Synthesis and Properties of the Metallo-Supramolecular Polymer Hydrogel
Poly[methyl vinyl ether-alt-mono-sodium maleate]-AgNO₃

Thesis by

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EXAMINATION COMMITTEE APPROVAL FORM

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ABSTRACT

Gels are a special class of materials which are composed of 3D networks of crosslinked polymer chains that encapsulate liquid/air in the matrix. They can be classified into organogels or hydrogels (organic solvent for organogel and water for hydrogel). For hydrogels that contain metallic elements in the form of ions, the term of metallo-supramolecular polymer hydrogel (MSPHG) is often used. The aim of this project is to develop a kind of new MSPHG and investigate its properties and possible applications. The commercial polymeric anhydride poly(methyl vinyl ether-alt-maleic anhydride) (PVM/MA) is converted by reaction with NaOH to give poly(methyl vinyl ether-alt-monosodium maleate) (PVM/Na-MA). By addition of AgNO₃-solution, the formation of the silver(I) supramolecular polymer hydrogel poly[methyl vinyl ether-alt-mono-sodium maleate]∙AgNO₃ is obtained. Freeze-dried samples of the hydrogel show a mesoporous network of polycarboxylate ligands that are crosslinked by silver(I) cations. The supercritical CO₂ dried silver(I) hydrogel was characterized by FT-IR, SEM-EDAX, TEM, TGA and Physical adsorption (BET) measurements. The intact silver(I) hydrogel was characterized by cryo-SEM. In the intact hydrogel, ion-exchange studies are reported and it is shown that Ag⁺ ions can be exchanged by copper(II) cations without disintegration of the hydrogel. The silver(I) hydrogel shows effective antibacterial activity and potential application as burn wound dressing.
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<td>3D</td>
<td>Three-dimensional</td>
</tr>
<tr>
<td>1D</td>
<td>One-dimensional</td>
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<tr>
<td>CPD</td>
<td>Critical point drier</td>
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<tr>
<td>DTGA</td>
<td>Differential thermal analysis</td>
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<tr>
<td>Dex-MA-SA</td>
<td>methacrylated and succinic derivative of dextran</td>
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<td>EDS</td>
<td>Energy dispersive spectroscopy</td>
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<td>FTIR</td>
<td>Four transform infrared spectroscopy</td>
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<tr>
<td>ICP</td>
<td>Inductively coupled plasma</td>
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<tr>
<td>LB broth</td>
<td>Luria-bertani broth</td>
</tr>
<tr>
<td>MIC</td>
<td>Minimum inhibitory concentration</td>
</tr>
<tr>
<td>MOF</td>
<td>Metal organic frameworks</td>
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<tr>
<td>MSPG</td>
<td>Metallo-supramolecular polymer gels</td>
</tr>
<tr>
<td>MSPS</td>
<td>Metallo-supramolecular polymers</td>
</tr>
<tr>
<td>NaBH₄</td>
<td>Sodium borohydride</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear magnetic resonance</td>
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<tr>
<td>NP’S</td>
<td>Nano particle’s</td>
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<tr>
<td>PAA</td>
<td>Poly acrylic acid</td>
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<tr>
<td>PBS</td>
<td>Phosphate buffer saline</td>
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<td>PHEA–SA</td>
<td>methacrylated and succinic derivative of α,β-poly(N-2-hydroxyethyl)-DL-aspartamide</td>
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<td>PXRD</td>
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CHAPTER 1: Introduction

Gels are defined by being a special state of matter with intermediate properties between solid and liquid states. They have been interesting materials over the past decades due to their many important characteristics that allows them to be potential candidates in many technologies.\textsuperscript{1-3} Although gels are mainly composed from liquid, yet they show mechanical strength very similar to solids. This is explained by their structure that is a cross-linked network that extends through the liquids and more specifically, it depends on the strength of bonding. Gels are classified and defined as follows:

1. Chemical gels:

This type of chemical gels is defined by the crosslinking polymeric chain which creates strong bonds of covalent nature between chains. Generally, many crosslinking methods have been used to obtain the chemical gels where crosslinking can either occur during the polymerization or in a consequent step following polymer synthesis. One example of formation of chemical gels is through condensation polymerization reaction of multifunctional monomers. During this reaction, the monomers grow to form polymers and then molecular networks and the gel is obtained when the molecular weights of these networks become infinite. An example of condensation polymerization is the reaction between adipic acid and ethylene glycol to form the ester bond which is the crosslinking point.\textsuperscript{4} The Figure 1.1 shows the schematic diagram for crosslinking mechanism showing the the crosslinking points.\textsuperscript{4}
2. Physical gels:

Physical gels are formed when non-covalent bonds are involved such as hydrogen bonding, ionic or chelate formation, or Van-der-weal interactions forces. These type of interactions are called secondary forces which yield relatively unstable gels. Physical gels can undergo sol-gel transition upon change in pH, temperature, or ionic strength. During these transitions, the structure of the gel is collapsed reversibly transforming into solution state and back to gel state when original gel formation conditions are back in place. These types of gels are therefore called reversible gels. They are of great importance in many applications.
Another classification is based on the encapsulated medium. That can be organic solvent (organo gel), air (aerogels), and water (hydrogel).

3. Aerogel:

Aerogels are defined being a special class of gels and solid materials. Their main constituent is air where it forms almost 95% of their volume. Air is surrounded by solid network which offers these type of gels many fascinating properties such as low densities, and wide pores, and high inner surface area. This affords them special physical properties such as extremely low thermal conductivity, and high optical transparency. In general they are prepared using the conventional sol-gel method starting from molecular precursors, and then the obtained gel which forms a solid 3D network filled with liquid. Then it is dried using mainly supercritical drying which keeps the solid network of the aerogels rigid and the pores are retained. Recently new drying techniques were used which involve chemically treating the gels before removing the liquid. The characteristics of gel depend generally on the molecular structure of starting precursors.

4. Hydrogels:

In the last 50 years, hydrogels received much attention due to their interesting properties, which make them amenable to many technologies and applications. In the coming parts the definition of hydrogels, structure, characteristics and most famous applications are explained.
1.1 Hydrogels

In general, a hydrogel is defined as a network of polymer chains that are dispersed in water without being dissolved therefore forming colloidal suspensions. However, many definitions have been assigned to these materials throughout the past decades such as water-swollen and cross-linked polymeric network which may be produced by simple reaction of various monomers. Furthermore, hydrogels are known as polymeric materials which can store large amounts of water in their structure causing them to swell. The structure of the hydrogel mainly determines its properties such as flexibility and mobility. Due to solvation and incorporation of water, the degree of flexibility is comparable to natural tissues. The gel constituents (polymer macromolecules) are spread in the 3D structure and therefore enhance the mobility of polymer macromolecules. The swelling ability of hydrogels is mainly controlled by the hydrophilic functional groups which are attached on the polymeric backbone. The crosslinking between polymer chains makes them resistible to dissolution and offers them hardness. The polymers can be either synthetic or natural. Natural polymers have been widely used previously due to their nontoxic and biocompatible nature some examples include collagen, gelatin, fibrin and alginate and many others. Nonetheless, synthetic polymers have been replacing natural polymers in hydrogels due to their long life-time, mechanical strength and high water absorption capacity. Due to their well-defined structure, synthetic polymers provide the advantage of designing hydrogels with both degradability and functionality.
1.2 Classification of hydrogels

Hydrogels can be classified into several categories depending on various characteristics. These characteristics are:

1. Source: Hydrogels can contain natural or synthetic polymer.\textsuperscript{12}

Polymeric composition: The preparation method results in distinct of structure polymers resulting in homopolymeric, copolymeric, or multipolymer hydrogels. Homopolymer hydrogels are synthesized starting from one type of monomer.\textsuperscript{13} The homopolymer strands are cross-linked using different crosslinking techniques. Copolymer molecules hydrogels are formed when combining two or more different monomer species. Finally, multipolymer interpenetrating polymeric hydrogels (IPN), are synthesized from two independent cross-linked polymer components which are crosslinked together to form a network structure.

2. Configuration: 1) amorphous (non-crystalline), 2) semi-crystalline (mixture of crystalline and amorphous, or crystalline).

3. Type of crosslinking (chemical or physical interactions), where chemical interactions include covalent bonding which is permanent and physical interactions include hydrogen bonding, ionic or hydrophobic interactions.

4. Physical appearances (matrix, film, or microsphere). The physical appearance is related to the preparation method.

5. Network electrical charge: Hydrogels may be categorized into four groups on the basis of presence or absence of electrical charge located on the cross-linked chains.\textsuperscript{14}
a. Nonionic (neutral).

b. Ionic (including anionic or cationic).

c. Amphoteric electrolyte (ampholytic) containing both acidic and basic groups.

d. Zwitterionic (polybetaines) containing both anionic and cationic groups in each structural repeating unit.

The characteristics of hydrogels obviously show that they possess great potential for several applications. However, designing a hydrogel system with specific predefined physicochemical parameters requires the knowledge with of polymer network synthesis and chemistry.

1.3 **Hydrogel technical features**

Due to many technical features of hydrogels, this family of attracted lots of attention in several applications. This is observed by an increase of studies and development. Some desirable characterizations of hydrogels are listed below: 15

1) High swelling capacity of water into 3D structure of gels.

2) Low price

3) High mechanical and photo stability

4) Non-toxic and degradable

5) Porous structure of some materials

6) Colorless, odorless, and biocompatible.
Not all of these characteristics are usually found in a particular gel. However, depending on the application the compromise between parameters should be optimized to be suitable for that specific application. Hydrogels have been employed in several applications such as hygienic products, agriculture, drug delivery systems, sealing, coal dewatering, artificial snow, food additives, pharmaceuticals, biomedical applications, tissue engineering and regenerative medicines, diagnostics, wound dressing, separation of biomolecules or cells, and barrier materials to regulate biological adhesions, and as biosensor.

