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Research Article

Synthesis and characterisation of citric acid-based disulphide polymers for potential drug delivery application

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Abstract

Polymers containing disulphide bonds have recently been recognised as an invaluable material in delivering anticancer drugs. These polymers show promise in overcoming the non-targeted distribution of drugs in the upper parts of the gastrointestinal tract before reaching the targeted disease site. Their redox-sensitive nature and resistance to the harsh environment of the stomach and small intestine make polymers containing disulphide bonds a potential solution. The objective of this study was to synthesise and characterise citric acid-based disulphide polymer. The monomer was synthesised using amide coupling reaction between citric acid and 2-(tritylthio)ethaneamine (1) utilising a two-step synthesis method. The s-trityl group of N^1,N^2,N^3-tris(2-(tritylthio)ethyl)-2-((2(tritylthio)ethyl)amino)propane-1,2,3-tricarboxamide (2) was deprotected using trifluoroacetic acid and triethylsilane to produce N^1,N^2,N^3-tris(2-mercaptoethyl)-2-((2-mercaptoethyl)amino)propane-1,2,3-tricarboxamide (3). The successful synthesis of three compounds was confirmed using FT-IR, NMR, and CHNS analysis. Subsequently, three disulphide polymers (P10, P15, and P51) were synthesised by the oxidative polymerisation of various molar ratios of tetra-thiol monomers and dithiol monomers. The polymers were analysed using spectrometric analysis (FT-IR, FE-SEM-EDX, SEM mapping, and Raman spectrometry). The FT-IR results indicated the presence of the C–O–C stretch peak of dithiol monomer in polymers P15 and P51, whereas it was absent in polymer P10 since polymerisation involves only tetra-thiol monomer. According to SEM analysis, polymers P51 resulted in rougher and coarser surfaces, but polymer P15 has more porous surfaces. The EDX and mapping findings indicated a uniform distribution of carbon, oxygen, sulphur, and nitrogen throughout all polymers. These findings indicated that the polymerisation of all disulphide bond polymers occurred homogeneously.

Keywords: synthesis, characterisation, citric acid, disulphide polymer, oral targeted drug delivery system

Introduction

Oral drug delivery, also referred to as peroral delivery, involves taking a dosage form by mouth for either local action or systemic absorption throughout the gastrointestinal tract (GIT) [1]. Over decades, oral drug delivery has been regarded as a preferable option compared to other novel drug-delivery systems (NDDS) [2]. Patients highly favor the oral route because of its various advantages, including its user-friendly nature, lack of discomfort, non-invasiveness, inexperience, and convenience for self-administration [3]. This preference has been proven by the Generic Drugs Market Report published by Precedence Research, which reveals that oral formulations make up roughly 90% of the global market share for all human-use pharmaceutical formulations. It is worth noting that approximately 84% of the top-selling pharmaceutical products are taken orally and are currently valued at $35 billion, with an annual growth rate of 10% [4].

Despite the high level of patient compliance, the delivery of anti-cancer drugs, vaccines, and proteins via the oral route remains limited and has recorded a very low bioavailability [5]. The oral route for drug delivery continues to be challenging due to the GIT microenvironment and numerous physiological barriers, including gastrointestinal, anatomical, biochemical, and physiological factors [3]. For instance, conventional drug delivery systems, such as normal tablets, capsules, and sterile drug preparations, are associated with limitations,

including low site-specific accumulation of drugs, unfavorable body distribution, and adverse side effects [6]. Therefore, strategies have been explored by researchers and drug manufacturers to develop targeted drug delivery systems to improve the absorption and bioavailability of orally administered drugs targeted to specific regions within the GIT for the localized treatment of colonic diseases such as colorectal cancer, irritable bowel syndrome, Crohn's disease, inflammatory bowel disease, amebiosis, and local treatment of colonic pathologies.

Administration of drugs through the oral route can be classified into immediate release and controlled release based on therapeutic intentions. Controlled release systems have been extensively studied for delivering drugs to specific target sites, such as the intestines and colon. The colon, as a particular region in the gastrointestinal tract, represents an attractive environment for targeted drug delivery due to its relatively low levels of diversity and intensity for digestive enzymes compared to the small intestine. The proteolytic activity of the colon mucosa is also minimal compared to that of the small intestine, making it an ideal absorption site for sensitive drugs such as peptide-based and protein-based drugs [7]. The drugs formulated as delay-release drug systems were liberated at different time intervals (delay release) compared to the immediate-release drug systems. The delayed-release formulations were usually coated, especially with polymers to protect their active ingredients against the harsh upper gastrointestinal environment [8].

The disulphide bond is a crucial component in targeted drug delivery systems and is considered one of the most promising tools in targeted drug delivery systems. The disulphide bond is a covalent bond that is mainly produced by the oxidation of the thiol (–SH) group, particularly in cysteine residues of amino acids [9]. Disulphide polymers have been referred to as smart polymers and shown "smart" characteristics in different physical environments. Recently, reduction-responsive carriers based on disulphide bond-containing polymers have gained significant attention. As redox-sensitive polymers, disulphide reduction can be manipulated by the intracellular reductive environment. However, disulphide bonds are broken by the reduced form of glutathione (GSH) through the thiol-disulphide exchange reaction [10]. Interestingly, GSH levels in tumor tissues are known to be at least four times higher than those in normal tissues [11]. The disulphide bond remains stable in the blood circulation for an extended period, but it is broken when entering tumor cells due to the high level of GSH [10]. Therefore, disulphide polymers are capable of withstanding harsh acidic conditions in the stomach and enzymatic degradation in the small intestine, preventing premature drug release during transmission through the GIT [10]. The encapsulation of hydrophobic chemotherapeutic drugs into disulphide polymers could protect and effectively deliver the drug to the target site.

The disulphide cross-linked polymers were prepared using a self-assembly technique in which thiol groups were air-oxidized to form disulphide linkage in the polymer matrix. In this study, a disulphide cross-linked polymer with three amide bonds was synthesized. The lower solubility of the amide-containing polymer can be attributed to the increase in the ratio of amide chains [12]. The disulphide cross-linked polymer developed by Mat Yusuf et al. is characterized by the presence of three thiol groups [13]. In contrast, this study focuses on the synthesis of a cross-linked polymer that contains four thiol groups. From a structural perspective, disulphide bonds play a crucial role in facilitating polymer folding by safeguarding the hydrophobic core and stabilizing the folded configuration. These bonds are instrumental in linking multiple chains of monomers to create a cohesive polymer structure [14]. The obje ctive of enhancing the stability of polymers while reducing their solubility is to ensure that these materials do not disintegrate prematurely before reaching the designated sites in the colon. This study employs citric acid as the adhesive and coating backbone for disulphide-containing polymers. Citric acid has attracted significant interest due to its remarkable attributes, including its environmentally friendly nature, biocompatibility, affordability, biodegradability, and wide availability in commercial markets [15]. Citric acid was selected for this study due to its capability to facilitate the creation of prepolymers through a straightforward, cost-effective, and catalyst-free thermal polycondensation process that encourages the formation of ester bonds and allows for hydrolysis degradation. During the synthesis of the prepolymers, the intrinsic chemistry of the pendent hydroxyl and carboxyl groups can be partially preserved, enhancing the versatility of these materials for the conjugation of bioactive compounds [16].

