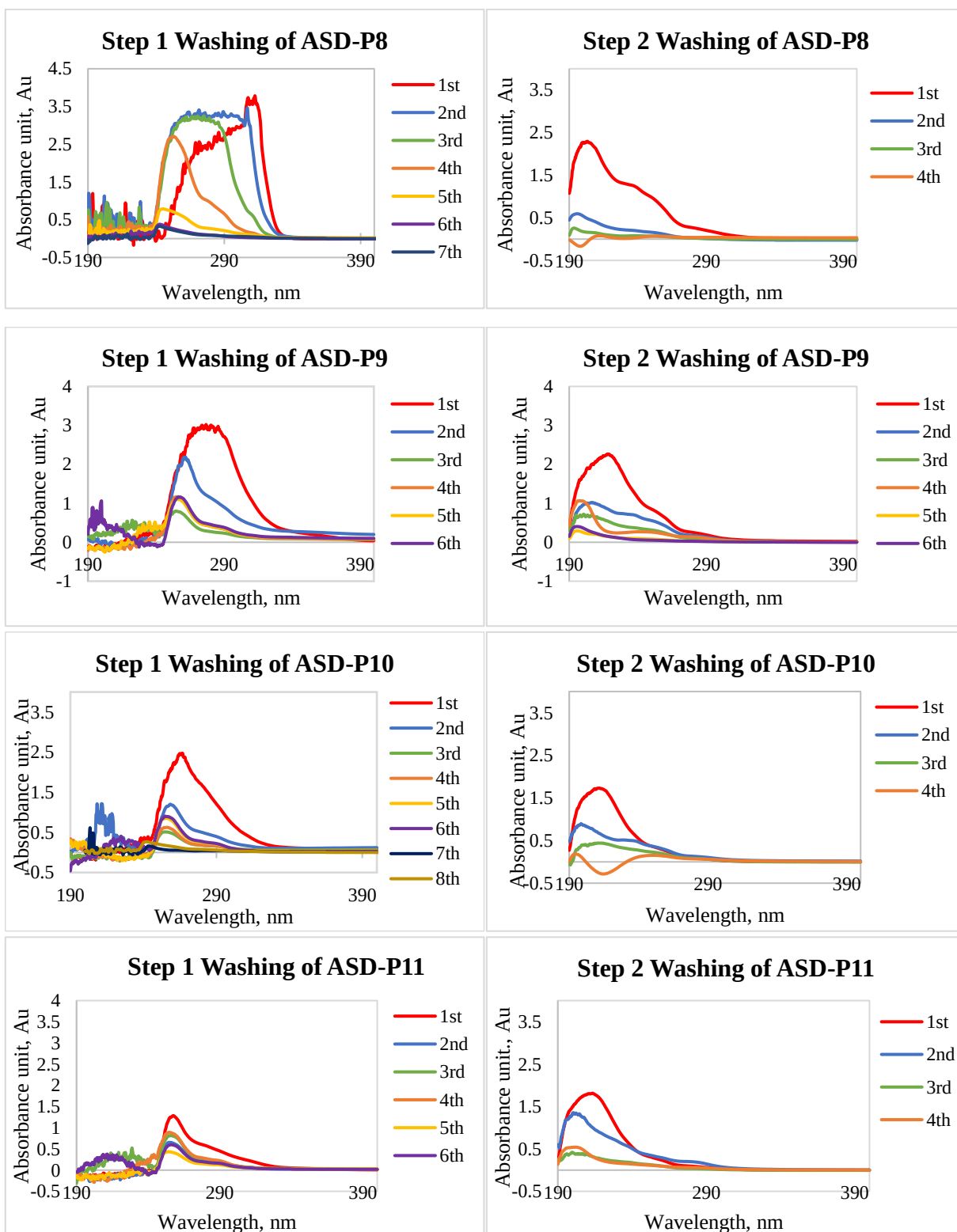
Polymer ID	Factors		Yield (g)			
	Monomer (mmol)	Crosslinker (mmol)	ASD	s.d	NIP	s.d
P1	4	16	0.72	0.03	0.56	0.15
P2	10	16	1.02	0.05	0.96	0.13
P3	4	20	0.86	0.01	0.80	0.04
P4	10	20	1.15	0.03	1.17	0.12
P5	2.76	18	0.59	0.04	0.63	0.09
P6	11.24	18	1.09	0.06	0.99	0.11
P7	7	15.17	0.80	0.09	0.56	0.11
P8	7	20.83	1.04	0.12	0.96	0.16
P9	7	18	0.99	0.03	0.87	0.09
P10	7	18	0.91	0.06	0.88	0.12
P11	7	18	0.95	0.07	0.88	0.06
P12	7	18	0.93	0.09	0.87	0.06
P13	7	18	0.92	0.06	0.87	0.19

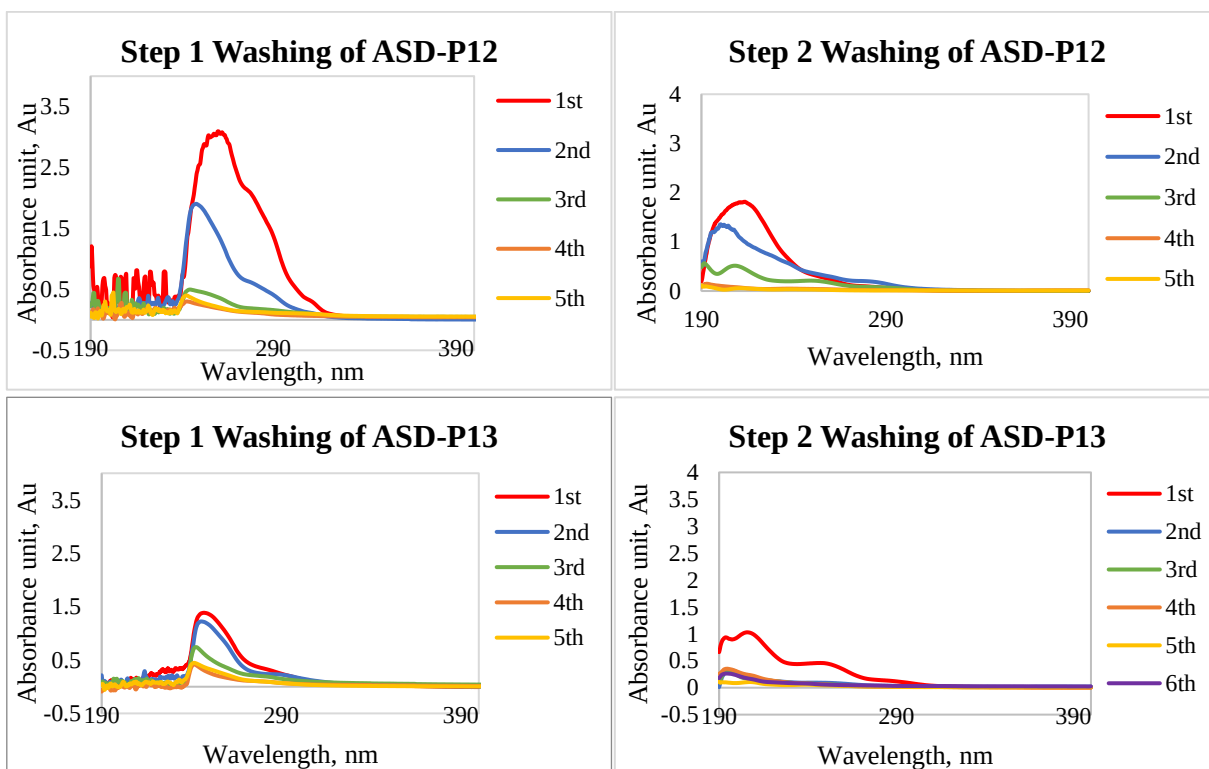
Appendix A2: Template Removal: UV-Vis Graph Monitoring

Appendix A2.1: Template Removal (Washing) Monitoring of MIP

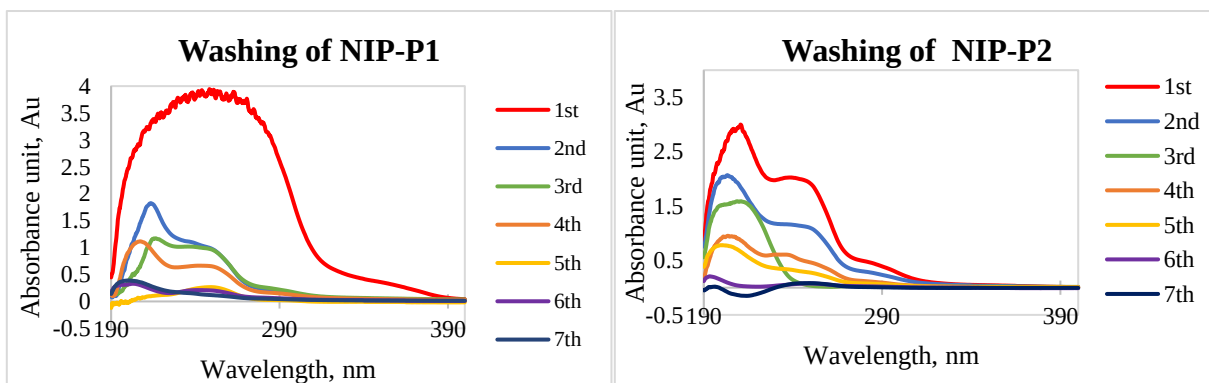


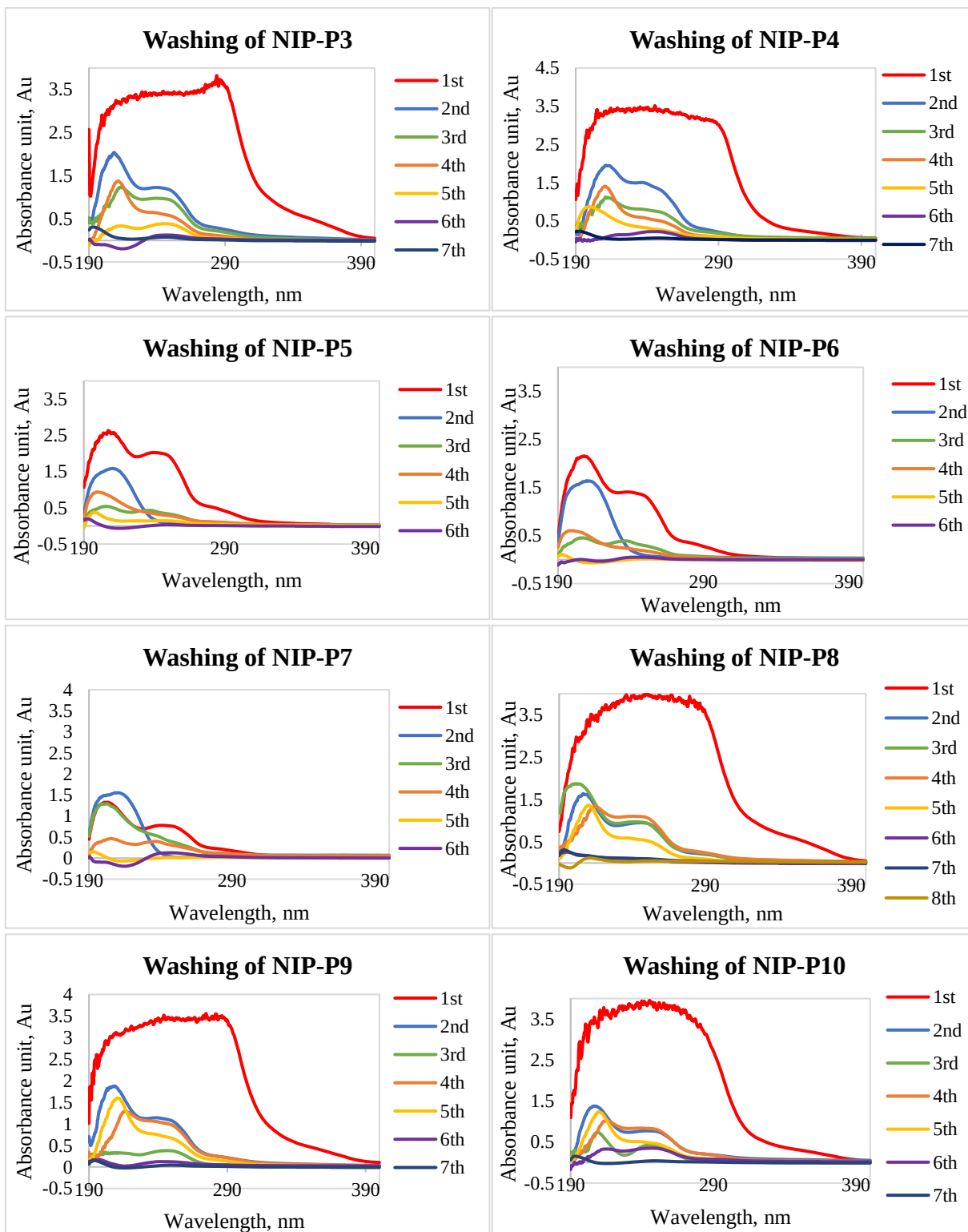


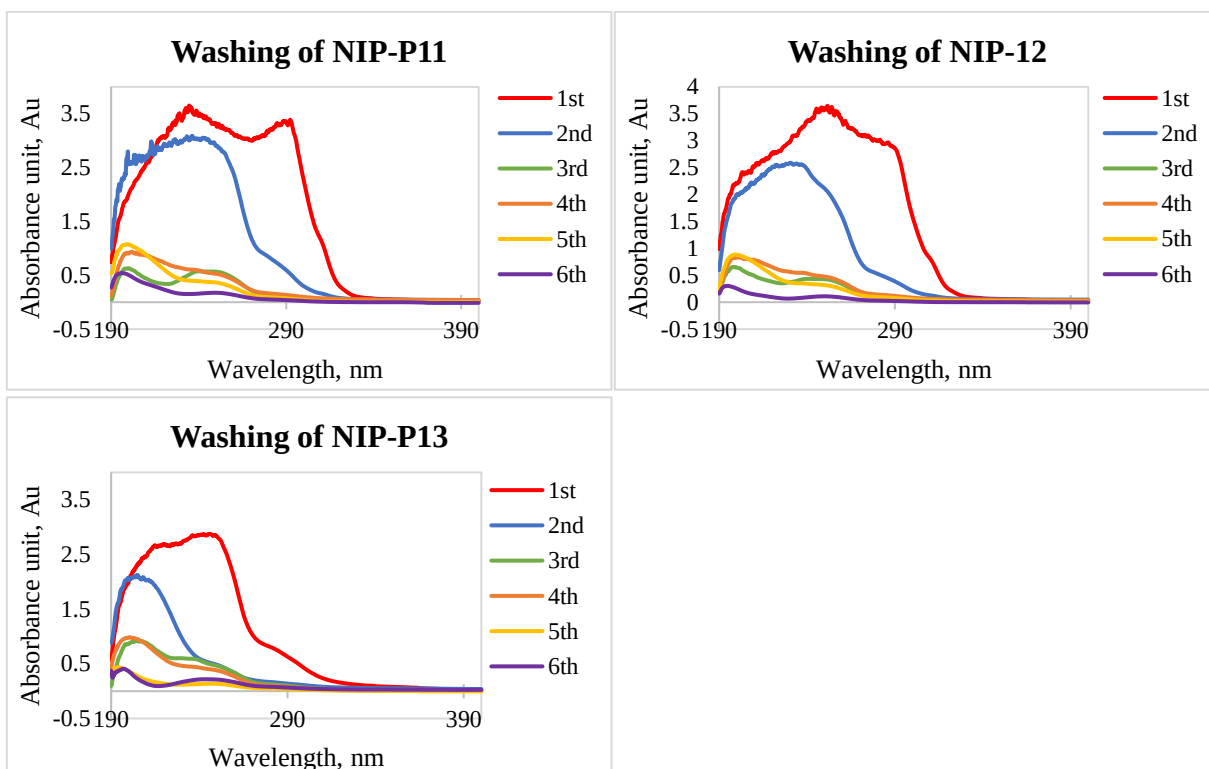




Appendix A2.2: Template Removal (Washing) Monitoring of NIP







Appendix A3: Particle size data.

Polymer ID	Length, μm							
	ASD				NIP			
	Ave.	s.d	Min	Max	Ave.	s.d	Min	Max
P1	1.98	0.43	0.83	2.65	3.12	0.82	0.95	4.06
P2	0.66	0.13	0.42	1.07	1.75	0.38	1.14	2.89
P3	2.02	0.51	1.25	4.14	1.84	0.18	1.45	2.18
P4	2.07	0.70	0.80	4.99	2.47	0.60	1.68	3.95
P5	0.88	0.23	0.46	1.56	1.76	0.67	0.58	3.74
P6	3.65	1.27	1.28	7.36	1.81	1.20	0.72	6.87
P7	1.78	0.83	0.62	5.22	2.53	0.08	2.31	2.72
P8	1.92	0.69	0.44	3.58	1.86	0.52	0.55	3.30
P9	2.57	0.59	1.37	4.13	1.69	0.71	1.26	4.36
P10	1.18	0.33	0.40	1.87	2.02	0.31	0.97	2.77
P11	2.75	0.96	0.75	4.75	1.75	0.15	1.53	2.09
P12	3.26	1.06	1.72	6.00	2.72	0.63	1.04	3.87
P13	1.50	0.42	0.68	2.56	2.27	0.68	1.06	3.80

Ave., average; s.d, standard deviation ; Min, minimum ; Max, maximum

Appendix A4: Preliminary Rebinding Study Data of ASD-MIP

Std	Polymer ID	Calibration data		Absorbance unit			Final Concentration (mg/L)					Rebinding capacity (mg/g)				
		Equation	R ²	T1	T2	T3	C1	C2	C3	Mean	s.d	Q1	Q2	Q3	Mean	s.d
1	ASD-1	$y = 186.73x - 22.404$	0.9888	0.59	0.60	0.58	88.33	89.07	86.65	88.02	1.24	2.92	2.73	3.34	3.00	0.31
2	ASD-2	$y = 186.73x - 22.404$	0.9888	0.60	0.61	0.59	89.07	91.87	87.95	89.63	2.02	2.73	2.03	3.01	2.59	0.50
3	ASD-3	$y = 183.96x - 15.294$	0.9943	0.56	0.57	0.58	88.46	90.12	91.22	89.93	1.39	2.89	2.47	2.20	2.52	0.35
4	ASD-4	$y = 186.73x - 22.404$	0.9888	0.61	0.61	0.60	90.94	91.50	90.19	90.88	0.66	2.26	2.12	2.45	2.28	0.16
5	ASD-5	$y = 198.31x - 7.7443$	0.9955	0.49	0.50	0.49	89.23	91.01	88.44	89.56	1.32	2.69	2.25	2.89	2.61	0.33
6	ASD-6	$y = 198.31x - 7.7443$	0.9955	0.49	0.49	0.49	89.03	88.44	89.63	89.03	0.59	2.74	2.89	2.59	2.74	0.15
7	ASD-7	$y = 183.96x - 15.294$	0.9943	0.57	0.57	0.57	89.75	89.38	90.12	89.75	0.37	2.56	2.66	2.47	2.56	0.09
8	ASD-8	$y = 198.31x - 7.7443$	0.9955	0.50	0.49	0.50	90.82	90.02	90.62	90.49	0.41	2.30	2.49	2.35	2.38	0.10
9	ASD-9	$y = 186.73x - 22.404$	0.9888	0.64	0.63	0.63	96.17	94.86	95.42	95.48	0.66	0.96	1.28	1.14	1.13	0.16
10	ASD-10	$y = 148.55x - 0.649$	0.9990	0.64	0.64	0.64	94.87	95.02	94.27	94.72	0.39	1.28	1.25	1.43	1.32	0.10
11	ASD-11	$y = 186.73x - 22.404$	0.9888	0.63	0.63	0.63	95.24	94.68	95.98	95.30	0.66	1.19	1.33	1.00	1.18	0.16
12	ASD-12	$y = 186.73x - 22.404$	0.9888	0.64	0.63	0.63	96.17	95.98	95.42	95.86	0.39	0.96	1.00	1.14	1.04	0.10
13	ASD-13	$y = 183.96x - 15.294$	0.9943	0.60	0.60	0.60	94.53	94.71	95.82	95.02	0.70	1.37	1.32	1.05	1.24	0.17

Std, standard order; s.d, standard deviation;

Appendix A4.1: Design Expert Results Summary for the Response of Preliminary Rebinding Study of ASD-MIP

Appendix A4.1.1: Fit Summary

*** WARNING: The Cubic Model and higher are Aliased! ***

Sequential Model Sum of Squares [Type I]

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Mean vs Total	54.39	1	54.39			
Linear vs Mean	0.16	2	0.082	0.13	0.8793	
2FI vs Linear	7.225E-003	1	7.225E-003	0.010	0.9211	
<u>Quadratic vs 2FI</u>	<u>6.10</u>	<u>2</u>	<u>3.05</u>	<u>126.09</u>	<u>< 0.0001</u>	<u>Suggested</u>
Cubic vs Quadratic	0.12	2	0.061	6.60	0.0396	Aliased
Residual	0.047	5	9.306E-003			
Total	60.83	13	4.68			

Lack of Fit Tests

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Linear	6.23	6	1.04	91.72	0.0003	
2FI	6.22	5	1.24	109.94	0.0002	
<u>Quadratic</u>	<u>0.12</u>	<u>3</u>	<u>0.041</u>	<u>3.65</u>	<u>0.1215</u>	<u>Suggested</u>
Cubic	1.250E-003	1	1.250E-003	0.11	0.7563	Aliased
Pure Error	0.045	4	0.011			

Model Summary Statistics

Source	Std. Dev.	R-Squared	Adjusted R-Squared	Predicted R-Squared	PRESS	
Linear	0.79	0.0254	-0.1695	-0.5456	9.95	
2FI	0.83	0.0265	-0.2980	-1.2153	14.26	
<u>Quadratic</u>	<u>0.16</u>	<u>0.9737</u>	<u>0.9549</u>	<u>0.8521</u>	<u>0.95</u>	<u>Suggested</u>

Cubic	0.096	0.9928	0.9827	0.9766	0.15	Aliased
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Appendix A4.1.2: ANOVA for Response Surface Quadratic Model

Analysis of variance table [Partial sum of squares - Type III]

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	6.27	5	1.25	51.85	< 0.0001	significant
<i>A-Monomer</i>	0.027	1	0.027	1.12	0.3244	
<i>B-Cross-linker</i>	0.14	1	0.14	5.64	0.0493	
<i>AB</i>	7.225E-003	1	7.225E-003	0.30	0.6016	
<i>A²</i>	3.94	1	3.94	163.00	< 0.0001	
<i>B²</i>	2.94	1	2.94	121.63	< 0.0001	
Residual	0.17	7	0.024			
<i>Lack of Fit</i>	0.12	3	0.041	3.65	0.1215	not significant
<i>Pure Error</i>	0.045	4	0.011			
Cor Total	6.44	12				

The Model F-value of 51.85 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise.

Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case B, A², B² are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

The "Lack of Fit F-value" of 3.65 implies the Lack of Fit is not significant relative to the pure error. There is a 12.15% chance that a "Lack of Fit F-value" this large could occur due to noise. Non-significant lack of fit is good -- we want the model to fit.

Std. Dev.	0.16	R-Squared	0.9737
Mean	2.05	Adj R-Squared	0.9549
C.V. %	7.60	Pred R-Squared	0.8521
PRESS	0.95	Adeq Precision	15.470
-2 Log Likelihood	-19.54	BIC	-4.15
		AICc	6.46

The "Pred R-Squared" of 0.8542 is in reasonable agreement with the "Adj R-Squared" of 0.9550; i.e. the difference is less than 0.2. "Adeq Precision" measures the signal to noise ratio.

A ratio greater than 4 is desirable. Your ratio of 15.470 indicates an adequate signal. This model can be used to navigate the design space.

Factor	Coefficient	df	Standard	95% CI		VIF
	Estimate		Error	Low	High	
Intercept	1.18	1	0.070	1.02	1.35	
A-Monomer	-0.058	1	0.055	-0.19	0.072	1.00
B-Cross-linker	-0.13	1	0.055	-0.26	-5.616E-004	1.00
AB	0.043	1	0.078	-0.14	0.23	1.00
A ²	0.75	1	0.059	0.61	0.89	1.02
B ²	0.65	1	0.059	0.51	0.79	1.02

Final Equation in Terms of Coded Factors:

$$\begin{aligned} \text{Binding Capacity MIP} = & \\ & +1.18 \\ & -0.058 * A \\ & -0.13 * B \\ & +0.043 * AB \\ & +0.75 * A^2 \\ & +0.65 * B^2 \end{aligned}$$

The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels of the factors are coded as -1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.

Final Equation in Terms of Actual Factors:

$$\begin{aligned} \text{Binding Capacity MIP} = & \\ & +60.15414 \\ & -1.31787 * \text{Monomer} \\ & -5.96712 * \text{Cross-linker} \\ & +7.08333\text{E-}003 * \text{Monomer} * \text{Cross-linker} \\ & +0.083639 * \text{Monomer}^2 \\ & +0.16256 * \text{Cross-linker}^2 \end{aligned}$$

The equation in terms of actual factors can be used to make predictions about the response for given levels of each factor. Here, the levels should be specified in the original units

for each factor. This equation should not be used to determine the relative impact of each factor because the coefficients are scaled to accommodate the units of each factor and the intercept is not at the center of the design space.