The following paragraph gives an overview about hydrogel applications in tissue engineering, antibacterial activity and drug delivery.

1.4 Applications of hydrogels

1.4.1 Tissue engineering

For a hydrogel to be considered as a candidate for tissue engineering, it must possess several important parameters. These include suitable physical properties (such as mechanical flexibility and strength as well as low rate of degradation. Furthermore, the hydrogel must mimic the behavior of the human tissue in terms of cell adhesion and function. Most importantly the hydrogel must possess biocompatibility to the body. This is defined by its ability to adapt to human cells and hence act as “self-cells” and not to destroy the adjacent cells or trigger the immune system. Otherwise, it will cause inflammation and the body
will attack the transplanted cells on addition to distribution of body’s physical state.\(^{30,31}\) Natural polymers are more biocompatible than synthetic polymers, but they possess less-stable structures than the synthetic polymers. However, one should select the synthetic method that balances between biocompatibility and stability. For example, although ionic crosslinking with multivalent ions provides easy access to hydrogels, such gels are prone to degradation due to ion exchange with ions from aqueous solutions.\(^{32}\) On the other hand, although covalent crosslinking provides specific control into the crosslinking density of hydrogels, most of the cross-linkers are toxic and non-degradable which is a disadvantage. Therefore, it is important to utilize other synthetic methods such as \textit{in situ} crosslinking \(^{33}\) and utilizing phase transition behavior or specific polymers.\(^{34}\) Eventually, mechanical properties of hydrogels are crucial parameters in tissue engineering as they depend on rigidity the polymer, crosslinker, as well as the swelling of the gel.\(^{35}\) The mechanical properties should be chosen with care since the gel should preserve space for tissue to grow. Furthermore, the adhesion and gene expression of cells are strongly influenced by hydrogel mechanical properties.\(^{36}\) Another very important factor is gel degradation. The ultimate scenario is to coincide the rate of degradation of polymer with the rate of tissue growth.
1.4.2 Antibacterial activity

Hydrogels with have been recognized as suitable host materials for metal nanoparticle composites especially silver nanoparticles which affords them antibacterial properties. Silver nanoparticles emerged as antibacterical agents with improved properties due to their electronic effects which modifies the local electronic structure on the surface of nanosized particles thus increasing the reactivity of these nanoparticles.\textsuperscript{37,38} Ag nanoparticles attack the bacterial enzyme (DNA Polymerase) by inhibiting its growth through preventing DNA replication.\textsuperscript{39} They can also attach to the bacteria and destabilize the plasma membrane. These nanoparticles are delivered to the body in many forms such as thin films, sol-gels and polyelectrolyte and porous polymers and Ag-NPs doped biocompatible polymers.\textsuperscript{40} Hydrogels are suitable growth media for metal nanoparticles where the 3D network structure provides free space for nucleation of nanoparticles and their growth as well. This approach prevents the aggregation of nucleated nanoparticles thus keeping their size distribution in required range (less than 3 nm).\textsuperscript{41} This approach has been used for antibacterial as well as wound dressings.\textsuperscript{3}

1.4.3 Controlled release drug delivery systems

Hydrogels are attractive materials in field of controlled release and drug delivery systems. Due to their unique physical properties of hydrogels, they are able to swell without dissolving in biological fluids. This characteristic is attributed to the
crosslinking within the hydrogel matrix structure.\textsuperscript{42} In addition to being hydrophilic, it allows them to swell, absorbing a huge amount of water (approximately 1000 times their dry weight). The presence of hydrophilic groups such as hydroxyl, carboxyl, amide and thioester groups provides strong interaction between the hydrogel network and the solvent. The covalent and physical interactions within the hydrogel matrix add stability to the hydrogel in the presence of solvents.\textsuperscript{43} The porosity of network structure can be tailored by modifying the sensitivity of crosslinkers in the gel matrix. The drug can be incorporated into the gel matrix generally by two methods: 1) “In- situ loading” where the polymer precursors and the drugs are mixed together. Hence, the crosslinking occurs simultaneously to drug loading. The other method is “post loading”. It involves encapsulation of drug into the already formed gel matrix through diffusion of the bioactive molecules.\textsuperscript{44} The drug trapping into the gel matrix is determined by diffusion coefficient of bioactive molecules.\textsuperscript{45} The release of the drug from the gel into the body can be due to many factors such as diffusion, swelling of gel, and changes in gel structure due to pH sensitivity of biodegradability. Hence to control the rate of drug release, many parameters can be controlled most importantly the network structure, the diffusion property of drug and mesh size. Recently, various forms of hydrogels were reported for controlled drug delivery systems. Some of these hydrogels showed bioadhessiveness do incorporation of molecular adhesion promoters.\textsuperscript{46} This enhances the drug immobilization to the targeted site consequently makes it suitable for protein and drug delivery. As mentioned earlier many hydrogels are
biocompatible in nature with a controllable degradation property. One example of degradable hydrogels is the “polysaccharide/polyaminoacid composite hydrogel, obtained by UV irradiation, without radical initiators. A mixture of a methacrylated and succinic derivative of dextran, Dex-MA (named Dex-MA-SA) and methacrylated and succinic derivative of α,β-poly(N-2-hydroxyethyl)-DL-aspartamide (PHEA) named PHEA–SA were photocrosslinked to obtain the desired hydrogel named PHM-SA. This hydrogel showed release of anticancer drug (2-methoxyestradiol) due to biodegradability by dextranase and esterase in the colon exclusively. This makes it a promising alternative as a drug delivery candidate for treating colon-rectal cancer. This is due to the biodegradability and the pH sensitivity of the drug in the conditions of intestinal fluid. The degradability is further enhanced by the presence of specific enzymes in the colon. This special feature makes them suitable to act as protein covers to protect the drug as well as injectable devices into specific sites. The ability to tune the degradation behavior of hydrogel is attractive in field of drug delivery since it allows control the rate of drug release and thus keeping constant levels of drug while treatment. This maximizes the absorption of drug and widens the therapeutic window thus increasing the safety margin of doses while keeping less frequent doses.
1.5 Synthetic routes to hydrogels

Hydrogels as defined above are mainly formed by incorporation of water into polymer networks. The polymer chains can be synthesized starting from hydrophilic monomers in general or can be provided naturally. Synthetic polymers must be cross-linked in a way to provide elasticity to the gel as shown in (Figure 1.2). Based on the type of crosslinking, they can be divided as classified above into chemical and physical gels.

![Diagram](image)

Figure 1.2: General scheme for hydrogel formation.

Many techniques have been known to crosslink the polymers and therefore forming the hydrogel through covalent bonding which makes them hard and irreversible. Crosslinking in chemical gels may occur either during polymerization or in a separate step after polymerization. There are various polymerization techniques can be used to form gels, including bulk, solution, and suspension polymerization. Mainly, the three critical parts of the hydrogels preparation are
monomer, initiator, and cross-linker. Another approach to synthesize hydrogels is through ionic bonding and hydrogen bonding which creates physical gels. Physical gels respond to changes in pH, temperature and composition of the solvent. Since the last decade, hydrogel preparation by self-assembly has been an active research area and it lead to a unique family of ion-coordination hydrogels which will be discussed in the following paragraph.

1.5.1 Polymerization by coordination

Synthesis of hydrogels by self-assembly has attracted lots of attention in the past decade especially in supramolecular chemistry. This crosslinking technique involves coordination bonding between the metal cation and functional groups on polymer chains such as carboxylic acid or amino groups by a supramolecular approach. This is known as metal-ligand coordination which is appealing due to special characteristics of the bond itself. Being non-covalent in nature but still strong enough, highly directional, reversible and dynamic. A wide variety of metal ions and organic ligands can be used for hydrogel formation. By choosing the suitable combination of low-molecular weight ligand/polymer and metal ions, many modifications on structure and thermodynamic stabilities can be achieved in the resulting compounds. Specifically, metallo-supramolecular polymers are of special interest. They are considered as a branch of metal-containing polymers. To obtain these type of structures, self-assembly between the metal ion with various forms of polymers which can be linear,
intercrosslinked MSPS\textsuperscript{60}, macrocyclic MSPs\textsuperscript{61}, or side chains MSPs is employed.\textsuperscript{62-68} Hence different shapes of structural motifs can be obtained depending on starting polymers. It is reported that MSPGs of first row transition metals are more labile in nature\textsuperscript{69} than second and third-row metals which show increased strength and stability of the polymer. Especially, when using organic polydentate ligands like bipridine for example.\textsuperscript{70} Eventually, it is worth to note that not all metal-containing polymers exhibit polymer-like characteristics. For example, "MOF" metal organic frameworks are composed of an infinite coordination network in the mostly crystalline solid state.\textsuperscript{71,72} They are considered to be coordination polymers although they don’t have any polymer-like properties.

MSPGs are divided into three types based on the form of crosslinking within the supramolecular structure. The first type MSPG (I) is composed of ditopic ligands and metal ions which can bind to more than two ligands. The second type of MSPG (II) is formed upon combination of stoichiometric mixtures of multitopic ligand and metal ions. MSPG (III) involves metal-ligand coordination in addition to secondary interactions. Therefore the type of gelation changes from crosslinking through metal ligand interaction in MSPG(I) into more covalent crosslinking in MSPG (II). In the following part we will discuss MSPG (I) and (II). In the following part we will focus on Metallo-supramolecular gels in specific after overviewing some examples from metal containing polymers in general.
1.5.1.1 Metal-containing polymers

As discussed earlier, metal nanoparticles in hydrogels offered several applications such as magnetic properties but most importantly antibacterial activity is observed in silver hydrogels. In literature, many approaches have been reported where metal nanoparticles are part of the polymer network in several forms such as spherical nanoparticles, nanocubes, nanorods, etc. Due to various advantages and applications of polymer-assisted synthesis of metal nanoparticles, these synthetic techniques have widely been used.\textsuperscript{73}

The incorporation of metal nanoparticle into (polymer/ligand) matrix can be achieved by two ways:

1. Starting from the metal salt, the metal cation can be incorporated into the polymer gel matrix, and then reduced through a reducing agent to obtain the metal particale.