In this research, a branched-chain disulphide bond polymer was prepared through amide coupling and oxidative polymerisation using citric acid. After each subproduct synthesis, the resulting compounds were subjected to spectrometric analysis, including ^{1}H-NMR, ^{13}C-NMR, CHNS, and FT-IR. Whereas the final product, synthesised polymers will be characterised by FE-SEM-EDX, SEM mapping to observe the distribution of disulphide bonding, and Raman spectrometry which is used to validate the formation of disulphide bond. Disulphide-containing polymers (P10, P15, and P51) were successfully

synthesised.

Materials and Methods

Materials

Cysteamine hydrochloride, N, N-diisopropylethylamine (DIPEA), triethylsilane (TES), diethyl ether, ethyl acetate and dichloromethane (DCM) were purchased from Merck (Darmstadt, Germany). 1-hydroxybenzotriazole hydrate (HOBT), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDAC), 2,2-(ethylenedioxy) diethanethiol, Ellman's reagent, and silica gel (40–63 µm particle size) were purchased from Sigma Chemical (St. Louis, USA). Triphenylmethanol, trifluoroacetic acid (TFA), and ammonium hydrogen carbonate were obtained from Alfa Aesar (Massachusetts, US), Thermo-scientific chemicals (Massachusetts, US), and Nacalai Tesque (Kyoto, Japan) respectively. Sodium chloride, calcium chloride, sodium bicarbonate, and sodium hydroxide were obtained from Bendosen (Selangor, Malaysia). Dimethyl sulfoxide (DMSO), citric acid, and petroleum ether were purchased from Chemiz (Selangor, Malaysia).

Preparation of monomers

Synthesis of 2-(tritylthio)ethaneamine (1)

Equal mole of cysteamine hydrochloride (0.05 mol; 5.685 g) and triphenylmethanol (0.05 mol; 13.0165 g) were mixed in 40 mL of trifluoroacetic acid (TFA) for 3 h at room temperature. A drying tube filled with calcium chloride was attached to the round bottom flask containing the reaction mixture to protect it from moisture. The acid was evaporated using Eyela N-1200BV-WD rotary evaporator (Tokyo, Japan) at 65 °C until thick and brownish oil formed. Then, the product was washed with a volume of diethyl ether until a white precipitate formed, and no more brown colour oil could be seen. The white precipitate was filtered out using filter paper and left to dry overnight. The white precipitate was partitioned between diethyl ether and 1 mol L^{-1} NaOH. The ether phase was collected, and diethyl ether was evaporated off using a rotary evaporator. The purified white colour compound (1) was collected, and it was used to synthesise a compound (2). Spectroscopic analysis (^{1}H-NMR, ^{13}C-NMR, CHNS, FT-IR) was also conducted on the purified compound. Compound (1) had the following characterisations: FT-IR (KBr disk, cm^{-1}): 3344 (primary–N–H stretching), 3262, 3038, 2923 (–C–H stretching), 1691–2098 (aromatic overtone), 1590 (–N–H bending), 1462 (aromatic ring); ^{1}H-NMR (500 MHz, $CDCl_3$); δ_H ppm, 7.41–7.66 (m, 15 H, aromatic Ar–H), 2.78–2.80 (t, 2 H, J = 6.5 Hz, CH_2–NH_2), 2.53–2.56 (t, 2 H, J = 6.5 Hz, S–CH_2); ^{13}C-NMR (125 MHz, $CDCl_3$): δ_C ppm, 144.94 (6 C, Ar–C), 129.68 (6 C, Ar–C), 127.99 (3 C, Ar–C), 126.78 (3 C, Ar–C), 66.67 (1 C, S–C–Ar_3), 40.99 (1 C, CH_2–NH_2), 36.13 (1 C, S–CH_2).

Synthesis of N^1,N^2,N^3-tris(2-(tritylthio)ethyl)-2-((2(tritylthio)ethyl)amino)propane-1,2,3-tricarboxamide(2)

First, citric acid (1 mol; 0.4803 g) was dissolved in dimethyl sulfoxide (DMSO). The citric acid solution and 4 moles (3.1946 g) of compound (1) were mixed with 50 mL of dichloromethane (DCM) and 1 mol of N, N-diisopropylethylamine (DIPEA). After ensuring the reactants were completely dissolved, the reaction flask was put into an ice bucket to decrease the reaction temperature to 0 °C. (N-(3-dimethylamine propyl)-N'-ethylcarbidiamide hydrochloride (EDAC) (1 mol) was introduced into the reaction and continued stirring at 0 °C under an inert atmosphere for 3 h with the attachment of calcium chloride filling drying tube. After this, the reactant was stored at 0 °C overnight to ensure a complete reaction. The unwanted resulting white precipitate was filtered out and then washed with 5% citric acid, 1 M of sodium bicarbonate, and 1 M of sodium chloride employing the liquid-liquid extraction method. The DCM phase was collected, and silica gel was added to the solution. A rotary evaporator evaporated the DCM solvent. The desired compound (2) was purified using column chromatography with an appropriate mobile phase of ethyl acetate: petroleum ether (65:35). The eluent which consists of the desired compound (2) was dried and collected. Spectroscopic methods (^{1}H-NMR, ^{13}C-NMR, CHNS, and FT-IR) were done on the eluate. The following data were the characteristics of compound (2): FT-IR (KBr disk, cm^{-1}): 3286 (secondary–N–H stretching), 3042 (–C–H stretching), 1729–1963 (aromatic overtone), 1670 (–C=O), 1523 (–N–H bending), 1442 (aromatic ring); ^{1}H-NMR (500 MHz, $CDCl_3$): δ_H ppm, 7.05–7.41 (m, 60 H, aromatic Ar–H), 6.33-6.47 (t, 3 H, J = 6 Hz, C=ONH), 2.89–3.05 (m, 8 H, J = 7 Hz, CH_2–NH, –CH_2–C=O), 2.29–2.55 (m, 12 H, J = 7 Hz, S–CH_2), 1.77 (s, 1 H, CH_2–NH); ^{13}C-NMR (125 MHz, $CDCl_3$): δ_C ppm, 170.30–173.44 (3C, C=ONH), 144.60 (8 C, Ar–C), 129.52 (29 C, Ar–C), 127.98 (38 C, Ar–C), 126.80 (15 C, Ar–C), 66.77, 66.81, 75.22 (3 C, S–C–Ar_3), 42.56 (4 C, CH_2–NH), 38.06–38.20 (2 C, O=C–CH_2), 31.62–31.76 (4 C, S–CH_2).

