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Supramolecular Gels Derived from Biomolecules

Bachelor's thesis
7.6.2021
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Abstract

This Bachelor's thesis consists of a literature part and an experimental part.

The literature part first presents the terminology of supramolecular chemistry followed by the concept of a supramolecular gel. After introducing selected biomolecules, some applications based on supramolecular gels derived from them are reviewed.

In the experimental part the gel formation abilities of three bile acid derivatives were examined.

Preface

This Bachelor's thesis was made in spring of 2021 at the Chemistry Department of University of Jyväskylä. The literature part displays the features and applications of supramolecular gels. In the experimental part the gel forming abilities of three different bile acid derivatives were examined. The databases of JYKDOK and Google Scholar were the primary sources when searching for the articles.

Acknowledgments for docent Elina Sievänen for checking my thesis thoroughly and giving me much needed feedback. I also want to thank doctoral student Riikka Kuosmanen for guiding me with the experimental part. Both of you were always available when I had questions.

I am grateful for the university for arranging the opportunity to perform the experimental part despite the pandemic.

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Milka Poimala

Contents

1. Introduction

Supramolecular chemistry is a relatively new area of research, which has rapidly expanded in the recent 40 years. Supramolecules are held together by non-covalent bonds, which makes them more labile than macromolecules with covalent bonding. Hydrogen bond is an example of non-covalent weak interaction.

Gels are topical substances in our everyday lives, in the forms of hygiene products and food to name a few. Conventional gels, known as chemical gels, are formed by a covalent polymer network entrapping the fluid. Supramolecular gels, also known as physical gels, differ from polymer assembled gels due to their capability to hierarchical self-assembly and consequent thermoreversible nature. These responsive supramolecular gels possess potential for huge range of applications, from water purification to tissue engineering, even acting as parts in electronical devices.

Amino acids, peptides, and steroids are of interest as supramolecular gelators due to their endogeneity and biodegradability. These are just some factors that make them great candidates for targeted drug delivery and other pharmaceutical uses.

2. Supramolecules

Jean-Marie Lehn, who won the Nobel prize for his work in the area, defined supramolecular chemistry as ‘chemistry of molecular assemblies and of the intermolecular bond’. More colloquially term ‘chemistry beyond molecule’ can be used. Supramolecules are held together by non-covalent bonding. Individually these interactions are much weaker than covalent bonding. Supramolecular chemistry was originally defined as non-covalent interactions between a ‘host’ and a ‘guest’ molecule. Figure 1 illustrates the differences between molecular and supramolecular chemistry according to Lehn.[1]

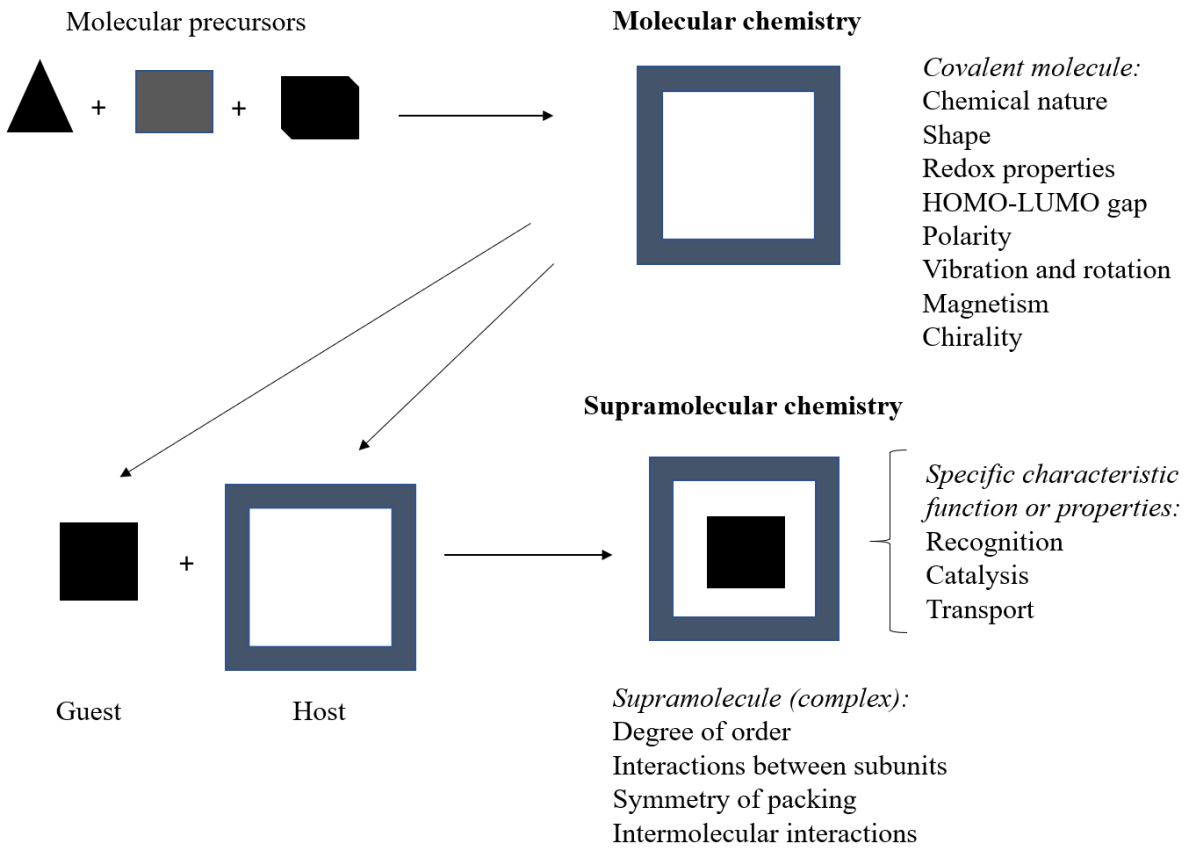

Figure 1. Comparison of molecular and supramolecular chemistry according to Lehn.[1]

The host-guest complex can be expressed in two different manners, *via* a cavitate or a clathrate. Cavitate, which is a box-shaped host molecule where the host accommodates the guest, can appear in solid or liquid form. In this case the host is assembled with covalent bonds, and the interaction with the small molecule takes place by non-covalent bonding. In a clathrate, where the host is a larger molecule and the guest is a smaller molecule, the lattice inclusion complex forming *via* crystallization appears in solid state only. However, the spontaneous self-assembled aggregate between DNA-strands does not correspond to the classical host-guest description. In addition of host-guest analogy terms lock-key or receptor-substrate can be used.[2]

In addition of guest-host systems, work in modern supramolecular chemistry includes molecular recognition called 'self-processes', such as self-assembly. Supramolecular chemistry interfaces with the emergence of complex matter and nanochemistry. [1]

Supramolecules can also be called supermolecules, which in one definition means the supramolecular assembly of two or more molecules *via* non-covalent bonding. Term supermolecule is also used in biochemistry for biomolecules, such as peptides. In some cases,

it can also describe ternary systems formed by ionic interactions or by incorporation of solvent molecules within a lattice.[3]

The term non-covalent bonding encompasses a huge range of attractive and repulsive effects. The interplay of all these interactions and effects relating both to the host and the guest as well as their surroundings is vital when considering a supramolecular system.[4] The nine most important non-covalent binding forces in supramolecular chemistry according to Anslyn and Dougherty[4] are:

1) Ion-ion interactions; ionic bonding is as strong as covalent bonding. Though, the lattice structure breaks down in solution due to solvation.

2) Ion-dipole interactions, such as between a water molecule and a Na^+ ion; these can appear in solid state and in solutions. Some ion-dipole complexes can be stabilized with the chelate effect. Ion-dipole interactions also include coordinative bonds.