Appendix A4.1.3: Diagnostic of Quadratic Model in Design Expert

Diagnostics Case Statistics

Run Order	Actual Value	Predicted Value	Residual	Leverage	Internally Studentized Residual	Externally Studentized Residual	Cook's Distance	Influence on Fitted Value DFFITS	Standard Order
6	3.00	2.82	0.18	0.625	1.929	2.608	1.033 ¹	3.367 ¹	1
3	2.59	2.61	-0.025	0.625	-0.260	-0.242	0.019	-0.313	2
8	2.52	2.47	0.050	0.625	0.523	0.494	0.076	0.638	3
4	2.28	2.44	-0.16	0.625	-1.666	-1.986	0.771	-2.564 ¹	4
13	2.61	2.77	-0.16	0.625	-1.679	-2.012	0.783	-2.597 ¹	5
12	2.74	2.61	0.13	0.625	1.417	1.553	0.557	2.005	6
9	2.56	2.67	-0.11	0.625	-1.125	-1.151	0.352	-1.486	7
11	2.38	2.30	0.082	0.625	0.863	0.845	0.207	1.091	8
5	1.13	1.18	-0.052	0.200	-0.374	-0.350	0.006	-0.175	9
10	1.32	1.18	0.14	0.200	0.992	0.991	0.041	0.495	10
1	1.18	1.18	-2.000E-003	0.200	-0.014	-0.013	0.000	-0.007	11
2	1.04	1.18	-0.14	0.200	-1.021	-1.025	0.043	-0.512	12
7	1.24	1.18	0.058	0.200	0.417	0.391	0.007	0.195	13

¹ Exceeds limits

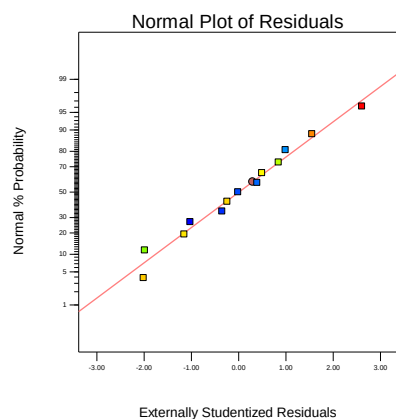
Box-Cox Power Transformation

Constant k	95% CI Low	95% CI High	Best Lambda	Rec. Transform
0.000	-0.71	2.11	0.61	None

Design-Expert® Software
ASD-MIP

Color points by value of
ASD-MIP:

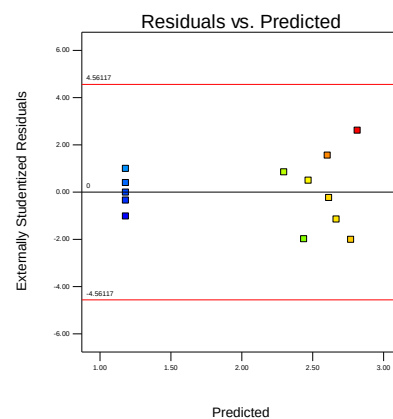
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1.04



Design-Expert® Software
ASD-MIP

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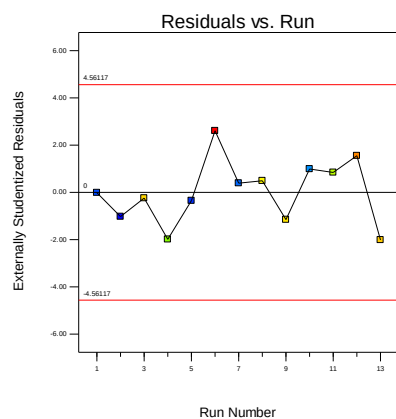
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Design-Expert® Software
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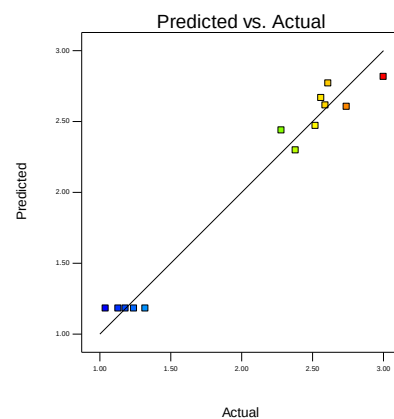
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Design-Expert® Software
ASD-MIP

Color points by value of
ASD-MIP:

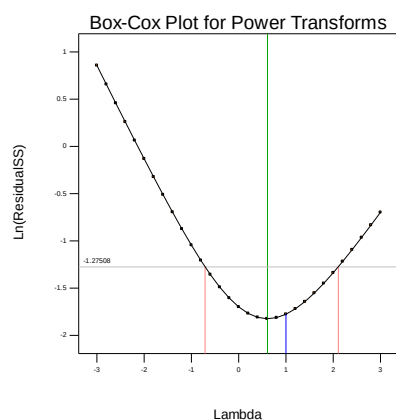
3.00
1.04



Design-Expert® Software
ASD-MIP

Lambda
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Best = 0.61
Low C.I. = -0.71
High C.I. = 2.11

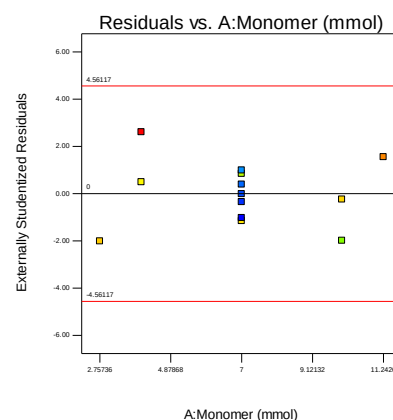
Recommend transform:
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(Lambda = 1)



Design-Expert® Software
ASD-MIP

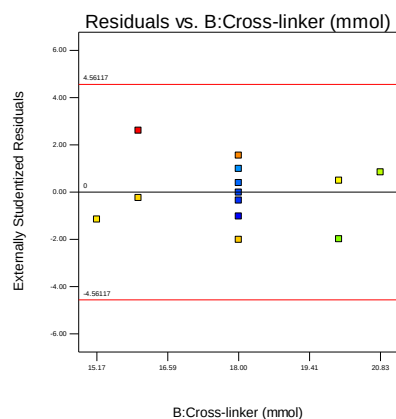
Color points by value of
ASD-MIP:

3.00
1.04



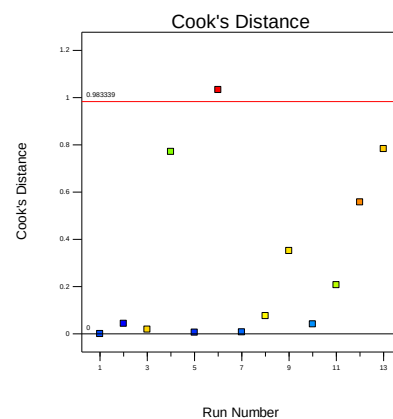
Design-Expert® Software
ASD-MIP

Color points by value of
ASD-MIP:
3.00
1.04



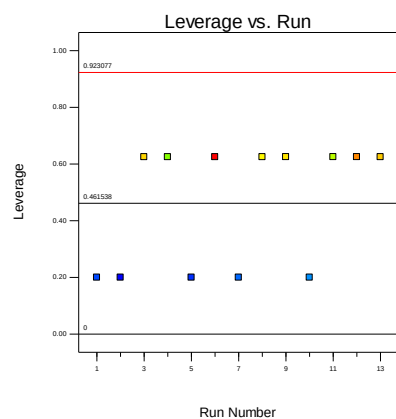
Design-Expert® Software
ASD-MIP

Color points by value of
ASD-MIP:
3.00
1.04



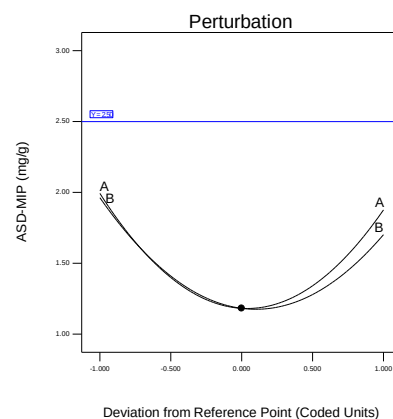
Design-Expert® Software
ASD-MIP

Color points by value of
ASD-MIP:
3.00
1.04



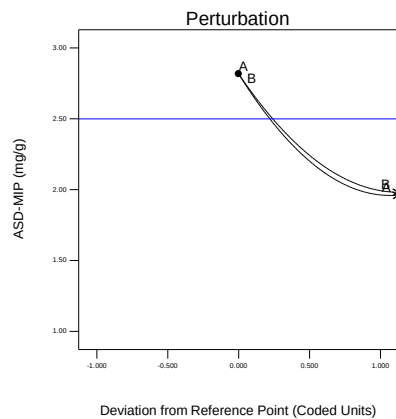
Design-Expert® Software
Factor Coding: Actual
ASD-MIP (mg/g)

Actual Factors
A: Monomer = 7.00
B: Cross-linker = 18.00



Design-Expert® Software
Factor Coding: Actual
ASD-MIP (mg/g)

Actual Factors
A: Monomer = 4.00
B: Cross-linker = 16.00



Appendix A5: Preliminary Rebinding Study Data of NIP

Std	Polymer ID	Calibration data		Absorbance unit			Final Concentration (mg/L)				Rebinding capacity (mg/g)					
		Equation	R ²	T1	T2	T3	C1	C2	C3	Mean	s.d	Q1	Q2	Q3	Mean	s.d
1	ASD1	y = 150.96x - 5.2791	0.9957	0.67	0.67	0.67	95.56	95.52	95.64	95.57	0.06	1.11	1.12	1.09	1.11	0.02
2	ASD2	y = 150.96x - 5.2791	0.9957	0.65	0.65	0.66	93.56	93.48	93.72	93.59	0.12	1.61	1.63	1.57	1.60	0.03
3	ASD3	y = 150.96x - 5.2791	0.9957	0.66	0.66	0.66	94.12	93.88	93.72	93.91	0.20	1.47	1.53	1.57	1.52	0.05
4	ASD4	y = 150.96x - 5.2791	0.9957	0.67	0.67	0.67	95.68	95.28	95.64	95.53	0.22	1.08	1.18	1.09	1.12	0.06
5	ASD5	y = 150.96x - 5.2791	0.9957	0.67	0.66	0.67	95.48	94.84	95.32	95.21	0.33	1.13	1.29	1.17	1.20	0.08
6	ASD6	y = 150.96x - 5.2791	0.9957	0.66	0.66	0.65	93.88	93.64	93.28	93.60	0.30	1.53	1.59	1.68	1.60	0.08
7	ASD7	y = 150.96x - 5.2791	0.9957	0.66	0.65	0.65	93.72	93.48	93.28	93.49	0.22	1.57	1.63	1.68	1.63	0.06
8	ASD8	y = 150.96x - 5.2791	0.9957	0.67	0.67	0.67	96.08	95.40	95.72	95.73	0.34	0.98	1.15	1.07	1.07	0.09
9	ASD9	y = 150.96x - 5.2791	0.9957	0.67	0.67	0.67	96.12	95.96	96.40	96.16	0.22	0.97	1.01	0.90	0.96	0.06
10	ASD10	y = 150.96x - 5.2791	0.9957	0.67	0.67	0.67	96.36	96.28	96.32	96.32	0.04	0.91	0.93	0.92	0.92	0.01
11	ASD11	y = 150.96x - 5.2791	0.9957	0.67	0.67	0.67	96.16	95.88	96.04	96.03	0.14	0.96	1.03	0.99	0.99	0.04
12	ASD12	y = 150.96x - 5.2791	0.9957	0.67	0.67	0.67	96.12	96.08	96.00	96.07	0.06	0.97	0.98	1.00	0.98	0.02
13	ASD13	y = 150.96x - 5.2791	0.9957	0.67	0.67	0.67	96.44	96.36	95.96	96.25	0.26	0.89	0.91	1.01	0.94	0.06

Std, standard order; s.d, standard deviation;

Appendix A6: Optimisation on Rebinding Capacity of ASD-P1 using RSM.

Appendix A6.1: Rebinding Capacity Data

Std.	Run	Calibration data		Absorbance unit	Concentration (mg/L)			Dosage (g)	Rebinding capacity (mg/g)
		Equation	R ²		Initial, C _i	Final, C _f	C _i - C _f		
1	B14	$y = 153.45x - 5.5501$	0.9967	0.322	50	46.32	3.68	0.02	1.84
2	B3	$y = 153.45x - 5.5501$	0.9967	0.272	50	41.71	8.29	0.04	2.07
3	B12	$y = 153.45x - 5.5501$	0.9967	1.951	300	296.29	3.71	0.02	1.86
4	B7	$y = 153.45x - 5.5501$	0.9967	1.841	300	286.16	13.84	0.04	3.46
5	B4	$y = 153.45x - 5.5501$	0.9967	0.315	50	45.55	4.45	0.02	2.23
6	B1	$y = 153.45x - 5.5501$	0.9967	0.258	50	40.33	9.67	0.04	2.42
7	B17	$y = 153.45x - 5.5501$	0.9967	1.876	300	289.99	10.01	0.02	5.00
8	B19	$y = 153.45x - 5.5501$	0.9967	1.746	300	277.41	22.59	0.04	5.65
9	B13	$y = 153.45x - 5.5501$	0.9967	1.14	175	171.53	3.47	0.01	2.63
10	B20	$y = 153.45x - 5.5501$	0.9967	1.025	175	160.94	14.06	0.05	3.00
11	B9	$y = 153.45x - 5.5501$	0.9967	0.039	10	4.12	5.88	0.03	1.96
12	B10	$y = 153.45x - 5.5501$	0.9967	2.319	385.22	366.41	18.81	0.03	6.27
13	B5	$y = 153.45x - 5.5501$	0.9967	1.129	175	170.61	4.39	0.03	1.46
14	B2	$y = 153.45x - 5.5501$	0.9967	1.069	175	165.09	9.91	0.03	3.30
15	B15	$y = 153.45x - 5.5501$	0.9967	1.092	175	167.08	7.92	0.03	2.64
16	B16	$y = 153.45x - 5.5501$	0.9967	1.106	175	168.46	6.54	0.03	2.18
17	B8	$y = 153.45x - 5.5501$	0.9967	1.09	175	166.93	8.07	0.03	2.69
18	B6	$y = 153.45x - 5.5501$	0.9967	1.108	175	168.62	6.38	0.03	2.13
19	B18	$y = 153.45x - 5.5501$	0.9967	1.105	175	168.31	6.69	0.03	2.23
20	B11	$y = 153.45x - 5.5501$	0.9967	1.089	175	166.93	8.07	0.03	2.69

Appendix A6.2: Design Expert Results Summary for the Response of Rebinding Capacity of ASD-P1

Appendix A6.2.1: Fit Summary

Sequential Model Sum of Squares [Type I]

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Mean vs Total	166.52	1	166.52			
Linear vs Mean	23.00	3	7.67	13.43	0.0001	
2FI vs Linear	3.17	3	1.06	2.31	0.1243	
Quadratic vs 2FI	<u>5.11</u>	<u>3</u>	<u>1.70</u>	<u>20.09</u>	<u>0.0001</u>	Suggested
Cubic vs Quadratic	0.48	5	0.095	1.28	0.3962	Aliased
Residual	0.37	5	0.074			
Total	198.65	20	9.93			

Lack of Fit Tests

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Linear	8.76	11	0.80	10.71	0.0085	
2FI	5.59	8	0.70	9.39	0.0122	
Quadratic	<u>0.48</u>	<u>5</u>	<u>0.095</u>	<u>1.28</u>	<u>0.3962</u>	Suggested
Cubic	0.000	0				Aliased
Pure Error	0.37	5	0.074			

Model Summary Statistics

Source	Std. Dev.	R-Squared	Adjusted R-Squared	Predicted R-Squared	PRESS	
Linear	0.76	0.7157	0.6624	0.4972	16.15	
2FI	0.68	0.8145	0.7289	0.4914	16.34	
Quadratic	<u>0.29</u>	<u>0.9736</u>	<u>0.9499</u>	<u>0.8513</u>	<u>4.78</u>	Suggested
Cubic	0.27	0.9884	0.9560		+	Aliased

+ Case(s) with leverage of 1.0000: PRESS statistic not defined

Appendix A6.2.2: ANOVA for Response Surface of Quadratic Model

Analysis of variance table [Partial sum of squares - Type III]

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	31.28	9	3.48	40.99	< 0.0001	significant
<i>A-Adsorbent dosage</i>	0.83	1	0.83	9.84	0.0106	
<i>B-Initial concentration</i>	12.66	1	12.66	149.34	< 0.0001	
<i>C-Contact time</i>	6.36	1	6.36	74.94	< 0.0001	
AB	0.42	1	0.42	4.94	0.0505	
AC	0.12	1	0.12	1.44	0.2571	
BC	2.63	1	2.63	31.06	0.0002	
A ²	0.27	1	0.27	3.22	0.1030	
B ²	4.79	1	4.79	56.48	< 0.0001	
C ²	0.18	1	0.18	2.13	0.1747	
Residual	0.85	10	0.085			not significant
<i>Lack of Fit</i>	0.48	5	0.095	1.28	0.3962	
<i>Pure Error</i>	0.37	5	0.074			
Cor Total	32.13	19				

The Model F-value of 40.99 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise.

Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A, B, C, BC, B² are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

The "Lack of Fit F-value" of 1.28 implies the Lack of Fit is not significant relative to the pure error. There is a 39.62% chance that a "Lack of Fit F-value" this large could occur due to noise.

Non-significant lack of fit is good -- we want the model to fit.

Std. Dev.	0.29	R-Squared	0.9736
Mean	2.89	Adj R-Squared	0.9499
C.V. %	10.09	Pred R-Squared	0.8513
PRESS	4.78	Adeq Precision	22.757
-2 Log Likelihood	-6.46	BIC	23.50
		AICc	37.99

"The ""Pred R-Squared"" of 0.8501 is in reasonable agreement with the ""Adj R-Squared"" of 0.9496;" i.e. the difference is less than 0.2.

"Adeq Precision"" measures the signal to noise ratio. A ratio greater than 4 is desirable. Your "ratio of 22.676 indicates an adequate signal. This model can be used to navigate the design space.

Factor	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	2.42	1	0.12	2.16	2.67	
A-Adsorbent dosage	0.25	1	0.080	0.072	0.43	1.00
B-Initial concentration	1.02	1	0.084	0.84	1.21	1.04
C-Contact time	0.74	1	0.086	0.55	0.94	1.07
AB	0.23	1	0.10	-6.484E-004	0.46	1.00
AC	-0.12	1	0.10	-0.35	0.11	1.00
BC	0.57	1	0.10	0.34	0.80	1.00
A ²	0.14	1	0.078	-0.034	0.32	1.01
B ²	0.67	1	0.089	0.47	0.87	1.04
C ²	-0.14	1	0.093	-0.34	0.071	1.07

Final Equation in Terms of Coded Factors:

$$\begin{aligned}
 \text{Rebinding capacity of ASD-P1} = & +2.42 \\
 & +0.25 * A \\
 & +1.02 * B \\
 & +0.74 * C \\
 & +0.23 * AB \\
 & -0.12 * AC \\
 & +0.57 * BC \\
 & +0.14 * A^2 \\
 & +0.67 * B^2 \\
 & -0.14 * C^2
 \end{aligned}$$

The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels of the factors are coded as -1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.

Final Equation in Terms of Actual Factors:

$$\begin{aligned} \text{Rebinding capacity of ASD-P1} = & +3.15214 \\ & -0.075601 * \text{Adsorbent dosage} \\ & -0.018210 * \text{Initial concentration} \\ & +6.28821\text{E-}003 * \text{Contact time} \\ & +1.83000\text{E-}004 * \text{Adsorbent dosage} * \text{Initial concentration} \\ & -1.17857\text{E-}004 * \text{Adsorbent dosage} * \text{Contact time} \\ & +4.37143\text{E-}005 * \text{Initial concentration} * \text{Contact time} \\ & +1.40728\text{E-}003 * \text{Adsorbent dosage}^2 \\ & +4.28580\text{E-}005 * \text{Initial concentration}^2 \\ & -1.22871\text{E-}005 * \text{Contact time}^2 \end{aligned}$$

The equation in terms of actual factors can be used to make predictions about the response for given levels of each factor. Here, the levels should be specified in the original units for each factor. This equation should not be used to determine the relative impact of each factor because the coefficients are scaled to accommodate the units of each factor and the intercept is not at the center of the design space.

Appendix A6.2.3: Diagnostic of Quadratic Model in Design Expert

Diagnostics Case Statistics

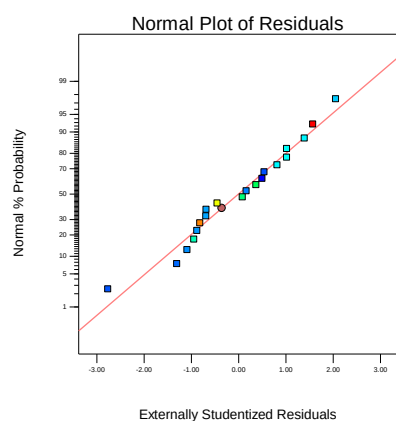
Run Order	Actual Value	Predicted Value	Residual	Leverage	Internally Studentized Residual	Externally Studentized Residual	Cook's Distance	Influence on Fitted Value DFFITS	Standard Order
14	1.84	1.76	0.085	0.733	0.564	0.543	0.087	0.900	1
3	2.07	2.04	0.026	0.737	0.173	0.165	0.008	0.276	2
12	1.86	2.20	-0.34	0.710	-2.142	-2.763	1.126 ¹	-4.329 ¹	3
7	3.46	3.40	0.060	0.716	0.389	0.372	0.038	0.590	4
4	2.23	2.34	-0.11	0.699	-0.707	-0.688	0.116	-1.047	5
1	2.42	2.14	0.28	0.705	1.789	2.057	0.763	3.178 ¹	6
17	5.00	5.08	-0.079	0.669	-0.469	-0.450	0.045	-0.640	7
19	5.65	5.79	-0.14	0.676	-0.830	-0.816	0.144	-1.179	8
13	2.63	2.40	0.23	0.650	1.333	1.395	0.330	1.899	9
20	3.00	3.18	-0.18	0.588	-0.948	-0.942	0.128	-1.126	10
9	1.96	2.23	-0.27	0.441	-1.261	-1.304	0.125	-1.159	11
10	6.27	6.03	0.24	0.674	1.469	1.573	0.447	2.264 ¹	12
5	1.46	1.34	0.12	0.372	0.520	0.500	0.016	0.384	13
2	3.30	3.29	0.014	0.686	0.088	0.084	0.002	0.124	14
15	2.64	2.42	0.22	0.157	0.832	0.818	0.013	0.353	15
16	2.18	2.42	-0.24	0.157	-0.889	-0.879	0.015	-0.380	16
8	2.69	2.42	0.27	0.157	1.019	1.021	0.019	0.441	17
6	2.13	2.42	-0.29	0.157	-1.076	-1.086	0.022	-0.469	18
18	2.23	2.42	-0.19	0.157	-0.702	-0.683	0.009	-0.295	19
11	2.69	2.42	0.27	0.157	1.019	1.021	0.019	0.441	20

Box-Cox Power Transformation

Constant	95% CI	95% CI	Best	Rec.
k	Low	High	Lambda	Transform
0.000	-0.33	1.54	0.71	None

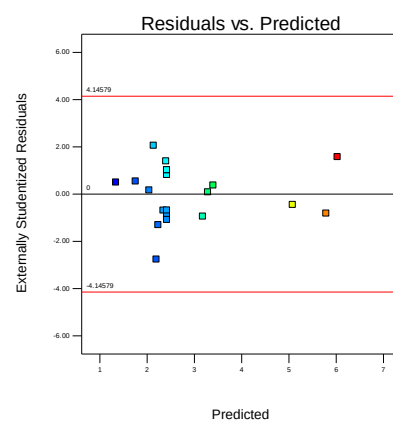
Design-Expert® Software
ASD-P1

Color points by value of
ASD-P1:
6.27
1.46



Design-Expert® Software
ASD-P1

Color points by value of
ASD-P1:
6.27
1.46

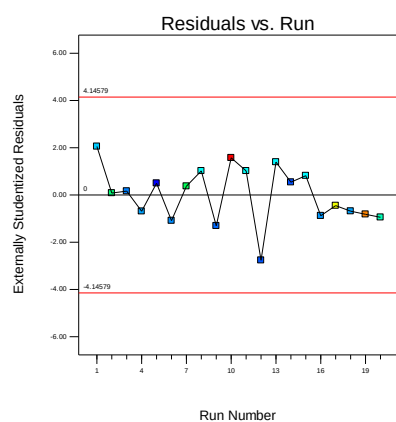


1

2

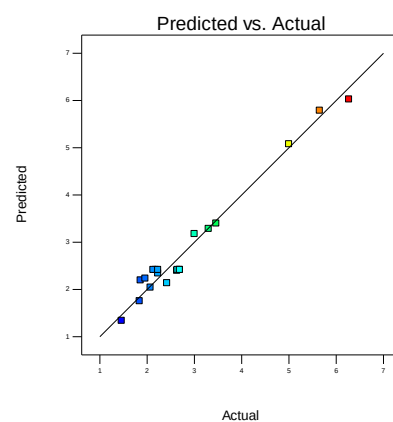
Design-Expert® Software
ASD-P1

Color points by value of
ASD-P1:
6.27
1.46



Design-Expert® Software
ASD-P1

Color points by value of
ASD-P1:
6.27
1.46

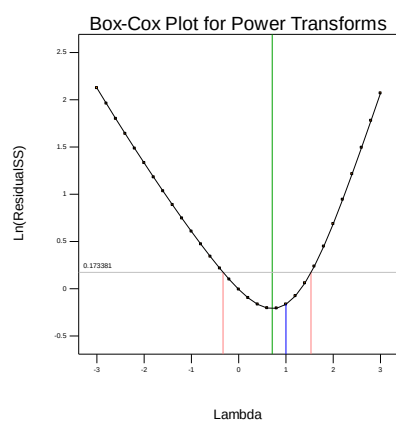


3

Design-Expert® Software
ASD-P1

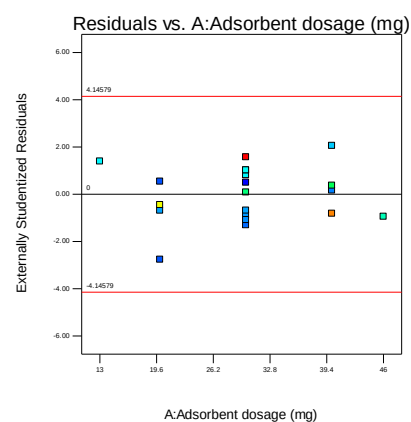
Lambda
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Best = 0.71
Low C.I. = -0.33
High C.I. = 1.53