Deprotection of N^1,N^2,N^3-tris(2-(tritylthio)ethyl)-2-((2(tritylthio)ethyl)amino)propane-1,2,3-tricarboxamide (2) to N^1,N^2,N^3-tris(2-mercaptoethyl)-2-((2-mercaptoethyl)amino)propane-1,2,3-tricarboxamide (3)

The protective group (trityl group) of purified compound (2) was deprotected in the following steps and that particular reactive functional group (thiol group) was regenerated. Compound (2) was suspended in anhydrous dichloromethane (DCM) and was treated with trifluoroacetic acid (TFA). Triethylsilane (TES) was added to the reaction

mixture and left to stir for 5 h. Evaporation of the solvent was done under a vacuum using a rotary evaporator to yield a white precipitate. The reactants were washed with copious diethyl ether. The resulting compound was subjected to spectrometric analysis (^{1}H-NMR, ^{13}CNMR, CHNS, and FT-IR). The following were the characteristics of compound (3): FT-IR (KBr disk, cm^{-1}): 3391, 3291 (secondary N–H stretching), 3071, 2928 (–C–H stretching), 2550 (–S–H), 1643 (–C=O) 1523 (–N–H bending); ^{1}H-NMR (500 MHz, DMSO-d_6): δ_H ppm, 7.84-8.23 (t, 3H, C=ON<u>H</u>, J = 5.5 Hz), 6.20 (s, 1H, –N<u>H</u>, J = 5 Hz), 2.15–2.99 (q, 12H, C<u>H</u>$_2$N–, C<u>H</u>$_2$–C=ONH J =7.5Hz), 3.05–3.67 (q, 8H, C<u>H</u>$_2$S, J =6.5Hz); ^{13}C-NMR (125 MHz, DMSO-d_6): δ_C ppm, 173.24, 171.32 (3 C, <u>C</u>=O), 44.50, 43.68, 42.79, 41.33 (4 C, <u>C</u>H$_2$–NH), 32.74, 34.78 (3 C, <u>C</u>H$_2$–C=O), 26.45, 27.32 27.96, 28.71, (4 C, <u>C</u>H$_2$–SH).

Physical characterisation of synthesised compounds and monomers

Melting point analysis

The melting points of the synthesised compound (1), (2), and (3) were determined using a Stuart MP10 melting point analyser (Staffordshire, UK). The samples were prepared and placed into sealed capillary tubes. These tubes were then inserted into the melting point apparatus, where the samples were rapidly heated to a predetermined temperature. Observations were made to record both the temperature at which the samples began to melt and the temperature at which complete melting occurred.

Fourier transform infrared resonance (FT-IR)

Fourier-transform infrared spectroscopy (FT-IR) spectra of the synthesised compounds were recorded from potassium bromide (KBr) disks using a Nexus 670 FTIR spectrometer (Massachusetts, US) with spectra region from 500 to 4000 cm^{-1}.

CHNS elemental analysis

The elemental analysis was conducted using a combustion analysis using PerkinElmer 2400 CHNS/O elemental analyser (Madison, WI, USA). The combustion temperature was 950 °C and the reduction occurred at 550 °C.

Nuclear magnetic resonance (NMR)

Samples of compound (1), (2), and (3) were prepared for both ^{1}H nuclear magnetic resonance (^{1}H-NMR) and ^{13}C nuclear magnetic resonance (^{13}C-NMR) using Bruker Avance 500 spectrometer (Stuttgart, Germany) which operated at 500 MHz and 125 MHz respectively. Deuterated chloroform was used as the solvent for compounds (1) and (2), whereas deuterated DMSO was used for compound (3). Both deuterated solvents for NMR were purchased from Sigma Chemical (St. Louis, US).

Polymerisation of tetra-thiol-tetra-thiol monomers and tetra-thiol-dithiol monomers

Oxidation of compound 3

A mixture of compound (3) was stirred in ammonium bicarbonate buffer. Dimethyl sulphoxide (DMSO) was added dropwise until the solids were approximately 50% dissolved in solution. The reaction was allowed to proceed in the open air for 120 h and terminated when no more thiols could be detected with 5% sodium nitroprusside reagent which no purple colour appeared for the nitroprusside reaction. The end product was filtered and washed with distilled water and ethanol to produce a solid powder named P10.

Oxidation of compound 3 with dithiol monomer

A mixture of compound (3) and dithiol monomer (2,2’ (ethylenedioxy)diethanethiol) was stirred in an ammonium bicarbonate buffer. DMSO was added dropwise until the solids were approximately 50% dissolved in the solution. The reaction was allowed to proceed in the open air for 120 h and terminated when no more thiols could be detected with 5% sodium nitroprusside reagent which no purple colour appeared for the nitroprusside reaction. The end product was filtered and washed with water and ethanol. Two polymers were synthesised by employing different molar ratios of compound (3) and 2,2’ (ethylenedioxy)diethanethiol in the reaction as shown below:

Polymer P15 - 1.0 compound 3: 5.0 dithiol monomer
Polymer P51 - 5.0 compound 3: 1.0 dithiol monomer

Physical characteristics of synthesised polymers

Field Emission-Scanning Electron Microscopy-Energy Dispersion X-Ray (FE-SEM-EDX)

Field Emission-Scanning Electron Microscopy-Energy Dispersion X-ray (FE-SEM-EDX) was used to allow targeted analysis of sample surfaces of different polymers. Each sample of polymer was first coated with platinum using a Quorum sputter coater (Oxford, UK). The morphological images of polymers were taken using FEI Nova NanoSEM™ 450 FE-SEM microscopy (Oregon, US) with a magnification of up to 1,000 ×. Energy-dispersive X-ray (EDX) was applied using the Oxford Instruments PLC detection microanalysis system (Bucks, UK), and it is to determine the chemical composition of the polymer sample, including what elements are present along with their concentration and distribution.