3) Dipole-dipole interactions are expressed well in solid state organic carbonyl compounds. However, in solutions these interactions are relatively weak. Hydrogen bonding is a specific type of dipole-dipole interaction. It is best known between water molecules, being the main reason of such a small molecule having such a high boiling point. Hydrogen bonds are also the reason behind the large solubility of polar molecules in water.[5]

4) Cation-π interactions appear in complexes of transition metal cations forming bonds with olefinic or aromatic carbohydrates. For example, in ferrocene $[Fe(C_5H_5)_2]$, the bonding is strong and in no means could be considered non-covalent, whereas the bond between alkaline and alkaline earth metal cations with a C=C double bond is clearly a non-covalent, weak interaction.[6]

5) Anion-π interactions have arisen interest just recently. Intuitively the interaction between an anion and π-electron density should be repulsive. However, a possibility for electrostatic attraction remains, due to the charge difference in neutral aromatic ring and an anion.[7]

6) Aromatic π-π interactions occur between aromatic rings, often in cases where the other is electron rich and the other electron poor. There are two main types of these interactions: face-to-face and edge-to-face. The latter is responsible for the slippery feel of graphite. These interactions also stabilize the DNA double helix.[8]

7) and 8) Polarization of an electron cloud arises when van der Waals interactions form weak electrostatic attraction. Solid state (*i.e.* crystal) structures have a need to achieve a closely

packed arrangement. According to Kitaigorodsky's[9] theory molecules are trying to achieve maximum number of intermolecular contacts (van der Waals), and they go through a shape simplification to achieve that.

9) Closed shell interaction includes secondary bonding interactions, metallophilic interactions, and halogen bonding. Against intuition in some cases atoms with closed shells or with neutral charges do form significant interactions. The strength of these interactions is equivalent to moderate strength hydrogen bonding.[10]

3. Amino acids, peptides, and proteins

Amino acids are the building blocks of life. There are 20 different amino acids, of which every occurring peptide and protein is built from. With these precursors different organisms can make wide range of different products like hormones, antibodies, muscle fibres, feathers, antibiotics, and poisons of mushroom. Every amino acid has its own alphabetical identification that makes the language for protein structures. Proteins are found in all cells and in all parts of the cell. They vary greatly, so there can be a thousand different kinds of proteins in one cell.[11]

All amino acids are α-amino acids, meaning that they have a carboxyl group and an amino group bonded to the same carbon, known as the α-carbon. Amino acids differ from each other in their side chains, which have different structure, size, electric charge, and solubility in water. Except glycine, all amino acids have four different kinds of groups attached to the α-carbon, which makes them chiral molecules. Two isomers, called enantiomers, can be found for every amino acid. The isomers possess either L-configuration (levorotatory) or D-configuration (dextrorotatory). The amino acid residues found in proteins are exclusively L-isomers; only few D-isomers are naturally found in some bacterial cell wall and certain peptide antibiotics. The sizes of amino acids vary from the smallest one, glycine, having a width of 0,5 nm to the largest one, tryptophan, whose width is 1 nm. Figure 2 shows the structure of all the naturally occurring amino acids.[11]

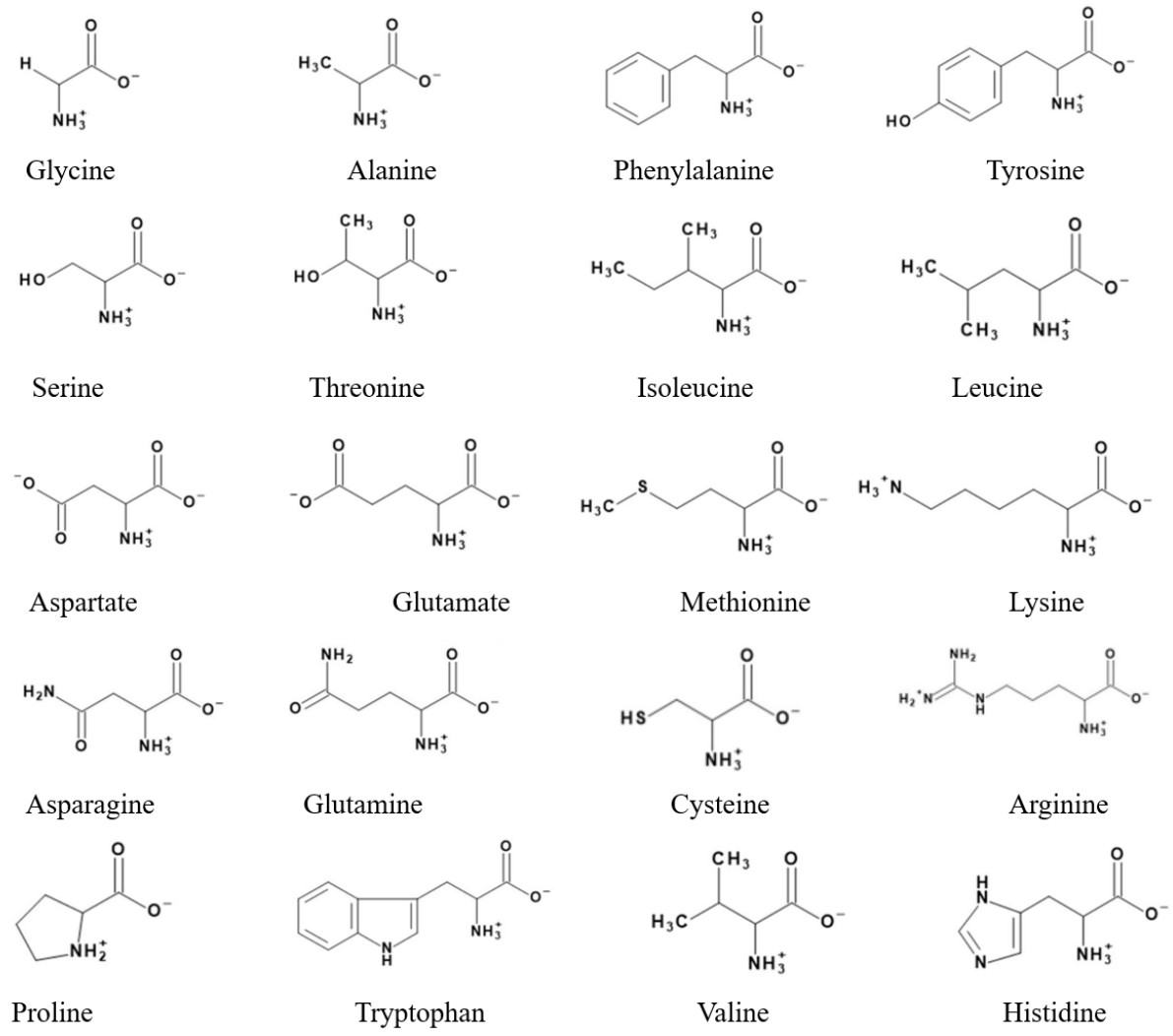

Figure 2. Structure of all the naturally occurring 20 amino acids.

Peptides and proteins are polymers of amino acids. Peptides are formed by covalent bonds, called peptide bonds, between amino acids. Figure 3 shows a peptide with the peptide bond highlighted. A molecule consisting of a few amino acids is called oligopeptide, whereas a molecule with more amino acids is called a polypeptide. It can hold thousands of amino acids.[11]

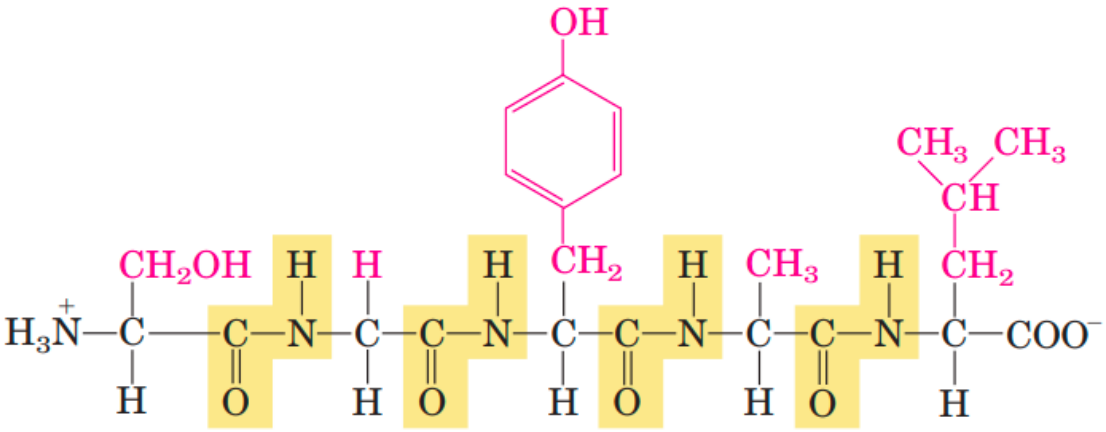

Figure 3. A peptide constructed of the amino acids serine, glycine, tyrosine, alanine, and leucine (from left to right). Peptide bonds are highlighted with yellow, and the side groups of the amino acids are displayed in pink colour. (Nelson, D. and Cox, M., *Lehninger Principles of Biochemistry*, 5th Ed., W. H. Freeman and Company, New York, **2008**, p. 82.)