Recommend transform:
None
(Lambda = 1)



Design-Expert® Software
ASD-P1

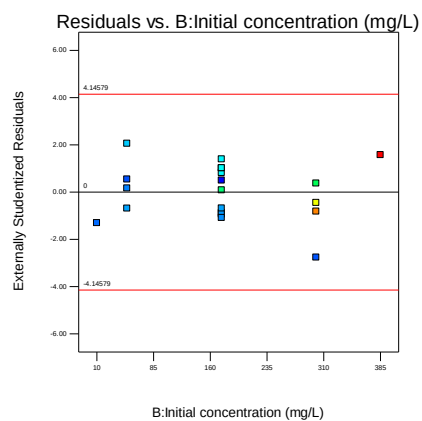
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ASD-P1:
6.27
1.46



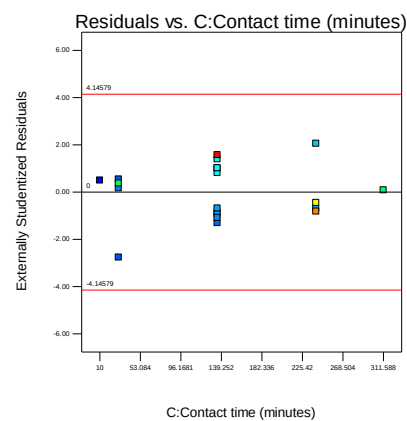
4

1

Design-Expert® Software
ASD-P1
Color points by value of
ASD-P1:
6.27
1.46

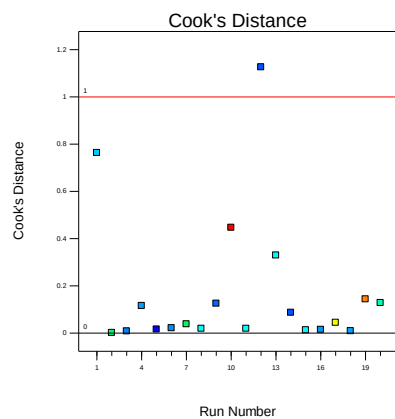


Design-Expert® Software
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Color points by value of
ASD-P1:
6.27
1.46

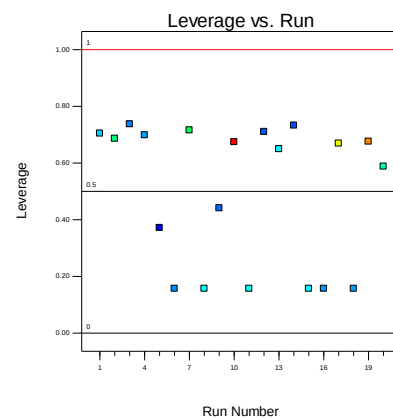


2

Design-Expert® Software
ASD-P1
Color points by value of
ASD-P1:
6.27
1.46

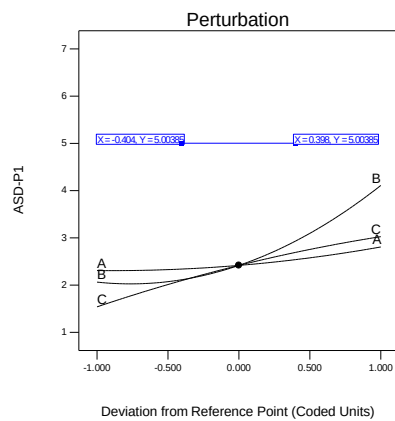


Design-Expert® Software
ASD-P1
Color points by value of
ASD-P1:
6.27
1.46



3

Design-Expert® Software
Factor Coding: Actual
ASD-P1
Actual Factors
A: Adsorbent dosage = 30
B: Initial concentration = 175
C: Contact time = 135



4

5

Appendix A7: In vitro Release Profiles of ASD-P1

Linearity:

$$y = 16.503x - 1.1408$$

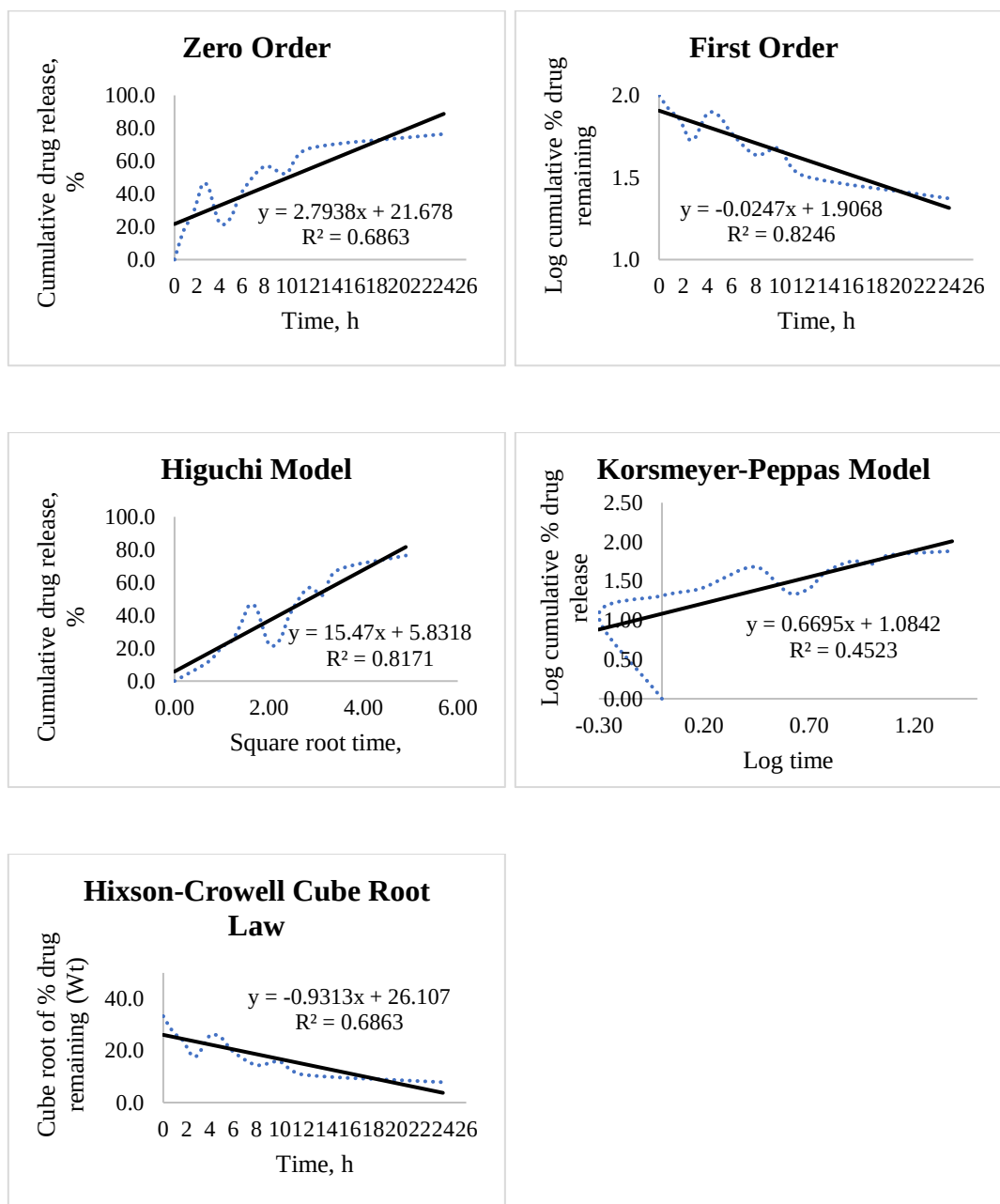
$$R^2 = 0.9865$$

Loading capacity = 4.97 mg/g

Appendix A7.1: Calculated data on the release profile of MIP in medium pH 2.2

Time (h)	Square root time	Log time	Abs.	Conc., mg/L	Amount drug release, mg/g	Drug release, %	Cumulative drug release, %	Drug remaining %	Log cumulative % drug remaining	Log cumulative % drug released	Cube root of % drug remaining (Wt)	Wo-Wt
0	0.00	0.00	0.000	0.00	0.00	0.00	0.00	100.00	2.00	0.00	33.33	0.00
0.5	0.71	-0.30	0.104	0.58	0.01	11.58	11.58	88.42	1.95	1.06	29.47	3.86
1	1.00	0.00	0.136	1.10	0.02	18.87	20.61	79.39	1.90	1.31	26.46	6.87
1.5	1.22	0.18	0.147	1.29	0.02	21.98	25.07	74.93	1.87	1.40	24.98	8.36
2	1.41	0.30	0.181	1.85	0.03	31.58	35.34	64.66	1.81	1.55	21.55	11.78
2.5	1.58	0.40	0.214	2.39	0.04	40.89	46.19	53.81	1.73	1.66	17.94	15.40
3	1.73	0.48	0.203	2.21	0.04	37.78	44.71	55.29	1.74	1.65	18.43	14.90
4	2.00	0.60	0.125	0.92	0.02	15.77	22.48	77.52	1.89	1.35	25.84	7.49
5	2.24	0.70	0.147	1.29	0.02	21.98	25.35	74.65	1.87	1.40	24.88	8.45
6	2.45	0.78	0.125	0.92	0.02	15.77	41.02	58.98	1.77	1.61	19.66	13.67
8	2.83	0.90	0.248	2.95	0.05	50.49	56.64	43.36	1.64	1.75	14.45	18.88
10	3.16	1.00	0.225	2.57	0.04	43.99	52.49	47.51	1.68	1.72	15.84	17.50
12	3.46	1.08	0.281	3.50	0.06	59.80	67.67	32.33	1.51	1.83	10.78	22.56
24	4.90	1.38	0.304	3.88	0.07	66.29	76.44	23.56	1.37	1.88	7.85	25.48

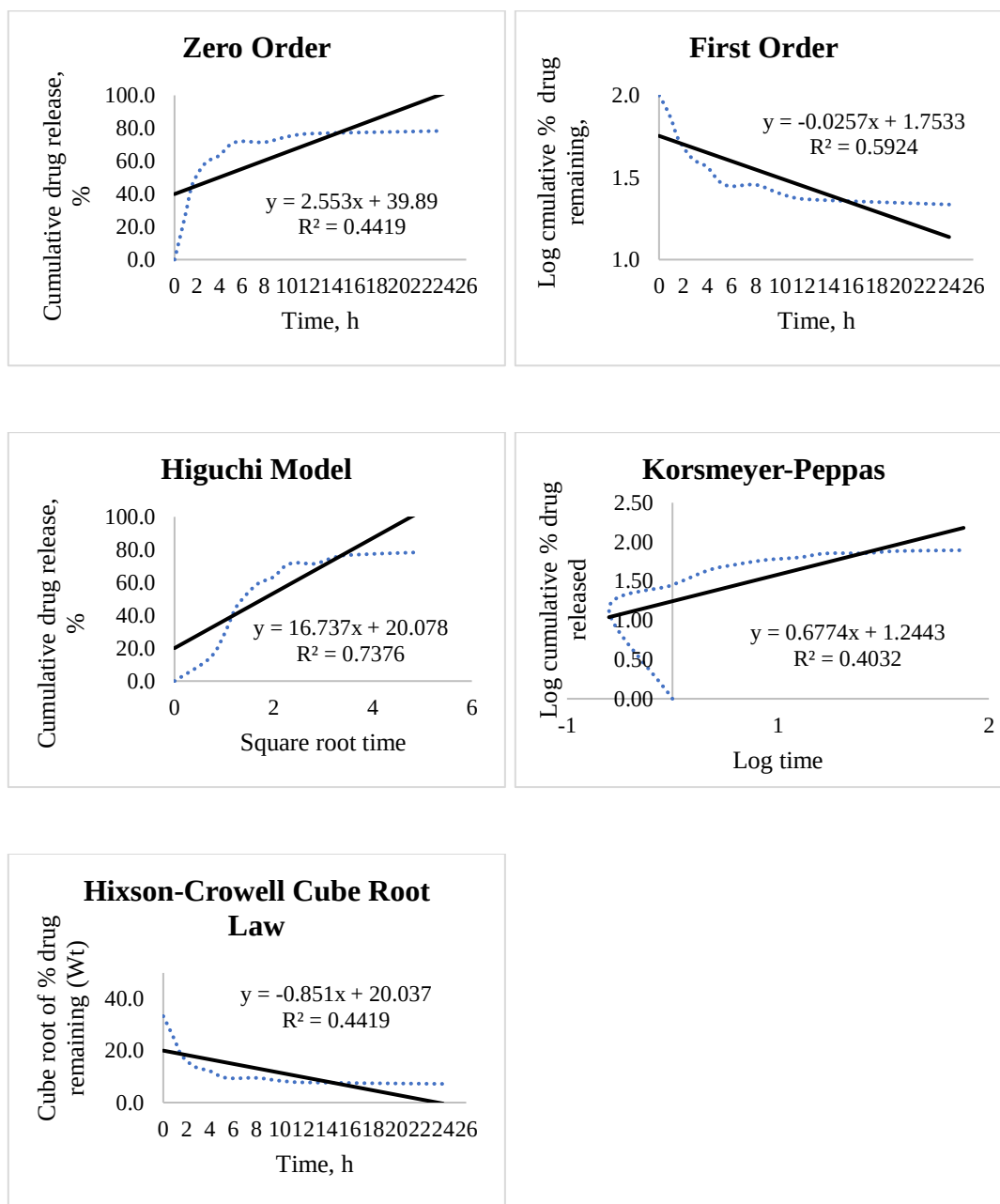
Appendix A7.1.1: Release Kinetics Modelling Graphs for pH 2.



Appendix A7.2: Calculated data on the release profile of MIP in medium pH 7

Time (h)	Square root time	Log time	Abs.	Conc., mg/L	Amount drug release, mg/g	Drug release, %	Cumulative drug release, %	Drug remaining %	Log cumulative % drug remaining	Log cumulative % drug released	Cube root of % drug remaining (Wt)	Wo-Wt
0	0	0	0.000	0.00	0.00	0.00	0.00	100.00	2.00	0.00	33.33	0.00
0.5	0.71	0.00	0.111	0.69	0.01	13.90	13.90	86.10	1.93	1.14	28.70	4.63
1	1.00	0.00	0.162	1.53	0.03	26.21	28.30	71.70	1.86	1.45	23.90	9.43
1.5	1.22	0.18	0.210	2.32	0.04	39.76	44.01	55.99	1.75	1.64	18.66	14.67
2	1.41	0.30	0.228	2.62	0.04	44.84	51.44	48.56	1.69	1.71	16.19	17.15
2.5	1.58	0.40	0.243	2.87	0.05	49.07	56.79	43.21	1.64	1.75	14.40	18.93
3	1.73	0.48	0.252	3.02	0.05	51.61	60.13	39.87	1.60	1.78	13.29	20.04
4	2.00	0.60	0.262	3.18	0.05	54.44	63.46	36.54	1.56	1.80	12.18	21.15
5	2.24	0.70	0.284	3.55	0.06	60.65	70.17	29.83	1.47	1.85	9.94	23.39
6	2.45	0.78	0.287	3.60	0.06	61.49	72.02	27.98	1.45	1.86	9.33	24.01
8	2.83	0.90	0.284	3.55	0.06	60.65	71.45	28.55	1.46	1.85	9.52	23.82
10	3.16	1.00	0.296	3.74	0.06	64.03	74.75	25.25	1.40	1.87	8.42	24.92
12	3.46	1.08	0.301	3.83	0.07	65.44	76.66	23.34	1.37	1.88	7.78	25.55
24	4.90	1.38	0.306	3.91	0.07	66.86	78.35	21.65	1.34	1.89	7.22	26.12

Appendix A7.2.1: Release Kinetics Modelling Graphs for pH 7.



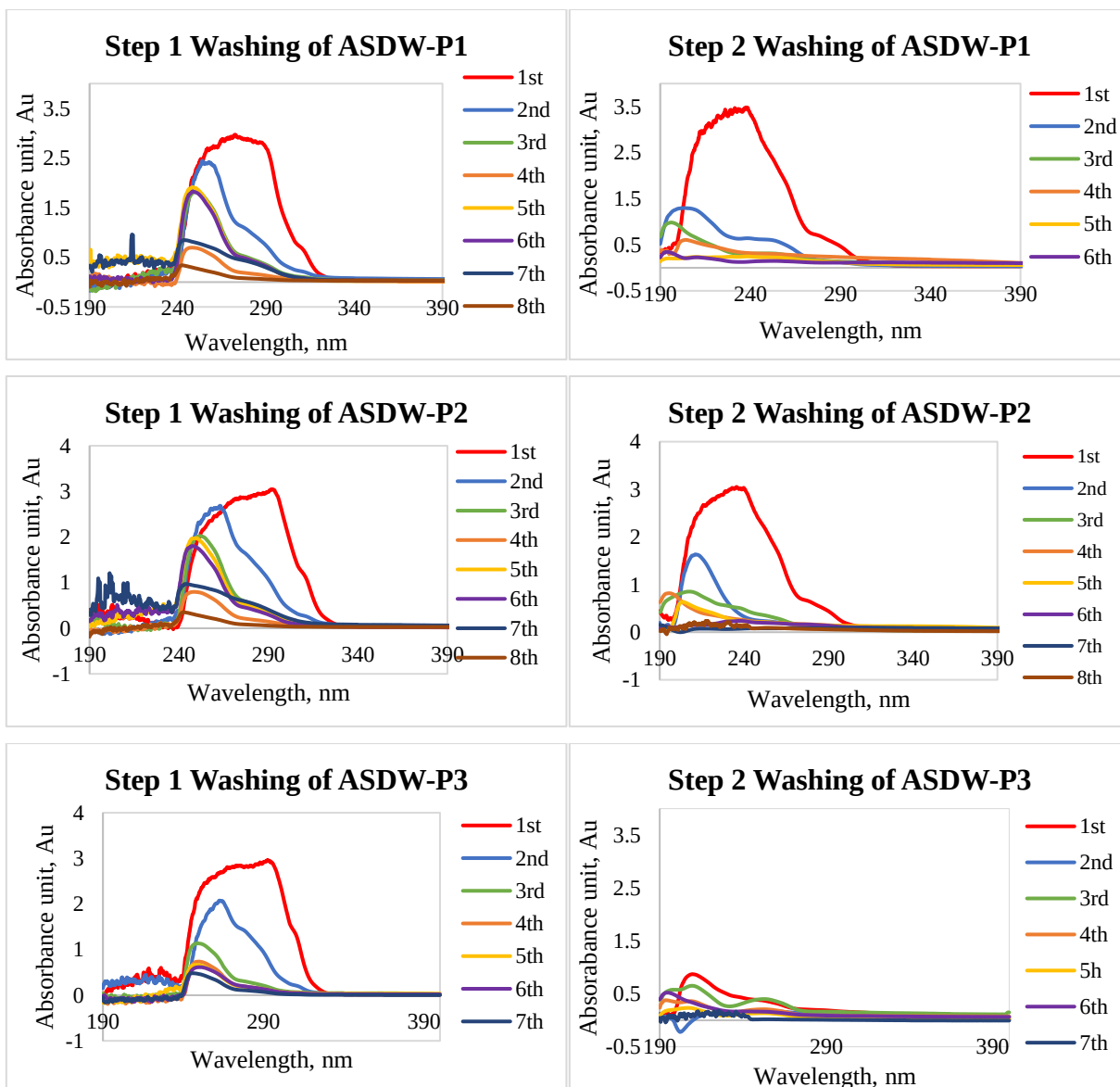
Appendix B: Microwave-assisted Synthesis of MIP using Aspirin (ASDW) as Template for Drug Delivery Application

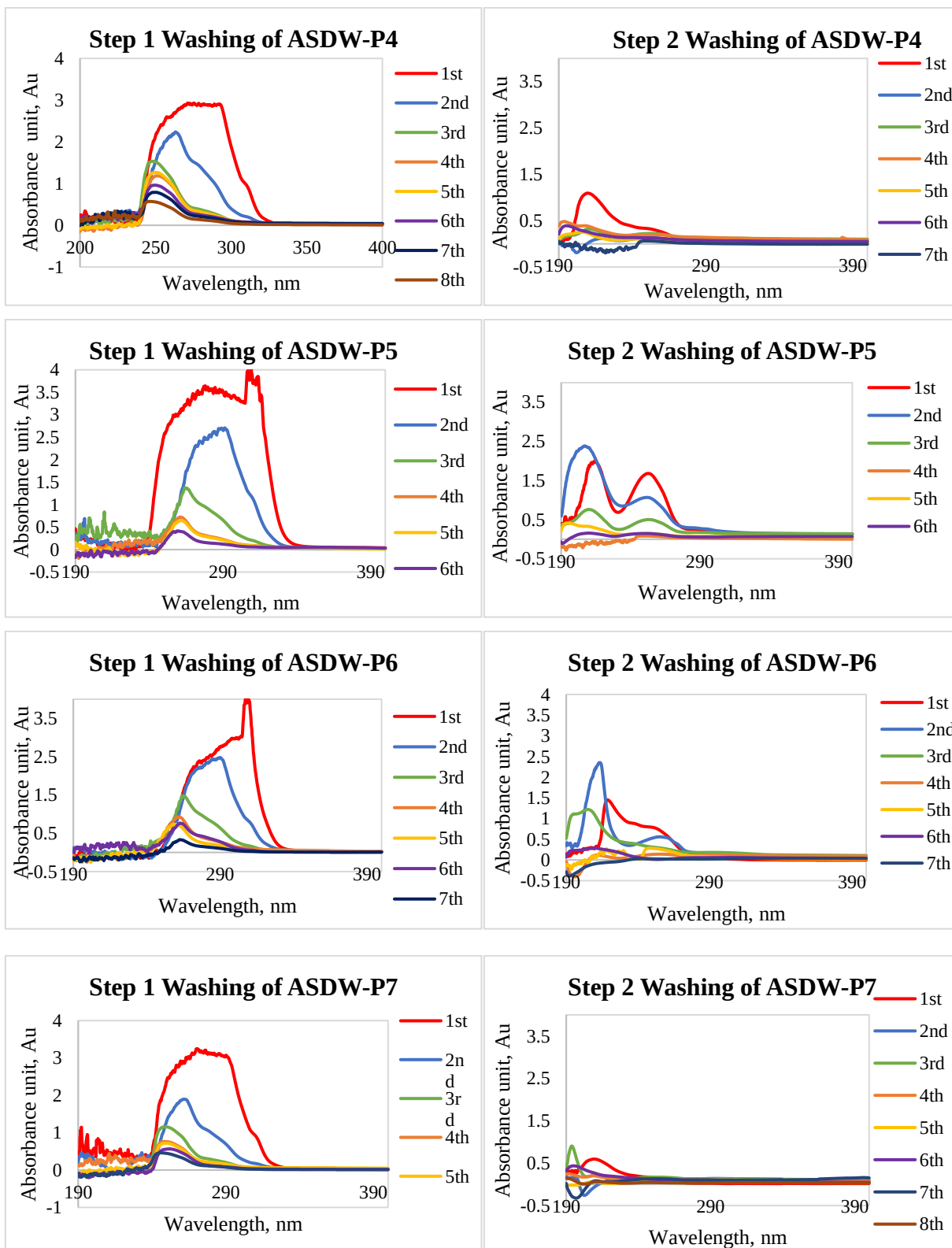
Appendix B1: Polymer Yield

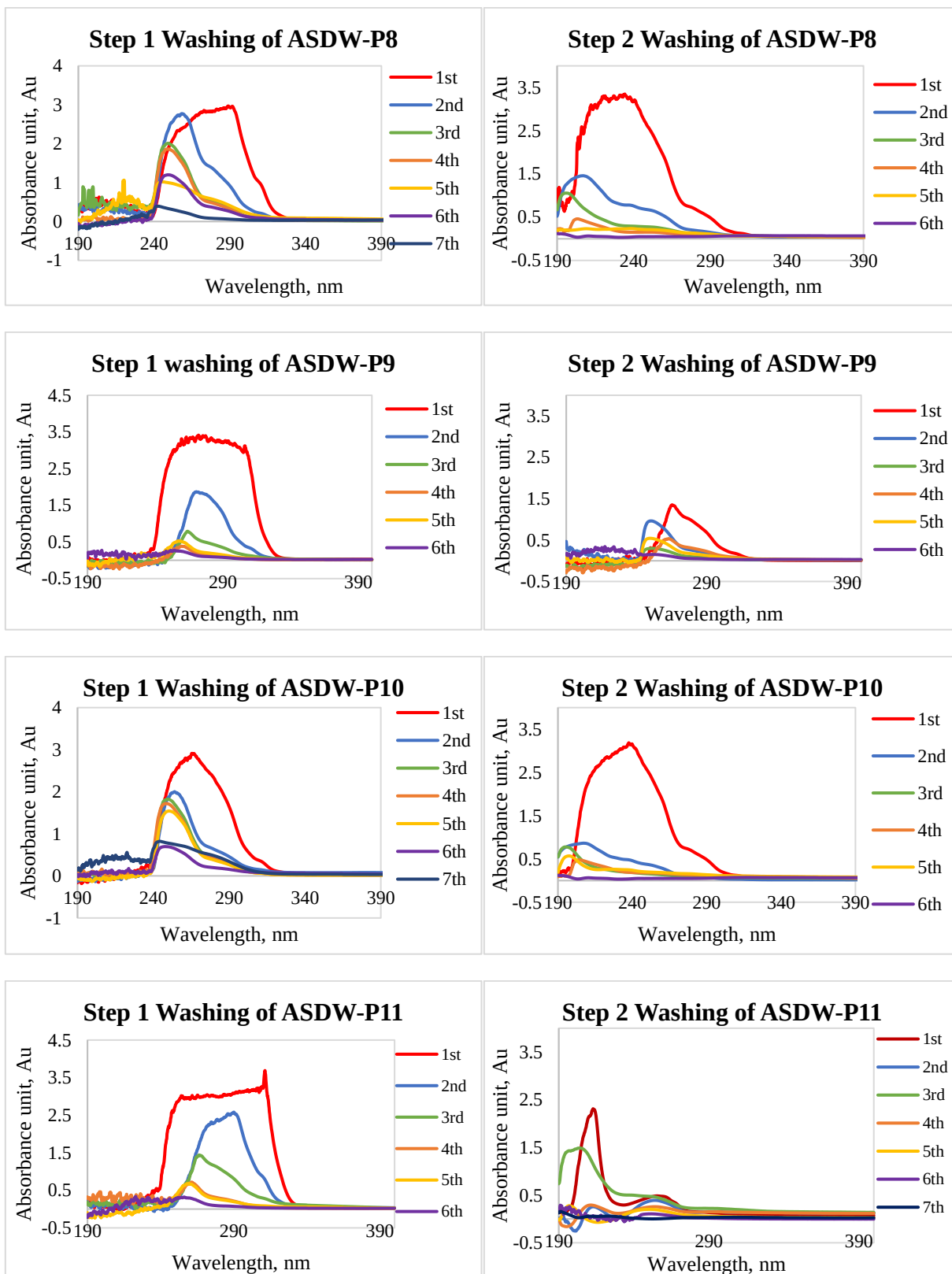
Polymer ID	Factors		Yield (g)	
	Monomer (mmol)	Crosslinker (mmol)	ASDW	NWP
P1	4	16	0.5312	0.704
P2	10	16	0.7560	0.892
P3	4	20	0.6220	0.750
P4	10	20	0.8940	0.815
P5	2.76	18	0.6878	0.630
P6	11.24	18	0.9862	0.995
P7	7	15.17	0.5270	0.592
P8	7	20.83	0.9258	0.979
P9	7	18	0.8639	0.627
P10	7	18	0.8413	0.683
P11	7	18	0.8713	0.629
P12	7	18	0.8335	0.684
P13	7	18	0.8534	0.644