2. The coordination between functional groups of polymer/ligand (mostly carboxylate) and the metal cation enables the crosslinking and formation of the hydrogel (ion coordination polymer hydrogel) simultaneously with the metal reduction without any external reducing agent (NaBH\textsubscript{4} or UV radiation).

The first approach is to incorporate the metal nanoparticles into the already formed hydrogel consists of three steps:
1. Preparation of hydrogel through polymerization techniques described earlier starting either from monomer or from two different types of polymers crosslinked together.

2. Loading the metal cations into the hydrogel matrix by diffusion from the metal salt solution.

3. Reduction of the metal cation using reducing agents.

In this context, polyvinyl /polyacrylamide hydrogels were reported with silver ions as an easy, fast and practical approach to form silver nanoparticle composites. These nanocomposites are used in antibacterial bionanotechnology. Another interesting approach using polyacrylamide was the “Breath in – Breath out” approach to load the silver nanoparticles into the polymer matrix.

![Figure 1.3: The scheme for the “Breath in – Breath out” swelling mechanism.](image)
It involves three steps as shown in (Figure1.3): the first is de-swelling the gel by removing the water content through the usage of acetone. Then the shrunken gel is immersed into an aqueous solution containing the nanoparticles. The polymer matrix will swell again absorbing the nanoparticles. The third step is removing the water from the polymer network by immersing again in acetone.\textsuperscript{75}

Another interesting approach to incorporate silver ions into poly[N-isopropyl acrylamide-co-(sodium acrylate)] yielded into uniformly distributed silver nanoparticles with narrow size distribution after reduction as shown in (Figure 1.4) with sodium borohydride.\textsuperscript{73}

![Figure 1.4: Schematic representation of the preparation of Ag nanoparticles in hydrogel networks.](image)

From this study it was concluded that the hydrogel network structure determines the shape and size distribution of nanoparticles. Another study involved nano-
tree" polymer which was encapsulated within poly(vinyl alcohol) through disulfone reaction. This compositied served as host for silver nanoparticles. The resulting hybrid structure showed good catalytic activity.\textsuperscript{76}

1.5.1.2 Metallo-supramolecular polymer hydrogels

1.5.1.2.1 Low molecular weight ligand

In general the formation of metal nanoparticles inside the gel offers suitable growth conditions of nanoparticles. For example, the cavities act as nanoscopic pots which are perfect for nanoparticles growth. Furthermore, the gel prevents nanoparticles from oxidation. In literature, many approaches have used low molecular weight hydrogelators in coordination with metal cations to form hydrogels through self-assembly.\textsuperscript{77,78} These hydrogels contained several types of metals (alkaline earth metals and transition metals) such as Mg, Cu, and Ag. Magnesium (Mg\textsuperscript{2+}) in coordination with a pyridyl ligand without a long alkyl chain resulted in a hydrogel that showed fluorescent properties.\textsuperscript{79} Also flexible and fluorescent fibers containing Mg\textsuperscript{+} ions were self-assembled starting from N-(7-hydroxyl-4-methyl-8-coumarinyl-alanine in coordination with Mg\textsuperscript{2+}.\textsuperscript{80} The 3D structure trapped water forming a hydrogel through non covalent interactions between 1D coordination polymers aggregation.\textsuperscript{80} Copper based coordination polymeric hydrogels were reported as well starting from pyridine based ligands and Cu\textsuperscript{2+} salt.\textsuperscript{81} These type of copper based hydrogel showed ability to act as drug delivery gels through encapsulating
chemo preventive curcumin which is a natural agent used to inhibit or delay or reverse carcinogenesis and triggered its release due to pH change. Silver-based hydrogels attracted lots of attention for their antibacterial activity as discussed earlier as well various applications such as pH and ion sensitivity. Many silver-coordinated polymer hydrogels have arised starting from Ag⁺ salts most commonly (AgNO₃). Melamine was reported to form a hydrogel through coordination with Ag⁺ via its heteroatoms which are nitrogen. The resultant gel showed special sensitivity towards pH and ions. Furthermore, it was utilized for dye absorption. Another very interesting silver based supra-molecular hydrogel with glutathione polymer showed sensitivity towards iodide ions which facilities their detection. This was achieved due to the reversible sol-gel transition of the Silver-Glutathione coordination polymer (Ag-GTH) as response to iodide ions. The transition can be observed by color change.

1.5.1.2.2 Polymeric ligand

Metalo supramolecular polymer gels can contain crosslinked MSPs Intra-or inter-
Cross-linked MSP’s, Macrocyclic MSP;s, Side-Chain MSP’s or Branched MSPs depending on the nature of the polymeric ligand (Figure 1.5)
In general the use of polymeric ligands i.e. polymer with many binding sites along the backbone is attractive. It was reported that starting from polyacrylic acids, polysaccharide and cellulose derivative, stable gels are formed in presence of metal ions through coordination Nijenhuis et al. reported the coordination of partially hydrolyzed polyacrylamide and chromium ions. The coordination of acid moieties with a metal ion is observed to be strong under high pH where the acid is deprotonated hence acting as a good ligand. However, these complexes suffered from slow formation at room temperature which required increase in temperature. Another example was reported by Jong and coworkers in which redox-responsive hydrogels were formed in aqueous solution of poly(acrylic acid) in combination with Fe$^{3+}$. Due to change in oxidation state (Figure 1.6), Fe$^{3+}$/Fe$^{2+}$, a reversible sol-gel transition occurred. The gel is dissolved upon reduction of Fe$^{3+}$ into Fe$^{2+}$ under illumination. Then under oxygen atmosphere and in absence of light the gel is formed again.

Figure 1.5: Schematic representation of possible metallo-supramolecular polymer architectures.
Figure 1.6: a) gel-sol-gel transition and b) redox-reactions in the poly acrylic acid iron hydrogel

This shows an example of the importance of gel sensitivity to external factors such as light and oxygen. As a response, to these factors the hydrogel changes its oxidation state and consequently changes its structure. This type of gel may be a good candidate for controlled drug delivery for example, or for encapsulating cells or bioactive molecules within the gel matrix.

Polymeric ligands were reported as well with nitrogen donor groups which offers facile route towards MSPG. Lewis and Miller\textsuperscript{89-93} reported using nitrogen in polyvinyl pyridine and polyvinyl bipyridine through non covalent bond with metal ions. Another approach using polymeric ligand with a bifunctional ligand was reported.\textsuperscript{94,95} In this context, polyvinyl pyridine was also reported to form a highly cross-linked gel system when used with another spacer ligand which is a bifunctional spincer.\textsuperscript{94} Together with a transition metal, such as platinum (II) or palladium(II). Metal-supramolecular gels were reported incorporations such
Another interesting approach is to obtain MSPGs starting from conjugated polymeric ligands with metal ions.\textsuperscript{96-100} One example is using π-conjugated organometallic polymer networks based on styrene soluble poly(p-phenylene ethynylene). The functional group is ethynylene group of the polymer leading to formation of photoluminescent gels. Finally, another paper started from poly (p-phenyl ethynylene) but adding 2,2' bipyridine moieties in the backbone.\textsuperscript{99} This approach important as it opened the door to wide variety of metallo-supramolecular polymer network and gels by utilizing conjugated polymers and first row transition metals. These include Co(II), Ni(II), Zn(II) and Cd(II). It is worth to note that the obtained gels showed different photophysical properties depending on the type of metal.

1.6 Aim of the thesis:

1) Investigate the reaction of PVM/MA with metal salts in order to access polymeric metal carboxylates that could show exciting properties similar to those crystalline MOFS.

2) Investigate properties of polymeric metal carboxylates e.g. surface area, chemical and physical stability and potential applications.
CHAPTER 2: Experimental

2.1 Instrumentation

2.1.1 Thermogravimetric analysis (TGA)
The TGA measurements were performed on a NETZSCH STA 449 F3 Jupiter
thermogravimetric analyzer using Al Pan under N2 flow (20 mL/min) with heating
rate 20 K/min. The sample amount was ca. 10 mg.

2.1.2 FTIR spectroscopy
FTIR analysis was performed on a Nicolet iS10 infrared spectrometer (Thermo
Electron Corporation, UK). The spectrometer has a KBr beam splitter and an
EverGlo IR source. The spectrometer has a mercury cadmium telluride (MCT-B).
Attenuated Total Reflectance (ATR) mode was performed using Smart iTR
accessory having a diamond crystal as sample holder window. The FTIR spectra
were recorded with the nominal resolution of 4 cm\(^{-1}\) over the range 525 – 4000
\(\text{cm}^{-1}\) and each spectrum corresponded to an average of 64 individual scans.

2.1.3 \(^1\text{H} \text{NMR and } ^{13}\text{C NMR spectra}
\(^1\text{H} \text{NMR and } ^{13}\text{C NMR spectra were recorded at 298 K on a Bruker 700 AVANAC
III spectrometer equipped with a 5 mm Bruker TCI CryoProbe using D}_2\text{O as
solvent.}
2.1.4 Scanning electron microscope (SEM) and energy dispersive spectroscopy (EDS)

Scanning electron microscopy (SEM) investigations were carried out in a FEI Quanta 3D scanning electron microscope operating at a 5 kV accelerating voltage. The Energy Dispersive Spectroscopy (EDS) (EDS Inc., N.J, USA) is a 40 mm$^2$ Silicon Drift detector with 135.0 eV resolutions on MnKα. EDS Genesis software is used to collect signals for the analysis. The hydrogel was dried by vacuum oven or CO$_2$ critical point drier. They were grounded in an agate mortar and used for SEM/EDS analysis. A 10 mm diameter aluminum pin (SEM holder) was used as a sample holder, and then a piece of double-side carbon tape was fixed to the aluminum SEM holder. The powder was fixed to the carbon tape using a spatula and excess powder was removed. The sample was transferred to a vacuum chamber of the electron microscope for measurement.