The structure of proteins can be divided into primary, secondary, tertiary, and quaternary structures. The structures are displayed in Figure 4. The most important element of the primary structure is the sequence of the amino acid residues. Every protein has a three-dimensional structure that reflects its function. The three-dimensional structure of a protein is stabilized with weak interactions, like hydrogen bonds and ionic interactions. The secondary structure includes the most prominent α-helix and β-sheet conformations, in which *e.g.* the primary structure has coiled into an α-helix by hydrogen bonds. The tertiary structure comprehends the overall three-dimensional arrangement of all atoms in a protein. The quaternary structure occurs when there are two or more separate polypeptide chains in a protein. The oxygen binding haemoglobin in red blood cells is an example of quaternary structure.[11]

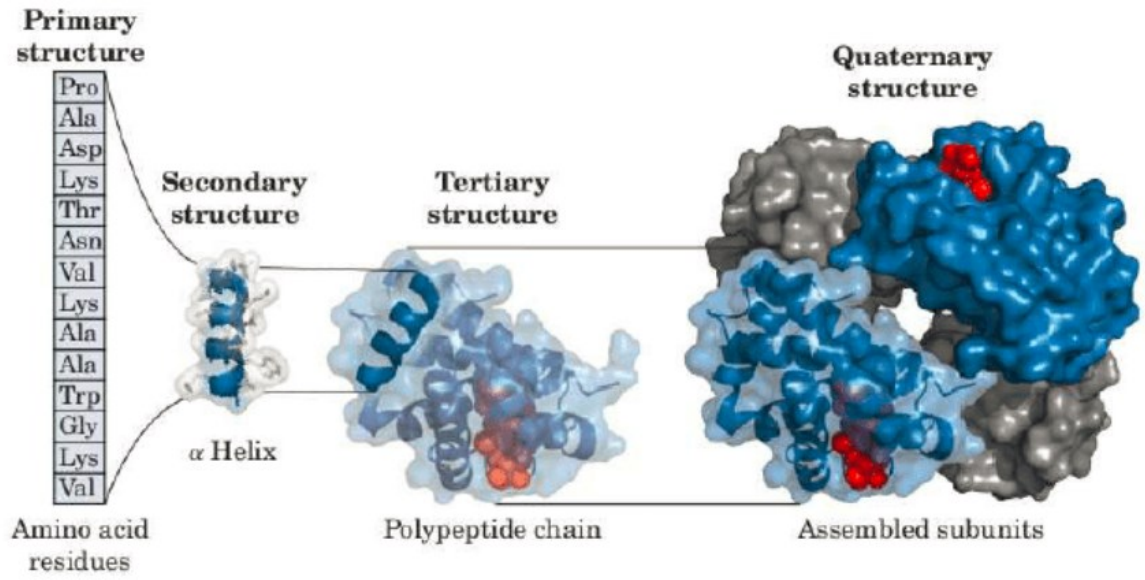

Figure 4. Protein's primary, secondary, tertiary, and quaternary structures. (Nelson, D. and Cox, M., *Lehninger Principles of Biochemistry*, 5th Ed., W. H. Freeman and Company, New York, **2008**, p. 92.)

The hierarchical mechanism of protein folding allows them to find their active conformation in minutes. On contrast, a random search of lowest energy conformer with a 100-amino-acid-protein would take 10^{27} years.[12]

Due to the importance of self-assembly of proteins, supramolecular chemists have tried to mimic this property in artificial systems and biological models. *Foldamers* are artificial or biomimetic molecules that self-assembly into a particular conformation. They can be, for example, amino acid based short polypeptide sequences.[13]

4. Gels

Gels are familiar to all of us from our everyday lives in the forms of hair gels, toothpaste, contact lenses, jelly, and much more. These gels are typically formed by a permanent polymer network, with covalent bonding, as the gelator and liquid as the swelling agent.

Gels are colloidal state of matter and are usually characterised by the following: "A two or more component system comprising a fibrous solid-like phase immobilising a much larger liquid volume (~1 % by weight per volume). A continuous structure with macroscopic dimensions

that is permanent on the timescale of an analytical experiment. Solid-like in its rheological behaviour."[14] On macroscale dimension gels usually do not flow, so a simple test confirming a substance to be a gel is to invert its container to see how it acts (Figure 5). Some gels flow when shortly shaken but re-form on standing, a phenomenon called *thixotrpy*. Despite solid appearance the liquid component of the gel is mobile and held only by capillary and surface forces. The solid network is formed with covalent polymer or supramolecular assembly by small molecules. Usually, gels are manufactured by melting the gelator above their melting point, T_{gel}, and allowing it to cool down.[15]

Figure 5. A polymer gel passing the 'inversion test'.

For gels the rheology is often studied. It examines the deformation and flow of matter under the influence of applied stress. By this manner, several categories of rheological behaviour can be defined. Matter can be defined as solid or liquid, but a range of substances falls between these categories and can be elastic, viscous or both. In the last case the matter is defined to be viscoelastic. In rheology the quantity G, called the complex dynamic modulus, is needed for examining properties of viscoelastic materials. It describes the stress-strain relationship by the quantities elastic storage modulus G' and elastic loss modulus G''. With the complex dynamic modulus supramolecular gels can be described to be soft glassy materials, cellular solids, or fractal/colloidal systems.[16]

4.1 Supramolecular gels

Supramolecular gels assemble between low-molecular-weight gelators (LMWGs) by non-covalent interactions. They form a solid-like nanoscale network spanning a liquid-like continuous phase. LMWGs are often difficult to manipulate, easily destroyed, and have a poor rheological performance. Only a limited range of supramolecular gels have the rheological strength of to be unmoulded and retain their shape.[17] Many of the supramolecular gels are viscoelastic materials.[16] The crosslinked network of the gel can be reversed by input of energy.[18] In Figure 6 is a scanning electron microscope (SEM) picture of the supramolecular gel network of lithocholic acid derivative as the LMWG.

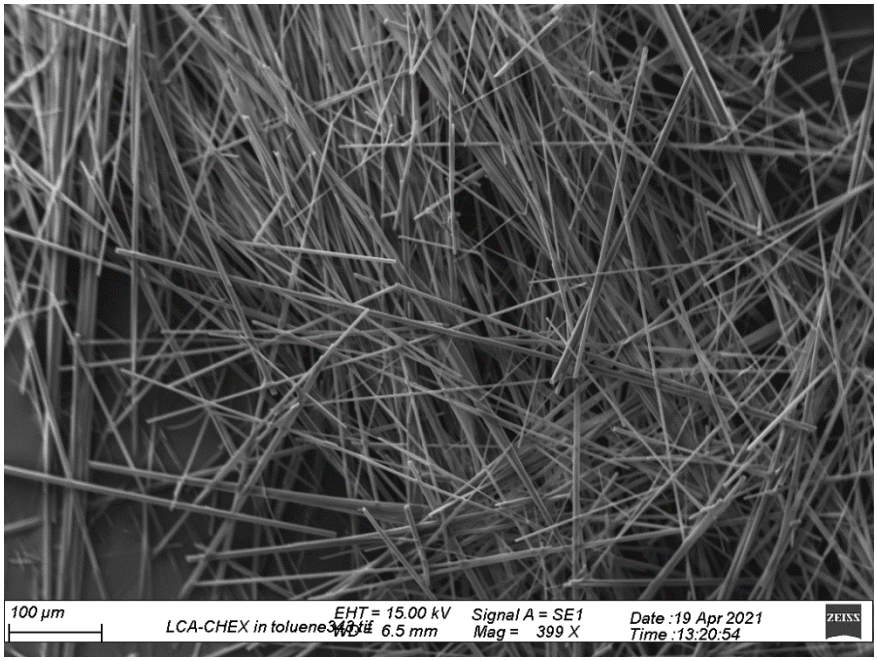

Figure 6. SEM micrograph presenting the fibrous gel network formed by lithocholic acid derivative as the gelator in toluene. R. Kuosmanen *et al*., unpublished results.