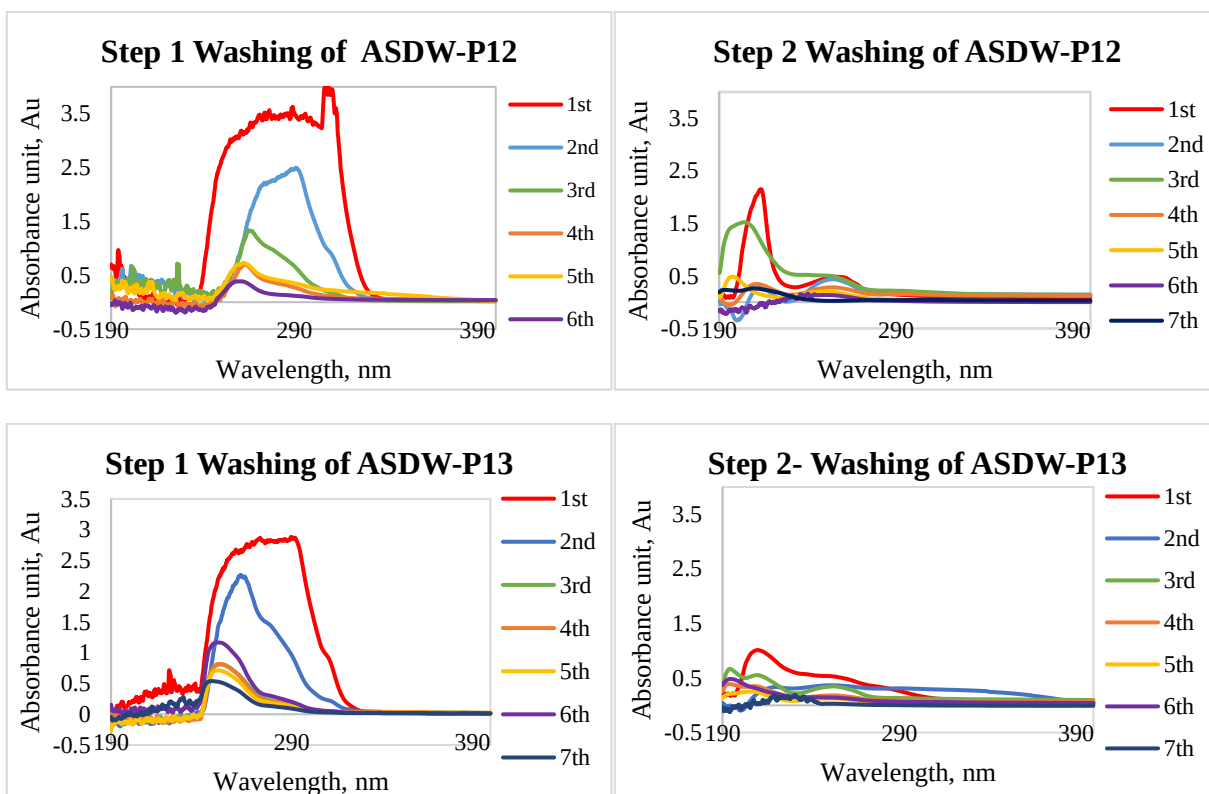
Appendix B2: Template Removal: UV-Vis Graph Monitoring

Appendix B2.1: Template Removal (Washing) Monitoring of MIP

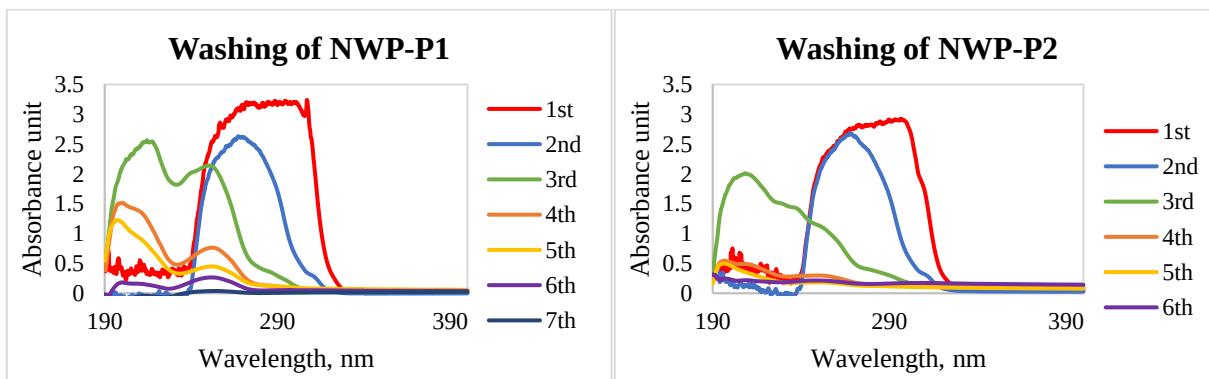


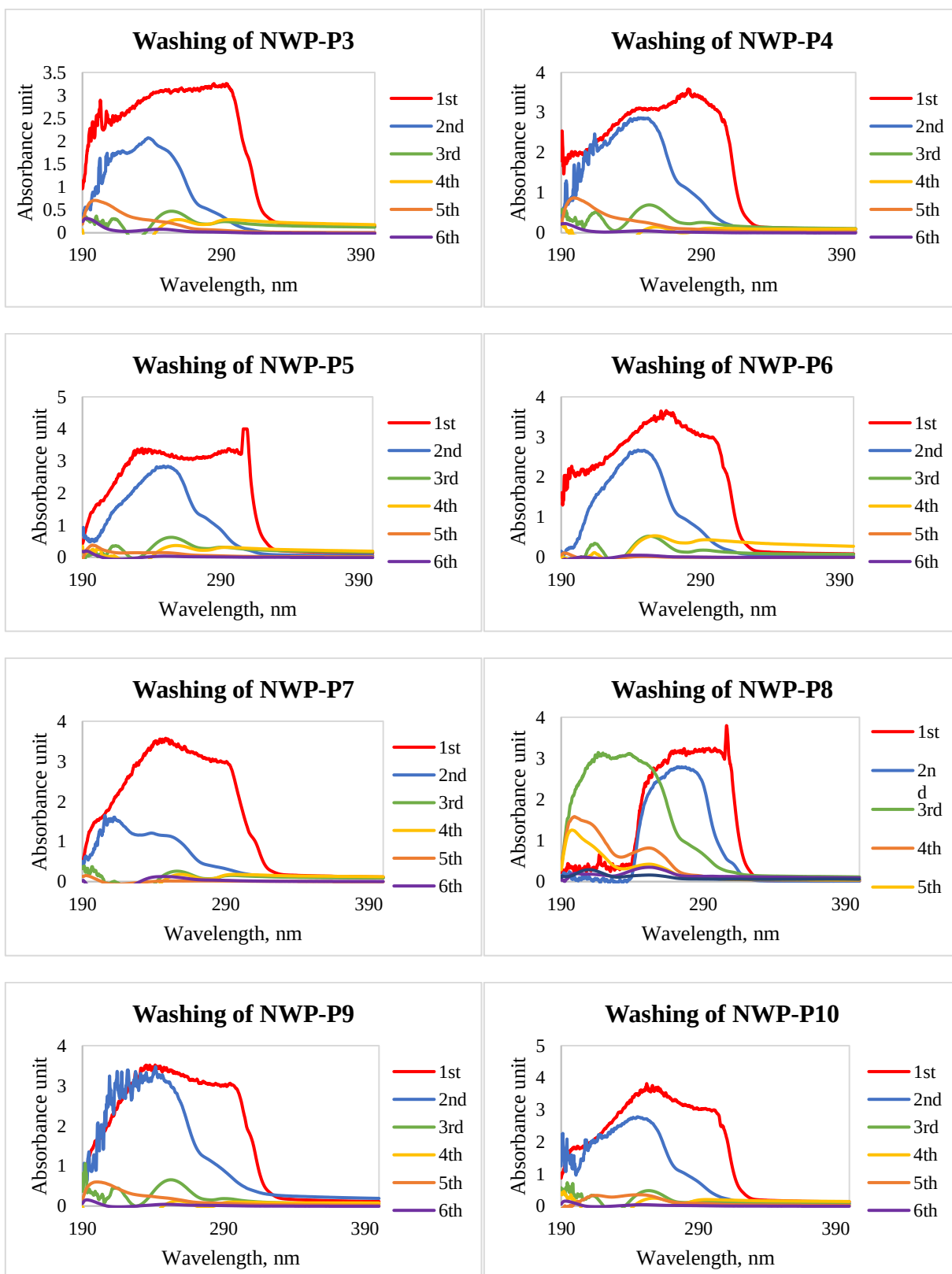


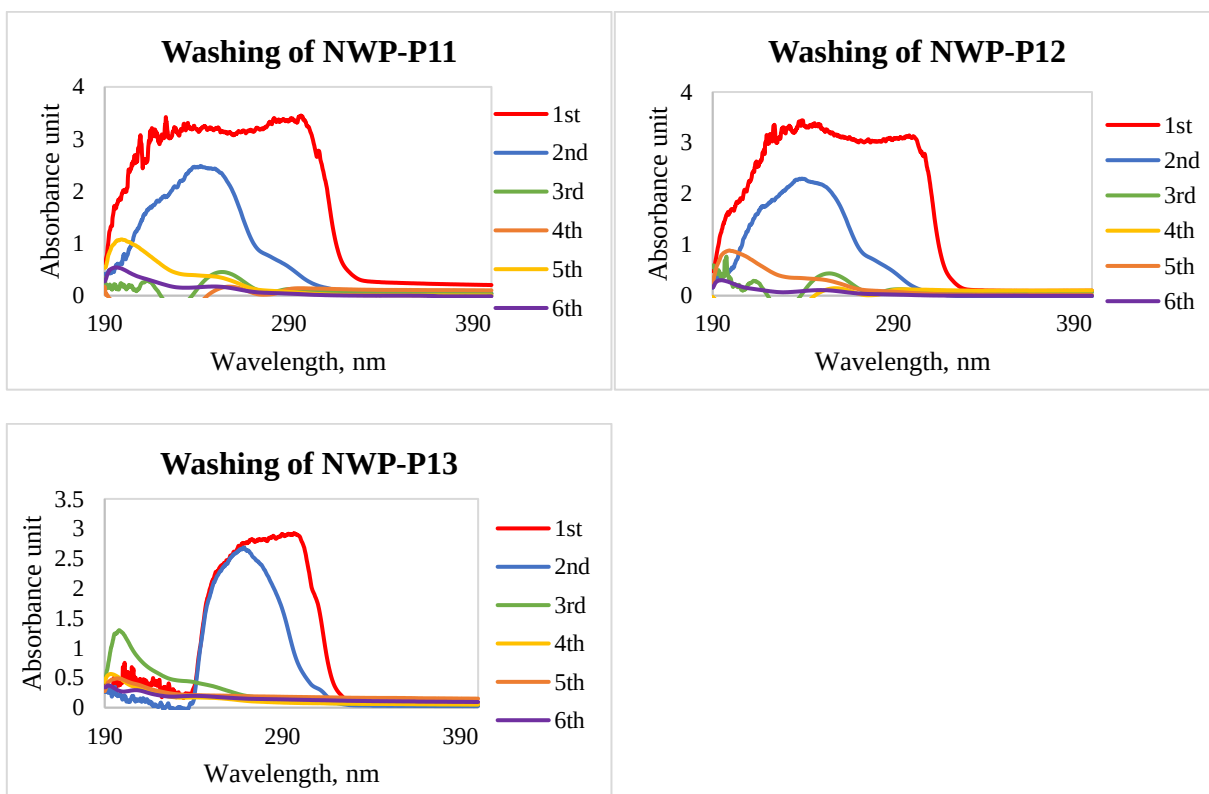




Appendix B2.2: Template Removal (Washing) Monitoring of NIP







Appendix B3: Particle size data

Polymer ID	Length, μm							
	ASDW				NWP			
	Ave.	std.	Min	Max	Ave.	std.	Min	Max
P1	0.50	0.10	0.22	0.74	1.95	0.34	0.90	2.93
P2	0.85	0.22	0.49	1.44	1.55	0.52	0.79	2.95
P3	1.15	0.35	0.60	2.35	0.87	0.30	0.41	1.53
P4	0.94	0.36	0.54	1.55	1.21	0.42	0.60	2.38
P5	1.11	0.25	0.42	1.86	1.22	0.34	0.52	1.92
P6	0.83	0.32	0.40	1.50	1.10	0.38	0.57	1.95
P7	1.45	0.35	0.93	2.38	1.37	0.40	0.66	2.75
P8	1.17	0.15	0.51	2.12	1.40	0.51	0.68	2.67
P9	0.56	0.29	0.30	0.87	1.12	0.31	0.68	1.80
P10	1.02	0.41	0.37	1.57	1.15	0.26	0.66	1.71
P11	1.28	0.24	0.66	2.75	1.08	0.30	0.65	1.82
P12	1.02	0.18	0.52	1.61	1.61	0.34	0.88	2.45
P13	0.59	0.35	0.26	1.07	1.21	0.37	0.57	1.96

Appendix B4: Preliminary Rebinding Data of ASDW

Polymer ID	Calibration data		Absorbance unit			Final Concentration (mg/L)					Rebinding capacity (mg/g)				
	Equation	R ²	T1	T2	T3	C1	C2	C3	Mean	s.d	Q1	Q2	Q3	Mean	s.d
ASDW-P1	$y = 144.83x + 2.2294$	0.9982	0.48	0.48	0.48	71.66	71.51	71.02	71.59	0.10	7.09	7.12	7.24	7.15	0.08
ASDW-P2	$y = 144.83x + 2.2294$	0.9982	0.50	0.52	0.52	74.80	77.55	77.25	76.18	1.95	6.30	5.61	5.69	5.87	0.38
ASDW-P3	$y = 144.83x + 2.2294$	0.9982	0.61	0.58	0.59	89.86	86.24	88.26	88.05	2.56	2.53	3.44	2.94	2.97	0.45
ASDW-P4	$y = 144.83x + 2.2294$	0.9982	0.55	0.55	0.56	82.48	82.27	83.20	82.37	0.15	4.38	4.43	4.20	4.34	0.12
ASDW-P5	$y = 144.83x + 2.2294$	0.9982	0.50	0.49	0.49	73.93	73.10	73.65	73.52	0.59	6.52	6.73	6.59	6.61	0.11
ASDW-P6	$y = 144.83x + 2.2294$	0.9982	0.49	0.50	0.50	73.50	74.08	73.98	73.79	0.41	6.63	6.48	6.51	6.54	0.08
ASDW-P7	$y = 144.83x + 2.2294$	0.9982	0.59	0.59	0.59	87.35	87.16	87.40	87.25	0.14	3.16	3.21	3.15	3.17	0.03
ASDW-P8	$y = 144.83x + 2.2294$	0.9982	0.58	0.59	0.59	86.53	87.50	87.30	87.02	0.68	3.37	3.13	3.17	3.22	0.13
ASDW-P9	$y = 144.83x + 2.2294$	0.9982	0.53	0.53	0.53	79.29	78.80	79.19	79.05	0.35	5.18	5.30	5.20	5.23	0.07
ASDW-P10	$y = 144.83x + 2.2294$	0.9982	0.56	0.55	0.56	83.78	81.63	83.15	82.70	1.53	4.05	4.59	4.21	4.29	0.28
ASDW-P11	$y = 144.83x + 2.2294$	0.9982	0.50	0.53	0.52	74.86	78.92	76.86	76.89	2.87	6.29	5.27	5.78	5.78	0.51
ASDW-P12	$y = 144.83x + 2.2294$	0.9982	0.55	0.57	0.56	81.38	84.62	82.81	83.00	2.29	4.66	3.84	4.30	4.27	0.41
ASDW-P13	$y = 144.83x + 2.2294$	0.9982	0.56	0.56	0.56	83.52	83.68	83.44	83.60	0.11	4.12	4.08	4.14	4.11	0.03

s.d, standard deviation

Appendix B4.1: Design Expert Results Summary for the Response of Rebinding Capacity of ASDW

Appendix B4.1.1: Fit Summary

Sequential Model Sum of Squares [Type I]

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Mean vs Total	<u>310.66</u>	<u>1</u>	<u>310.66</u>			<u>Suggested</u>
Linear vs Mean	3.98	2	1.99	1.01	0.4001	
2FI vs Linear	1.76	1	1.76	0.88	0.3734	
<u>Quadratic vs 2FI</u>	<u>11.61</u>	<u>2</u>	<u>5.81</u>	<u>6.35</u>	<u>0.0267</u>	<u>Suggested</u>
Cubic vs Quadratic	4.18	2	2.09	4.71	0.0708	Aliased
Residual	2.22	5	0.44			
Total	334.41	13	25.72			

Lack of Fit Tests

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Linear	17.63	6	2.94	5.49	0.0607	
2FI	15.87	5	3.17	5.93	0.0547	
<u>Quadratic</u>	<u>4.26</u>	<u>3</u>	<u>1.42</u>	<u>2.65</u>	<u>0.1848</u>	<u>Suggested</u>
Cubic	0.078	1	0.078	0.15	0.7221	Aliased
Pure Error	2.14	4	0.54			

Model Summary Statistics

Source	Std. Dev.	R-Squared	Adjusted R-Squared	Predicted R-Squared	PRESS	
Linear	1.41	0.1674	0.0009	-0.7395	41.31	
2FI	1.41	0.2413	-0.0115	-0.8953	45.01	
<u>Quadratic</u>	<u>0.96</u>	<u>0.7304</u>	<u>0.5379</u>	<u>-0.4166</u>	<u>33.64</u>	<u>Suggested</u>
Cubic	0.67	0.9065	0.7756	0.6488	8.34	Aliased

Appendix B4.1.2: ANOVA for Response Surface Reduced Cubic Model

Analysis of variance table [Partial sum of squares - Type III]

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	21.52	6	3.59	9.68	0.0071	significant
<i>A-Monomer</i>	<i>1.011E-005</i>	<i>1</i>	<i>1.011E-005</i>	<i>2.728E-005</i>	<i>0.9960</i>	
<i>B-Cross-linker</i>	<i>1.250E-003</i>	<i>1</i>	<i>1.250E-003</i>	<i>3.372E-003</i>	<i>0.9556</i>	
<i>AB</i>	<i>1.76</i>	<i>1</i>	<i>1.76</i>	<i>4.74</i>	<i>0.0724</i>	
<i>A²</i>	<i>6.53</i>	<i>1</i>	<i>6.53</i>	<i>17.61</i>	<i>0.0057</i>	
<i>B²</i>	<i>3.62</i>	<i>1</i>	<i>3.62</i>	<i>9.76</i>	<i>0.0205</i>	
<i>A²B</i>	<i>4.18</i>	<i>1</i>	<i>4.18</i>	<i>11.27</i>	<i>0.0153</i>	
Residual	2.22	6	0.37			
<i>Lack of Fit</i>	<i>0.082</i>	<i>2</i>	<i>0.041</i>	<i>0.077</i>	<i>0.9272</i>	not significant
<i>Pure Error</i>	<i>2.14</i>	<i>4</i>	<i>0.54</i>			
Cor Total	23.75	12				
Model	21.52	6	3.59	9.68	0.0071	

The Model F-value of 9.68 implies there is a 0.71% chance that an F-value this large could occur due to noise. "Values of "Prob > F" less than 0.0500 indicate model terms are significant. " In this case A^2 , B^2 , A^2B are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

The "Lack of Fit F-value" of 0.08 implies the Lack of Fit is not significant relative to the pure error. There is a 92.72% chance that a "Lack of Fit F-value" this large could occur due to noise. Non-significant lack of fit is good -- we want the model to fit.

Std. Dev.	0.61	R-Squared	0.9063
Mean	4.89	Adj R-Squared	0.8126
C.V. %	12.46	Pred R-Squared	0.7722
PRESS	5.41	Adeq Precision	9.356
-2 Log Likelihood	13.94	BIC	31.90
		AICc	50.34

A negative "Pred R-Squared" implies that the overall mean may be a better predictor of your response than the current model. "Adeq Precision" measures the signal to noise ratio. A ratio

greater than 4 is desirable. Your ratio of 6.740 indicates an adequate signal. This model can be used to navigate the design space.

Factor	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	4.74	1	0.27	4.07	5.40	
A-Monomer	-1.124E-003	1	0.22	-0.53	0.53	1.00
B-Cross-linker	0.018	1	0.30	-0.73	0.76	2.00
AB	0.66	1	0.30	-0.082	1.41	1.00
A ²	0.97	1	0.23	0.40	1.53	1.02
B ²	-0.72	1	0.23	-1.29	-0.16	1.02
A ² B	-1.45	1	0.43	-2.50	-0.39	2.00

Final Equation in Terms of Coded Factors:

$$\begin{aligned} \text{Rebinding capacity of ASDW} = & +4.74 \\ & -1.124\text{E-}003 * A \\ & +0.018 * B \\ & +0.66 * AB \\ & +0.97 * A^2 \\ & -0.72 * B^2 \\ & -1.45 * A^2B \end{aligned}$$

The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels of the factors are coded as -1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.

Final Equation in Terms of Actual Factors:

$$\begin{aligned} \text{Rebinding capacity of ASDW} = & +36.16959 \\ & -23.72750 * \text{Monomer} \\ & +1.79195 * \text{Cross-linker} \\ & +1.23444 * \text{Monomer} * \text{Cross-linker} \\ & +1.55283 * \text{Monomer}^2 \\ & -0.18028 * \text{Cross-linker}^2 \\ & -0.080288 * \text{Monomer}^2 * \text{Cross-linker} \end{aligned}$$

The equation in terms of actual factors can be used to make predictions about the response for given levels of each factor. Here, the levels should be specified in the original units for each factor. This equation should not be used to determine the relative impact of each factor because the coefficients are scaled to accommodate the units of each factor and the intercept is not at the center of the design space.

Appendix B4.1.3: Diagnostic of Reduced Cubic Model in Design Expert

Diagnostics Case Statistics

Run Order	Actual Value	Predicted Value	Residual	Leverage	Internally Studentized Residual	Externally Studentized Residual	Cook's Distance	Influence on Fitted Value DFFITS	Standard Order
6	7.15	7.07	0.075	0.750	0.247	0.226	0.026	0.392	1
3	5.87	5.75	0.12	0.750	0.402	0.372	0.069	0.644	2
8	2.97	2.89	0.075	0.750	0.247	0.226	0.026	0.392	3
4	4.34	4.22	0.12	0.750	0.402	0.372	0.069	0.644	4
13	6.61	6.68	-0.065	0.625	-0.175	-0.160	0.007	-0.207	5
12	6.54	6.67	-0.13	0.625	-0.354	-0.327	0.030	-0.422	6
9	3.17	3.27	-0.099	0.875	-0.459	-0.426	0.210	-1.128	7
11	3.22	3.32	-0.099	0.875	-0.459	-0.426	0.210	-1.128	8
5	5.23	4.74	0.49	0.200	0.907	0.891	0.029	0.446	9
10	4.29	4.74	-0.45	0.200	-0.819	-0.793	0.024	-0.397	10
1	5.78	4.74	1.04	0.200	1.917	2.811	0.131	1.406	11
2	4.27	4.74	-0.47	0.200	-0.856	-0.834	0.026	-0.417	12
7	4.11	4.74	-0.63	0.200	-1.149	-1.188	0.047	-0.594	13

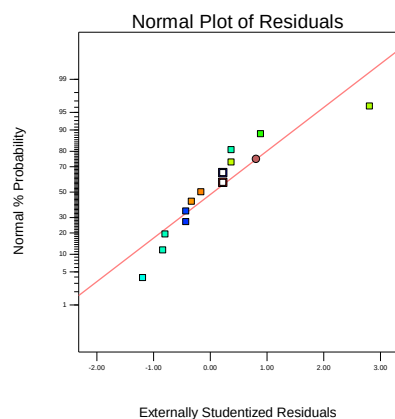
^1 Exceeds limits.