2.1.5 Cryo SEM:

Low temperature experiments were carried out using a Quorum PP2000T cryo-transfer system (Qurorum Technologies, Newhaven, UK) that was fitted to an FEI Quanta 3DFEG SEM with a field emission electron source. To do cryo-SEM on the hydrogels, a small amount of sample was mounted in a 1.5 mm in diameter machined-hole on an aluminum stub that was secured on the specimen holder. The sample holder, attached to a transfer rod, was then rapidly plunged into liquid nitrogen slush and under vacuum transferred into the PP2000T preparation chamber, pre-cooled to -185°C and allowed to equilibrate for 2 minutes. The
sample temperature was raised to -90°C for 2 min to sublime residual ice contamination on the surface of sample that happened during transfer to preparation chamber. The temperature was then lowered to -150°C and the surface of frozen gel was sputter-coated with 5 nm thick platinum in an argon atmosphere. The fractured surface of hydrogel was obtained by hitting the top of frozen gel kept at -150°C with a knife cooled at -185°C. The sample was then transferred to the SEM cryo-stage which was held at -130°C and the revealed fractured plane of frozen hydrogel was imaged. In order to remove the water from the fractured plane and reveal further surface details, samples were sublimed inside the SEM chamber at -90°C for up to 37 min and SEM images from the same areas were captured during the sublimation process. To avoid charging problems, the sample was transferred back to the preparation chamber and sputter-coated with 3 nm thick platinum in an argon atmosphere at -150°C. The sample was then transferred back to the SEM cryo-stage, which was held at -130°C and high quality SEM images were captured. In all cases, the imaging was performed using an accelerating voltage of 3-5 kV and a working distance of 7-10 mm.

2.1.6 Transmission electron microscopy (TEM)

Transmission electron microscopy (TEM) investigations were carried out in a FEI 80-300 transmission electron microscope operating at 300 kV accelerating voltage.
2.1.7 Powder X-ray diffraction measurements:
Powder-XRD of PVM/MA and polymer hydrogel poly[methyl vinyl ether-alt-mono-sodium maleate]·AgNO$_3$-vacuum dried powder were measured by Bruke D8 Powder X-Ray Differactometer.

2.1.8 CO$_2$ Critical point drier
The solvent-exchanged polymer hydrogel was supercritically dried with CO$_2$ using the Tousimis Autosamdri 815B.

2.1.9 Physical absorption analysis
Low-pressure N$_2$ gas sorption experiments at 77 K were carried out on a Micrometrics ASAP 2020 volumetric gas sorption instrument. The BET specific surface area was calculated from the BET equation using the transform plot in the relative pressure range of 0.05 and 0.3. The pore size distribution profiles were obtained from BJH adsorption isotherm data reduction. Before any analysis, all samples ware outgassed at 90°C on the ultrahigh vacuum degasing port of the instrument.

2.1.10 Inductively coupled plasma mass spectroscopy
The silver release experiments were carried out in PBS solution. Briefly, 12.5 grams of polymer hydrogel poly[methyl vinyl ether-alt-mono-sodium maleate]·AgNO$_3$
(silver content was 1.1% by weight) were immersed in 200 mL PBS. The solution samples were taken out at different time, filtered and diluted 1,000 times before analysis.

The release rate of silver cation from the polymer hydrogel poly[methyl vinyl ether-alt-mono-sodium maleate]-AgNO₃ in phosphate buffered saline (PBS) solution was measured on ICP-MS Elan DRC II with the condition of nebulizer gas flow: 0.92 L/min, auxiliary gas flow: 1.2 L/min, plasma gas flow: 15 L/min, ICP RF Power: 1300 w and I v: 7.5 v.

2.1.11 Optical microscopy
Microscope images were recorded on a Leica M60 Microsystem and a IC80 HD camera.

2.2 Synthesis
2.2.1 Preparation of poly(methyl vinyl ether-alt-monosodium maleate) (PVM/Na-MA) stock solution
Poly (methyl vinyl ether-alt-maleic anhydride) (PVM/MA, 10.0 g, containing 64 mmol of cyclo anhydride) and sodium hydroxide aqueous solution (42.7 mL, 1.5 mol/L, 64 mmol) were mixed in a 250 mL flask. The reaction was sonicated at ca. 30°C to form a viscous solution of poly(methyl vinyl ether-alt-monosodium maleate) (PVM/Na-MA). The solution was transferred into a 250-mL-volumetric
flask and diluted to volume with deionized water. The diluted PVM/Na-MA solution (254.62g) was used as a stock solution. The diluted PVM/Na-MA solution (10.0 g) was dried under vacuum at 50°C for 24 hours.

2.2.2 Preparation of polymer hydrogel poly[methyl vinyl ether-alt-mono-sodium maleate]-AgNO₃

The PVM/Na-MA stock solution (10.0 g, containing 2.5 mmol of sodium carboxylate groups) was poured into a scintillation vial. Silver nitrate aqueous solution (2.0 mL, 0.64 mol/L, 1.28 mmol) was added to the PVM/Na-MA solution and stirred. A colorless hydrogel formed within 5 minutes. The hydrogel turned into the color of light yellow, yellow, brown and dark brown gradually. The obtained polymer hydrogel poly[methyl vinyl ether-alt-mono-sodium maleate]-AgNO₃ was taken out and dried first in air and then in the vacuum oven at 80°C for 24 hours. 0.69 grams of yellow items were obtained and the items were grounded into fine powder. It was termed “polymer hydrogel poly[methyl vinyl ether-alt-mono-sodium maleate]-AgNO₃ -vacuum dried powder”.

2.3 Cation-exchange of the hydrogels

The polymer hydrogel poly[methyl vinyl ether-alt-mono-sodium maleate]-AgNO₃ -vacuum dried powder (at 80°C in a vacuum oven for 1d) and the intact polymer hydrogel poly[methyl vinyl ether-alt-mono-(Ag/Na) maleate] were used as examples to show the cation-exchange of the hydrogels.
2.3.1 Cation-exchange in poly[methyl vinyl ether-alt-mono-sodium maleate]∙AgNO₃

The PVM/Na-MA stock solution (5.0 g) and AgNO₃ aqueous solution (1.0 mL, 0.64 mol/L, 0.64 mmol) were combined in a 50 mL-beaker resulting in the formation of the poly[methyl vinyl ether-alt-mono-sodium maleate]∙AgNO₃ hydrogel. Ethanol (8.0 mL) and Cu(NO₃)₂ aqueous solution (5.1 mL, 0.63 mol/L, 3.2 mmol) were added into the vial and the system was stirred with a magnetic stirrer for 24 hours. Then the upper clear solution was decanted. The remainder in the beaker was washed by (8.0 mL of ethanol and 5.1 mL of water) for three times. After each wash, the upper clear solution was decanted. The ion-exchanged gel was dried under air and the residue analyzed by SEM-EDX.

2.3.2 Cation exchange of polymer hydrogel the poly[methyl vinyl ether-alt-mono-sodium maleate]∙AgNO₃-vacuum dried powder

The polymer hydrogel poly[methyl vinyl ether-alt-mono-sodium maleate]∙AgNO₃-vacuum dried powder (0.055 g, containing 0.10 mmol of silver cations) was put into a scintillation vial. Ethanol (8.0 mL) and copper nitrate aqueous solution (0.8 mL, 0.63mol/L, 0.50 mmol) were added into the vial and the system was stirred with a magnetic stirrer. The system was stirred for 24 hours. Then the upper clear solution was decanted. The remainder in the vial was washed with (8.0 mL of ethanol and 0.80 mL of water) for three times. After each wash, the upper clear solution was decanted. The ion-exchanged gel was dried and analyzed by SEM-EDX.
2.4 Antibacterial activity test of polymer hydrogel poly[methyl vinyl ether-\textit{alt}-mono-sodium maleate]\cdot AgNO_3

Antibacterial properties of the hydrogel poly[methyl vinyl ether-\textit{alt}-mono-sodium maleate]\cdot AgNO_3 were investigated in the Stingl group at KAUST.

**Antibacterial activity assay:** The Kirby-Bauer Zone of Inhibition test method was used\textsuperscript{101} to examine the antibacterial activity against the three bacterial strains\textsuperscript{102}: \textit{E.coli} (CGSC 5240), \textit{S. epidermidis} (ATCC 12228), and \textit{P. aeruginosa} (ATCC 14885). Details of the antibacterial tests were as follows.

LB agar broth was prepared by mixing of LB Broth (Lennox, 45 g), Difco\textsuperscript{TM} Agar Granulated (5.4 g) and deionized water (360 mL) and sterilized at 121°C for 20 minutes. The sterilized LB agar broth was cooled to 60°C and poured into aseptic Petri dishes to solidify. Then, 100 µL of the freshly grown bacteria (about $1 \times 10^8$ cfu/mL (cfu, colony-forming unit)) were dropped on the surface of the solidified LB broth and spread evenly on the surface. Small holes (inner diameter 2mm) were made on the surface of the solidified LB broth. The intact polymer hydrogel poly[methyl vinyl ether-\textit{alt}-mono-sodium maleate]\cdot AgNO_3 (silver content was 1.1% by weight, 5 µL) was injected carefully into the small holes. The plates were incubated at 37°C for 24 hours. Colonies were visualized and digital images were captured. Antibacterial Activity Assay was also carried out by the aqueous solution of polymer hydrogel poly[methyl vinyl ether-\textit{alt}-mono-sodium maleate]\cdot AgNO_3.
The aqueous solution was obtained by dissolving intact polymer hydrogel poly[methyl vinyl ether-alt-mono-sodium maleate]-AgNO₃ in deionized water and the solution was transferred into a 250 mL of volumetric flask and dilute to volume with water. The silver concentration was 0.55mg/mL. Small round filter paper (inner diameter 5 mm) was submerged into the solution for half an hour and then taken out. Solidified LB broth aseptic Petri dishes freshly grown bacteria were prepared as the above method. The filter paper which had been submerged into aqueous solution of polymer hydrogel poly[methyl vinyl ether-alt-mono-sodium maleate]-AgNO₃ was carefully put on the surface of the solidified LB broth. The plates were incubated at 37°C for 24 hours. Colonies were visualized and digital images were captured.