Typically, LMWGs include strong hydrogen bond donors or acceptors, such as bis(ureas), amides, fatty acids, steroids, and nucleobases. In organic solvents hydrogen bonding dominates as the mechanism of aggregation of the LMWG, whereas in water hydrophobic interactions dominate. The gel aggregation, like many other formations of supramolecular structures, is hierarchical. One property that makes LMWG systems very interesting is the reversibility of the supramolecular interactions between the gelator molecules leading to the possibility of dynamic behaviour, *i.e.* self-healing and slow release.[15]

Understanding and probing the behaviour of supramolecular gels is difficult, because the gels arise from assembly across many length scales. One-dimensional growth must be favoured for formation of suitable aggregates that can eventually entangle. Predicting the gel formation of a molecule is often extremely difficult. Thus, gelation has been described as an empirical science.[19]

Properties of gels are highly process-dependent, so by using one gelator molecule materials with very different properties can be accessed. The use of multiple gelators offers an opportunity to obtain materials with a wide range of properties and high degree of information.[18]

Multicomponent systems have significant opportunities for LMWGs. Three classes of multicomponent systems according to Buerkle and Rowan[20] are: 1) a two-component gel-phase, where both components are needed for gel formation, 2) a two-gelator system, where both gelator molecules can gelate individually, and 3) a system comprising of a gelator and non-gelling additive.

4.1.1 Supramolecular hydrogels

Hydrogels are semi-colloidal systems having a three-dimensional configuration of polymeric network with the capability of imbibing high amounts of water or biological fluids.[21] Even as low a gelator concentration as 0,02 % w/v has been reported.[22] The high amount of water provides liquid-like behaviour to these solid-like rheological materials. This feature leads to great resemblance to human tissues. Hydrogels have received a designation of *Smart Materials* due to their use in targeted and controlled drug delivery based on their responsiveness to the environment.[23]

When gelators are mixed with ionic solutions, hydrogels can be formed through ionic interactions. Networks with positive and negative charges attracting each other results. The resulting gels are called supramolecular ionogels.[24]

4.1.2 Other types of supramolecular gels

In *organogels* the liquid component of the gel is an organic solvent, such as isooctane, oil, DMSO, or ethanol. When using LMWGs, the nature of the intermolecular interactions in the organogels are physical, which can lead either to solid-fibre matrix or fluid-fibre matrix.[25]

In *metallogels* the fibres are held together *via* metal-ligand or metal-metal interactions. The metal-gelator coordination mimics biological metal-peptide bonds, which influence self-assembly and mechanical properties of the gels. Mechanical properties include biodegradability, biocompatibility, tunability, and recyclability.[26]

In *aerogels* instead of liquid a gas component, for example carbon dioxide, is used.[15] The highly porous aerogels have a great thermal and acoustic insulation, transparency, and strength per unit weight. Though, the preparation of aerogels is not feasible due to the framework collapse.[27]

Xerogel is the network of fibrils of a dried gel. The evaporation of solvent in xerogels leaves only the gelator usually in the form of tangled mesh of fibres.[27]

4.1.3 Stimuli-responsiveness of supramolecular gels

In addition of the rational design of LMWGs, there have been extensive efforts for creating stimuli-responsive molecular gels. For supramolecular gels, the gel-solution transition is reversible. With appropriate LMWGs the induction of the gel-solution transition can be triggered with physical and chemical stimuli. Physical stimuli include heat, mechanical forces, ultrasound waves, and UV-vis light, whereas chemical stimuli cover acid-base reagents, salts, neutral molecules, redox reagents, and enzymes.[28]

Physiological stimuli can trigger changes in the structural and physicochemical properties of supramolecular gels, which may lead responses such as drug release and/or alteration of shape.[29]

4.1.4 Applications of supramolecular gels

Supramolecular gels offer a huge variety of opportunities for different applications. They can be used as lithium greases, within a blend of natural oil and lithium salt, which is a commonly used lubricant.[22] Modern *napalm* uses a polymer additive to achieve gelation. The long, fibrillar nature of these gels suggest a use for molecular electronics.[15] The self-assembly, which leads to aggregates, can be suitable for optoelectronic devices when using multiple gelators.[18]

The porosity, the solvent presence or absence, and the interconnectivity makes aero- and xerogels distinctive from other gels. Aerogels can be used in nanomaterials; for example carbon-based aerogels can be used as "aero-capacitors". The randomly criss-crossing mat of xerogels, for one, gives an opportunity for magnetic coupling and the processes of excited or ground-state electron transport.[27]

Organogelators can be used in safer disposal of used domestic oils and in oil spill recovery.[22]

5. Amino acid and peptide based supramolecular gels

The amphiphilic character of amino acids gives them a great potential for hydrogelation. The hydrophilic parts create opportunities for hydrogen bonding and stabilization of water molecules, whereas the hydrophobic parts may prompt aggregation.[29]

The structure and chemical properties of peptides with the growing knowledge of the fundamental aspects of proteins has led to structurally diverse designs of peptide-based supramolecular gels. Examples ranging from dipeptides to high-molecular-weight protein-like analogues have been reported.[30]

The simplest derivatives of peptide-based gelators are *N*-protected amino-acids. In these the polar fragments of the amino acid backbone are combined with hydrophobic groups either as *N*-capping residues or as side groups. Many of the *N*-protected amino acids bearing aromatic rings are reported to be efficient hydrogelators, where π-stacking is crucial interaction of gel network formation.[31]

The design opportunities increase by growing the size of the gelator molecule by adding more amino acids. The gelator molecule should be at least partially preorganized in order to function

similarly to the secondary structure of the protein.[32] It is not easy to predict in advance the final structure of a supramolecule by tertiary or particularly by quaternary assembly.[33]

One of the essential amino acids for humans, phenylalanine (Phe, F), is the smallest molecule reported to date to form gel networks in water. The L-conformation of the amino acid has a crystalline gel state. Ramalhete *et al.*[29] have used single and multi-component hydrogels of L-phenylalanine as model materials to develop an NMR-based approach for deeper understanding of supramolecular gelation. They discovered that adding amino acid L-serine resulted in weaker materials, but addition of L-tryptophan stabilized the 3D-network of the gel. Their study confirmed that characterizing the multiphase character of supramolecular gels requires combination of complementary analytical techniques.

5.1 Applications of peptide and protein based supramolecular gels

Protein-based hydrogels are widely used in pharmaceutical industry as drug delivery systems. Other biomedical applications for these gels can be found in tissue engineering, such as development on artificial bone substitutes.[21] In addition to the biomaterial science, self-assembling peptides can be used in many other areas of nanoscience.[30] Figure 7 collects together applications of peptide-based supramolecular gels.

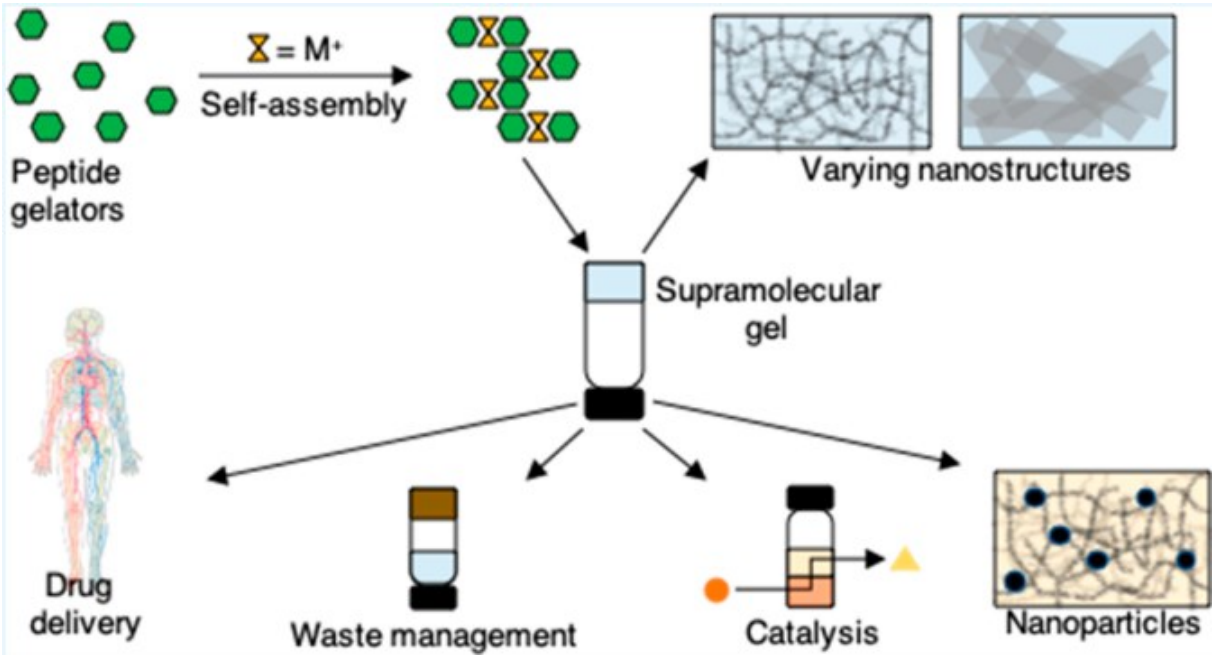

Figure 7. Applications of peptide-based supramolecular gels. Reproduced from (Shao, T.; Falcone, N. and Kraatz, H. B., Supramolecular Peptide Gels: Influencing Properties by Metal Ion Coordination and Their Wide-Ranging Applications, ACS Omega, **2020**, 5, 1312–1317.) with the permission of ACS.