Box-Cox Power Transformation

Constant k	95% CI Low	95% CI High	Best Lambda	Rec. Transform
0.000	-3.29	4.07	0.39	None

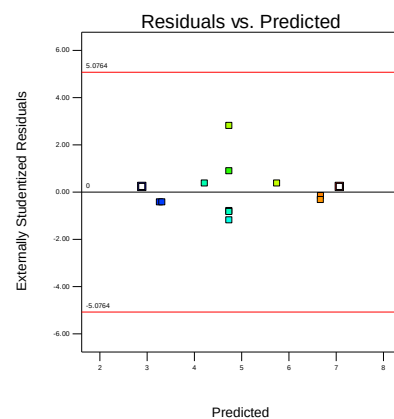
Design-Expert® Software
ASDW-MIP2

Color points by value of
ASDW-MIP2:



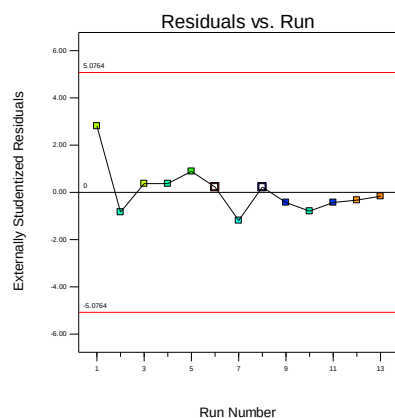
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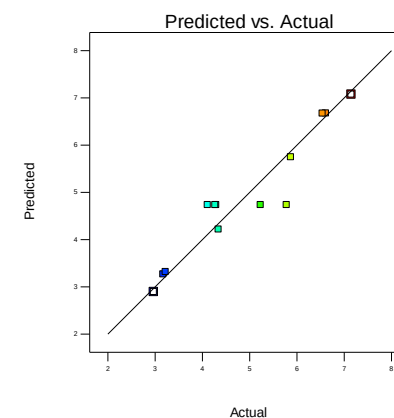
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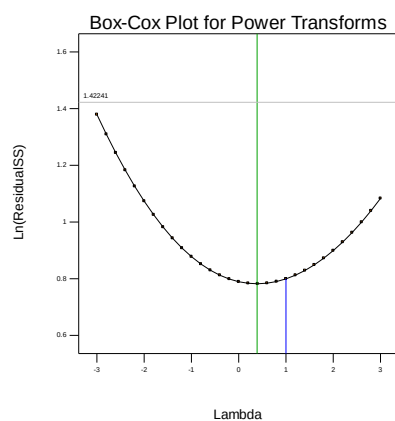
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ASDW-MIP2

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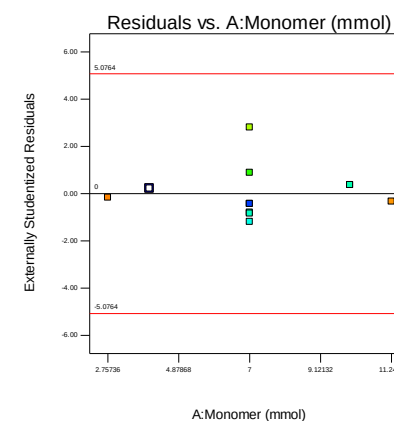
Design-Expert® Software
ASDW-MIP2

Lambda
Current = 1
Best = 0.39
Low C.I. = -3.29
High C.I. = 4.07
Recommend transform:
None
(Lambda = 1)



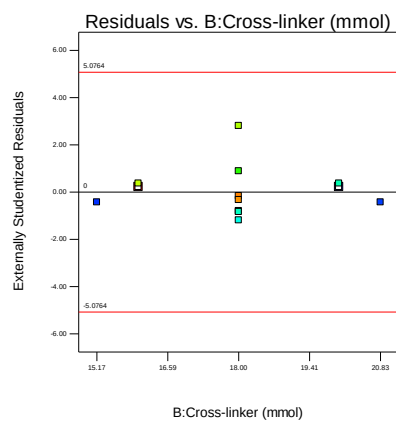
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ASDW-MIP2

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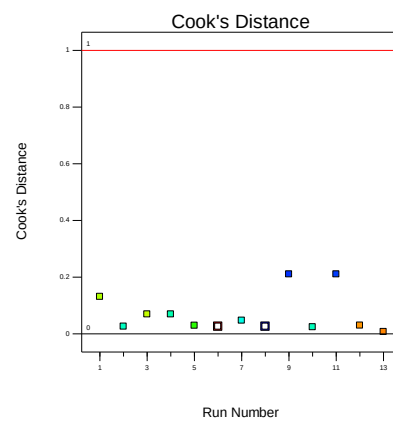
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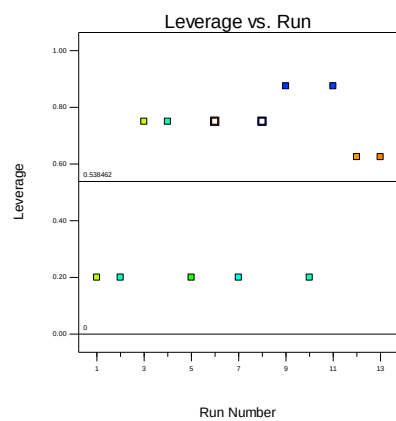
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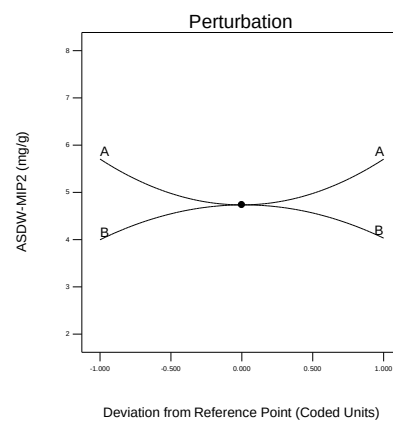
Design-Expert® Software
ASDW-MIP2

Color points by value of
ASDW-MIP2:



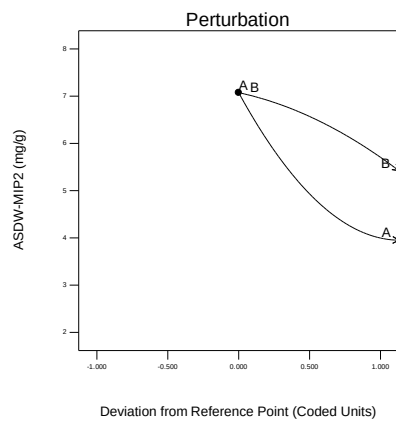
Design-Expert® Software
Factor Coding: Actual
ASDW-MIP2 (mg/g)

Actual Factors
A: Monomer = 7.00
B: Cross-linker = 18.00



Design-Expert® Software
Factor Coding: Actual
ASDW-MIP2 (mg/g)

Actual Factors
A: Monomer = 4.00
B: Cross-linker = 16.00



Appendix B5: Preliminary Rebinding Study Data of NWP

Polymer ID	Calibration data		Absorbance unit			Final Concentration (mg/L)					Rebinding capacity (mg/g)				
	Equation	R ²	T1	T2	T3	C1	C2	C3	Mean	s.d	Q1	Q2	Q3	Mean	std.
NWP-P1	$y = 150.96x - 5.2791$	0.9957	0.64	0.64	0.65	90.88	90.58	92.71	91.39	1.15	2.28	2.35	1.82	2.15	0.29
NWP-P2	$y = 150.96x - 5.2791$	0.9957	0.62	0.62	0.62	88.25	88.07	88.60	88.31	0.27	2.94	2.98	2.85	2.92	0.07
NWP-P3	$y = 150.96x - 5.2791$	0.9957	0.62	0.62	0.62	88.42	88.70	88.50	88.54	0.15	2.90	2.83	2.87	2.87	0.04
NWP-P4	$y = 150.96x - 5.2791$	0.9957	0.65	0.65	0.64	92.19	92.10	91.14	91.81	0.58	1.95	1.98	2.22	2.05	0.15
NWP-P5	$y = 150.96x - 5.2791$	0.9957	0.66	0.65	0.66	93.75	93.55	94.01	93.77	0.23	1.56	1.61	1.50	1.56	0.06
NWP-P6	$y = 150.96x - 5.2791$	0.9957	0.62	0.61	0.62	88.05	87.14	88.51	87.90	0.70	2.99	3.21	2.87	3.02	0.17
NWP-P7	$y = 150.96x - 5.2791$	0.9957	0.61	0.62	0.62	87.14	87.65	88.09	87.63	0.47	3.22	3.09	2.98	3.09	0.12
NWP-P8	$y = 150.96x - 5.2791$	0.9957	0.62	0.63	0.62	88.55	89.25	87.95	88.58	0.65	2.86	2.69	3.01	2.85	0.16
NWP-P9	$y = 150.96x - 5.2791$	0.9957	0.64	0.65	0.64	91.93	92.50	91.59	92.01	0.46	2.02	1.87	2.10	2.00	0.12
NWP-P10	$y = 150.96x - 5.2791$	0.9957	0.64	0.65	0.65	92.05	92.50	92.50	92.35	0.26	1.99	1.88	1.88	1.91	0.06
NWP-P11	$y = 150.96x - 5.2791$	0.9957	0.65	0.65	0.64	92.49	92.97	92.06	92.51	0.46	1.88	1.76	1.98	1.87	0.11
NWP-P12	$y = 150.96x - 5.2791$	0.9957	0.65	0.65	0.65	92.18	92.50	92.45	92.38	0.17	1.95	1.88	1.89	1.91	0.04
NWP-P13	$y = 150.96x - 5.2791$	0.9957	0.65	0.65	0.65	92.50	92.83	92.41	92.58	0.22	1.87	1.79	1.90	1.86	0.05

Std, standard order; s.d, standard deviation

Appendix B6: Optimisation on Rebinding Capacity of ASDW-P1 Using RSM

Appendix B6.1: Rebinding Capacity Data

Std.	Run	Calibration data		Absorbance unit	Concentration (mg/L)			Dosage (g)	Rebinding capacity (mg/g)
		Equation	R ²		Initial	Final	Ci - Cf		
1	B14	$y = 152.93x - 2.0065$	0.9984	0.299	50	43.72	6.28	0.02	3.14
2	B3	$y = 152.93x - 2.0065$	0.9984	0.248	50	35.92	14.08	0.04	3.52
3	B12	$y = 152.93x - 2.0065$	0.9984	1.924	300	292.23	7.77	0.02	3.88
4	B7	$y = 152.93x - 2.0065$	0.9984	1.847	300	280.46	19.54	0.04	4.89
5	B4	$y = 152.93x - 2.0065$	0.9984	0.268	50	38.98	11.02	0.02	5.51
6	B1	$y = 152.93x - 2.0065$	0.9984	0.159	50	22.31	27.69	0.04	6.92
7	B17	$y = 152.93x - 2.0065$	0.9984	1.864	300	283.06	16.94	0.02	8.47
8	B19	$y = 152.93x - 2.0065$	0.9984	1.724	300	261.64	38.36	0.04	9.59
9	B13	$y = 152.93x - 2.0065$	0.9984	1.119	175	169.12	5.88	0.01	4.46
10	B20	$y = 152.93x - 2.0065$	0.9984	0.908	175	136.85	38.15	0.05	8.15
11	B9	$y = 152.93x - 2.0065$	0.9984	0.022	10	1.36	8.64	0.03	2.88
12	B10	$y = 152.93x - 2.0065$	0.9984	2.35	385.22	357.38	27.85	0.03	9.28
13	B5	$y = 152.93x - 2.0065$	0.9984	1.107	175	167.29	7.71	0.03	2.57
14	B2	$y = 152.93x - 2.0065$	0.9984	0.998	175	150.62	24.38	0.03	8.13
15	B15	$y = 152.93x - 2.0065$	0.9984	1.069	175	161.48	13.52	0.03	4.51
16	B16	$y = 152.93x - 2.0065$	0.9984	1.085	175	163.92	11.08	0.03	3.69
17	B8	$y = 152.93x - 2.0065$	0.9984	1.068	175	161.32	13.68	0.03	4.56
18	B6	$y = 152.93x - 2.0065$	0.9984	1.086	175	164.08	10.92	0.03	3.64
19	B18	$y = 152.93x - 2.0065$	0.9984	1.083	175	163.62	11.38	0.03	3.79
20	B11	$y = 152.93x - 2.0065$	0.9984	1.063	175	160.56	14.44	0.03	4.81

Appendix B6.2: Design Expert Results Summary for the Response Rebinding Capacity of ASDW-P1

Appendix B6.2.1: Fit Summary

Sequential Model Sum of Squares [Type I]

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Mean vs Total	565.94	1	565.94			
Linear vs Mean	75.51	3	25.17	19.50	< 0.0001	
2FI vs Linear	1.73	3	0.58	0.40	0.7586	
<u>Quadratic vs 2FI</u>	<u>13.24</u>	<u>3</u>	<u>4.41</u>	<u>7.77</u>	<u>0.0057</u>	<u>Suggested</u>
Cubic vs Quadratic	4.35	5	0.87	3.26	0.1102	Aliased
Residual	1.33	5	0.27			
Total	662.10	20	33.11			

Lack of Fit Tests

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Linear	19.32	11	1.76	6.59	0.0248	
2FI	17.59	8	2.20	8.25	0.0163	
<u>Quadratic</u>	<u>4.35</u>	<u>5</u>	<u>0.87</u>	<u>3.26</u>	<u>0.1102</u>	<u>Suggested</u>
Cubic	0.000	0				Aliased
Pure Error	1.33	5	0.27			

Model Summary Statistics

Source	Std. Dev.	R-Squared	Adjusted R-Squared	Predicted R-Squared	PRESS	
Linear	1.14	0.7853	0.7450	0.6638	32.33	
2FI	1.21	0.8032	0.7124	0.4141	56.34	
<u>Quadratic</u>	<u>0.75</u>	<u>0.9409</u>	<u>0.8878</u>	<u>0.6065</u>	<u>37.84</u>	<u>Suggested</u>
Cubic	0.52	0.9861	0.9473		+	Aliased

+ Case(s) with leverage of 1.0000: PRESS statistic not defined.

Appendix B6.2.2: ANOVA for Response Surface Quadratic Model

Analysis of variance table [Partial sum of squares - Type III]

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	90.48	9	10.05	17.70	< 0.0001	significant
<i>A-Adsorbent dosage</i>	7.99	1	7.99	14.07	0.0038	
<i>B-Initial concentration</i>	20.18	1	20.18	35.54	0.0001	
<i>C-Contact time</i>	40.24	1	40.24	70.85	< 0.0001	
<i>AB</i>	0.014	1	0.014	0.025	0.8764	
<i>AC</i>	0.16	1	0.16	0.29	0.6045	
<i>BC</i>	1.55	1	1.55	2.73	0.1297	
<i>A²</i>	7.21	1	7.21	12.69	0.0052	
<i>B²</i>	5.84	1	5.84	10.28	0.0094	
<i>C²</i>	0.52	1	0.52	0.92	0.3611	
Residual	5.68	10	0.57			
<i>Lack of Fit</i>	4.35	5	0.87	3.26	0.1102	not significant
<i>Pure Error</i>	1.33	5	0.27			
Cor Total	96.16	19				

The Model F-value of 17.70 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise.

Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A, B, C, A², B² are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

The "Lack of Fit F-value" of 3.26 implies the Lack of Fit is not significant relative to the pure error. There is a 11.02% chance that a "Lack of Fit F-value" this large could occur due to noise. Non-significant lack of fit is good -- we want the model to fit.

Std. Dev.	0.75	R-Squared	0.9409
Mean	5.32	Adj R-Squared	0.8878
C.V. %	14.17	Pred R-Squared	0.6065
PRESS	37.84	Adeq Precision	15.304
-2 Log Likelihood	31.58	BIC	61.54
		AICc	76.03

The "Pred R-Squared" of 0.6065 is not as close to the "Adj R-Squared" of 0.8878 as one might normally expect; i.e. the difference is more than 0.2. This may indicate a large block effect or a possible problem with your model and/or data. Things to consider are model reduction, response transformation, outliers, etc. All empirical models should be tested by doing confirmation runs.

"Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 15.304 indicates an adequate signal. This model can be used to navigate the design space.

Factor	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	4.16	1	0.30	3.50	4.83	
A-Adsorbent dosage	0.77	1	0.21	0.31	1.23	1.00
B-Initial concentration	1.29	1	0.22	0.81	1.77	1.04
C-Contact time	1.87	1	0.22	1.38	2.37	1.07
AB	0.042	1	0.27	-0.55	0.64	1.00
AC	0.14	1	0.27	-0.45	0.74	1.00
BC	0.44	1	0.27	-0.15	1.03	1.00
A ²	0.72	1	0.20	0.27	1.18	1.01
B ²	0.74	1	0.23	0.23	1.25	1.04
C ²	0.23	1	0.24	-0.31	0.76	1.07

Final Equation in Terms of Coded Factors:

$$\begin{aligned}
 \text{Rebinding capacity of ASDW-P1} = & +4.16 \\
 & +0.77 * A \\
 & +1.29 * B \\
 & +1.87 * C \\
 & +0.042 * AB \\
 & +0.14 * AC \\
 & +0.44 * BC \\
 & +0.72 * A^2 \\
 & +0.74 * B^2 \\
 & +0.23 * C^2
 \end{aligned}$$

The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1 and the low

levels of the factors are coded as -1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.

Final Equation in Terms of Actual Factors:

$$\begin{aligned} \text{Rebinding capacity ASDW-} &= +7.48902 \\ \text{P1} & \\ &-0.38093 \quad * \text{ Adsorbent dosage} \\ &-0.011781 \quad * \text{ Initial concentration} \\ &+2.26527\text{E-} \quad * \text{ Contact time} \\ &003 \\ &+3.40000\text{E-} \quad * \text{ Adsorbent dosage} * \text{ Initial} \\ &005 \quad \text{concentration} \\ &+1.35714\text{E-} \quad * \text{ Adsorbent dosage} * \text{ Contact time} \\ &004 \\ &+3.35238\text{E-} \quad * \text{ Initial concentration} * \text{ Contact time} \\ &005 \\ &+7.23118\text{E-} \quad * \text{ Adsorbent dosage}^2 \\ &003 \\ &+4.73299\text{E-} \quad * \text{ Initial concentration}^2 \\ &005 \\ &+2.08323\text{E-} \quad * \text{ Contact time}^2 \\ &005 \end{aligned}$$

The equation in terms of actual factors can be used to make predictions about the response for given levels of each factor. Here, the levels should be specified in the original units for each factor. This equation should not be used to determine the relative impact of each factor because the coefficients are scaled to accommodate the units of each factor and the intercept is not at the center of the design space.

Appendix B6.2.3: Diagnostic of Quadratic Model in Design Expert

Diagnostics Case Statistics

Run Order	Actual Value	Predicted Value	Residual	Leverage	Internally Studentized Residual	Externally Studentized Residual	Cook's Distance	Influence on Fitted Value DFFITS	Standard Order
14	3.14	2.54	0.60	0.733	1.528	1.656	0.640	2.742 ¹	1
3	3.52	3.72	-0.20	0.737	-0.515	-0.495	0.074	-0.829	2
12	3.88	4.16	-0.28	0.710	-0.696	-0.677	0.119	-1.060	3
7	4.89	5.51	-0.62	0.716	-1.535	-1.665	0.594	-2.644 ¹	4
4	5.51	5.12	0.39	0.699	0.934	0.928	0.202	1.413	5
1	6.92	6.87	0.052	0.705	0.128	0.121	0.004	0.187	6
17	8.47	8.50	-0.031	0.669	-0.072	-0.068	0.001	-0.097	7
19	9.59	10.42	-0.83	0.676	-1.924	-2.301	0.773	-3.324 ¹	8
13	4.46	4.94	-0.48	0.650	-1.076	-1.085	0.215	-1.478	9
20	8.15	7.25	0.90	0.588	1.862	2.186	0.495	2.612 ¹	10
9	2.88	3.75	-0.87	0.441	-1.538	-1.670	0.187	-1.484	11
10	9.28	8.42	0.86	0.674	2.002	2.453	0.830	3.531 ¹	12
5	2.57	2.26	0.31	0.372	0.520	0.500	0.016	0.384	13
2	8.13	7.96	0.17	0.686	0.402	0.384	0.035	0.569	14
15	4.51	4.16	0.35	0.157	0.502	0.482	0.005	0.208	15
16	3.69	4.16	-0.47	0.157	-0.683	-0.664	0.009	-0.287	16
8	4.56	4.16	0.40	0.157	0.574	0.554	0.006	0.239	17
6	3.64	4.16	-0.52	0.157	-0.755	-0.738	0.011	-0.319	18
18	3.79	4.16	-0.37	0.157	-0.539	-0.518	0.005	-0.224	19
11	4.81	4.16	0.65	0.157	0.936	0.929	0.016	0.401	20

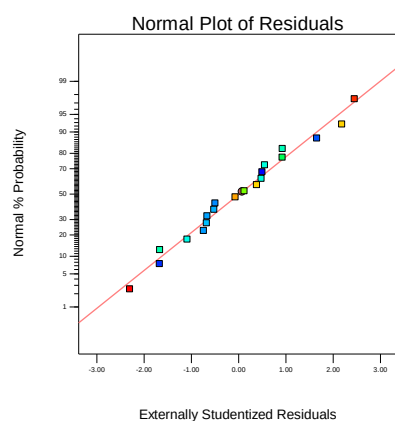
¹ Exceeds limits.

Box-Cox Power Transformation

Constant k	95% CI Low	95% CI High	Best Lambda	Rec. Transform
0.000	-0.79	1.99	0.53	None

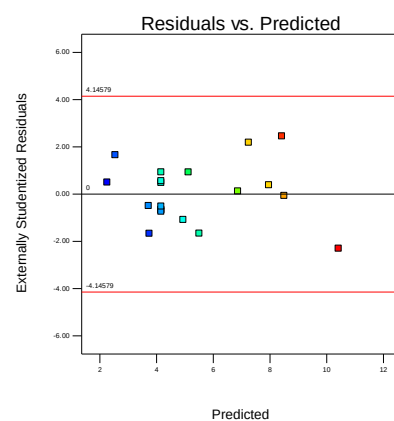
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ASDW-P1

Color points by value of
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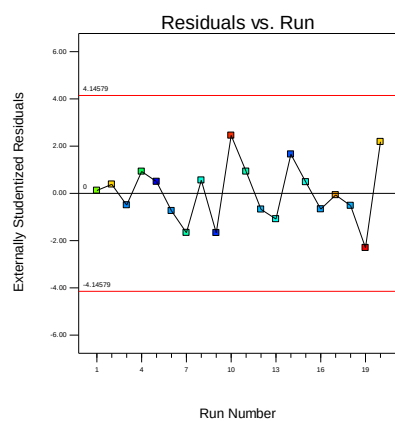
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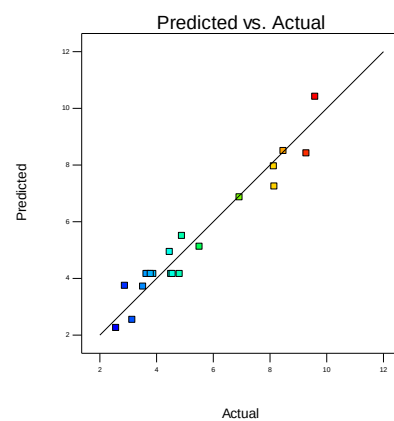
Design-Expert® Software
ASDW-P1

Color points by value of
ASDW-P1:



Design-Expert® Software
ASDW-P1

Color points by value of
ASDW-P1:



Design-Expert® Software
ASDW-P1

Lambda

Current = 1

Best = 0.53

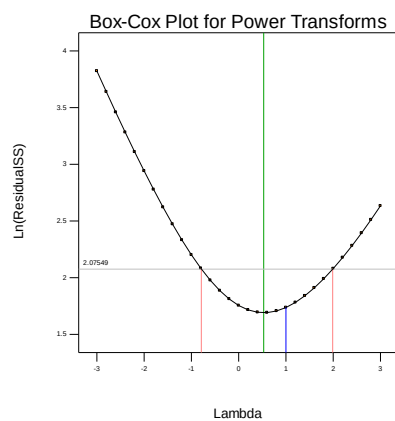
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High C.I. = 1.99

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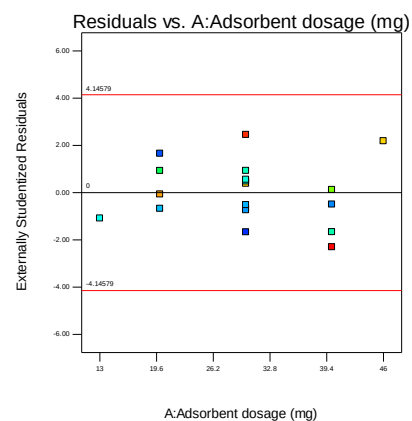
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(Lambda = 1)



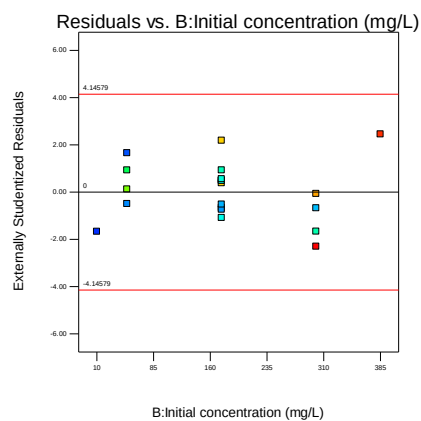
Design-Expert® Software
ASDW-P1

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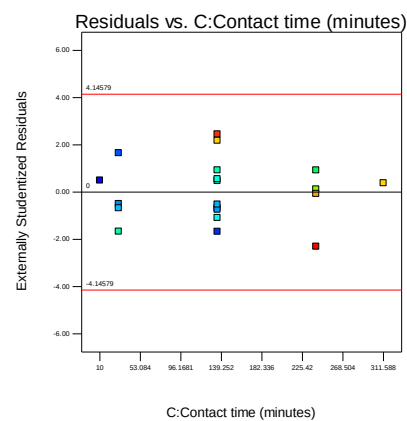
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Color points by value of
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2.57