2.4.1 Minimum inhibitory concentration (MIC) testing:

Minimum Inhibitory Concentrations (MIC) of the polymer hydrogel poly[methyl vinyl ether-alt-mono-sodium maleate]-AgNO₃, AgNO₃ and PVM/MA against *E. coli* (DSM 10198), *S. epidermidis* (DSM 1798), and *P. aeruginosa* (DSM 11148) were determined by the broth microdilution method¹⁰³. First, solutions with various concentration gradients of the polymer hydrogel poly[methyl vinyl ether-alt-mono-sodium maleate]-AgNO₃, AgNO₃ and PVM/MA were prepared with LB broth in the 15 mL of cultivation tubes. Then, 5 µL of the microorganism culture (about 1 × 10⁶ cfu/mL) was added into each of the cultivation tube. The optical density values of microorganism cultures were measured after 72 h of incubation at 37°C. The concentration at which no growth of microorganism was observed
with the naked eyes and microplate reader (Tecan Infinite M200) and recorded as MIC. The tests were repeated three times. For PVM/MA, solutions of 9,500 µg/mL, 6,500 µg/mL, 4,000 µg/mL, 3,500 µg/mL, 3,000 µg/mL and 2,500 µg/mL polymer LB broth were prepared and tested. For polymer hydrogel poly[methyl vinyl ether-alt-mono-sodium maleate]-AgNO₃, solutions of 55 µg/mL, 28 µg/mL, 11 µg/mL, 5.5 µg/mL, 5.0 µg/mL, 4.5 µg/mL, 4.0 µg/mL, 3.5 µg/mL, 3.0 µg/mL and 2.5 µg/mL LB broth were prepared and tested. For AgNO₃, solutions of 10.0 µg/mL, 9.0 µg/mL, 8.0 µg/mL, 7.0 µg/mL, 6.0 µg/mL, 5.5 µg/mL, 5.0 µg/mL, 4.5 µg/mL, 4.0 µg/mL, 3.5 µg/mL and 3.0 µg/mL LB broth were prepared and tested.
CHAPTER 3: Results and discussion

3.1 Insertion reactions

Our interest in coordination polymer hydrogels has developed from investigations of insertion reactions between organic anhydrides and late transition metal and main group metal alkoxides (Figure 3.1). By moving from molecular organic anhydrides to polymeric anhydrides such as poly(methyl vinyl ether-alt-maleic anhydride) PVM/MA ([CAS: 9011-16-9], av. molecular weight: 1,080,000), it was planned to open the cyclic anhydride groups with metal alkoxides and construct polymeric metal carboxylates/esters (Figure 3.1). Polymeric metal carboxylates are commonly synthesized by polymerization of unsaturated metal carboxylates. Starting from a polyanhydride or polyacid, however, avoids interference of the metal ion during polymerization and allows diverse metal functionalization of the polymer and the study of resulting properties.

Figure 3.1: Insertion reaction of a polymeric organic anhydride into the metal-oxygen M-O bond of a metal alkoxide MOR (R = alkyl).
3.2 Synthesis of the poly[methyl vinyl ether-alt-mono-sodium maleate]·AgNO₃

As a starting material the commercially available copolymer poly(methyl vinyl ether-alt-maleic anhydride) (PVM/MA) was chosen because it has a defined amount of anhydride groups for stoichiometric reactions with metal salts and it is slightly soluble in organic solvents and water. Furthermore, PVM/MA is non-toxic and has been used for various pharmaceutical purposes.¹⁰⁴

Dissolving PVM/MA in organic solvents, however, takes several hours and a nucleophilic ring-opening reaction of the anhydride groups with NaOH followed by a metathesis reaction with an aqueous transition metal salt solution seemed a more practical option than the reaction scheme outlined in (Figure 3.1). The hydrolysis/metathesis route (Figure 3.2) gave rise to a variety of novel metallo-supramolecular polymer hydrogels containing Cu²⁺, Co²⁺, Ni²⁺, Al³⁺ and Ag⁺. In this thesis, we focus on the synthesis and properties of the poly[methyl vinyl ether-alt-mono-sodium maleate]·AgNO₃ hydrogel. In the first reaction step (Figure 3.2), one equivalent of sodium hydroxide was used to open the cyclo anhydride in the polymer chain to give PVM/Na-MA. Silver nitrate aqueous solution (2.0 mL, 0.64 mol/L, 1.28 mmol) was added into PVM/Na-MA stock solution (10.0 g, containing 2.5 mmol of sodium carboxylate groups) and mixed in a scintillation vial.
Figure 3.2: Reaction of PVM/Ma with NaOH resulting in the formation of poly(methyl vinyl ether-alt-monosodium maleate) PVM/Na-MA (only one of the carboxylate/carboxyl isomers is shown) and synthesis of the silver hydrogel poly[methyl vinyl ether-alt-mono-sodium maleate]-AgNO₃.

The pale yellow hydrogel forms within 5 minutes (Figure 3.3). The hydrogel in (Figure 3.3) is 3 days old and as the gel ages, a gradual colour-change from colourless-yellow to brown is observed.

Figure 3.3: Left: AgNO₃ aq. solution; middle: PVM/MA aq. solution; right: polymer hydrogel poly[methyl vinyl ether-alt-mono-sodium maleate]-AgNO₃

3.2.1 Influence of AgNO₃ content on hydrogel formation.

The PVM/Na-MA stock solution (10.0 g, containing 2.5 mmol of sodium carboxylate groups) was added into a 20 mL of vial. Different amount of silver nitrate aqueous solution (0.64 mol/L) was added into the vial and stirred. For each of the stock solutions, one equivalent of sodium hydroxide was used to
open the cyclo anhydride in the polymer chain. Hydrogel formation conditions were listed in (Table 3.1). it was observed that when 1.0 ml of \( \text{AgNO}_3 \) was added no hydrogel formation was observed. This may be attributed to low silver ions amount (polymer: \( \text{Ag}^+ \) ratio is 3.9:1), and since silver acts as crosslinker in this type of coordination polymer hydrogel then hydrogel will not form due to not sufficient crosslinker. When the amount of silver increases (1.5, 2, and 3 ml), colorless hydrogel forms and then turns into dark brown color within 1 month. This is due to the reduction of silver ions into silver metal nanoparticles. With a ratio of around (1:1 or 1.1:1) no hydrogel formation was observed. This may be due to low amount of polymer so that there is more metal centers than carboxylate ligands. Two carboxylates will bind to metal center and form the gel, therefore with a ratio around 1:1 no crosslinking will occur.
Table 3.1: Investigation of hydrogel formation condition of PVM/Na-MA solution (10.0 g) with different amounts of AgNO₃ aq. solution (0.64 mol/L)

<table>
<thead>
<tr>
<th>No.</th>
<th>AgNO₃ Amount</th>
<th>Hydrogel Formation</th>
<th>Hydrogel Formation Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.0 mL</td>
<td>3.9 : 1</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Colorless solution but no hydrogel formed.</td>
</tr>
<tr>
<td>2</td>
<td>1.5 mL</td>
<td>2.6 : 1</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Colorless hydrogel formed in 2 hours and changed into dark brown color in 1 month</td>
</tr>
<tr>
<td>3</td>
<td>2.0 mL</td>
<td>2.0 : 1</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Colorless hydrogel formed within 5 minutes and changed into dark brown color in 1 month</td>
</tr>
<tr>
<td>4</td>
<td>3.0 mL</td>
<td>1.3 : 1</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Colorless hydrogel formed within 5 minutes and changed into dark brown color in 1 month</td>
</tr>
<tr>
<td>5</td>
<td>3.5 mL</td>
<td>1.1 : 1</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Something like hydrogel formed but it was not real hydrogel. The items could not stand at the bottom of the vial while the vial was put up-side-down.</td>
</tr>
<tr>
<td>6</td>
<td>3.9 mL</td>
<td>1 : 1</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Some cloudy items but not hydrogel formed.</td>
</tr>
</tbody>
</table>

3.2.2 Influence on PVM/Na-MA amount on hydrogel formation

Changing the polymer concentration has significant effect on gel formation. While low polymer concentration (20 g/L) did not yielded the hydrogel network, higher ratios of 80 and 120 g/L resulted in hydrogel formation with too viscous solution
with AgNO₃ as precipitate. The optimal concentration was found to be 40 g/L where a colorless hydrogel was formed in 10 minutes as shown in (Table 3.2)

Table 3.2: Investigation of hydrogel formation at different polymer concentrations

<table>
<thead>
<tr>
<th>No.</th>
<th>Polymer Concentration (g/L)</th>
<th>Hydrogel Formation Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20</td>
<td>White solution but no hydrogel formed.</td>
</tr>
<tr>
<td>2</td>
<td>40</td>
<td>Colorless hydrogel formed within 10 minutes.</td>
</tr>
<tr>
<td>3</td>
<td>80</td>
<td>The solution was too viscous and AgNO₃ appeared as precipitate. Hydrogel did form but some solution still remained.</td>
</tr>
<tr>
<td>4</td>
<td>120</td>
<td>The solution was too viscous and AgNO₃ appeared as precipitate. Hydrogel did form but some solution still remained.</td>
</tr>
</tbody>
</table>

* PVM/Na-MA stock solution was prepared by the method described in the Experimental chapter. For each of the stock solutions, one equivalent of sodium hydroxide was used to open the cyclo anhydride in the polymer chain.