Peptide-based supramolecular metallogels also have a wide range of applications, such as[26]: drug applications, waste management, scaffolds for nanoparticle formation, and catalysis. They also possess a great potential in drug delivery systems due to their resemblance of biological structures. Moreover, peptide-based supramolecular metallogels could be used as target specific and efficient cancer drugs.[34] They are waste-sensitive, reusable, and biodegradable, which makes them useful in the fight against pollution. Their peptide basis can aid metal absorption, and their porous structures can trap pollutants.[35] They can be used as scaffolds for nanoparticle formation due to their three-dimensional matrix. Peptide-based supramolecular metallogels can also be functionalized to fabricate metal nanoparticles. This gel-nanoparticle hybrid material could be used as recyclable catalyst.[36] By incorporating inorganic components into protein-based hydrogels, unique properties such as redox, catalysis, and photochemical properties, can be introduced to the resulting material. By constructing metallogel-based soft, functional materials with appropriate functionality and suitable metal ion, catalytic activity can be shown.[37]

6. Bile acids

The bile acid science, *cholanology*, has been around for over a century and still it is important in medicine and biology. The primary function of cholesterol-derived bile acids is to emulsify the lipids of food. Bile salts together with lipids, fats, or cholesterol form mixed micelles. All bile acids consist of a rigid steroid nucleus and a short aliphatic side chain, which makes them amphiphiles. They are biosynthesized in the liver and stored in the gall bladder.[22] Due to their structural uniqueness, bile acids are pharmacologically interesting and have a potential in carrying, enhancing, and absorbing of drugs. They are also the major regulators of cholesterol homeostasis.[22, 38] The human bile acid pool consists of the primary bile acids cholic acid (CA) and chenodeoxycholic acid (CDCA), which are biosynthesized in the liver cells, and the secondary bile acid deoxycholic acid (DCA), and traces of lithocholic acid (LCA).[39] The structure of the most abundant bile acid, cholic acid, is shown in Figure 8.

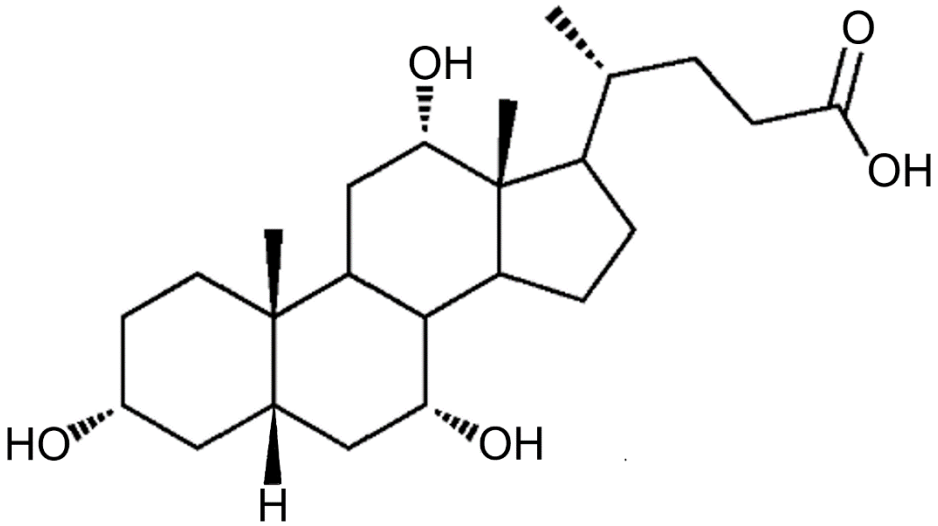

Figure 8. Cholic acid.

6.1 Bile acid-derived supramolecular gels

The availability and organ specificity of the enterohepatic pathway of bile acids are just a few reasons why bile acids have such a great potential in pharmaceutical industry.[38] They have a potential to be used as therapeutic agents for treating metabolic deceases of the liver.[39] They have also shown antiviral properties and bacterial swelling features.[40]

Some bile acids and their derivatives are potential gelators in both organo- and hydrogels, in which the latter has a markable water-holding ability.[22] Gundiah *et al.*[41] have demonstrated an application from tripodal cholamide-derived gels, an organic template for creating inorganic nanotubes.

Expression of supramolecular properties seems to be a result from facial amphiphilicity and the rigid backbone of bile acids.[22] Kuosmanen *et al.*[42] have studied the self-assembly properties of the bile acid alkyl amide-based LMWGs, which varied with respect of the amount of hydroxyl groups attached to the steroidal backbone as well as with the length and branching of the attached alkyl chain. They concluded that the number of hydroxyl groups plays a major role in gelation.

7. Aim of the experimental work

In the experimental part of this bachelor's thesis the aim was to examine the gel formation abilities of three different derivatives of lithocholic acid. The structures of the gelator compounds are shown in Figure 9. The bile acid derivatives used in the study had been synthesized in the laboratory earlier. The gel formation was examined in different solvents and with three different metal salts. The used metal salts were copper chloride, nickel chloride, and zinc chloride. Also, different ratios of solvent and the metal salt solution were inspected; 1:1, 3:7, and 1:9. In total 27 series of tests and 260 gelation experiments were conducted.

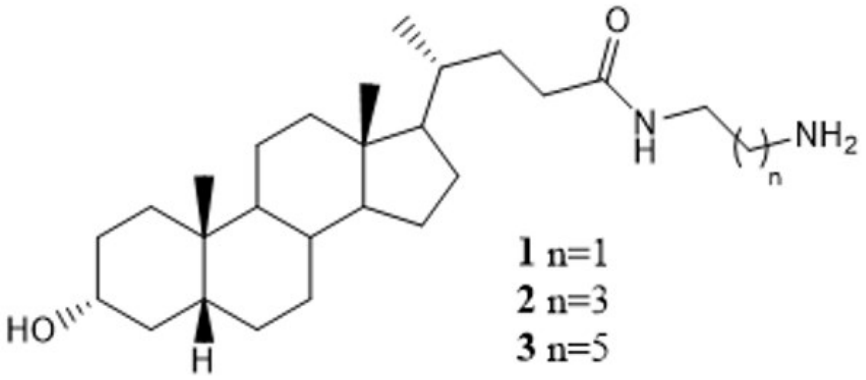

Figure 9. Lithocholic acid derivatives used as gelators in the experiment part.

8. Methods

The gelation tests were carried out by weighing precisely 5 mg of the gelator molecule in a 2 ml glass vial. It was dissolved into the chosen solvent and the metal salt solution was added. In each vial, the amount of the metal salt was one third of the amount of substance of the gelator molecule, but the concentration and consequently the volume of the metal salt solution added varied. Total volume of the solution was 500 µl. The intention was to examine, how the ratio of water in the solution affected on the gel formation. The sample was treated with ultrasound bath for one minute. The used ultrasound bath was Ultrasonic Cleaner BRANSON 200, which operates with the frequency of 45 kHz. After ultrasound treatment, the sample was heated with a hot air gun until the gelator was fully dissolved or the boiling point of the solvent was reached. After the treatment, the sample was let to cool down, and the possible gel formation was followed after cooling and in the following days.

The used solvents and reagents are listed in Tables 1 and 2.