Raman spectrometry

The structural compound of synthesised polymer samples was identified using the Jobin–Yvon HR 800 UV Raman spectrometer (Lower Hutt, New Zealand). The incident laser excitation wavelength was 514.5 nm with an output of 20 mW. The sample

was then placed on a highly reflective slide which is a glass microscope slide coated with gold substrate. The Raman spectra were recorded from 100 to 3000 cm^{-1} for identification of S–H and S–S peaks.

Solubility test

Solubility tests were conducted on various synthesised polymers to assess their solubility in a range of organic solvents. The solvents evaluated included acetic acid, acetone, chloroform, cyclohexane, dichloromethane, dimethyl sulfoxide, ethanol, formic acid, toluene, trifluoroacetic acid, and water. Additionally, buffer systems were prepared to simulate gastric, intestinal, and colonic conditions, excluding pepsin, pancreatin, and glutathione, respectively. For each test, approximately 0.03 g of the synthesised polymer was combined with 1 mL of dichloromethane in a 2 mL microcentrifuge tube. The mixture was then homogenised for 5 min and subsequently examined under bright light to evaluate the solubility of the polymer. These procedures were repeated using various organic solvents and phosphate buffers with different polymer samples to ensure comprehensive analysis.

Results and Discussion

Synthetic route for synthesis tetra-thiol monomer

The synthesis route for the citric acid-based tetra-thiol monomer, N^1,N^2,N^3-tris(2-mercaptoethyl)-2-((2-mercaptoethyl)amino)propane-1,2,3-tricarboxamide (3), is illustrated in Scheme 1. Compound (1) was generated through a protection reaction involving cysteamine and triphenylmethanol to commence the synthesis process. Subsequently, compound (1) was reacted with citric acid to produce N^1,N^2,N^3-tris(2-(tritylthio)ethyl)-2-((2(tritylthio) ethyl)amino)propane-1,2,3-tricarboxamide (2). Lastly, N^1,N^2,N^3-tris(2-mercaptoethyl)-2-((2-mercaptoethyl)amino)propane-1,2,3-tricarboxamide (3) was synthesised by deprotecting compound (2) to remove the trityl protecting group.

Synthesis and physical characterisation of Compound (1), (2), and (3) [tetra-thiol monomer]

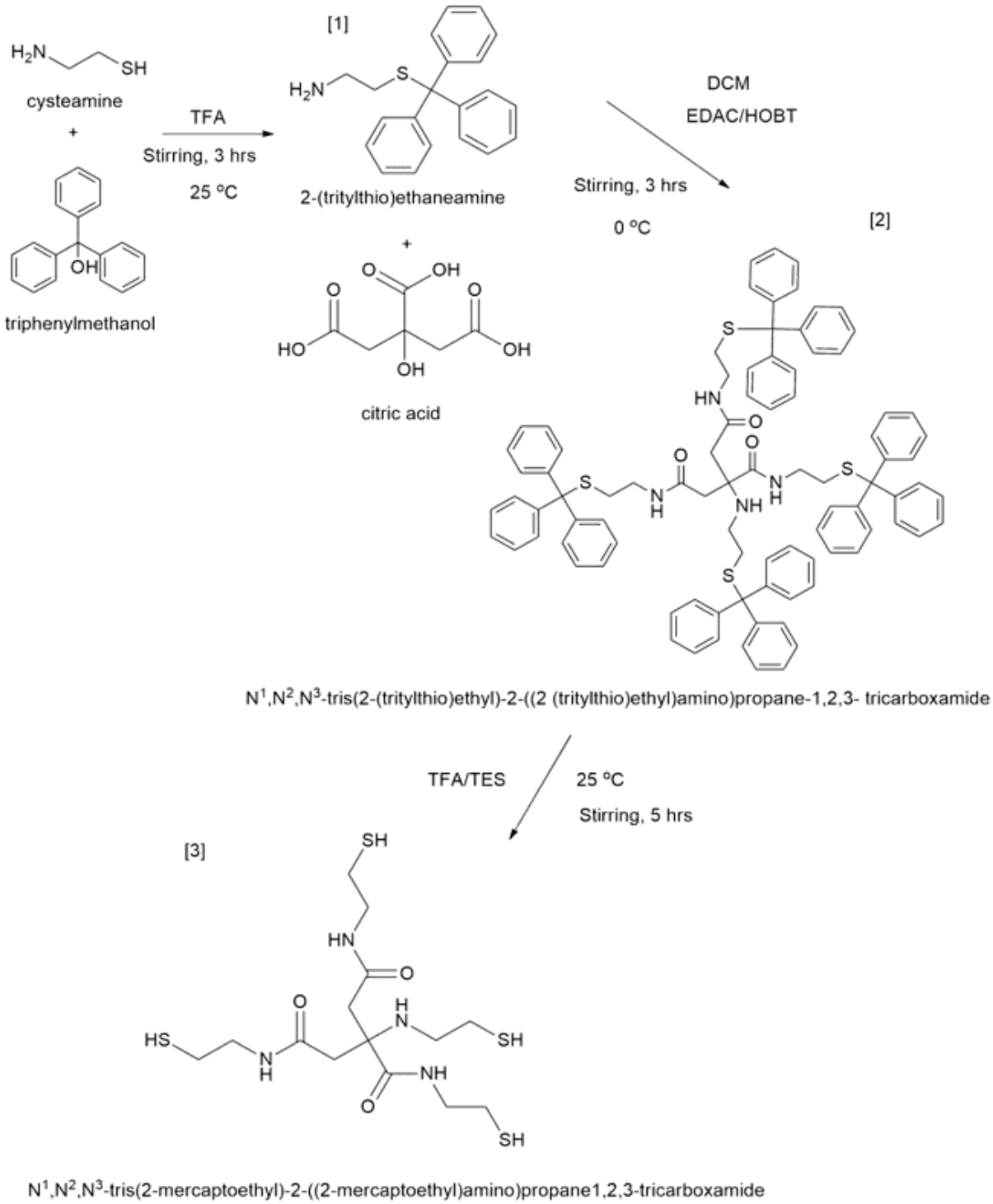
2-(tritylthio)ethaneamine (1)