PVM/2Na-MA stock solution was used to make polymer hydrogel [Ag, 2Na][poly(methyl vinyl ether-alt-maleate)]. Two equivalents of sodium hydroxide to the cyclo anhydride in the polymer chain were also used to open the cyclo anhydride and it was found that poly(methyl vinyl ether-alt-disodium maleate) (PVM/2Na-MA) solution can also be used for hydrogel preparation. But the optimal ratio for hydrogel formation is (polymer: Ag⁺, 1:1) where the Hydrogel
formed after 2 hours. The color changed into dark yellow and black within 1 week. As shown in Table 3.3. In general the hydrogel formation of (PVM/2Na-MA) with Ag⁺ followed same trend as (PVM/Na-MA) where below the optimal concentration minor hydrogel ratio was formed and higher than optimal concentration no hydrogel was formed due to excess Ag.

Table 3.3: Investigation of hydrogel formation condition of PVM/2Na-MA stock solution (10.0 g) with different amounts of AgNO₃ aq. solution.

<table>
<thead>
<tr>
<th>No.</th>
<th>AgNO₃ Amount</th>
<th>Ratio</th>
<th>Hydrogel Formation</th>
<th>Hydrogel Formation Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.0 mL</td>
<td>2.0 : 1</td>
<td>Yes</td>
<td>Only 10% of the total solution formed hydrogel 12 hours later. Solution remained.</td>
</tr>
<tr>
<td>2</td>
<td>3.0 mL</td>
<td>1.3 : 1</td>
<td>Yes</td>
<td>Only 20% of the total solution formed hydrogel 12 hours later. Solution remained.</td>
</tr>
<tr>
<td>3</td>
<td>4.0 mL</td>
<td>1 : 1</td>
<td>Yes</td>
<td>Hydrogel formed after 2 hours. The color changed into dark yellow and black within 1 week.</td>
</tr>
<tr>
<td>4</td>
<td>5.0 mL</td>
<td>1 : 1.3</td>
<td>No</td>
<td>Something like hydrogel formed but it was not real hydrogel. The items could not stand at the bottom of the vial while the vial was put up-side-down.</td>
</tr>
</tbody>
</table>
3.2.3 Influence of alkali metal bases on hydrogel formation

There are no noticeable differences in hydrogel formation when other alkali metal hydroxides are used for similar experiments as shown in (Table 3.4).

Table 3.4: Investigation of hydrogel formation condition of “polymer-alkali metal base stock solution” (10.0 g) with AgNO₃ aq. solution.

<table>
<thead>
<tr>
<th>No.</th>
<th>Alkali metal base</th>
<th>Hydrogel Formation</th>
<th>Hydrogel Formation Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>LiOH</td>
<td>Yes</td>
<td>Colorless hydrogel formed within 5 minutes and changed into dark brown color in 1 month.</td>
</tr>
<tr>
<td>2</td>
<td>KOH</td>
<td>Yes</td>
<td>Colorless hydrogel formed within 5 minutes and changed into dark brown color in 1 month.</td>
</tr>
<tr>
<td>3</td>
<td>RuOH</td>
<td>Yes</td>
<td>Colorless hydrogel formed within 5 minutes and changed into dark brown color in 1 month.</td>
</tr>
<tr>
<td>4</td>
<td>CsOH</td>
<td>Yes</td>
<td>Colorless hydrogel formed within 5 minutes and changed into dark brown color in 1 month.</td>
</tr>
</tbody>
</table>

3.3 Characterization of Poly(methyl vinyl ether-alt-mono-sodium maleate) and Poly[methyl vinyl ether-alt-mono-sodium maleate]-AgNO₃

3.3.1 FT-IR spectroscopy

The starting material- PVM/MA, the intermediate product PVM/Na-MA and the polymer hydrogel poly[methyl vinyl ether-alt-mono-sodium maleate]-AgNO₃- vacuum dried powder were characterized by FT-IR (Figure 3.4). It can be observed that the CO bond shows a sharp peak in the pure polymer, but the peak intensity decreases and shifts to right due to the breaking down of the cyclic
anhydride and formation of carboxylate functional groups. The addition of Ag ions showed further decrease of CO peak intensity and peak corresponding to NO$_3^-$ appears. This confirms together with NMR.

Figure 3.4: FT-IR ATR of the starting material PVM/MA, PVM/Na-MA and of the polymer hydrogel poly[methyl vinyl ether-alt-mono-(Ag/Na) maleate]-AgNO$_3$ vacuum-dried powder showing the carbonyl shift upon ring-opening of the cyclic anhydride group.

3.3.2 $^1$HNMR of starting material and intermediate

$^1$HNMR of poly(methyl vinyl ether-alt-maleic anhydride) (PVM/MA) [CAS: 9011-16-9] (Mw: 1,080,000, M$_n$ ~ 311,000) and (PVM/Na-MA) were measured and the images were shown in (Figure 3.5 and 3.6).
$^1$HNMR of PVM/MA:

Figure 3.5: $^1$H NMR of PVM/MA

$^1$HNMR (D$_2$O, 700 MHz) $\delta$ ppm:

1.95 (s, 2H), 2.83 $\sim$2.92 (m, 1H), 3.08 (s, 1H), 3.25 $\sim$3.31 (m, 3H), 3.54 $\sim$3.65 (m, 1H).

$^1$H-NMR of the starting material-poly(methyl vinyl ether-alt-maleic anhydride) (PVM/MA) is shown in (Figure 3.5). The peaks in the NMR spectrum can be assigned to the corresponding hydrogens bonded to the numbered carbons in the structure. The singlet peak at 1.95 ppm corresponds to the 2 hydrogen of CH$_2$ on carbon 1, the multiplet at 2.83 $\sim$2.92 corresponds to hydrogens connected to carbon number 4 where it partially overlaps the singlet at 3.08 ppm.
which is coming from 1H bonded to carbon 3. It was observed that the methoxy group the (CH$_3$ at carbon 7) showed a peak at 3.25 ~3.31 ppm. The hydrogen bonded to carbon 2 showed a multiplet at 3.54 ~3.65.

$^1$H NMR of PVM/Na-MA

![NMR spectrum image]

Figure 3.6: $^1$H NMR of PVM/Na-MA

$^1$HNMR (D$_2$O, 700 MHz) $\delta$ ppm: 1.74 ~ 1.92 (m, 2H), 2.62 ~ 2.76 (m, 1H), 3.21 ~ 3.32 (s, 3H), 3.53 ~ 3.62 (m, 1H)

The reaction system of PVM/MA and NaOH was dried and analyzed by $^1$H-NMR, which was shown in (Figure 3.6). The peaks in the NMR spectrum can be assigned to the corresponding hydrogens bonded to the numbered carbons in
the structure. The multiplet peak at 1.74 ~ 1.92 ppm corresponds to the 2 hydrogen of CH$_2$ on carbon 1, the multiplet at 2.62 ~ 2.76 corresponds to hydrogens connected to both Carbon number 4 and carbon 3 where they completely overlap which indicates the opening of the ring. It was observed that the methoxy group the (CH$_3$ at carbon 7) showed a peak at 3.21 ~ 3.32 ppm. The hydrogen bonded to carbon 2 showed a multiplet at 3.53 ~ 3.62.

The comparison of NMR results of the starting material (Figure 3.5) shows 5 groups of signals however that of the reaction system PVM/MA and NaOH (Figure 3.6) shows only 4 groups of signals, this change indicated PVM/MA has changed into another chemical compound after reacting with NaOH. In this process of PVM/Na-MA modification the charge of molecule changes the chemical environment so that the chemical shift of proton has changed.

### 3.3.3 $^{13}$C NMR of starting material and intermediate

$^{13}$C NMR of poly(methyl vinyl ether-alt-maleic anhydride) (PVM/MA) [CAS: 9011-16-9] (Mw: 1,080,000, M$_n$ ~ 311,000) and (PVM/ Na-MA) were measured and the images were shown in (Figure 3.7 and 3.8).

$^{13}$C NMR of PVM/MA (D$_2$O, 175 MHz) δ ppm: 31.33, 32.21, 40.72, 41.34, 42.17, 48.80, 49.15, 49.89, 50.86, 51.48, 56.52, 57.23, 57.48, 58.54, 76.44, 76.72, 77.71, 128.46, 175.37, 176.26, 177.50, 178.26.
"\(^{13}\)C NMR of PVM/MA

Before ring openings the chemical shifts (J) of C1, C4, C3, C7, C2, C5, and C6 where (31-32), (41-42), (49-51), (57-59), (76-78), (175-178) ppm respectively. (Fig. 3.7). However after ring opening from \(^{13}\)C NMR it was observed that all peaks were shifted low field to higher frequencies due to presence of additional oxygen atom after ring opening which makes carbons more deshielded. Thus confirms the ring opening which affords the chemical shifts J (PVN/Na-MA) corresponding to C1, C4, C3, C7, C2, C5, C6 are (33-35), (42-43.5), (53-55), (57-58), (78.5-80), (178-180) respectively. (Fig. 3.8)
The NMR results together with IR data confirm the ring opening of the cyclic anhydride.

$^{13}$C NMR of PVM/Na-MA

$^{13}$C NMR of (PVN/Na-MA) (D$_2$O, 175 MHz) δ ppm: 33.07, 34.45, 34.74, 42.29, 42.78, 42.93, 43.06, 43.53, 53.00, 53.31, 53.78, 54.19, 54.67, 56.89, 57.36, 58.07, 78.51, 79.21, 80.07, 178.02, 178.33, 178.59, 178.96, 179.80, 180.26
3.3.4 Thermogravimetric analysis (TGA)

The thermal properties of the hydrogel poly[methyl vinyl ether-alt-mono-sodium maleate]·AgNO₃, the dried hydrogel and PVM/MA were investigated by thermogravimetric analysis (TGA). Commercial PVM/MA shows some weight loss at 84°C and 160°C that could be evaporation of residual organic solvent/starting material from synthesis and decomposition at around 306°C. The hydrogel shows weight loss of 95% at 120°C and the dry poly[methyl vinyl ether-alt-mono-sodium maleate]·AgNO₃ gel decomposes at 207°C as shown in (Figure 3.9 and 3.10).