Table 1. Solvents used

Solvent	Purity (%)	Manufacturer
Methanol	≥99.8	Honeywell
Ethanol	99.5	
1-Propanol	>99.5	VWR
1-Butanol	100	VWR
1-Pentanol	99	Fluka
1-Hexanol	98	Riedel de haën
1-Heptanol	99	Merck
1-Octanol	>98	Prolabo
DMSO		
DMF	99.9	AnalaR

Table 2. The metal salts used

Metal salt	Purity (%)	Manufacturer
$CuCl_2$	97	Aldrich
$NiCl_2$	≥98	Merck
$ZiCl_2$	≥97	Fischer

9. Results

The formed permanent gels are listed in Table 3 and the formed temporary gels are listed in Table 4. The stability of the gel phase of the temporary gels varied from one hour to several days.

Table 3. Permanent gels

Gelator*	Solvent	Metal salt	Solvent : Salt solution (v/v)
1	DMF	$CuCl_2$	1:1
1	EtOH	$CuCl_2$	3:7
1	DMSO	$NiCl_2$	1:1
1	DMF	$NiCl_2$	1:1
1	DMF	$NiCl_2$	3:7
1	DMSO	$ZiCl_2$	1:1
1	DMF	$ZiCl_2$	1:1
1	MeOH	$ZiCl_2$	3:7
2	EtOH	$CuCl_2$	3:7
2	MeOH	$NiCl_2$	1:1
2	DMSO	$NiCl_2$	1:1
2	DMF	$NiCl_2$	1:1
2	MeOH	$NiCl_2$	3:7
2	DMF	$NiCl_2$	3:7
2	MeOH	$NiCl_2$	1:9
2	MeOH	$ZiCl_2$	1:1
2	DMF	$ZiCl_2$	1:1
2	MeOH	$ZiCl_2$	3:7
3	DMF	$CuCl_2$	1:9
3	MeOH	$NiCl_2$	3:7
3	MeOH	$ZiCl_2$	3:7
3	1-Propanol	$ZiCl_2$	1:9

* Compound number same as in Figure 9.

Table 4. Temporary gels

Gelator	Solvent	Metal salt	Solvent : Salt solution (v/v)
1*	MeOH	$ZiCl_2$	3:7
1*	DMF	$ZiCl_2$	3:7
3	DMF	$CuCl_2$	1:9
3	DMSO	$NiCl_2$	1:1
3	MeOH	$ZiCl_2$	1:1
3	DMF	$ZiCl_2$	1:1
3	DMF	$ZiCl_2$	1:9

* Formed gel with ultrasound treatment

Most of the permanent gel formed into a gel phase as soon as they cooled down. Overall success of permanent gel forming was 8.5 %. With respect of the gel formation, nickel chloride was the most favourable of the metal salts, whereas copper chloride was the least favourable one. Considering the length of the side chain, the butyl derivative formed more gels than the ethyl derivative, which - for one - formed more gels than the hexyl derivative. When comparing the ratios of the solvent and the metal salt solution, the ratio of 1:1 formed more gels than the ratio of 3:7, which - for one - formed more gels than the ratio of 1:9. This clearly shows that water does not favour the gel formation. Compound 2 with $NiCl_2$ with 1:1 solvent:metal salt solution (v/v) formed numerically most gels (in three solvents out of the tested 10 solvents). Methanol and DMF were the best solvents in inducing gel formation, followed by DMSO, ethanol, and 1-propanol in that order. Solvents, that did not form any gels, were 1-butanol, 1-pentanol, 1-hexanol, 1-heptanol and 1-octanol. The success rate of gel formation regarding these different aspects is collected in Table 5.

When considering the temporary gels, compound 3 and $ZiCl_2$ were the most favourable components. The compound 1 formed two gels with ultrasound treatment, but the gels collapsed within an hour. The gel phase of rest of the temporary gels lasted several days. The same solvents, methanol and DMF, were good for inducing the formation of both permanent and temporary gels.

Table 5. The success rate of permanent gel formation

Aspect	%	Gel out of all
All	8.5	22/260
Bile acid derivative		
1	9	8/90
2	12	10/85
3	5	4/85
Metal salt		
$CuCl_2$	5	4/80
$NiCl_2$	11	10/90
$ZiCl_2$	9	8/90
Solvent		
MeOH	32	8/25
DMF	32	8/25
DMSO	11	3/27
EtOH	7	2/25
1-Propanol	4	1/27
Solvent/ Salt solution ratio (v/v)		
1:1	13	10/80
3:7	10	9/90
1:9	3	3/90

Results from all of the gelation tests are listed in Appendix 1.

Examples presenting some of the permanent gels are shown in Figures 10- 12.

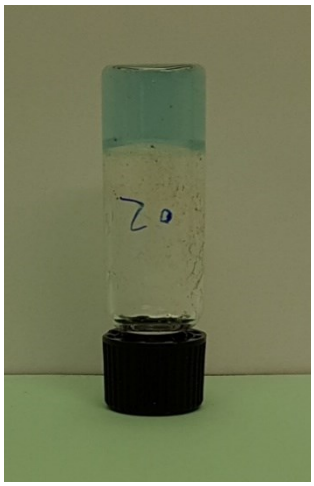

Figure 10. A blue, clear gel formed by compound 2 with ratio of 3:7 of ethanol and $CuCl_2$ solution.

Figure 11. A colourless, clear gel formed by compound 2 with ratio of 1:1 of DMF and $NiCl_2$ solution.

Figure 12. A white gel formed by compound 3 with ratio of 3:7 of methanol and $ZiCl_2$ solution.

10. Discussion

The overall success of gel formation, 8.5 %, in the current study can be considered relatively good. Compounds 2 and 3 formed more gels than compound 1 most likely because the side chains in the previous compounds are longer and therefore can have more interactions with one another, the solvent molecules, and the metal ions.

There might have been a problem with weighing the zinc chloride because the salt was snow-like in its texture. The number of the scale did not fluctuate, but zinc chloride is highly hygroscopic; therefore, it should have been dried before weighing it. Even though it formed a decent number of gels, there is no knowing of the actual molar equivalent of the zinc chloride versus the gelator. Consequently, for more reliable results the zinc chloride part should be redone.

Based on the results, in the future it might be beneficial to concentrate on the solvents methanol and DMF. Of the metal salts, nickel chloride proved to be the most successful and the amount of water should not exceed 50 % of the total volume of the solution. For possible studies of ultrasound responsive gels, compound 1 with zinc chloride and 3:7 solvent:salt solution (v/v) ratio with methanol and DMF as solvents would be beneficial considering the results of this experiment. For this, lower concentrations of the gelator molecule could be tried because all of the weighed amount of compound 1 was not dissolved, when the gel was formed in the ultrasound bath.

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Appendixes

Appendix 1: Tables related to the gelation tests

The gelation was inspected first after adding the salt solution into the solution of the gelator molecule in a given solvent, and then after the ultrasound treatment and heating.

Meanings of the shortenings: PS+ = partially soluble without heating, PS = partially soluble with heating, PG= partial gel, G= gel, G-= weak gel, S+= soluble without heating, S= soluble with heating.

Only five solvents were used in the test series #4 and #7, because the rest of the solvents had been studied already previously.

Table 1. Test series #1 Compound 1 with 1:1 solvent: $CuCl_2$ solution (v/v)

Solvent	Experiment	1 hour	Later
MeOH	PS+, PS+, PS	PS+	
EtOH	PS+, PS+, PS	PS+	
1-Propanol	PS+, PS+, P	PS+	
1-Butanol	PG, PS+, PS	PS+	
1-Pentanol	PS+, PS+, PS	PS+	
1-Hexanol	PS+, PS+, PS	PS+	
1-Heptanol	PS+, PS+, PS	PS+	
1-Octanol	PS+, PS+, PS	PS+	
DMSO	PS+, PS+, PS	PS+	
DMF	PS+, PS+, PS	G	G

Table 2. Test series #2 Compound 1 with 3:7 solvent: $CuCl_2$ solution (v/v)

Solvent	Experiment	1 hour	Later
MeOH	PS+, PS+, PS	PS+	
EtOH	PS+, PS+, PS	G	G
1-Propanol	PS+, PS+, PS	PS+	
1-Butanol	PS+, PS+, PS	PS+	
1-Pentanol	PS+, PS+, PS	PS+	
1-Hexanol	PS+, PS+, PS	PS+	
1-Heptanol	PS+, PS+, PS	PS+	
1-Octanol	PS+, PS+, PS	PS+	
DMSO	PS+, PS+, PS	PS+	
DMF	PS+, PS+, PS	PS+	

Table 3. Test series #3 Compound 1 with 1:9 solvent: $CuCl_2$ solution (v/v)