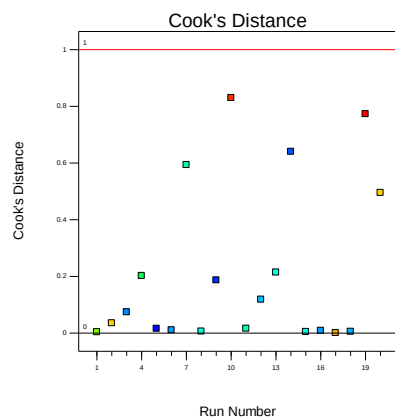
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Color points by value of
ASDW-P1:
9.59
2.57



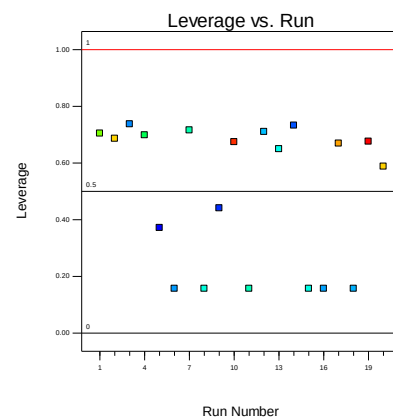
Design-Expert® Software
ASDW-P1

Color points by value of
ASDW-P1:
9.59
2.57



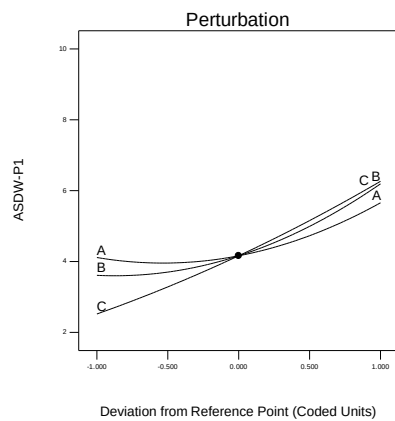
Design-Expert® Software
ASDW-P1

Color points by value of
ASDW-P1:
9.59
2.57



Design-Expert® Software
Factor Coding: Actual
ASDW-P1

Actual Factors
A: Adsorbent dosage = 30
B: Initial concentration = 175
C: Contact time = 135



Appendix B7: In vitro release profile of ASDW-P1

Linearity:

$$y = 16.503x - 1.1408$$

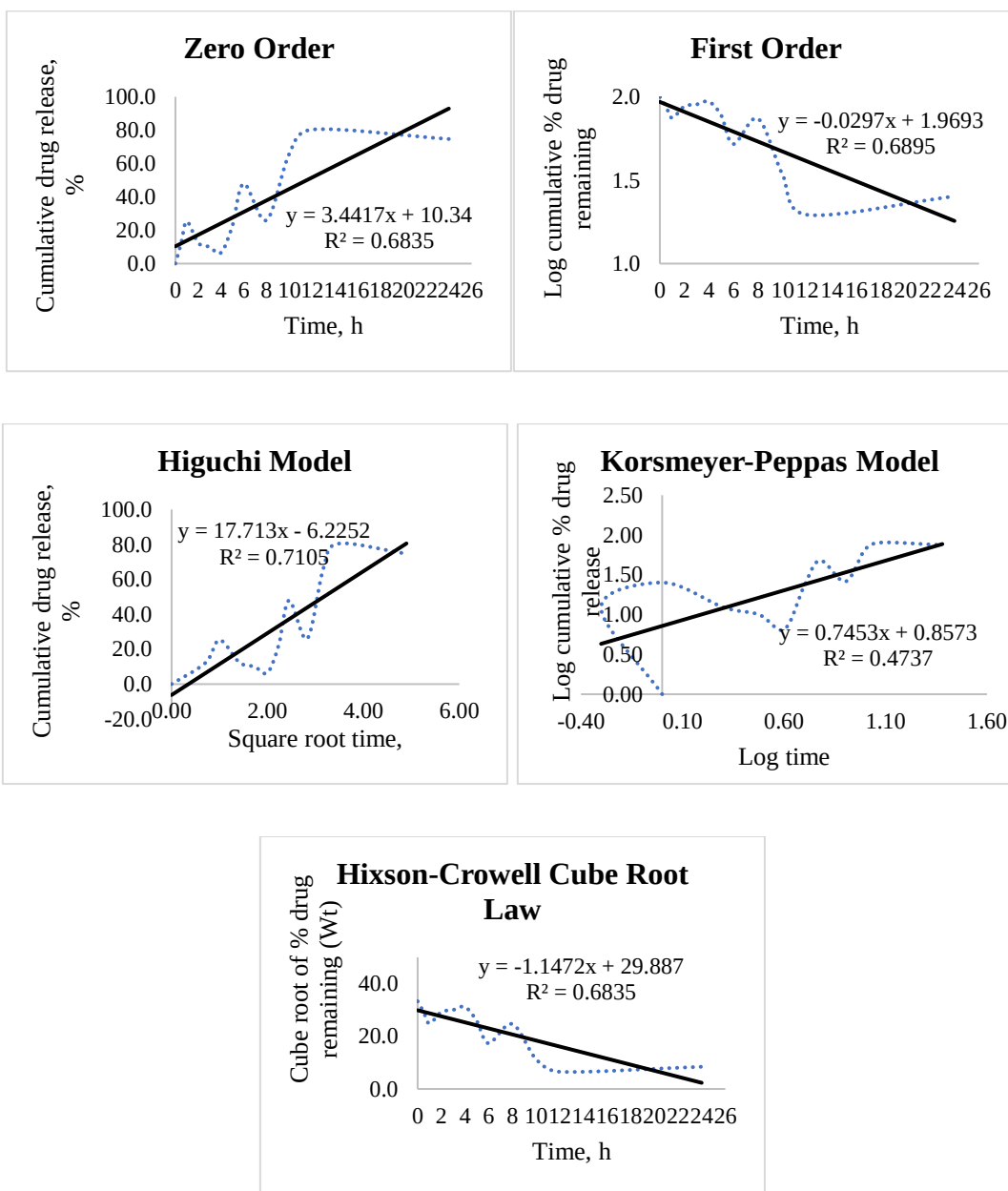
$$R^2 = 0.9865$$

Loading capacity = 9.45 mg/g

Appendix B7.1: Calculated data on the release profile of MIP in medium pH 2.2

Time (h)	Square root time	Log time	Abs.	Conc., mg/L	Amount drug release, mg/g	Drug release, %	Cumulative drug release, %	Drug remaining %	Log cumulative % drug remaining	Log cumulative % drug released	Cube root of % drug remaining (Wt)	Wo-Wt
0	0.00	0.00	0.000	0.00	0.00	0.00	0.00	100.00	2.00	0.00	33.33	0.00
0.5	0.71	-0.30	0.139	1.15	0.02	12.20	12.20	87.80	1.94	1.09	29.27	4.07
1	1.00	0.00	0.227	2.61	0.04	23.43	25.26	74.74	1.87	1.40	24.91	8.42
2	1.41	0.30	0.127	0.96	0.02	8.59	12.38	87.62	1.94	1.09	29.21	4.13
3	1.73	0.48	0.124	0.91	0.02	8.15	10.00	90.00	1.95	1.00	30.00	3.33
4	2.00	0.60	0.103	0.56	0.01	5.03	6.53	93.47	1.97	0.81	31.16	2.18
5	2.24	0.70	0.215	2.41	0.04	21.65	22.63	77.37	1.89	1.35	25.79	7.54
6	2.45	0.78	0.369	4.95	0.08	44.51	47.91	52.09	1.72	1.68	17.36	15.97
8	2.83	0.90	0.196	2.09	0.04	18.83	26.02	73.98	1.87	1.42	24.66	8.67
10	3.16	1.00	0.487	6.90	0.12	62.03	65.93	34.07	1.53	1.82	11.36	21.98
12	3.46	1.08	0.544	7.84	0.13	70.49	80.38	19.62	1.29	1.91	6.54	26.79
24	4.90	1.38	0.491	6.96	0.12	62.62	74.68	25.32	1.40	1.87	8.44	24.89

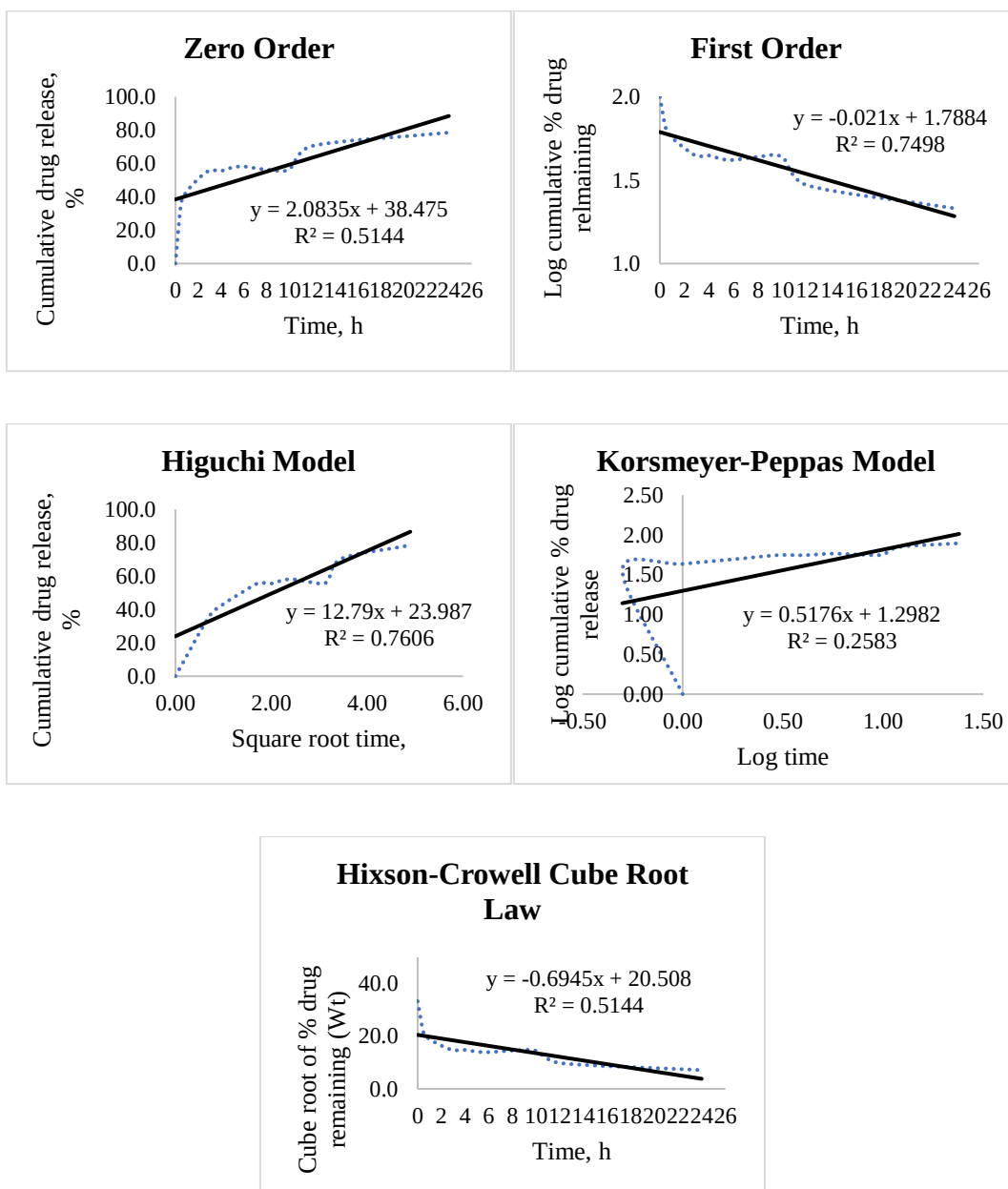
Appendix B7.1.1: Release Kinetics Modelling Graphs for pH 2.



Appendix B7.2: Calculated data on the release profile of MIP in medium pH 7

Time (h)	Square root time	Log time	Abs.	Conc., mg/L	Amount drug release, mg/g	Drug release, %	Cumulative drug release, %	Drug remaining %	Log cumulative % drug remaining	Log cumulative % drug released	Cube root of % drug remaining (Wt)	Wo-Wt
0	0.00	0.00	0.000	0.00	0.00	0.00	0.00	100.00	2.00	0.00	33.33	0.00
0.5	0.71	-0.30	0.288	4.13	0.08	0.69	35.95	64.05	1.81	1.56	21.35	11.98
1	1.00	0.00	0.285	1.69	0.03	0.24	43.23	56.77	1.75	1.64	18.92	14.41
2	1.41	0.30	0.289	4.94	0.08	0.70	50.70	49.30	1.69	1.71	16.43	16.90
3	1.73	0.48	0.288	4.13	0.07	0.59	55.83	44.17	1.65	1.75	14.72	18.61
4	2.00	0.60	0.286	2.50	0.04	0.36	55.56	44.44	1.65	1.74	14.81	18.52
5	2.24	0.70	0.286	2.50	0.04	0.36	57.60	42.40	1.63	1.76	14.13	19.20
6	2.45	0.78	0.285	1.69	0.03	0.24	58.20	41.80	1.62	1.76	13.93	19.40
8	2.83	0.90	0.286	2.50	0.04	0.36	56.36	43.64	1.64	1.75	14.55	18.79
10	3.16	1.00	0.286	2.50	0.04	0.36	56.53	43.47	1.64	1.75	14.49	18.84
12	3.46	1.08	0.286	2.50	0.04	0.36	70.51	29.49	1.47	1.85	9.83	23.50
24	4.90	1.38	0.285	1.69	0.03	0.24	78.54	21.46	1.33	1.90	7.15	26.18

Appendix B7.2.1: Release Kinetics Modelling Graphs for pH 7



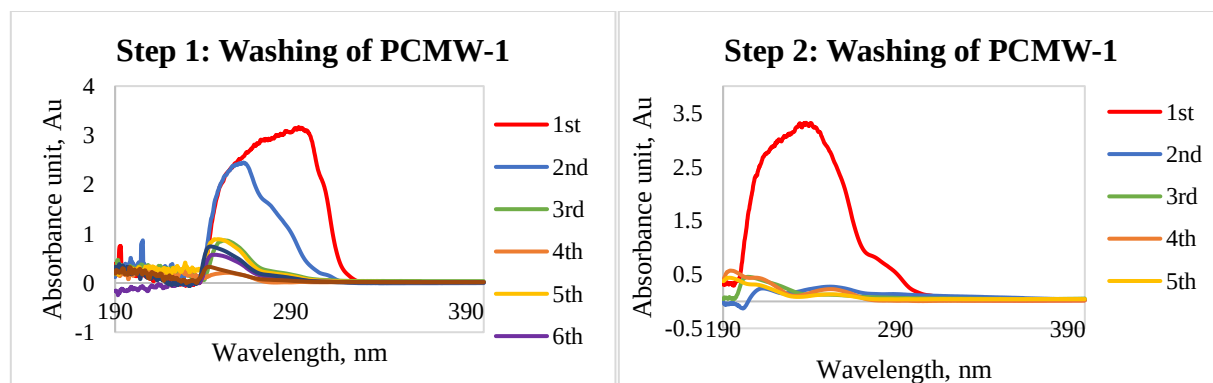
Appendix C: Microwave-assisted Synthesis of MIP using Paracetamol as Template for Drug Delivery Application

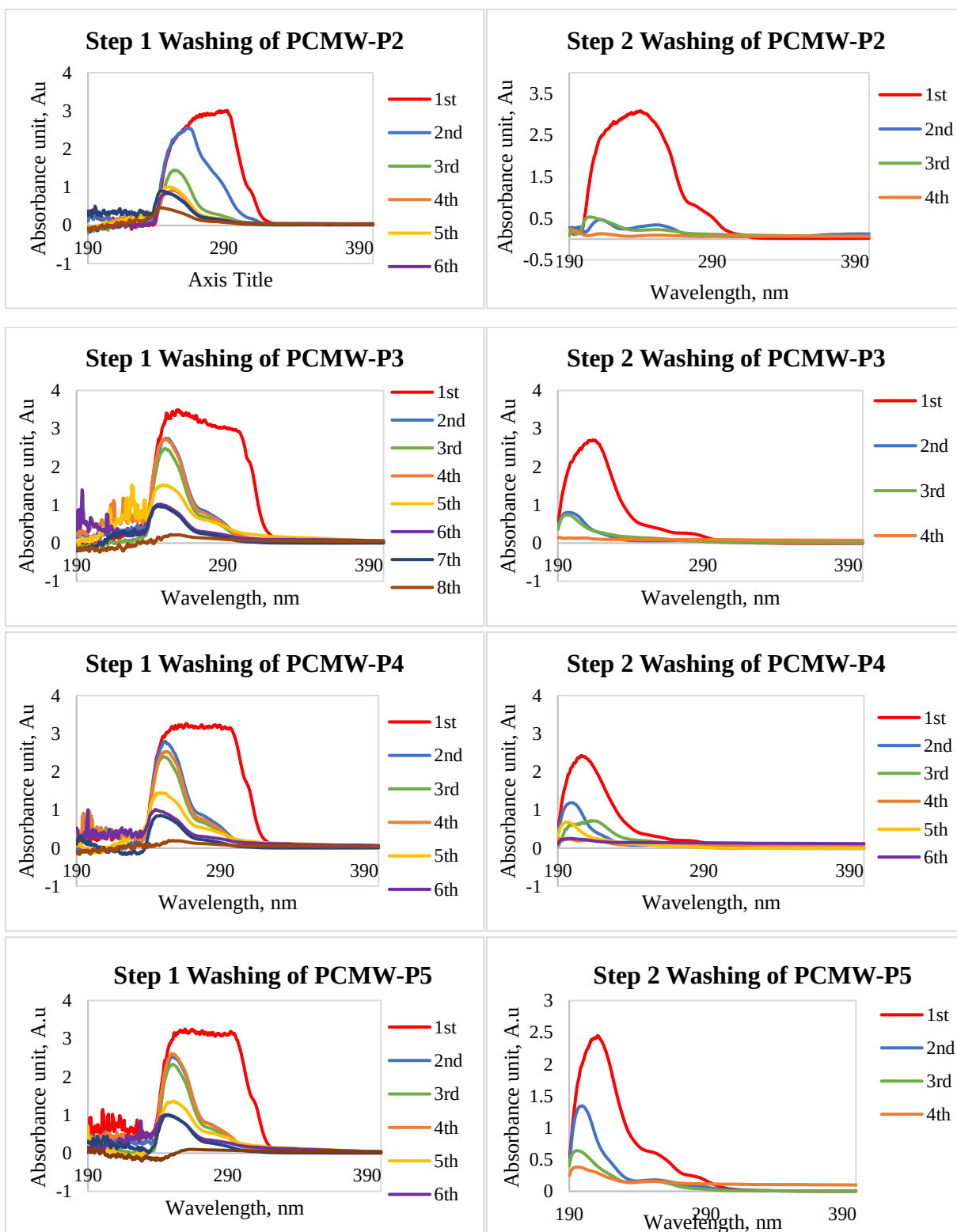
Appendix C1: Polymer Yield

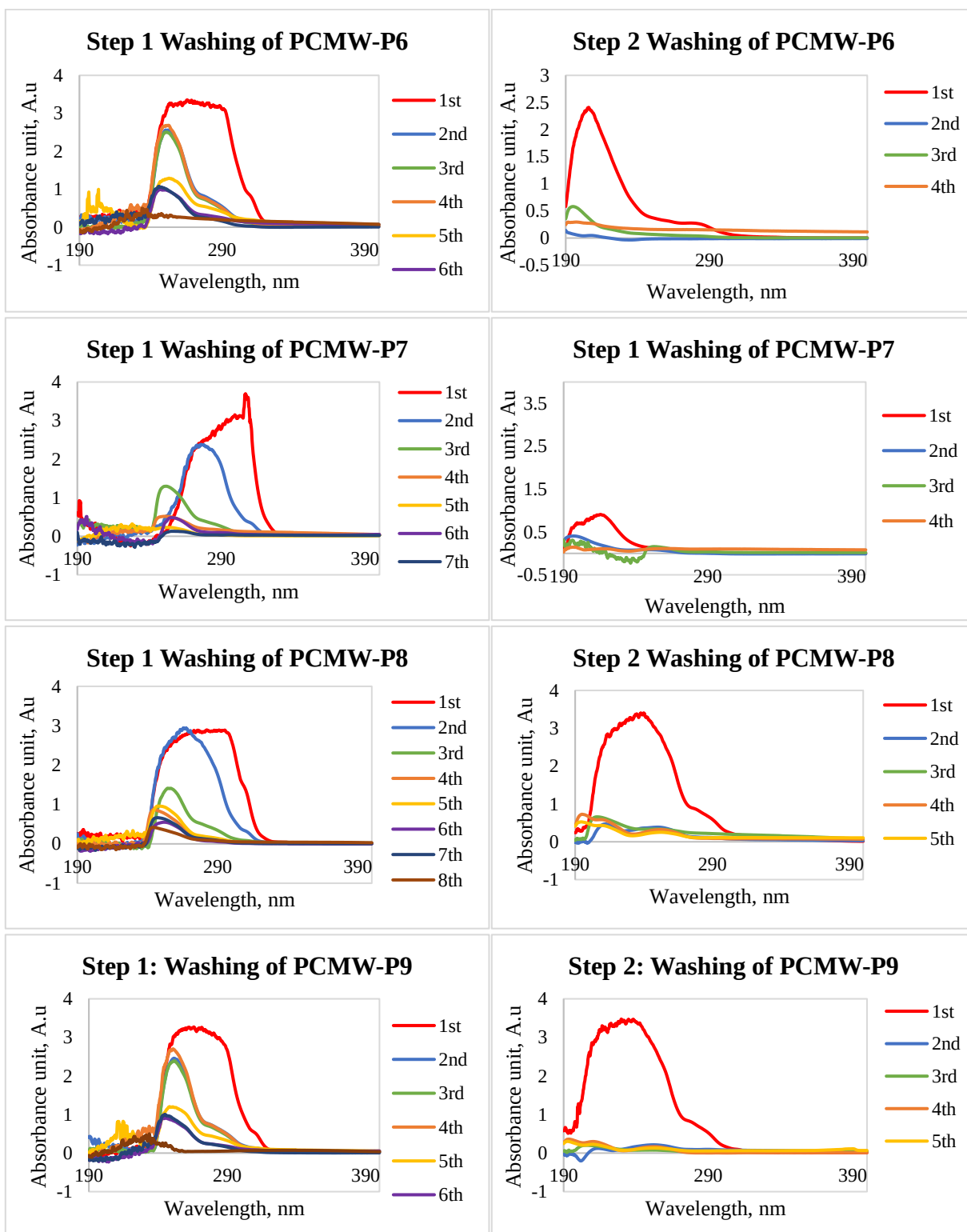
Polymer ID	Factors		Yield (g)
	Monomer (mmol)	Crosslinker (mmol)	PCMW
P1	4	16	0.5390
P2	10	16	0.5395
P3	4	20	0.7755
P4	10	20	0.6680
P5	2.76	18	0.8735
P6	11.24	18	0.6390
P7	7	15.17	0.6985
P8	7	20.83	0.6055
P9	7	18	0.7820
P10	7	18	0.6745
P11	7	18	0.7720
P12	7	18	0.7075
P13	7	18	0.7850

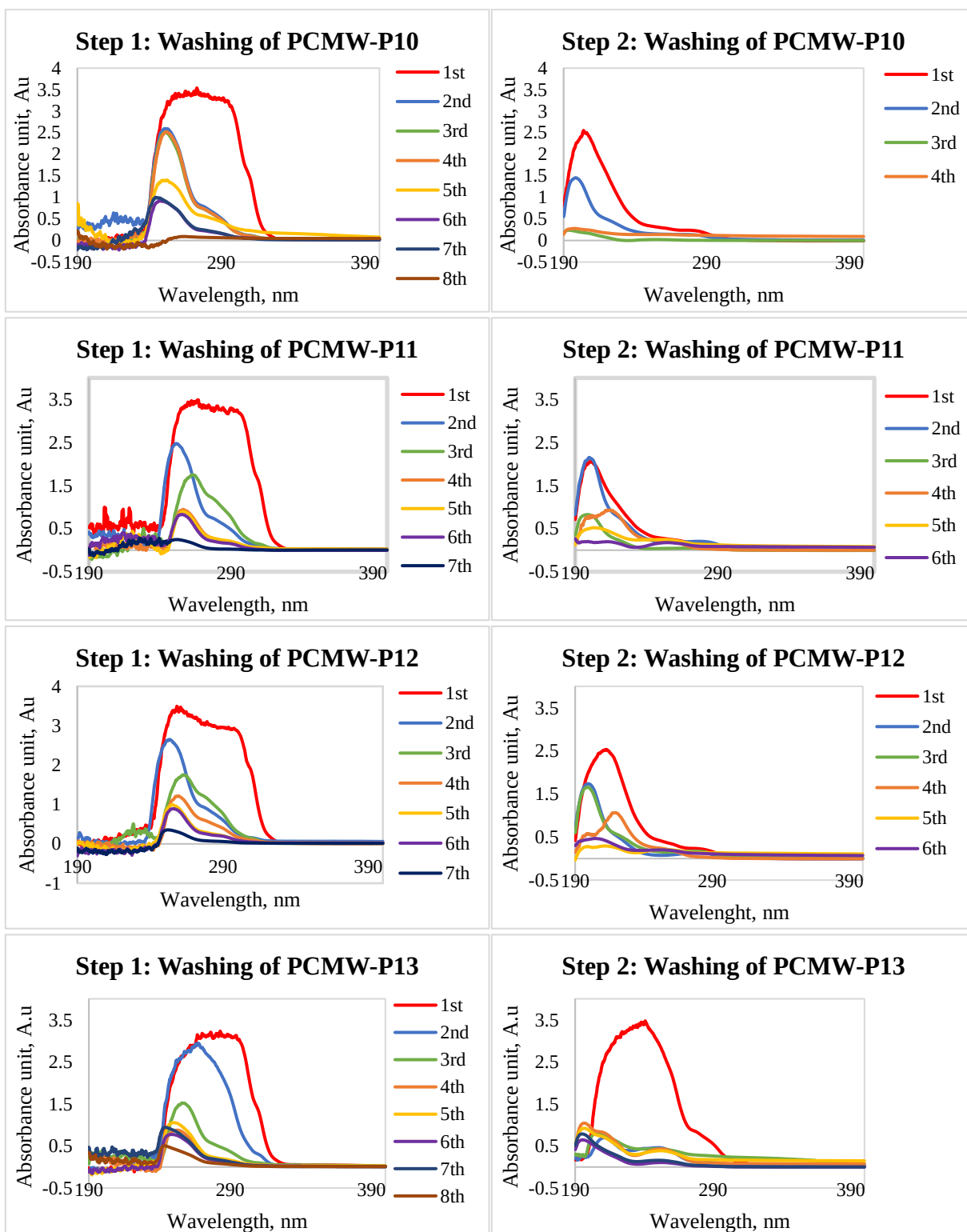
Appendix C2: Template Removal: UV-Vis Graph Monitoring