The synthesis of 2-(tritylthio)ethaneamine (1) was achieved by reacting cysteamine with triphenylmethanol. The resulting compound (1) was a white powder with a yield of 93–98%, obtained through liquid-liquid extraction with a 1 M sodium hydroxide (NaOH) solution to eliminate any excess trifluoroacetic acid (TFA) residue. The melting point of compound (1) was estimated to be between 94–96 °C. Thin-layer chromatography revealed a dark spot on the TLC plate at R_f 0.65 using a solvent system consisting of ethyl acetate: methanol: acetic acid (6:3:1) (v/v/v). The ninhydrin test was conducted on the spot, confirming the presence of a primary amine group. The successful synthesis of compound (1) was further validated through Fourier transform infrared resonance (FT-IR), CHNS elemental analysis, and nuclear magnetic resonance (^{1}H and ^{13}C-NMR). The FT-IR results showed peaks at 3262 cm^{-1} and 3344 cm^{-1}, indicating the presence of a primary amine group (–NH), while the peak at 1590 cm^{-1} indicated the presence of amine bending. The infrared spectra exhibit peaks at 3038 cm^{-1} and 2923 cm^{-1}, which correspond to the appearance of C–H stretching. Moreover, the weak combination and overtone absorptions present between 2000 cm^{-1} and 1691 cm^{-1} are indicative of the presence of aromatic groups. The synthesis of 2-(tritylthio)ethaneamine was successful, which was achieved by combining the triphenyl group from triphenylmethanol and the ethane skeleton with the free thiol group from cysteamine. The formation of the compound was further confirmed through ^{1}H-NMR analysis, which revealed the presence of a triphenyl methyl group as multiplets at δ 7.41–7.66 ppm (m, 15H aromatic). The elemental composition of the compound was determined using elemental analysis, which is an analytical technique used in this study to determine the composition of chemical compounds and their composites. The experimentally obtained weight percentages of carbon, hydrogen, nitrogen, and sulphur of compound (1) are similar to the theoretical values calculated based on the empirical formula of compound (1) ($C_{21}H_{21}NS$).

N^1,N^2,N^3-tris(2-(tritylthio)ethyl)-2-((2(tritylthio) ethyl)amino)propane-1,2,3-tricarboxamide (2)

A citric acid skeleton free of hydroxyl (–OH) groups was combined with compound (1) to synthesise N^1,N^2,N^3-tris(2-(tritylthio)ethyl)-2-((2(tritylthio) ethyl)amino)propane-1,2,3-tricarboxamide (2) which was protected with four trityl groups. A white powder form of compound (2) was obtained with a percentage yield of 33–38% after purifying it using column chromatography at R_f 0.75, with a mobile phase consisting of ethyl acetate and petroleum ether in a ratio of 6.5:3.5 (v/v). The melting point of the compound was recorded as 266 to 269 °C. The FT-IR spectrum of the compound showed a negative shift in the N–H bending peaks from 1590 cm^{-1} (**Figure 1 (1)**) to 1523 cm^{-1} (**Figure 1 (2)**), indicating the conversion of the primary amine group to a secondary amine group due to the reaction between compound (1) and citric acid. A new peak at 1670 cm^{-1} was observed, indicating the presence of a carboxyl group. Additionally, weak combination and overtone peaks indicated the appearance of an aromatic ring group between peaks 1729–1963 cm^{-1}. The successful synthesis of compound (2) was further confirmed by ^{1}H-NMR analysis, which showed the appearance of a proton peak at 6.33–6.47

ppm (t, 4H, CONH, J = 6.0 Hz) and 7.05–7.41 ppm (m, 60H, aromatic C–H), indicating that compound (1) and citric acid were successfully linked. The elemental analysis of compound (2) in **Table 1** showed a percentage of elements that agreed with the empirical formula of the structure ($C_{90}H_{84}N_4O_3S_4$).



Scheme 1. Synthetic route for preparing N^1,N^2,N^3-tris(2-mercaptoethyl)-2-((2-mercaptoethyl)amino)propane-1,2,3-tricarboxamide (3) (tetra-thiol monomer)

N^1,N^2,N^3-tris(2-mercaptoethyl)-2-((2-mercaptoethyl) amino)propane-1,2,3-tricarboxamide (3)

N^1,N^2,N^3-tris(2-mercaptoethyl)-2-((2-mercaptoethyl) amino)propane-1,2,3-tricarboxamide (3), also known as a tetra-thiol monomer, was synthesised by deprotecting the four trityl groups of compounds (2). Trifluoroacetic acid (TFA) was utilised to eliminate the trityl group, resulting in a yellowish solution upon addition. Subsequently, triethylsilane (TES) was added to prevent the reverse reaction by scavenging it, yielding a colourless triphenylmethane solution [17, 18, 19, 20, 21]. The reaction produced a white powdery solid with a yield of 81–87% and a melting point of 177–180 °C. A simple, rapid, and qualitative nitroprusside test was conducted to assess the presence of free thiol/sulfhydryl groups (–SH), with a positive result indicated by the appearance of a violet-coloured complex [22]. According to **Figure 1 (3)**, the weak combination and overtone peaks that signalled the presence of the aromatic ring group disappeared. A new peak emerged at 2550 cm^{-1}, which was attributed to the appearance of the thiol group in compound (3), and a peak at 1643 cm^{-1} showed the presence of the carbonyl group of amides. These results were confirmed by ^{1}H-NMR and ^{13}C-NMR analyses, which demonstrated the absence of protons and carbons from the aromatic rings. For elemental analysis, the experimental elemental percentage was almost identical to the theoretical elemental percentage of compound (3) with the empirical formula $C_{14}H_{28}N_4O_3S_4$. These findings validated the successful synthesis of the tetra-thiol monomer (3).