Solvent	Experiment	1 hour	Later
MeOH	PS+, PS+, PS	PS+	
EtOH	PS+, PS+, PS	PS+	
1-Propanol	PS+, PS+, PS	PS+	
1-Butanol	PS+, PS+, PS	PS+	
1-Pentanol	PS+, PS+, PS	PS+	
1-Hexanol	PS+, PS+, PS	PS+	
1-Heptanol	PS+, PS+, PS	PS+	
1-Octanol	PS+, PS+, PS	PS+	
DMSO	PS+, PS+, PS	PS+	
DMF	PS+, PS+, PS	PS+	

Table 4. Test series #4 Compound 2 with 1:1 solvent: $CuCl_2$ solution (v/v)

Solvent	Experiment	1 hour	Later
EtOH	PS+, PS+, PS	PS+	
1-Propanol	PS+, PS+, PS	PS+	
1-Heptanol	PS+, PS+, PS	PS+	
1-Octanol	PS+, PS+, PS	PS+	
DMSO	PS+, PS+, PS	PG	G-

Table 5. Test series #5 Compound 2 with 3:7 solvent: $CuCl_2$ solution (v/v)

Solvent	Experiment	1 hour	Later
MeOH	PS+, PS+, PS	PS+	
EtOH	PS+, PS+, PS	G	G
1-Propanol	PS+, PS+, PS	PS+	
1-Butanol	PS+, PS+, S	PS+	
1-Pentanol	PS+, PS+, PS	PS+	
1-Hexanol	PS+, PS+, PS	PS+	
1-Heptanol	PS+, PS+, PS	PS+	
1-Octanol	PS+, PS+, PS	PS+	
DMSO	PS+, PS+, PS	PS+	
DMF	PS+, PS+, PS	PG	

Table 6. Test series #6 Compound 2 with 1:9 solvent: $CuCl_2$ solution (v/v)

Solvent	Experiment	1 hour	Later
MeOH	PS+, PS+, PS	PS+	
EtOH	PS+, PS+, PS	PS+	
1-Propanol	PS+, PS+, PS	PG	PS+
1-Butanol	PS+, PS+, PS	PS+	
1-Pentanol	PS+, PS+, PS	PS+	
1-Hexanol	PS+, PS+, PS	PS+	
1-Heptanol	PS+, PS+, PS	PS+	
1-Octanol	PS+, PS+, PS	PS+	
DMSO	PS+, PS+, PS	PG	PS+
DMF	PS+, PS+, PS	PS+	

Table 7. Test series #7 Compound 3 with 1:1 solvent: $CuCl_2$ solution (v/v)

Solvent	Experiment	1 hour	Later
EtOH	PS+, PS+, PS	PS+	
1-Propanol	S+, PS+, PS	PS+	
1-Heptanol	PS+, PS+, PS	PS+	
1-Octanol	PS+, PS+, PS	PS+	
DMSO	PS+, PS+, PS	PG	PS+

Table 8. Test series #8 Compound 3 with 3:7 solvent: $CuCl_2$ solution (v/v)

Solvent	Experiment	1 hour	Later
MeOH	PS+, PS+, PS	PS+	PG
EtOH	PS+, PS+, PS	PS+	
1-Propanol	PS+, PS+, PS	PS+	
1-Butanol	PS+, PS+, S	PS+	
1-Pentanol	PS+, PS+, PS	PS+	
1-Hexanol	PS+, PS+, PS	PS+	
1-Heptanol	PS+, PS+, PS	PS+	
1-Octanol	PS+, PS+, PS	PS+	
DMSO	PS+, PS+, PS	PG	PS+
DMF	PS+, PS+, PS	PG	PS+

Table 9. Test series #9 Compound 3 with 1:9 solvent: $CuCl_2$ solution (v/v)

Solvent	Experiment	1 hour	Later
MeOH	PS+, PS+, PS	PG	G-
EtOH	PS+, PS+, PS	PS+	
1-Propanol	PS+, PS+, PS	PS+	
1-Butanol	PS+, PS+, PS	PS+	
1-Pentanol	PS+, PS+, PS	PS+	
1-Hexanol	PS+, PS+, PS	PS+	
1-Heptanol	PS+, PS+, PS	PS+	
1-Octanol	PS+, PS+, PS	PS+	
DMSO	PS+, PS+, PS	PG	
DMF	PS+, PS+, PS	G	G-

Table 10. Test series #10 Compound 1 with 1:1 solvent: $NiCl_2$ solution (v/v)

Solvent	Experiment	1 hour	Later
MeOH	PS+, PS+, PS	PS+	
EtOH	PS+, PS+, S	PS+	
1-Propanol	PS+, PS+, S	PS+	
1-Butanol	PS+, PS+, S	PS+	
1-Pentanol	PS+, PS+, PS	PS+	
1-Hexanol	PS+, PS+, PS	PS+	
1-Heptanol	PS+, PS+, PS	PS+	
1-Octanol	PS+, PS+, PS	PS+	
DMSO	PS+, PS+, S	G	G
DMF	PS+, PS+, PS	PG	G

Table 11. Test series #11 Compound 1 with 3:7 solvent: $NiCl_2$ solution (v/v)

Solvent	Experiment	1 hour	Later
MeOH	PS+, PS+, PS	PS+	
EtOH	PS+, PS+, PS	PS+	
1-Propanol	PS+, PS+, PS	PS+	
1-Butanol	PS+, PS+, S	PS+	
1-Pentanol	S+, PS+, S	PS+	
1-Hexanol	PS+, PS+, PS	PS+	
1-Heptanol	PS+, PS+, PS	PS+	
1-Octanol	PS+, PS+, PS	PS+	
DMSO	PS+, PS+, PS	PS+	
DMF	PS+, PS+, PS	G	G

Table 12. Test series #12 Compound 1 with 1:9 solvent: $NiCl_2$ solution (v/v)

Solvent	Experiment	1 hour	Later
MeOH	PS+, PS+, PS	PS+	
EtOH	PS+, PS+, PS	PS+	
1-Propanol	PS+, PS+, PS	PS+	
1-Butanol	PS+, PS+, S	PS+	PG
1-Pentanol	PS+, PS+, S	PS+	
1-Hexanol	PS+, PS+, PS	PS+	
1-Heptanol	PS+, PS+, PS	PS+	
1-Octanol	PS+, PS+, PS	PS+	
DMSO	PS+, PS+, S	PS+	
DMF	PS+, PS+, S	PS+	

Table 13. Test series #13 Compound 2 with 1:1 solvent: $NiCl_2$ solution (v/v)

Solvent	Experiment	1 hour	Later
MeOH	PS+, PS+, S	PS+	G
EtOH	PS+, PS+, S	PS+	
1-Propanol	PS+, PS+, S	S+	PS+
1-Butanol	PS+, PS+, S	S+	PS+
1-Pentanol	PS+, PS+, PS	PS+	
1-Hexanol	PS+, PS+, S	PS+	
1-Heptanol	S+, PS+, PS	PS+	
1-Octanol	PS+, PS+, PS	PS+	
DMSO	PS+, PS+, PS	G	G
DMF	PS+, PS+, PS	G	G

Table 14. Test series #14 Compound 2 with 3:7 solvent: $NiCl_2$ solution (v/v)

Solvent	Experiment	1 hour	Later
MeOH	PS+, PS+, PS	PG	G
EtOH	PS+, PS+, S	PS+	G-
1-Propanol	PS+, PS+, S	PS+	
1-Butanol	PS+, PS+, S	PS+	
1-Pentanol	S+, PS+, S	PS+	
1-Hexanol	PS+, PS+, PS	PS+	
1-Heptanol	PS+, PS+, PS	PS+	
1-Octanol	PS+, PS+, PS	PS+	
DMSO	PS+, PS+, S	PG	G-
DMF	PS+, PS+, S	G	G

Table 15. Test series #15 Compound 2 with 1:9 solvent: $NiCl_2$ solution (v/v)

Solvent	Experiment	1 hour	Later
MeOH	PS+, PS+, PS	G-	G
EtOH	PS+, PS+, PS	PS+	G-
1-Propanol	PS+, PS+, PS	PS+	G-
1-Butanol	PS+, PS+, S	PS+	
1-Pentanol	S+, PS+, PS	PS+	
1-Hexanol	PS+, PS+, PS	PS+	
1-Heptanol	S+, PS+, PS	PS+	
1-Octanol	PS+, PS+, PS	PS+	
DMSO	PS+, PS+, PS	PS+	
DMF	PS+, PS+, PS	PG	PG