Appendix C2.1: Template Removal (Washing) Monitoring of PCMW-MIP









Appendix C3: Particle size data

Polymer ID	Length, μm							
	PCMW				NWP			
	Ave.	std.	Min	Max	Ave.	std.	Min	Max
P1	1.31	0.27	0.66	1.82	1.95	0.34	0.90	2.93
P2	1.15	0.37	0.55	2.14	1.55	0.52	0.79	2.95
P3	1.58	0.44	1.00	2.80	0.87	0.30	0.41	1.53
P4	1.33	0.42	0.78	2.87	1.21	0.42	0.60	2.38
P5	1.38	0.41	0.43	1.99	1.22	0.34	0.52	1.92
P6	1.07	0.31	0.68	1.98	1.10	0.38	0.57	1.95
P7	1.11	0.31	0.60	1.72	1.37	0.40	0.66	2.75
P8	1.64	0.39	0.97	2.56	1.40	0.51	0.68	2.67
P9	1.51	0.70	0.39	3.70	1.12	0.31	0.68	1.80
P10	1.29	0.52	0.58	3.11	1.15	0.26	0.66	1.71
P11	1.23	0.35	0.75	2.27	1.08	0.30	0.65	1.82
P12	1.09	0.34	0.46	1.96	1.61	0.34	0.88	2.45
P13	1.15	0.36	0.57	1.99	1.21	0.37	0.57	1.96

Appendix C4: Preliminary Rebinding Data of PCMW

Polymer ID	Calibration data		Absorbance unit			Final Concentration (mg/L)					Rebinding capacity (mg/g)				
	Equation	R ²	T1	T2	T3	C1	C2	C3	Mean	s.d	Q1	Q2	Q3	Mean	s.d
PCMW-P1	$y = 80.039x + 0.1462$	0.9996	0.94	0.94	0.94	75.38	75.38	75.16	75.31	0.13	6.15	6.15	6.21	6.17	0.03
PCMW-P2	$y = 80.039x + 0.1462$	0.9996	0.95	0.96	0.97	76.12	76.72	77.48	76.77	0.68	5.97	5.82	5.63	5.81	0.17
PCMW-P3	$y = 80.039x + 0.1462$	0.9996	0.96	0.97	0.96	77.22	77.78	77.16	77.39	0.34	5.69	5.55	5.71	5.65	0.09
PCMW-P4	$y = 80.039x + 0.1462$	0.9996	0.99	0.98	1.00	79.48	78.88	80.08	79.48	0.60	5.13	5.28	4.98	5.13	0.15
PCMW-P5	$y = 80.039x + 0.1462$	0.9996	0.97	0.98	0.96	77.46	78.52	76.76	77.58	0.89	5.63	5.37	5.81	5.60	0.22
PCMW-P6	$y = 80.039x + 0.1462$	0.9996	1.00	1.00	1.00	80.35	80.03	80.52	80.30	0.25	4.91	4.99	4.87	4.93	0.06
PCMW-P7	$y = 80.039x + 0.1462$	0.9996	1.00	0.98	0.98	79.95	78.18	78.72	78.95	0.90	5.01	5.45	5.32	5.26	0.23
PCMW-P8	$y = 80.039x + 0.1462$	0.9996	1.00	1.00	1.01	80.52	80.28	81.32	80.71	0.54	4.87	4.93	4.67	4.82	0.14
PCMW-P9	$y = 80.039x + 0.1462$	0.9996	1.05	1.05	1.04	84.12	83.96	83.40	83.83	0.38	3.97	4.01	4.15	4.04	0.09
PCMW-P10	$y = 80.039x + 0.1462$	0.9996	1.07	1.06	1.07	85.64	84.76	85.44	85.28	0.46	3.59	3.81	3.64	3.68	0.12
PCMW-P11	$y = 80.039x + 0.1462$	0.9996	1.07	1.07	1.08	85.96	86.04	86.52	86.17	0.30	3.51	3.49	3.37	3.46	0.08
PCMW-P12	$y = 80.039x + 0.1462$	0.9996	1.06	1.06	1.06	84.88	84.72	84.96	84.85	0.12	3.78	3.82	3.76	3.79	0.03
PCMW-P13	$y = 80.039x + 0.1462$	0.9996	1.05	1.04	1.05	83.96	83.24	84.12	83.77	0.47	4.01	4.19	3.97	4.06	0.12

s.d, standard deviation

Appendix C4.1: Design Expert Results Summary for the Response of Rebinding Capacity of PCMW

Appendix C4.1.1: Fit Summary

Sequential Model Sum of Squares [Type I]

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Mean vs Total	299.52	1	299.52			
Linear vs Mean	0.83	2	0.42	0.46	0.6413	
2FI vs Linear	6.400E-003	1	6.400E-003	6.433E-003	0.9378	
<u>Quadratic vs 2FI</u>	<u>8.08</u>	<u>2</u>	<u>4.04</u>	<u>32.31</u>	<u>0.0003</u>	<u>Suggested</u>
Cubic vs Quadratic	0.042	2	0.021	0.13	0.8835	Aliased
Residual	0.83	5	0.17			
Total	309.31	13	23.79			

Lack of Fit Tests

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Linear	8.70	6	1.45	22.75	0.0047	
2FI	8.70	5	1.74	27.28	0.0035	
<u>Quadratic</u>	<u>0.62</u>	<u>3</u>	<u>0.21</u>	<u>3.24</u>	<u>0.1429</u>	<u>Suggested</u>
Cubic	0.58	1	0.58	9.06	0.0396	Aliased
Pure Error	0.26	4	0.064			

Model Summary Statistics

Source	Std. Dev.	R-Squared	Adjusted R-Squared	Predicted R-Squared	PRESS	
Linear	0.95	0.0850	-0.0980	-0.4712	14.41	
2FI	1.00	0.0857	-0.2191	-1.5705	25.17	
<u>Quadratic</u>	<u>0.35</u>	<u>0.9106</u>	<u>0.8468</u>	<u>0.5090</u>	<u>4.81</u>	<u>Suggested</u>
Cubic	0.41	0.9149	0.7959	-2.8170	37.38	Aliased

Appendix C4.1.2: ANOVA for Response Surface Quadratic Model

Analysis of variance table [Partial sum of squares - Type III]

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	8.92	5	1.78	14.26	0.0015	significant
<i>A-Monomer</i>	0.42	1	0.42	3.34	0.1104	
<i>B-Cross-linker</i>	0.42	1	0.42	3.32	0.1112	
<i>AB</i>	6.400E-003	1	6.400E-003	0.051	0.8275	
<i>A²</i>	5.19	1	5.19	41.52	0.0004	
<i>B²</i>	3.93	1	3.93	31.41	0.0008	
Residual	0.88	7	0.13			
<i>Lack of Fit</i>	0.62	3	0.21	3.24	0.1429	not significant
<i>Pure Error</i>	0.26	4	0.064			
Cor Total	9.79	12				

The Model F-value of 14.26 implies the model is significant. There is only a 0.15% chance that an F-value this large could occur due to noise.

Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A^2 , B^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

The "Lack of Fit F-value" of 3.24 implies the Lack of Fit is not significant relative to the pure error. There is a 14.29% chance that a "Lack of Fit F-value" this large could occur due to noise. Non-significant lack of fit is good -- we want the model to fit.

Std. Dev.	0.35	R-Squared	0.9106
Mean	4.80	Adj R-Squared	0.8468
C.V. %	7.37	Pred R-Squared	0.5090
PRESS	4.81	Adeq Precision	8.537
-2 Log Likelihood	1.82	BIC	17.21
		AICc	27.82

The "Pred R-Squared" of 0.5090 is not as close to the "Adj R-Squared" of 0.8468 as one might normally expect; i.e. the difference is more than 0.2. This may indicate a large block effect or possible problem with your model and/or data. Things to consider are model reduction, response transformation, outliers, etc. All empirical models should be tested by doing confirmation runs.

"Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 8.537 indicates an adequate signal. This model can be used to navigate the design space.

Factor	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	3.81	1	0.16	3.43	4.18	
A-Monomer	-0.23	1	0.13	-0.52	0.067	1.00
B-Cross-linker	-0.23	1	0.13	-0.52	0.068	1.00
AB	-0.040	1	0.18	-0.46	0.38	1.00
A ²	0.86	1	0.13	0.55	1.18	1.02
B ²	0.75	1	0.13	0.43	1.07	1.02

Final Equation in Terms of Coded Factors:

$$\begin{aligned} \text{Rebinding capacity of PCMW-MIP} = & +3.81 \\ & -0.23 * A \\ & -0.23 * B \\ & -0.040 * AB \\ & +0.86 * A^2 \\ & +0.75 * B^2 \end{aligned}$$

The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels of the factors are coded as -1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.

Final Equation in Terms of Actual Factors:

$$\begin{aligned} \text{Rebinding capacity of PCMW-MIP} = & +71.11376 \\ & -1.29995 * \text{Monomer} \\ & -6.82960 * \text{Cross-linker} \\ & -6.66667\text{E-}003 * \text{Monomer} * \text{Cross-linker} \\ & +0.095986 * \text{Monomer}^2 \\ & +0.18784 * \text{Cross-linker}^2 \end{aligned}$$

The equation in terms of actual factors can be used to make predictions about the response for given levels of each factor. Here, the levels should be specified in the original units for each factor. This equation should not be used to determine the relative impact of each factor because the coefficients are scaled to accommodate the units of each factor and the intercept is not at the center of the design space.

Appendix C4.1.3: Diagnostic for Response Surface Quadratic Model

Diagnostics Case Statistics

Run Order	Actual Value	Predicted Value	Residual	Leverage	Internally Studentized Residual	Externally Studentized Residual	Cook's Distance	Influence on Fitted Value DFFITS	Standard Order
6	6.17	5.84	0.33	0.625	1.536	1.746	0.655	2.254 ¹	1
3	5.81	5.46	0.35	0.625	1.614	1.885	0.723	2.434 ¹	2
8	5.65	5.46	0.19	0.625	0.869	0.851	0.210	1.099	3
4	5.13	4.93	0.20	0.625	0.947	0.939	0.249	1.212	4
13	5.60	5.86	-0.26	0.625	-1.186	-1.228	0.391	-1.586	5
12	4.93	5.21	-0.28	0.625	-1.296	-1.377	0.467	-1.777	6
9	5.26	5.63	-0.37	0.625	-1.713	-2.081	0.815	-2.686 ¹	7
11	4.82	4.99	-0.17	0.625	-0.769	-0.745	0.164	-0.961	8
5	4.04	3.81	0.23	0.200	0.740	0.713	0.023	0.357	9
10	3.68	3.81	-0.13	0.200	-0.398	-0.373	0.007	-0.187	10
1	3.46	3.81	-0.35	0.200	-1.094	-1.112	0.050	-0.556	11
2	3.79	3.81	-0.016	0.200	-0.051	-0.047	0.000	-0.023	12
7	4.06	3.81	0.25	0.200	0.803	0.780	0.027	0.390	13

¹ Exceeds limits.

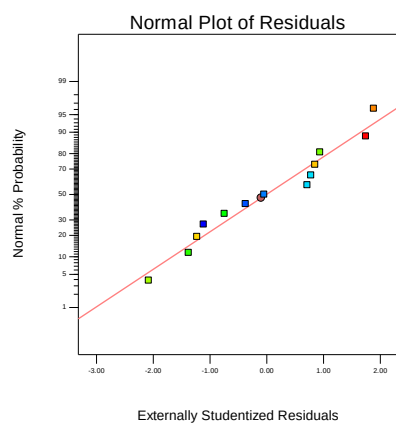
Box-Cox Power Transformation

Constant k	95% CI Low	95% CI High	Best Lambda	Rec. Transform
0.000	-2.48	3.48	0.35	None

Design-Expert® Software
PCMW-MIP

Color points by value of
PCMW-MIP:

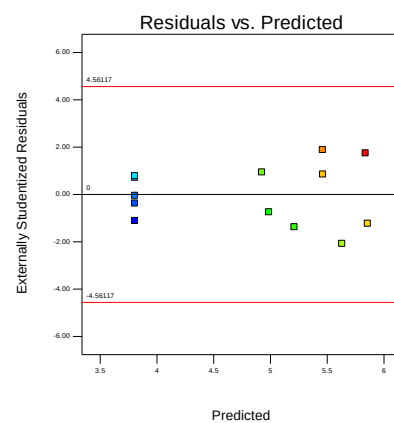
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3.46



Design-Expert® Software
PCMW-MIP

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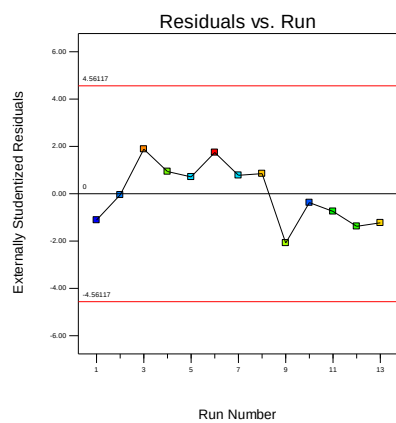
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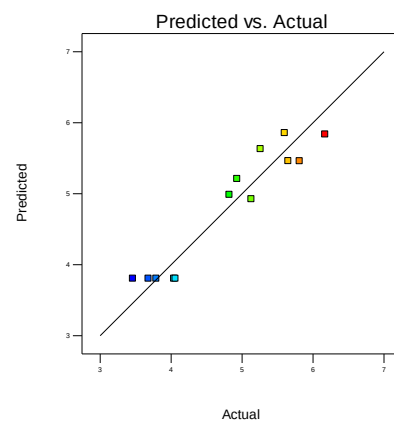
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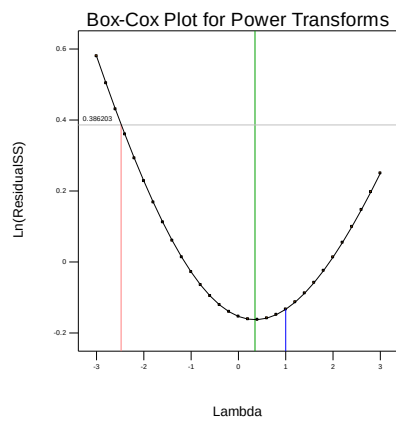
6.17
3.46



Design-Expert® Software
PCMW-MIP

Lambda
Current = 1
Best = 0.35
Low C.I. = -2.48
High C.I. = 3.48

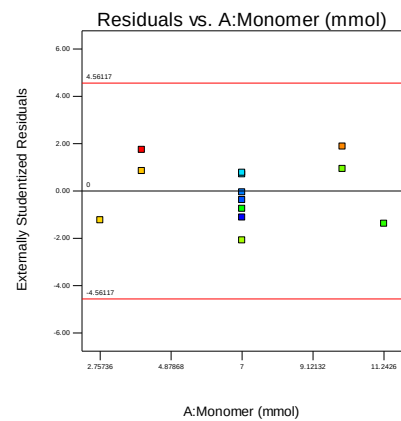
Recommend transform:
None
(Lambda = 1)



Design-Expert® Software
PCMW-MIP

Color points by value of
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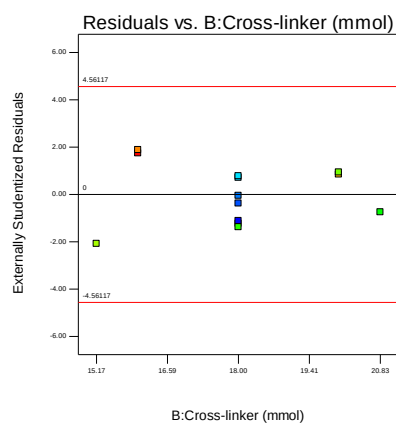
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Design-Expert® Software
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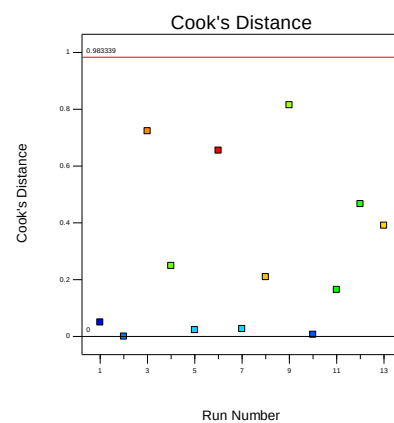
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Design-Expert® Software
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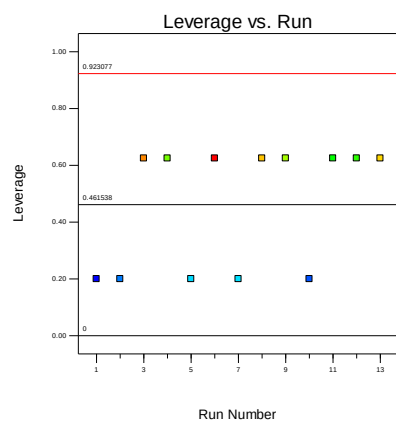
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Design-Expert® Software
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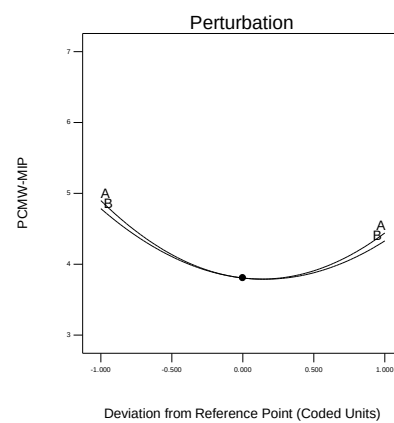
Color points by value of
PCMW-MIP:

6.17
3.46



Design-Expert® Software
Factor Coding: Actual
PCMW-MIP

Actual Factors
A: Monomer = 7.00
B: Cross-linker = 18.00



Appendix C5: Preliminary Rebinding Study Data of NWP

Polymer ID	Calibration data		Absorbance unit			Final Concentration (mg/L)					Rebinding capacity (mg/g)				
	Equation	R ²	T1	T2	T3	C1	C2	C3	Mean	s.d	Q1	Q2	Q3	Mean	s.d
NWP-P1	$y = 80.039x + 0.1462$	0.9996	1.26	1.26	1.25	95.39	95.39	94.64	95.14	0.43	1.15	1.15	1.34	1.21	0.11
NWP-P2	$y = 80.039x + 0.1462$	0.9996	1.24	1.21	1.23	93.87	91.39	92.52	92.59	1.24	1.53	2.15	1.87	1.85	0.31
NWP-P3	$y = 80.039x + 0.1462$	0.9996	1.29	1.30	1.29	97.23	97.79	97.16	97.40	0.35	0.69	0.55	0.71	0.65	0.09
NWP-P4	$y = 80.039x + 0.1462$	0.9996	1.28	1.29	1.29	96.75	97.55	97.28	97.20	0.41	0.81	0.61	0.68	0.70	0.10
NWP-P5	$y = 80.039x + 0.1462$	0.9996	1.29	1.29	1.29	97.47	97.07	97.16	97.24	0.21	0.63	0.73	0.71	0.69	0.05
NWP-P6	$y = 80.039x + 0.1462$	0.9996	1.25	1.26	1.26	94.52	94.84	94.92	94.76	0.21	1.37	1.29	1.27	1.31	0.05
NWP-P7	$y = 80.039x + 0.1462$	0.9996	1.31	1.30	1.30	98.48	98.19	98.36	98.34	0.14	0.38	0.45	0.41	0.41	0.04
NWP-P8	$y = 80.039x + 0.1462$	0.9996	1.26	1.26	1.26	95.15	94.88	95.36	95.13	0.24	1.21	1.28	1.16	1.22	0.06
NWP-P9	$y = 80.039x + 0.1462$	0.9996	1.27	1.31	1.28	96.15	98.51	96.68	97.12	1.24	0.96	0.37	0.83	0.72	0.31
NWP-P10	$y = 80.039x + 0.1462$	0.9996	1.28	1.29	1.29	96.83	97.15	97.08	97.02	0.17	0.79	0.71	0.73	0.74	0.04
NWP-P11	$y = 80.039x + 0.1462$	0.9996	1.27	1.28	1.28	96.03	96.75	96.68	96.49	0.40	0.99	0.81	0.83	0.88	0.10
NWP-P12	$y = 80.039x + 0.1462$	0.9996	1.29	1.29	1.30	97.63	97.47	98.04	97.72	0.29	0.59	0.63	0.49	0.57	0.07
NWP-P13	$y = 80.039x + 0.1462$	0.9996	1.30	1.28	1.29	98.11	96.67	97.04	97.28	0.75	0.47	0.83	0.74	0.68	0.19

s.d, standard deviation

Appendix C6: Optimisation on Rebinding Capacity of PCMW-P1 Using RSM

Appendix C6.1: Rebinding Study Data of PCMW-P1

Experimental		Calibration data		Absorbance unit	Concentration (mg/L)			Dosage (g)	Rebinding capacity (mg/g)
Std.	Run	Equation	R ²		Initial	Final	Ci - Cf		
1	B14	$y = 96.457x - 17.913$	0.9818	0.666	50	46.33	3.67	0.02	1.84
2	B3	$y = 96.457x - 17.913$	0.9818	0.618	50	41.70	8.30	0.04	2.08
3	B12	$y = 96.457x - 17.913$	0.9818	3.258	300	296.34	3.66	0.02	1.83
4	B7	$y = 96.457x - 17.913$	0.9818	3.153	300	286.22	13.78	0.04	3.45
5	B4	$y = 96.457x - 17.913$	0.9818	0.659	50	45.65	4.35	0.02	2.17
6	B1	$y = 96.457x - 17.913$	0.9818	0.605	50	40.44	9.56	0.04	2.39
7	B17	$y = 96.457x - 17.913$	0.9818	3.193	300	290.07	9.93	0.02	4.96
8	B19	$y = 96.457x - 17.913$	0.9818	3.062	300	277.44	22.56	0.04	5.64
9	B13	$y = 96.457x - 17.913$	0.9818	1.965	175	171.63	3.37	0.01	2.56
10	B20	$y = 96.457x - 17.913$	0.9818	1.855	175	161.01	13.99	0.05	2.99
11	B9	$y = 96.457x - 17.913$	0.9818	0.23	10	4.27	5.73	0.03	1.91
12	B10	$y = 96.457x - 17.913$	0.9818	3.985	385.224	366.47	18.76	0.03	6.25
13	B5	$y = 96.457x - 17.913$	0.9818	1.955	175	170.66	4.34	0.03	1.45
14	B2	$y = 96.457x - 17.913$	0.9818	1.897	175	165.07	9.93	0.03	3.31
15	B15	$y = 96.457x - 17.913$	0.9818	1.919	175	167.19	7.81	0.03	2.60
16	B16	$y = 96.457x - 17.913$	0.9818	1.933	175	168.54	6.46	0.03	2.15
17	B8	$y = 96.457x - 17.913$	0.9818	1.917	175	167.00	8.00	0.03	2.67
18	B6	$y = 96.457x - 17.913$	0.9818	1.935	175	168.73	6.27	0.03	2.09
19	B18	$y = 96.457x - 17.913$	0.9818	1.932	175	168.44	6.56	0.03	2.19
20	B11	$y = 96.457x - 17.913$	0.9818	1.926	175	167.86	7.14	0.03	2.38

Appendix C6.2: Design Expert Results Summary for the Response Rebinding Capacity of PCMW-P1

Appendix C6.2.1: Fit Summary

Sequential Model Sum of Squares [Type I]

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Mean vs Total	161.88	1	161.88			
Linear vs Mean	23.13	3	7.71	13.16	0.0001	
2FI vs Linear	3.28	3	1.09	2.33	0.1224	
<u>Quadratic vs 2FI</u>	<u>5.32</u>	<u>3</u>	<u>1.77</u>	<u>22.73</u>	<u>< 0.0001</u>	<u>Suggested</u>
Cubic vs Quadratic	0.48	5	0.096	1.60	0.3089	Aliased
Residual	0.30	5	0.060			
Total	194.39	20	9.72			

Lack of Fit Tests

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Linear	9.08	11	0.83	13.76	0.0048	
2FI	5.80	8	0.72	12.09	0.0069	
<u>Quadratic</u>	<u>0.48</u>	<u>5</u>	<u>0.096</u>	<u>1.60</u>	<u>0.3089</u>	<u>Suggested</u>
Cubic	0.000	0				Aliased
Pure Error	0.30	5	0.060			

Model Summary Statistics

Source	Std. Dev.	R-Squared	Adjusted R-Squared	Predicted R-Squared	PRESS	
Linear	0.77	0.7116	0.6575	0.4914	16.53	
2FI	0.68	0.8124	0.7258	0.4769	17.01	
<u>Quadratic</u>	<u>0.28</u>	<u>0.9760</u>	<u>0.9544</u>	<u>0.8546</u>	<u>4.73</u>	<u>Suggested</u>
Cubic	0.24	0.9908	0.9650		+	Aliased

+ Case(s) with leverage of 1.0000: PRESS statistic not defined.