![Thermogravimetric analysis of PVM/MA, the hydrogel poly[methyl vinyl ether-alt-mono-sodium maleate]·AgNO₃ and the dried hydrogel (20 mL/min N₂-flow, heating rate 20 K/min, sealed punctured Al crucibles).](image)

Figure 3.9: Thermogravimetric analysis of PVM/MA, the hydrogel poly[methyl vinyl ether-alt-mono-sodium maleate]·AgNO₃ and the dried hydrogel (20 mL/min N₂-flow, heating rate 20 K/min, sealed punctured Al crucibles).
Although structural information cannot be obtained directly, it is believed that the silver ions crosslink the polymer strands in the poly[methyl vinyl ether-alt-mono-sodium maleate]-AgNO₃ hydrogel. Structural models for the expected silver coordination modes are believed to be similar to those found in silver maleate complexes (typical metal carboxylate paddle-wheel structure motifs). Attempts to synthesize a molecular model that illustrates the bonding within the hydrogel were made. The synthesis of the mixed-metal Ag/Na maleate (Ag/Na)O₂C-(CH)₂-COOH from, maleic acid anhydride, NaOH and AgNO₃ in water, however, failed. Instead single crystals of the silver salt AgO₂C-(CH)₂-COOH were isolated.
3.3.5 Physical adsorption analysis

In order to get a better idea of the internal structure of the poly[methyl vinyl ether-alt-mono-sodium maleate]-AgNO₃ hydrogel, solvent exchange with ethanol followed by critical point drying of the gel with CO₂ was performed. Although this was aimed at removing the solvent whilst retaining the internal gel structure, subsequent BET surface area measurements resulted in a low-surface area material with 70 m²/g surface area as shown in (Figure 3.11). When treated with sc-CO₂, the dry silver polycarboxylate structure shows an average pore width of 14 nm.

Figure 3.11: N₂ desorption isotherm of polymer hydrogel poly[methyl vinyl ether-alt-mono-sodium maleate]-AgNO₃-CPD powder.
3.3.6 SEM and Cryo-SEM

The low surface area is an indicator for isolated pore formation during CO$_2$ evaporation and a collapse of the gelator structure during the procedure (Figure 3.12a). This results into a fibrous like morphology due to the breaking down of structure and closure of pores. As a second attempt to convert the polymer hydrogel poly[methyl vinyl ether-alt-mono-sodium maleate]-AgNO$_3$ into a porous polymer, freeze-drying/cryo-SEM (Figure 3.12b) of the hydrogel was performed which revealed a macroporous internal structure that could be the result of thermally induced face-separation during freeze-drying.$^{106}$ Diameters of the pores within the hydrogel are about 1 μm (Figure 3.12b). Larger amounts of the macroporous gelator structure were not prepared so far.

![Figure 3.12](image_url)

Figure 3.12: a) SEM of the hydrogel after sc-CO$_2$ drying; b) Cryo-SEM image of poly[methyl vinyl ether-alt-mono-sodium maleate]-AgNO$_3$. 

3.3.7 Powder-XRD (powder X-ray diffraction) measurements

From Figure 3.13 it can be confirmed that the crystalline component in the dried hydrogel poly[methyl vinyl ether-alt-mono-sodium maleate]-\( \text{AgNO}_3 \) is \( \text{AgNO}_3 \). This shows that the hydrogel poly[methyl vinyl ether-alt-mono-sodium maleate]-\( \text{AgNO}_3 \) is a composite of PVM/Na-MA and \( \text{AgNO}_3 \).

Figure 3.13: Powder-XRD (powder X-ray diffraction) analysis results of polymer hydrogel poly[methyl vinyl ether-alt-mono-sodium maleate]-\( \text{AgNO}_3 \)-vacuum dried powder, PVM/MA, NaOH, \( \text{AgNO}_3 \) and \( \text{NaNO}_3 \).
3.4 Properties of polymer hydrogel poly[methyl vinyl ether-alt-mono-sodium maleate]\(\cdot\)AgNO$_3$.

3.4.1 Formation of silver nanoparticles

The formation of silver nanoparticles within the gel matrix is deduced by three methods; 1) color change, 2) transmission electron microscopy TEM 3) particle size measurements.

![Figure 3.14: Color change ageing of intact polymer hydrogel poly[methyl vinyl ether-alt-mono-sodium maleate]\(\cdot\)AgNO$_3$.](image)

As observed, the color of the polymer hydrogel [Ag,Na][poly(methyl vinyl ether-alt-maleate)] changed gradually. (Figure 3.14) showed this color change. Newly formed polymer hydrogel poly[methyl vinyl ether-alt-mono-sodium maleate]\(\cdot\)AgNO$_3$ was almost colorless. Then the color changed into light-yellow, yellow, light brown, brown and dark-brown. Sometimes the color even changed into light-black. The change in colour is caused by Ag-nanoparticle formation with the polymer backbone acting as the reducing agent.$^{107}$
Transmission electron microscopy (TEM)

TEM test of the polymer poly[methyl vinyl ether-alt-mono-sodium maleate]-AgNO₃ vacuum dried powder showed there were silver nanoparticles deposited on the crosslinked polymer framework, as shown Figure 3.15.

Figure 3.15: TEM image of polymer hydrogel poly[methyl vinyl ether-alt-mono-sodium maleate]-AgNO₃ vacuum dried powder.

3.4.2 Cation-exchange of polymer hydrogel poly[methyl vinyl ether-alt-mono-sodium maleate]-AgNO₃

Cation-exchange by copper(II)

Ion-exchange materials have done great contributions to modern industry and brought a lot of conveniences to people’s daily lives. They are widely used in
analytical chemistry, antibiotic purification, separation of radioisotopes, hydrometallurgy, water treatment and pollution control.\textsuperscript{108}

Cation-exchange of the hydrogel poly[methyl vinyl ether-alt-mono-sodium maleate]-\textit{AgNO}_3 was tested using a variety of metals. For better visibility of silver ion exchange, the gel was suspended in an ethanol/water solution and treated with a five-fold excess of Cu(NO\textsubscript{3})\textsubscript{2} solution \([n(\text{Ag}^+)/n(\text{Cu}^{2+}) = 1:5]\) for 1d (Figure 3.16). A water/ethanol solvent mix was chosen in order to determine the ion-exchange-properties of the intact gel. The poly[methyl vinyl ether-alt-mono-sodium maleate]-\textit{AgNO}_3 hydrogel would dissolve in excess water.

![Figure 3.16](image.png)

Figure 3.16: Conversion of the intact polymer hydrogel poly[methyl vinyl ether-alt-mono-sodium maleate]-\textit{AgNO}_3 into poly[methyl vinyl ether-alt-mono-sodium maleate]-\textit{Cu(NO}_3)\textsubscript{2}.

When the poly[methyl vinyl ether-alt-mono-sodium maleate]-\textit{Cu(NO}_3)\textsubscript{2} gel (Figure 3.16 right) is treated with excess \textit{AgNO}_3-solution, \textit{Cu}^{2+} cannot be fully exchanged for \textit{Ag}^+ showing that the exchange reaction is irreversible.
under the chosen conditions (5-fold ion excess, 24 h stirring of the intact gel in an ethanol/water solution at room temperature). The ready release of silver ions from poly[methyl vinyl ether-alt-mono-sodium maleate]-AgNO₃ prompted us to investigate its antibacterial properties.

**SEM-EDX of Cation-exchange by copper(II)**

SEM-EDX results showed almost all the silver (I) cations in the intact hydrogel and powder could be exchanged by copper (II). This is clear through the increase in copper amount reaching a Cu: Ag ratio of 68:1 after the experiment as shown in (Figure 3.17).

![Figure 3.17: EDX of dried powder of intact polymer hydrogel poly[methyl vinyl ether-alt-mono-sodium maleate]-AgNO₃ after ion exchang by Cu⁺²](image)
(Table 3.5) shows the effect of Cu/Ag ratio and reaction time on the ion exchange reaction. It can be seen that at 1 eq. ratio, there is remaining silver and cation exchange with Cu\(^{2+}\) is not complete (Cu:Ag =1 :0.2). However as Cu increases to 5 equivalents or higher the exchange reaction is complete with no remaining silver. Thus to study the effect of time, the Cu:Ag was fixed at 5 equivalent and the time was modified for 8, 16, and 24 h. It was observed that 8 h is not sufficient for complete ion exchange where remaining silver is detected with Cu:Ag (1:0.1 ). At 16 or 24 h the ion exchange reaction was complete with no remaining silver.