Table 16. Test series #16 Compound 3 with 1:1 solvent: $NiCl_2$ solution (v/v)

Solvent	Experiment	1 hour	Later
MeOH	S+, PS+, S	PS+	
EtOH	PS+, PS+, S	PS+	
1-Propanol	S+, PS+, S	S+	
1-Butanol	PS+, PS+, S	PS+	
1-Pentanol	PS+, PS+, PS	PS+	
1-Hexanol	S+, PS+, PS	PS+	
1-Heptanol	PS+, PS+, PS	PS+	
1-Octanol	S+, PS+, PS	PS+	
DMSO	PS+, PS+, S	G	G-
DMF	PS+, PS+, S	PS+	

Table 17. Test series #17 Compound 3 with 3:7 solvent: $NiCl_2$ solution (v/v)

Solvent	Experiment	1 hour	Later
MeOH	PS+, PS+, S	G	G
EtOH	PS+, PS+, S	PS+	
1-Propanol	S+, PS+, S	S+	S+
1-Butanol	PS+, PS+, S	PS+	S+
1-Pentanol	PS+, PS+, S	PS+	
1-Hexanol	S+, PS+, PS	PS+	
1-Heptanol	PS+, PS+, PS	PS+	
1-Octanol	S+, PS+, PS	PS+	
DMSO	PS+, PS+, S	PG	PS+
DMF	PS+, PS+, S	PS+	

Table 18. Test series #18 Compound 3 with 1:9 solvent: $NiCl_2$ solution (v/v)

Solvent	Experiment	1 hour	Later
MeOH	PS+, PS+, PS	PG	PG
EtOH	PS+, PS+, PS	PS+	
1-Propanol	PS+, PS+, PS	PG	PS+
1-Butanol	PS+, PS+, S	S+	
1-Pentanol	S+, PS+, PS	PS+	
1-Hexanol	S+, PS+, PS	PS+	
1-Heptanol	PS+, PS+, PS	PS+	
1-Octanol	PS+, PS+, PS	PS+	
DMSO	PS+, PS+, PS	PS+	
DMF	PS+, PS+, PS	PS+	

Table 19. Test series #19 Compound 1 with 1:1 solvent: $ZiCl_2$ solution (v/v)

Solvent	Experiment	1 hour	Later
MeOH	PS+, PS+, S	PS+	
EtOH	PS+, PS+, S	PS+	
1-Propanol	PS+, PS+, S	PS+	
1-Butanol	PS+, PS+, S	PS+	
1-Pentanol	PS+, PS+, PS	PS+	
1-Hexanol	PS+, PS+, PS	PS+	
1-Heptanol	PS+, PS+, PS	PS+	
1-Octanol	PS+, PS+, PS	PS+	
DMSO	PS+, PS+, S	PG	G
DMF	PS+, PS+, PS	G	G

Table 20. Test series #20 Compound 1 with 3:7 solvent: $ZiCl_2$ solution (v/v)

Solvent	Experiment	1 hour	Later
MeOH	PS+, G-, G	G	G-
EtOH	PS+, PS+, PS	PS+	
1-Propanol	PS+, PS+, S	PS+	
1-Butanol	PS+, PS+, S	PS+	
1-Pentanol	PS+, PS+, S	PS+	
1-Hexanol	PS+, PS+, PS	PS+	
1-Heptanol	PS+, PS+, PS	PS+	
1-Octanol	PS+, PS+, PS	PS+	
DMSO	PS+, PS+, S	PS+	
DMF	PS+, G-, G	G-	PG

Table 21. Test series #21 Compound 1 with 1:9 solvent: $ZiCl_2$ solution (v/v)

Solvent	Experiment	1 hour	Later
MeOH	PS+, PS+, PS	PS+	
EtOH	PS+, PS+, PG	PS+	
1-Propanol	PS+, PS+, S	PS+	
1-Butanol	PS+, PS+, S	PS+	
1-Pentanol	PS+, PS+, S	PS+	
1-Hexanol	PS+, PS+, PS	PS+	
1-Heptanol	PS+, PS+, PS	PS+	
1-Octanol	PS+, PS+, PS	PS+	PG-
DMSO	PS+, PS+, S	PS+	
DMF	PS+, PS+, S	PS+	

Table 22. Test series #22 Compound 2 with 1:1 solvent: $ZiCl_2$ solution (v/v)

Solvent	Experiment	1 hour	Later
MeOH	PS+, PS+, S	PS+	G
EtOH	PS+, PS+, S	PS+	
1-Propanol	S+, S+, S	S+	
1-Butanol	PS+, PS+, S	PS+	
1-Pentanol	PS+, PS+, PS	PS+	
1-Hexanol	PS+, PS+, PS	PS+	
1-Heptanol	PS+, PS+, PS	PS+	
1-Octanol	PS+, PS+, PS	PS+	
DMSO	PS+, PS+, S	PS+	
DMF	PS+, PG, S	G	G

Table 23. Test series #23 Compound 2 with 3:7 solvent: $ZiCl_2$ solution (v/v)

Solvent	Experiment	1 hour	Later
MeOH	PS+, PS+, S	PS+	G
EtOH	PS+, PS+, S	PS+	
1-Propanol	PS+, PS+, S	PS+	
1-Butanol	PS+, PS+, S	PS+	
1-Pentanol	PS+, PS+, PS	PS+	
1-Hexanol	PS+, PS+, PS	PS+	
1-Heptanol	PS+, PS+, PS	PS+	
1-Octanol	PS+, PS+, PS	PS+	
DMSO	PS+, PS+, S	PS+	
DMF	PS+, PS+, PS	PS+	

Table 24. Test series #24 Compound 2 with 1:9 solvent: $ZiCl_2$ solution (v/v)

Solvent	Experiment	1 hour	Later
MeOH	PS+, PS+, PS	PG	PS+
EtOH	PS+, PS+, S	PS+	
1-Propanol	PS+, PS+, S	PS+	
1-Butanol	PS+, PS+, S	S+	PS+
1-Pentanol	PS+, PS+, S	PS+	
1-Hexanol	S+, PS+, PS	PS+	
1-Heptanol	S+, PS+, PS	PS+	
1-Octanol	PS+, PS+, PS	PS+	
DMSO	PS+, PS+, S	PS+	
DMF	PS+, PS+, S	PS+	

Table 25. Test series #25 Compound 3 with 1:1 solvent: $ZiCl_2$ solution (v/v)

Solvent	Experiment	1 hour	Later
MeOH	PS+, PS+, PS	PS+	
EtOH	PS+, PS+, PS	PS+	
1-propanol	PS+, PS+, PS	PS+	
1-Butanol	PS+, PS+, PS	PS+	
1-Pentanol	PS+, PS+, PS	PS+	
1-Hexanol	PS+, PS+, PS	PS+	
1-Heptanol	S+, PS+, PS	PS+	
1-Octanol	PS+, PS+, PS	PS+	
DMSO	PS+, PS+, PS	PS+	PG
DMF	PS+, PS+, S	G	PS+

Table 26. Test series #26 Compound 3 with 3:7 solvent: $ZiCl_2$ solution (v/v)

Solvent	Experiment	1 hour	Later
MeOH	PS+, PS+, PS	G	G
EtOH	PS+, PS+, S	PS+	
1-Propanol	PS+, PS+, S	PS+	S+
1-Butanol	PS+, PS+, PS	PS+	
1-Pentanol	S+, PS+, PS	PS+	
1-Hexanol	PS+, PS+, PS	PS+	
1-Heptanol	PS+, PS+, PS	PS+	
1-Octanol	PS+, PS+, PS	PS+	
DMSO	PS+, PS+, S	PS+	
DMF	PS+, PS+, S	PS+	

Table 27. Test series #27 Compound 3 with 1:9 solvent: $ZiCl_2$ solution (v/v)

Solvent	Experiment	1 hour	Later
MeOH	PS+, PS+, PS	PS+	
EtOH	PS+, PS+, PS	PS+	
1-Propanol	PS+, PS+, PS	G	G
1-Butanol	PS+, PS+, S	PS+	
1-Pentanol	S+, PS+, PS	PS+	
1-Hexanol	PS+, PS+, PS	PS+	
1-Heptanol	S+, PS+, PS	PS+	
1-Octanol	PS+, PS+, PS	PS+	
DMSO	PS+, PS+, PS	PS+	
DMF	PS+, PS+, PS	G	G-