Appendix C6.2.2: ANOVA for Response Surface Quadratic Model

Analysis of variance table [Partial sum of squares - Type III]

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	31.73	9	3.53	45.20	< 0.0001	significant
<i>A-Adsorbent dosage</i>	0.93	1	0.93	11.87	0.0063	
<i>B-Initial concentration</i>	12.70	1	12.70	162.80	< 0.0001	
<i>C-Contact time</i>	6.19	1	6.19	79.34	< 0.0001	
<i>AB</i>	0.42	1	0.42	5.43	0.0420	
<i>AC</i>	0.12	1	0.12	1.49	0.2501	
<i>BC</i>	2.74	1	2.74	35.07	0.0001	
<i>A²</i>	0.32	1	0.32	4.08	0.0710	
<i>B²</i>	5.02	1	5.02	64.40	< 0.0001	
<i>C²</i>	0.10	1	0.10	1.31	0.2798	
Residual	0.78	10	0.078			not significant
<i>Lack of Fit</i>	0.48	5	0.096	1.60	0.3089	
<i>Pure Error</i>	0.30	5	0.060			
Cor Total	32.51	19				

The Model F-value of 45.20 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise.

Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A, B, C, AB, BC and B² are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

The "Lack of Fit F-value" of 1.60 implies the Lack of Fit is not significant relative to the pure error. There is a 30.89% chance that a "Lack of Fit F-value" this large could occur due to noise. Non-significant lack of fit is good -- we want the model to fit.

Std. Dev.	0.28	R-Squared	0.9760
Mean	2.84	Adj R-Squared	0.9544
C.V. %	9.82	Pred R-Squared	0.8546
PRESS	4.73	Adeq Precision	23.669
-2 Log Likelihood	-8.13	BIC	21.83
		AICc	36.32

The "Pred R-Squared" of 0.8546 is not as close to the "Adj R-Squared" of 0.9544. i.e. the difference is less than 0.2.

"Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 23.669 indicates an adequate signal. This model can be used to navigate the design space.

Factor	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	2.34	1	0.11	2.09	2.59	
A-Adsorbent dosage	0.26	1	0.076	0.093	0.43	1.00
B-Initial concentration	1.02	1	0.080	0.85	1.20	1.04
C-Contact time	0.73	1	0.082	0.55	0.92	1.07
AB	0.23	1	0.099	0.010	0.45	1.00
AC	-0.12	1	0.099	-0.34	0.099	1.00
BC	0.58	1	0.099	0.36	0.80	1.00
A ²	0.15	1	0.075	-0.016	0.32	1.01
B ²	0.69	1	0.085	0.50	0.88	1.04
C ²	-0.10	1	0.089	-0.30	0.097	1.07

Final Equation in Terms of Coded Factors:

$$\begin{aligned}
 \text{Rebinding capacity of PCMW-P1} = & +2.34 \\
 & +0.26 * A \\
 & +1.02 * B \\
 & +0.73 * C \\
 & +0.23 * AB \\
 & -0.12 * AC \\
 & +0.58 * BC \\
 & +0.15 * A^2 \\
 & +0.69 * B^2 \\
 & -0.10 * C^2
 \end{aligned}$$

The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels of the factors are coded as -1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.

Final Equation in Terms of Actual Factors:

$$\begin{aligned} \text{Rebinding capacity PCMW-} &= +3.27048 \\ \text{P1} & -0.081593 \quad * \text{ Adsorbent dosage} \\ & -0.018707 \quad * \text{ Initial concentration} \\ & +5.12829\text{E-} \quad * \text{ Contact time} \\ & 003 \\ & +1.84096\text{E-} \quad * \text{ Adsorbent dosage} * \text{ Initial} \\ & 004 \quad \text{concentration} \\ & -1.14830\text{E-}004 \quad * \text{ Adsorbent dosage} * \text{ Contact time} \\ & +4.45539\text{E-} \quad * \text{ Initial concentration} * \text{ Contact time} \\ & 005 \\ & +1.51935\text{E-} \quad * \text{ Adsorbent dosage}^2 \\ & 003 \\ & +4.38942\text{E-} \quad * \text{ Initial concentration}^2 \\ & 005 \\ & -9.21675\text{E-}006 \quad * \text{ Contact time}^2 \end{aligned}$$

The equation in terms of actual factors can be used to make predictions about the response for given levels of each factor. Here, the levels should be specified in the original units for each factor. This equation should not be used to determine the relative impact of each factor because the coefficients are scaled to accommodate the units of each factor and the intercept is not at the center of the design space.

Appendix C6.2.3: Diagnostic of Quadratic Model in Design Expert

Diagnostics Case Statistics

Run Order	Actual Value	Predicted Value	Residual	Leverage	Internally Studentized Residual	Externally Studentized Residual	Cook's Distance	Influence on Fitted Value DFFITS	Standard Order
14	1.84	1.75	0.088	0.733	0.609	0.589	0.102	0.976	1
3	2.08	2.05	0.021	0.737	0.145	0.138	0.006	0.231	2
12	1.83	2.17	-0.34	0.710	-2.255	-3.051	1.248 ¹	-4.780 ¹	3
7	3.45	3.39	0.052	0.716	0.350	0.334	0.031	0.530	4
4	2.17	2.29	-0.11	0.699	-0.745	-0.728	0.129	-1.108	5
1	2.39	2.11	0.28	0.705	1.823	2.116	0.793	3.269 ¹	6
17	4.96	5.05	-0.083	0.669	-0.517	-0.497	0.054	-0.707	7
19	5.64	5.79	-0.15	0.676	-0.945	-0.940	0.187	-1.358	8
13	2.56	2.33	0.23	0.650	1.385	1.462	0.356	1.991	9
20	2.99	3.15	-0.16	0.588	-0.900	-0.891	0.116	-1.064	10
9	1.91	2.18	-0.27	0.441	-1.307	-1.361	0.135	-1.209	11
10	6.25	6.00	0.26	0.674	1.610	1.775	0.537	2.554 ¹	12
5	1.45	1.32	0.13	0.372	0.567	0.547	0.019	0.420	13
2	3.31	3.29	0.025	0.686	0.161	0.152	0.006	0.226	14
15	2.60	2.34	0.27	0.157	1.034	1.038	0.020	0.448	15
16	2.15	2.34	-0.19	0.157	-0.722	-0.704	0.010	-0.304	16
8	2.67	2.34	0.33	0.157	1.284	1.333	0.031	0.576	17
6	2.09	2.34	-0.25	0.157	-0.973	-0.970	0.018	-0.419	18
18	2.19	2.34	-0.15	0.157	-0.597	-0.576	0.007	-0.249	19
11	2.38	2.34	0.040	0.157	0.156	0.148	0.000	0.064	20

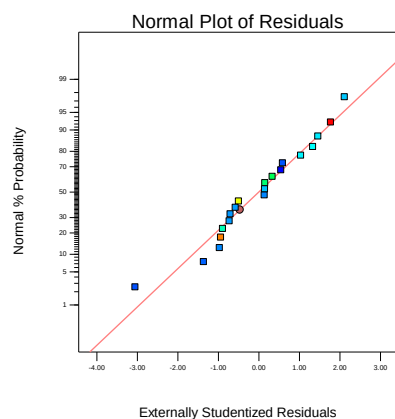
¹ Exceeds limits.

Box-Cox Power Transformation

Constant k	95% CI Low	95% CI High	Best Lambda	Rec. Transform
0.000	-0.32	1.48	0.69	None

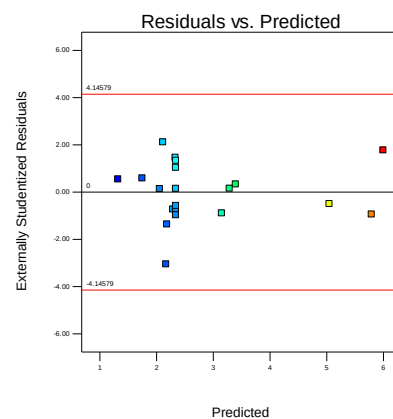
Design-Expert® Software
PCMW-P1

Color points by value of
PCMW-P1:
6.25195
1.44652



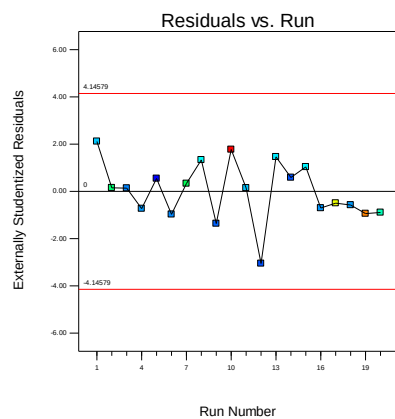
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PCMW-P1

Color points by value of
PCMW-P1:
6.25195
1.44652



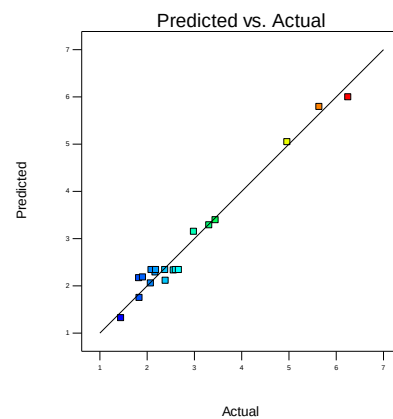
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Color points by value of
PCMW-P1:
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1.44652



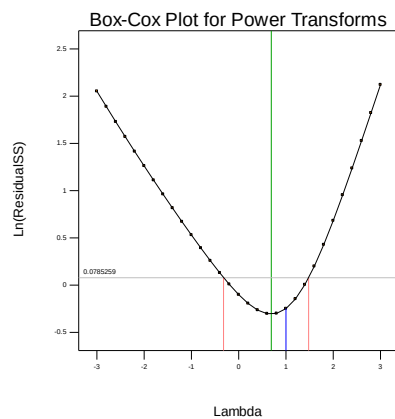
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PCMW-P1

Color points by value of
PCMW-P1:
6.25195
1.44652



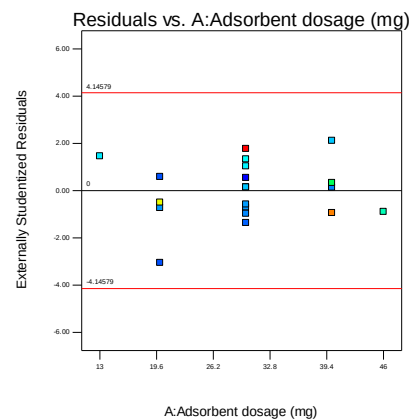
Design-Expert® Software
PCMW-P1

Lambda
Current = 1
Best = 0.69
Low C.I. = -0.32
High C.I. = 1.48
Recommend transform:
None
(Lambda = 1)



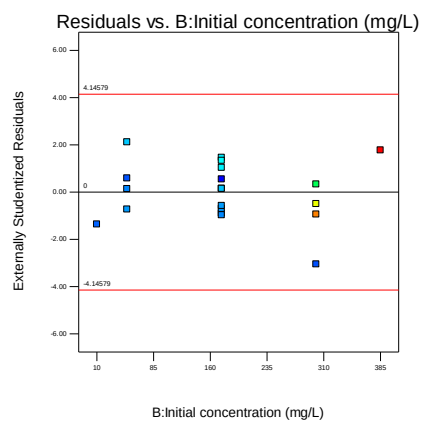
Design-Expert® Software
PCMW-P1

Color points by value of
PCMW-P1:
6.25195
1.44652



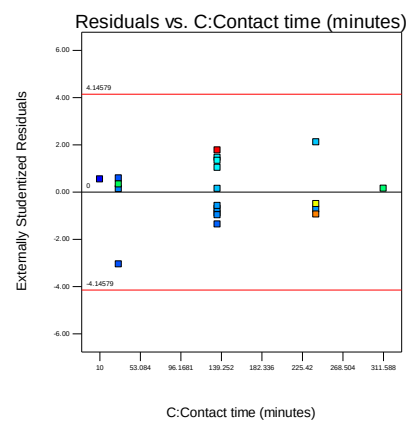
Design-Expert® Software
PCMW-P1

Color points by value of
PCMW-P1:
6.25195
1.44652



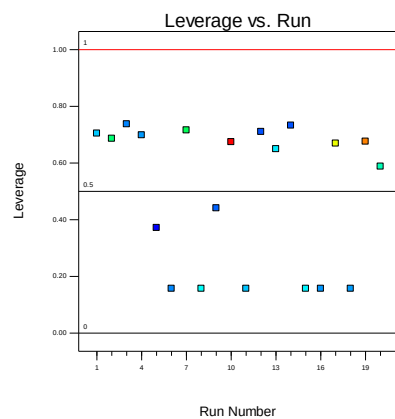
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PCMW-P1

Color points by value of
PCMW-P1:
6.25195
1.44652



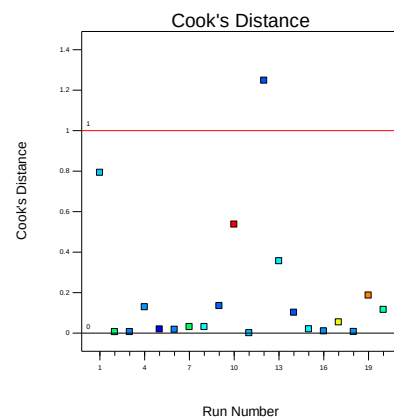
Design-Expert® Software
PCMW-P1

Color points by value of
PCMW-P1:
6.25195
1.44652



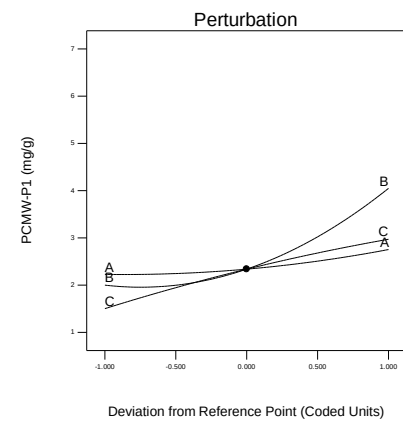
Design-Expert® Software
PCMW-P1

Color points by value of
PCMW-P1:
6.25195
1.44652



Design-Expert® Software
Factor Coding: Actual
PCMW-P1 (mg/g)

Actual Factors
A: Adsorbent dosage = 30
B: Initial concentration = 175
C: Contact time = 135



Appendix C7: In vitro release profile of PCMW-P1

Linearity:

$$y = 20.312 - 2.164$$

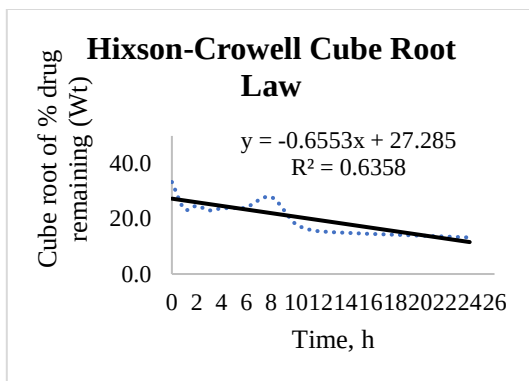
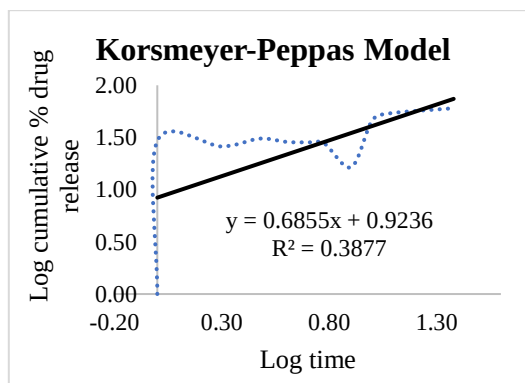
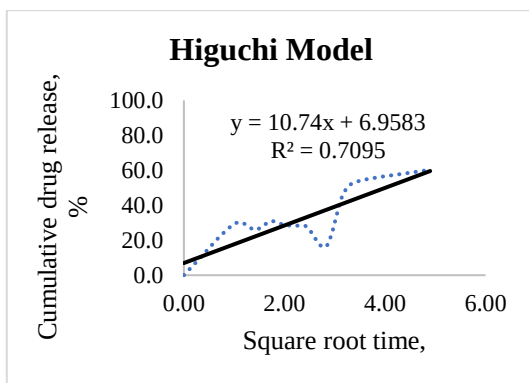
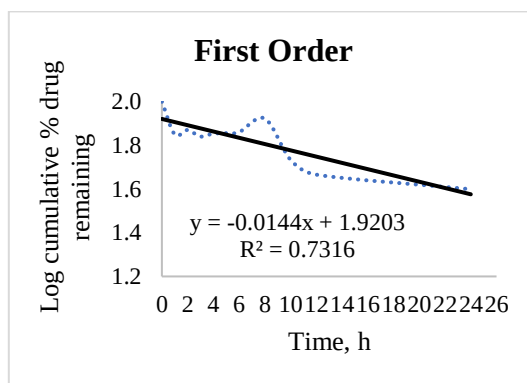
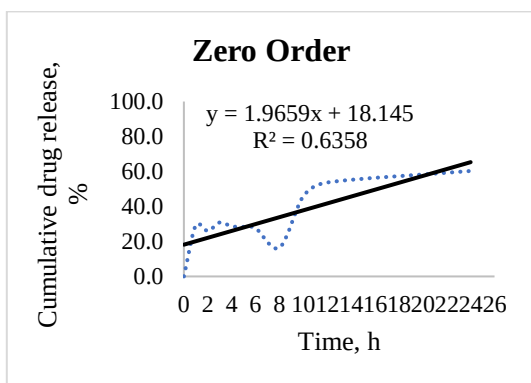
$$R^2 = 0.9898$$

Loading capacity = 7.65 mg/g

Appendix C7.1: Calculated data on the release profile of MIP in medium pH 2

Time (h)	Square root time	Log time	Abs.	Conc., mg/L	Amount drug release, mg/g	Drug release, %	Cumulative drug release, %	Drug remaining %	Log cumulative % drug remaining	Log cumulative % drug released	Cube root of % drug remaining (Wt)	Wo-Wt
0	0.00	0.00	0.000	0.00	0.00	0.00	0.00	100.00	2.00	0.00	33.33	0.00
1	1.00	0.00	0.217	2.24	0.04	29.33	29.33	70.67	1.85	1.47	23.56	9.78
2	1.41	0.30	0.202	1.94	0.03	21.54	25.94	74.06	1.87	1.41	24.69	8.65
3	1.73	0.48	0.226	2.43	0.04	26.96	30.85	69.15	1.84	1.49	23.05	10.28
4	2.00	0.60	0.213	2.16	0.04	24.03	28.66	71.34	1.85	1.46	23.78	9.55
5	2.24	0.70	0.213	2.16	0.04	24.03	28.33	71.67	1.86	1.45	23.89	9.44
6	2.45	0.78	0.21	2.10	0.04	23.35	27.60	72.40	1.86	1.44	24.13	9.20
8	2.83	0.90	0.161	1.11	0.02	12.29	16.43	83.57	1.92	1.22	27.86	5.48
10	3.16	1.00	0.299	3.91	0.07	43.44	45.90	54.10	1.73	1.66	18.03	15.30
12	3.46	1.08	0.314	4.21	0.07	46.82	53.71	46.29	1.67	1.73	15.43	17.90
24	4.90	1.38	0.338	4.70	0.08	52.24	60.29	39.71	1.60	1.78	13.24	20.10

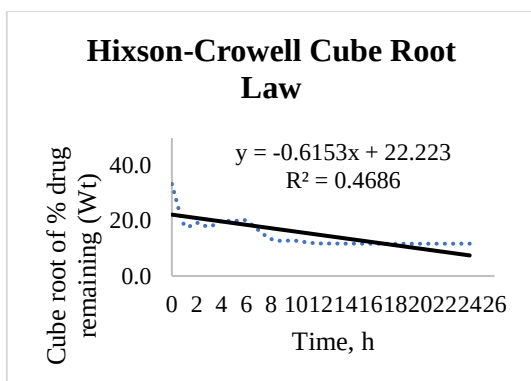
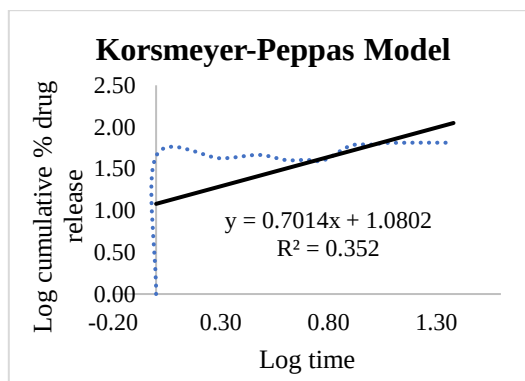
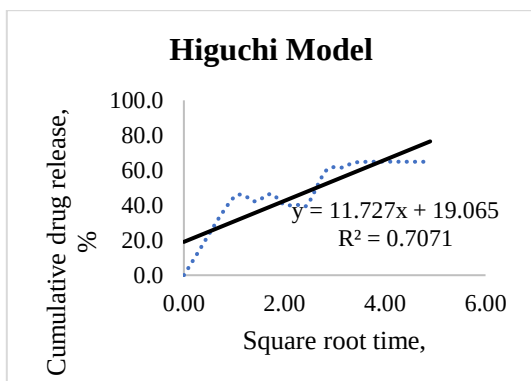
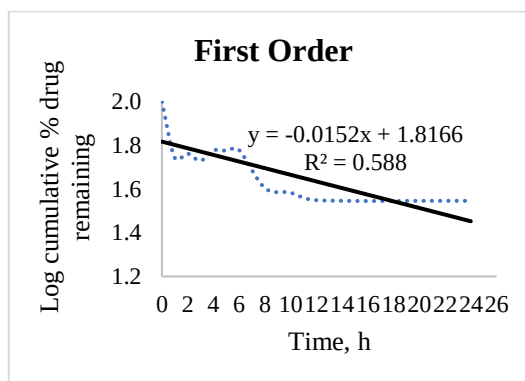
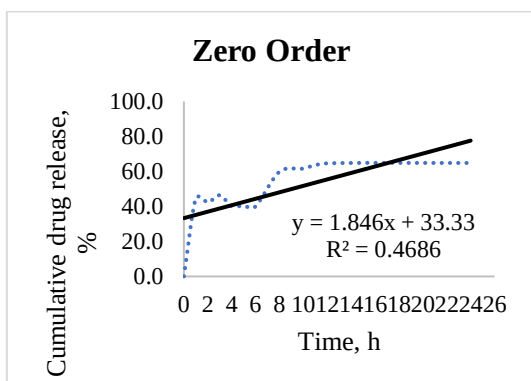
Appendix C7.1.1: Release Kinetics Modelling Graphs for pH 2.



Appendix C7.2: Calculated data on the release profile of MIP in medium pH 7

Time (h)	Square root time	Log time	Abs.	Conc., mg/L	Amount drug release, mg/g	Drug release, %	Cumulative drug release, %	Drug remaining %	Log cumulative % drug remaining	Log cumulative % drug released	Cube root of % drug remaining (Wt)	Wo-Wt
0	0.00	0.00	0.000	0.00	0.00	0.00	0.00	100.00	2.00	0.00	33.33	0.00
1	1.00	0.00	0.275	3.42	0.07	44.73	44.73	55.27	1.74	1.65	18.42	14.91
2	1.41	0.30	0.264	3.20	0.05	35.54	42.25	57.75	1.76	1.63	19.25	14.08
3	1.73	0.48	0.284	3.60	0.06	40.05	46.39	53.61	1.73	1.67	17.87	15.46
4	2.00	0.60	0.254	3.00	0.05	33.28	40.24	59.76	1.78	1.60	19.92	13.41
5	2.24	0.70	0.259	3.10	0.05	34.41	40.44	59.56	1.77	1.61	19.85	13.48
6	2.45	0.78	0.257	3.06	0.05	33.96	40.02	59.98	1.78	1.60	19.99	13.34
8	2.83	0.90	0.345	4.84	0.08	53.82	59.82	40.18	1.60	1.78	13.39	19.94
10	3.16	1.00	0.34	4.74	0.08	52.69	61.66	38.34	1.58	1.79	12.78	20.55
12	3.46	1.08	0.352	4.99	0.08	55.40	64.65	35.35	1.55	1.81	11.78	21.55
24	4.90	1.38	0.351	4.97	0.08	55.17	64.87	35.13	1.55	1.81	11.71	21.62

Appendix C7.2.1: Release Kinetics Modeling Graphs for pH 7.



Appendix D: List of Publications

1. Nordin, N. A., **Lawai, V.**, Ngaini, Z., Abd Halim, A. N., Hwang, S. S., Linton, R. E., ... Neilsen, P. M. (2020). In vitro cytotoxicity evaluation of thiourea derivatives bearing Salix sp. constituent against HK-1 cell lines. *Natural Product Research*, 34(11).
<https://doi.org/10.1080/14786419.2018.1517120>
2. **Lawai, V.**, & Ngaini, Z. (2021). Chitosan magnetic nanocomposites for gene delivery. In *Polysaccharide-based Nanocomposites for Gene Delivery and Tissue Engineering* (S., Bhawani, Z., Karim, M., Jawaidd), pp. 279-294. United Kingdom: Woodhead Publishing.
3. **Lawai, V.**, Ngaini, Z., Bhawani, S. A., Farooq, S., & Wahi, R. (2024). Microwave-assisted molecularly imprinted polymer synthesis for chemometric and rebinding mechanism of drug release applications. *Polymer Advanced Technologies*, 35(1), 1–12.
<https://doi.org/10.1002/pat.6291>
4. **Lawai, V.**, Ngaini, Z., Farooq, S., Wahi, R., & Bhawani, S. A. (2024). Current advances in molecularly imprinted polymers and their release mechanisms in drug delivery systems. *Polymer Advanced Technologies*, 35(January), 1–18.
<https://doi.org/10.1002/pat.6317>