Table 3.5: Results of intact polymer hydrogel [Ag,Na][poly(methyl vinyl ether-alt-maleate)] exchanged by Cu(NO\(_3\))\(_2\) aq. solution

<table>
<thead>
<tr>
<th>Entry</th>
<th>Amount of Cu: Ag</th>
<th>Exchange time</th>
<th>Cu/Ag after exchange</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1 eq.</td>
<td>24 h</td>
<td>1 : 0.2</td>
</tr>
<tr>
<td>2</td>
<td>5 eq.</td>
<td>24 h</td>
<td>1 : 0</td>
</tr>
<tr>
<td>3</td>
<td>10 eq.</td>
<td>24 h</td>
<td>1 : 0</td>
</tr>
<tr>
<td>4</td>
<td>5 eq.</td>
<td>8 h</td>
<td>1 : 0.1</td>
</tr>
<tr>
<td>5</td>
<td>5 eq.</td>
<td>16 h</td>
<td>1 : 0</td>
</tr>
<tr>
<td>6</td>
<td>5 eq.</td>
<td>24 h</td>
<td>1 : 0</td>
</tr>
</tbody>
</table>
Cation exchange of polymer hydrogel the poly[methyl vinyl ether-\textit{alt}-mono-sodium maleate]-\textit{AgNO}_3-vacuum dried powder

The cation-exchange of poly[methyl vinyl ether-\textit{alt}-mono-sodium maleate]-\textit{AgNO}_3-vacuum dried powder with Cu(NO\textsubscript{3})\textsubscript{2} was done after dissolving the powder in ethanol and addition of Copper nitrate aqueous solution. It was clearly observed that Ag\textsuperscript{+} was replaced with Cu\textsuperscript{2+} this is evident by color change of crystal from yellow in the poly[methyl vinyl ether-\textit{alt}-mono-sodium maleate]-\textit{AgNO}_3-vacuum dried powder into greenish blue after addition of copper as shown in (Figure 3.18)

![Image of polymer poly[methyl vinyl ether-\textit{alt}-mono-sodium maleate]-\textit{AgNO}_3-vacuum dried powder before and after ion exchange with Cu\textsuperscript{2+}]

Cation-exchange by aluminum(III)

Cation-exchange of the hydrogel poly[methyl vinyl ether-\textit{alt}-mono-sodium maleate]-\textit{AgNO}_3 was tested using a variety of metals such as Al. The gel was suspended in an ethanol/water solution and treated with a five-fold excess of Al(NO\textsubscript{3})\textsubscript{3} solution [n(Ag\textsuperscript{+})/n(Al\textsuperscript{3+}) = 1:5] for (Figure 3.19). It was
observed that the intact hydrogel color changed from yellow to brown which suggests that Ag\(^+\) exchange for Al\(^{3+}\) confirming Ag\(^+\) release of the hydrogel in the presence of harder cations than Ag\(^+\).

Figure 3.19: Conversion of the intact polymer hydrogel poly[methyl vinyl ether-alt-mono-sodium maleate]-AgNO\(_3\) into poly[methyl vinyl ether-alt-mono-sodium maleate]-Al(NO\(_3\))\(_3\).

SEM-EDX results showed almost all the silver (I) cations in the intact hydrogel and powder could be exchanged by aluminum (III). This is clear through the increase in aluminum amount reaching an Al: Ag ratio of 118.6:1 after the experiment as shown in (Figure 3.20).
The reason why silver (I) cations could be easily exchanged by copper(II) or aluminum(III) is probably due to the coordination between copper(II) and oxygen is much stronger than coordination between silver(I) and oxygen. Single crystal structures of maleic acid-cupric complexes have also been reported before.\textsuperscript{109,110} According to the single crystal structures, in one maleic acid molecular, two of the four oxygen atoms have coordination with one Cu (II) cation and the other two have coordination with another Cu (II) cation. The distances of the four oxygen-copper bonds in maleic acid-cupric complexes are 1.950Å, 1.953Å, 1.957Å and 1.966Å.\textsuperscript{110} And the distances of the three oxygen-silver bonds in maleic acid-silver complexes are 2.314Å, 2.388Å and 2.488Å.\textsuperscript{111} It is deduced that more and much stronger coordination lead to the cation exchange of silver(I) by copper(II).
The cation exchange of silver (I) cations by copper(II) cations allowed optical observation of silver ions release which prompted medical application studies.

3.4.3 Application of polymer hydrogel poly[methyl vinyl ether-\textit{alt}-mono-sodium maleate]\textperiodcentered AgNO$_3$

**Antibacterial activity test of polymer hydrogel poly[methyl vinyl ether-\textit{alt}-mono-sodium maleate]\textperiodcentered AgNO$_3$**

The antibacterial properties of silver-containing hydrogels or entrapped Ag nanoparticles have been investigated extensively.$^{75,112-117}$ An important aspect is the improved cytocompatibility recently found in the of coordination polymer hydrogel fabricated by self-assembly of silver(I)-glutathione.$^{118}$ This study combines favourable properties of a potential new wound dressing, e.g., the fast, scalable synthesis of the hydrogel poly[methyl vinyl ether-\textit{alt}-mono-sodium maleate]-AgNO$_3$ with a non-toxic polymer PVM/MA that is widely used for pharmaceutical purposes.$^{104}$ Mixing of PVM/Na-MA and AgNO$_3$-solutions could allow hydrogel formation when needed on the wound and it would solve the problem of unfavourable coloration due to nanoparticle formation in the ageing hydrogel or Ag$^+$/polymer solutions.
The antibacterial tests were carried out using intact polymer hydrogel poly[methyl vinyl ether-alt-mono-sodium maleate]·AgNO$_3$ against gram-negative $E. \ coli$, $P. \ aeruginosa$ and gram-positive $S. \ epidermidis$, that are frequently found on burn wounds.\textsuperscript{119} In this study, the zone-of-inhibition test method was used to examine the antibacterial activity of silver (I) hydrogel against the above-mentioned three bacteria on Luria–Bertani (LB) agar plates. The inhibition zones of the silver (I) hydrogel are shown in (Figure 3.21). It is clear that the silver (I) hydrogels show excellent antibacterial activities against the three bacteria.
Figure 3.22: Antibacterial activity assays with aqueous solution of polymer hydrogel poly[methyl vinyl ether-alt-mono-sodium maleate]-AgNO₃. The photographs were taken after incubation at 37 °C overnight.

The same observation was observed with aqueous solution of polymer hydrogel poly[methyl vinyl ether-alt-mono-sodium maleate]-AgNO₃ as shown in (Figure 3.22)

**Minimum inhibitory concentrations (MIC)**

Minimum inhibitory concentrations (MIC) tests of the polymer hydrogel [Ag,Na][poly(methyl vinyl ether-alt-maleate)], AgNO₃ and PVM/MA against *E. coli*, *S. epidermidis* and *P. aeruginosa* were carried out in LB broth in order to evaluate the antibacterial activities quantitatively. MIC values of the polymer hydrogel [Ag,Na][poly(methyl vinyl ether-alt-maleate)], AgNO₃ and PVM/MA were summarized in (Table 3.6). MIC values of silver (I) hydrogel against *E. coli*, *S. epidermidis*, and *P. aeruginosa* were 4.0, 3.5 and 4.0 µg mL⁻¹, respectively as shown in (Table 3.6). The MIC values were even lower than those of AgNO₃ (6.0, 5.5 and 6.0 µg mL⁻¹), rather
than those of the PVM/MA (4000, 2500 and 3000 µg mL\(^{-1}\)), which exhibited the effective antibacterial activity and property of the silver (I) hydrogel, and indicating that the antibacterial activity of the silver (I) hydrogel is mainly due to the active silver species released from the hydrogel.

Table 3.6: MIC values of polymer hydrogel [Ag,Na][poly(methyl vinyl ether-alt-maleate)], AgNO\(_3\) and PVM/MA against different microorganisms

<table>
<thead>
<tr>
<th></th>
<th>E. coli</th>
<th>P. aeruginosa</th>
<th>S. epidermidis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ag(I)-hydrogel</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(µg of Ag mL(^{-1}))</td>
<td>4.0</td>
<td>4.0</td>
<td>3.5</td>
</tr>
<tr>
<td>AgNO(_3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(µg of Ag mL(^{-1}))</td>
<td>6.0</td>
<td>6.0</td>
<td>5.5</td>
</tr>
<tr>
<td>PVE/MA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(µg mL(^{-1}))</td>
<td>4000</td>
<td>3000</td>
<td>2500</td>
</tr>
</tbody>
</table>

**Release amount of silver**

The release rate of soluble silver species is important to inhibit bacteria that have a relative long growth period. The release rate of silver ion from the hydrogel in phosphate buffered saline (PBS) was measured by inductively coupled plasma (ICP) method as shown in (Figure 3.23).
Figure 3.23: Amount of silver released from the intact polymer hydrogel poly[methyl vinyl ether-alt-mono-sodium maleate]-AgNO₃ in PBS solution as a function of immersion time.

The concentrations of the released silver species were 48.9 µg mL⁻¹ and 70.4 µg mL⁻¹ (from 8,640 µg of Ag mL⁻¹ hydrogel) after one hour and six hours, respectively, which were much higher than the MIC values against E. coli, S. epidermidis, and P. aeruginosa. The sustained release of silver species in PBS solution is beneficial to maintain long-term antibacterial activity.
CHAPTER 4: Conclusion

The formation, characterization and properties of the hydrogel poly[methyl vinyl ether-alt-mono-sodium maleate]-AgNO₃ were described. The intermediate product poly(methyl vinyl ether-alt-mono-sodium maleate) PVM/Na-MA has been characterized and it was shown that it can be converted to a new family of metallo-supramolecular polymer hydrogels by reactions with aqueous metal salt solutions. Ion exchange proceeds in poly[methyl vinyl ether-alt-mono-sodium maleate]-AgNO₃ by replacing Ag⁺ ions irreversibly with harder metal cations. By ion-exchanged reactions of poly[methyl vinyl ether-alt-mono-sodium maleate]-AgNO₃ with Cu²⁺ the Cu-hydrogel poly[methyl vinyl ether-alt-mono-sodium maleate]-Cu(NO₃)₂ was prepared. Antibacterial tests showed the hydrogel had effective antibacterial activity. The synthesis of poly[methyl vinyl ether-alt-mono-sodium maleate]-AgNO₃ can be scaled up and medical applications of this hydrogel for example as burn wound dressing are currently under investigation. In future it is planned to investigate the ageing of poly[methyl vinyl ether-alt- mono-sodium maleate]-AgNO₃ and silver nanoparticle formation by UV-VIS spectroscopy.

The findings in this thesis have been submitted for publication.¹²⁰
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