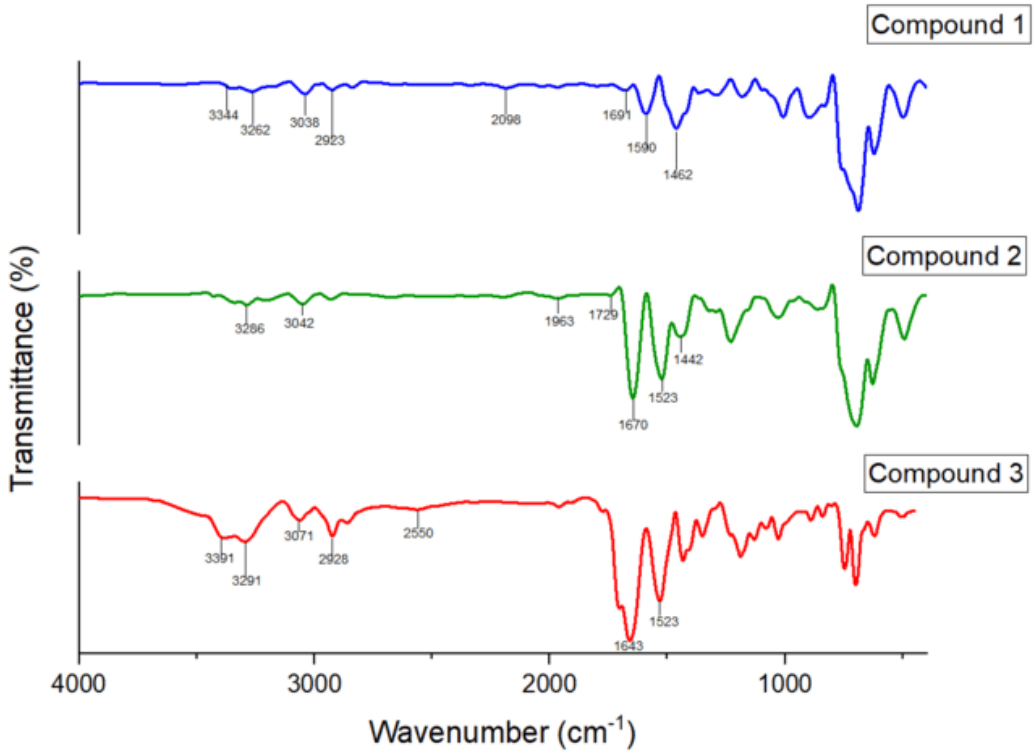


Figure 1. FTIR spectra of 2-(tritylthio)ethaneamine (1), N^1,N^2,N^3-tris(2-(tritylthio)ethyl)-2-((2 (tritylthio)ethyl)amino)propane-1,2,3-tricarboxamide (2), and N^1,N^2,N^3-tris(2-mercaptoethyl)-2-((2-mercapto ethyl)amino)propane-1,2,3-tricarboxamide (3)

Table 1. Percentage of elements carbon, hydrogen, nitrogen, and sulphur of compound (1), (2), (3)

Compound	Carbon (%)		Hydrogen (%)		Nitrogen (%)		Sulphur (%)	
	Theoretical	Actual	Theoretical	Actual	Theoretical	Actual	Theoretical	Actual
Compound 1	78.95	76.53	6.64	6.212	4.38	4.87	10.04	9.949
Compound 2	77.32	75.93	6.07	5.855	4.00	4.49	9.18	8.933
Compound 3	39.22	40.11	6.60	5.899	13.07	12.02	29.92	28.962

Synthesis and physical characterisation of disulphide containing polymers (P10, P15, and P51)

Disulphide-containing polymers such as P10, P15, and P51 were synthesised using oxidative polymerisation of varying molar ratios of tetra-thiol monomer (compound (3)) and dithiol monomer (2,2'(ethylenedioxy)diethanethiol). The resultant white suspension was created through oxidative polymerisation in the presence of an oxidising agent, dimethyl sulfoxide (DMSO) in ammonium bicarbonate buffer. The process of DMSO-promoted oxidation of thiols to disulphides was first described by Wallace and Mohan [23, 24]. This method is advantageous as it works over an extended pH range of 3–8, has faster reaction rates, and improves the solubility of the materials being oxidised [23, 25, 26]. Ammonium bicarbonate buffer served as both a base and catalyst, facilitating the oxidation of thiols to disulphides during the reaction [27]. A negative result in the nitroprusside test indicated the absence of a thiol group, which confirmed the successful completion of the oxidative polymerisation process.

Physical appearance of disulphide containing polymers

Table 2 below illustrates the physical characteristics of synthesised disulphide bond-containing polymers with various molar ratios.

Solubility test of disulphide containing polymer

A range of organic solvents with varying types and polarities was employed to evaluate the solubility of disulphide-containing polymers. The solvents utilised in the solubility assessment included acetone, acetonitrile, chloroform, dichloromethane (DCM), dimethylsulfoxide (DMSO), ethanol, toluene, and water. Besides, buffer systems which were prepared to simulate gastric, intestinal, and colonic conditions, excluding pepsin, pancreatin, and glutathione, respectively were also tested. As detailed in **Table 3**, polymers P10 and P51 exhibited insolubility across all tested solvents and buffer, whereas polymer P15 demonstrated partial solubility in DMSO. This observation suggests that an increased proportion of dithiol monomers correlates with enhanced solubility of the polymer, particularly in DMSO. As a result, DMSO was chosen as the oxidising reagent for the polymerisation of the tetra-thiol monomer, facilitating the effective formation of the disulphide bond polymer. DMSO is recognised as an excellent oxidising agent, capable of efficiently converting thiols into disulphides [28].

Table 2. Physical appearance of synthesised disulphide bond containing polymers (P10, P15, and P51)

Polymers	Physical Appearance
P10	White fine powder
P15	Thin white film
P51	Rugged white solid

Table 3. Result of solubility test of synthesised disulphide bond containing polymers (P10, P15 and P51)

Solvents	Polymer		
	P10	P15	P51
Acetone	Insoluble	Insoluble	Insoluble
Acetonitrile	Insoluble	Insoluble	Insoluble
Chloroform	Insoluble	Insoluble	Insoluble
Dichloromethane (DCM)	Insoluble	Insoluble	Insoluble
Dimethylsulfoxide (DMSO)	Insoluble	Partially soluble	Insoluble
Ethanol	Insoluble	Insoluble	Insoluble
Toluene	Insoluble	Insoluble	Insoluble
Water	Insoluble	Insoluble	Insoluble
Buffer system simulating gastric (without pepsin)	Insoluble	Insoluble	Insoluble
Buffer system simulating small intestinal (without pancreatin)	Insoluble	Insoluble	Insoluble
Buffer system simulating colon (without glutathione)	Insoluble	Insoluble	Insoluble

Fourier-transform infrared spectroscopy (FT-IR) analysis of synthesised disulphide-containing polymer

The outcomes of the FT-IR analysis for the three polymers are presented as follows: FT-IR (KBr disk, cm^{-1}): P10: 3349 (–NH stretching), 3062 ($–CH_2$), 1648 (–NHC=O), 1223 (–CN); P15: 3487 (–NH stretching), 2928 ($–CH_2$), 1652 (–NHC=O), 1303 (–CN), 1103 (C–O–C stretching); P51: 3320 (–NH stretching), 2928 ($–CH_2$), 1657 (–NHC=O), 1213 (–CN), 1098 (C–O–C stretching). Based on the FT-IR results of all three polymers, the peaks of the amide group were detected at 3062 cm^{-1}, 3487 cm^{-1}, and 3320 cm^{-1} for polymers P10, P15, and P51, respectively.

New peaks at 1103 cm^{-1} and 1098 cm^{-1}, indicating the presence of C–O–C stretching, were observed in the FT-IR spectra of polymers P15 and P51. However, the peak of C–O–C stretching was not observed in polymer P10 because only tetra-thiol monomers were involved in polymerisation. From the FT-IR results, the peak that indicates the presence of the thiol (–SH) group disappeared from all three polymers. These results demonstrated the successful synthesis of all polymers by oxidative polymerisation.

Raman spectrometry of disulphide containing polymer

The formation of disulphide bonds in three distinct polymers (P10, P15, and P51) was validated using Raman spectroscopy. The Raman spectroscopy revealed the presence of a disulphide peak in all three synthesised disulphide polymers, which was found to be located between 425 and 550 cm^{-1} (**Figure 2**). Additionally, the presence of C–S bonds in the polymers was indicated by some peaks observed between 670 and 780 cm^{-1}.

Field emission-scanning electron microscopy (FE-SEM) morphology, energy dispersion X-ray (EDX) micrograph and elemental mapping of disulphide containing polymer

Field emission scanning electron microscopy (FE-SEM) serves as a highly effective tool for the advanced characterisation of polymers. This technique enables the production of exceptionally detailed and magnified images by scanning the polymer surface. Additionally, energy-dispersive X-ray (EDX) analysis provides valuable insights into the elemental composition of the polymers, while elemental mapping offers a comprehensive overview of the composition across specific areas of the materials. The surface morphology of the polymers P10, P15, and P51 at a magnification of 1000× is illustrated in **Figure 3**. Notably, the surfaces of all examined polymers exhibit uneven characteristics.

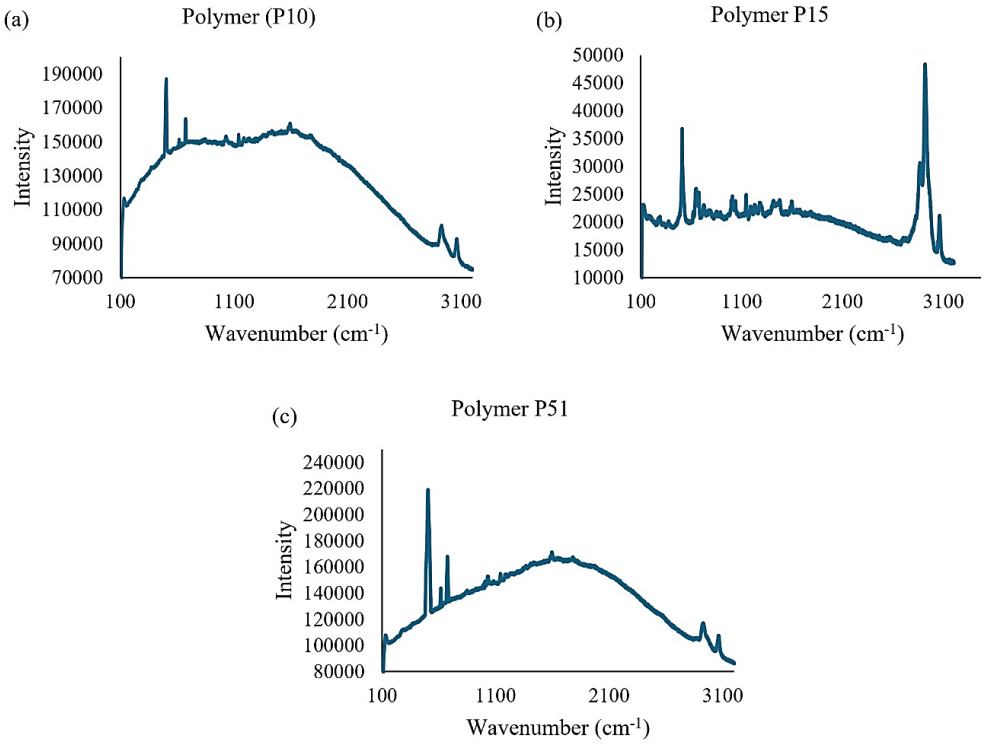


Figure 2. Raman spectra of polymer (a) P10 (b) P15 and (c) P51

Polymer P10 displayed a rough and coarse surface when observed under 1000× magnification (**Figure 3(a)**). This surface morphology resulted from the oxidative polymerisation process involving only tetra-thiol monomers, which led to the formation of a denser region within the polymer network. In comparison, the surfaces of polymers made from a combination of tetra-thiol and dithiol monomers appeared more porous than those polymers that were synthesised from only tetra-thiol monomers [29, 30]. Among these three polymers, P15 (**Figure 3(b)**) exhibited the most uneven surface and highest surface porosity. The polymer network of P15 is loosely structured due to the higher proportion of dithiol monomer, whereas the surface morphology of P51 is tighter because of the higher proportion of tetra-thiol monomer. According to multiple investigations, polymers with higher molecular weights exhibit a more irregular topography, which aligns with the findings of scanning electron microscopy [13, 29, 30].

The spectra for the polymers are depicted in **Figure 4**, which includes subfigures (a), (b), and (c). The elemental mapping clearly demonstrated the presence of carbon, nitrogen, oxygen, and sulphur in the disulphide polymer. The mapping revealed that the sulphur distribution was higher in the tightly structured polymer (P10) compared to the loosely structured polymers (P15 and P51). Moreover, the oxygen and sulphur maps exhibited similar intensity distributions for all the polymers, indicating that they reacted uniformly.

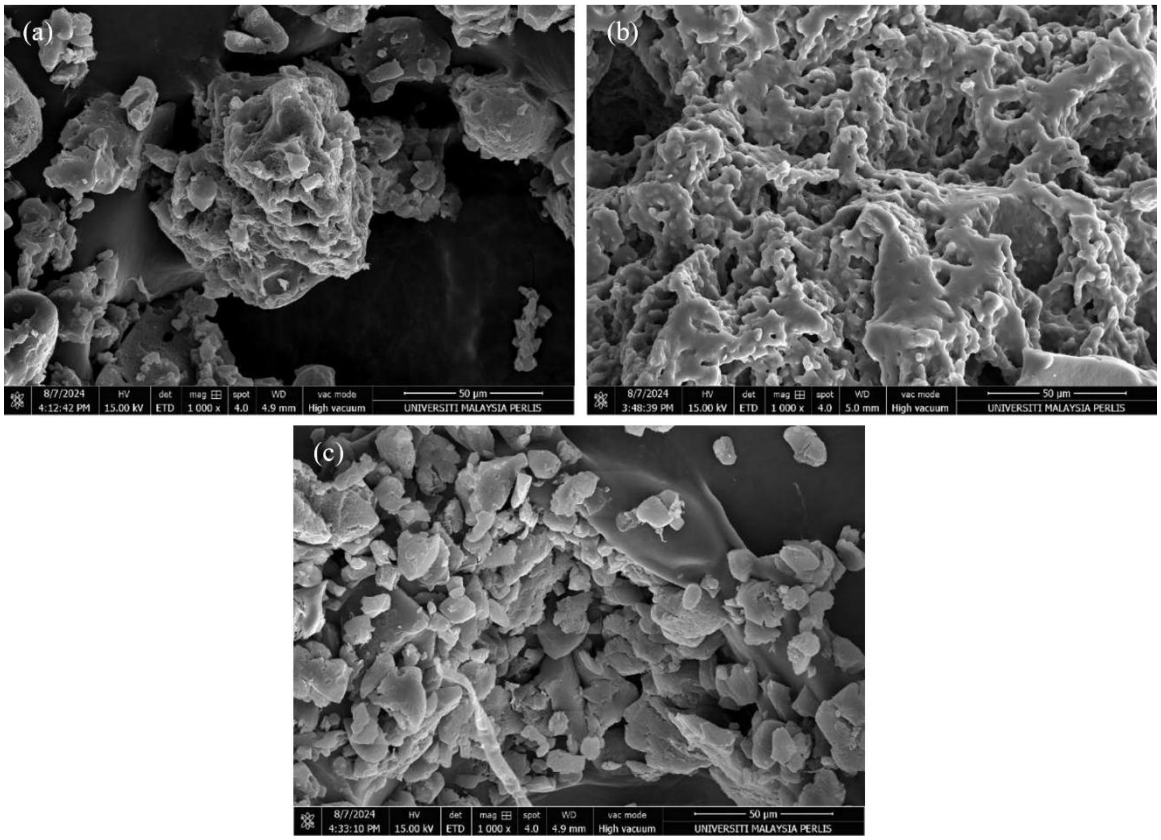


Figure 3. Field emission-scanning electron microscope (FE-SEM) micrographs of polymers (a) P10 (b) P15 and (c) P51 at 1000 × magnification

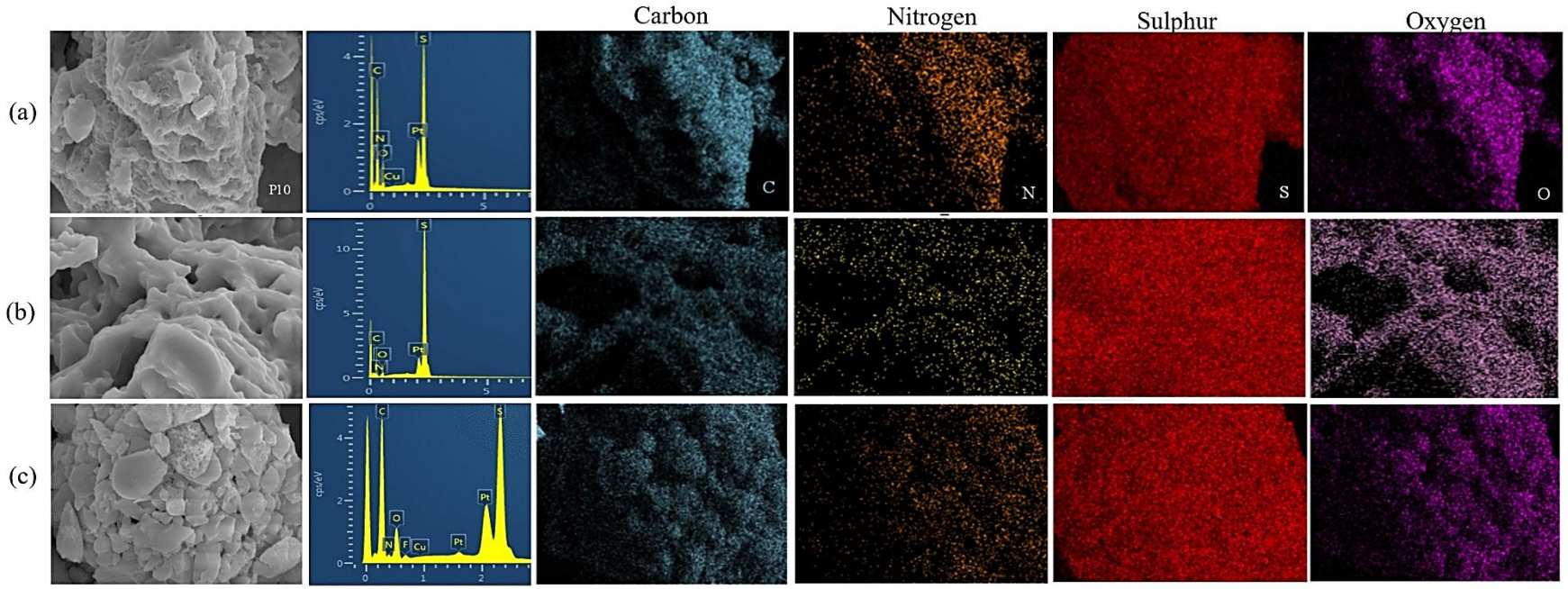


Figure 4. SEM micrograph, EDX, and elemental mapping for carbon, nitrogen, oxygen, and sulphur of polymer (a) P10, (b) P15, and (c) P51 at 2500× magnification

Conclusion

The synthesis of disulphide-containing polymers has been successfully achieved through the application of dimethylsulfoxide (DMSO)-promoted oxidative polymerisation. Three distinct types of disulphide polymers were developed by varying the molar ratios of tetra-thiol polymer and dithiol monomer. The resulting compounds and polymers underwent comprehensive characterisation utilising a variety of analytical techniques, including nuclear magnetic resonance (NMR), Fourier-transform infrared spectroscopy (FT-IR), CHNS elemental analysis, solubility testing, Raman spectrometry, field emission scanning electron microscopy with energy-dispersive X-ray spectroscopy (FE-SEM-EDX), and elemental mapping. The physical characterisation of the disulphide polymer was conducted successfully, revealing that the proportions of both tetra-thiol and dithiol monomers significantly influence the structural properties of the polymers. However, further investigation is warranted to assess the potential application of the synthesised disulphide polymer in oral drug delivery systems. This should include evaluations of the mechanisms and kinetics of drug encapsulation, as well as analyses of cytotoxic effects and cellular uptake associated with the drug-encapsulated polymers.

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