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Hydrogelators

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Chapter 1

An introduction to hydrogels

In this chapter the field of hydrogelators based on cyclohexane triamides and benzene triamides will be reviewed. Several systems and their unique properties will be discussed. An overview of the techniques that are useful for analysing gels at different length scales will be reported. This chapter concludes with a brief discussion on the challenges faced in developing new hydrogelators and an overview of the topics discussed in each chapter.

Introduction

Gels are as much a part of our day-to-day life as any other state of matter. We recognize a material as being a gel intuitively; however, take a moment to think about what a gel is – to define it – set boundary conditions. Therein lies the challenge as the boundaries of what is and is not a gel go beyond composition and physical properties; one has to delve deeper into how gels ‘work’. In reality the term gel covers a wide and diverse range of materials and substances and is in some ways impossible to define rigorously. Despite this, I still have to define what a gel is since the rest of this thesis is focused on gels!

Luckily others have, in the past, thought deeply about this overtly simple problem on many occasions. As early as 1861 Thomas Graham stated: “while the rigidity of the crystalline structure shuts out external expressions, the softness of the gelatinous colloid partakes of fluidity and enables the colloid to become a medium for liquid diffusion, like water itself.”²¹ This definition is not particularly clear, however. Dorothy Jordon Lloyd proposed: “only one rule seems to hold for all gels and that is that they must be built from two components, one which is a liquid at the temperature under consideration and the other which, the gelling substance proper, often spoken of as the gelator, is a solid. The gel itself has the mechanical properties of a solid, i.e. it can maintain its form under stress of its own weight and under any mechanical stress it shows the phenomenon of strain.”²² In the end the easiest definition to go by is the shorter definition by Dorothy Jordon Lloyd: “if it looks like a gel it must be a gel”. One might add to this: if it cannot be proven to not be a gel, then it must be a gel.

The basis for any type of gel is that a compound is dissolved in a liquid and upon a trigger, e.g. cooling, light, pH, vibration, etc. the dissolved gelator forms a network.³ This network gels the solvent, turning the free flowing solution to a solid-like material. Gelators that gelate water are called hydrogelators. Gelators can be divided into two classes, chemical and physical. Chemical gels undergo aggregation driven by covalent cross-links, leading to irreversible gelation. Physical gels undergo aggregation driven by non-covalent cross-links, leading to reversible gelation. All gelators described in this introduction and the rest of the thesis are physical gels.

Gelators are based on any of three distinct classes of building blocks: polymers^{4,5}, colloids,⁶ and small molecules. In the case of polymer gels the long chains directly form an interconnected network, while, for colloidal and small molecule gelators aggregation into stacks precedes formation of a network; a process that should not result in flocculation or crystallization. The focus of this thesis is on small molecules for gelling water, the so called low molecular weight hydrogelators (LMWHGs).

This introduction will not give an comprehensive overview of all LMWHGs and the interested reader is directed to the many excellent reviews on this topic.⁷⁻¹² Instead the focus will be on a specific class of hydrogelators and consideration of design aspects. It is known that LMWHGs first aggregate into stacks and then form a fibrous network. Once a gel has formed, its formation can be rational-

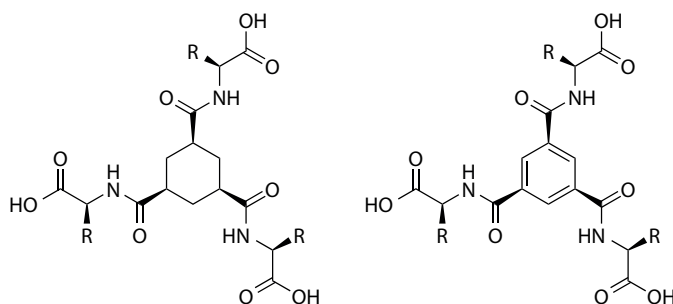


Figure 1. Basic structures of hydrogelators used in this thesis.

ized by analysis of intermolecular interactions between the individual building blocks. Designing and predicting the gelation properties of LMWHGs beforehand, however, is not trivial. The design of most LMWHG often relies on a method of unidirectional stacking, which can be achieved by incorporating hydrogen bonding units, e.g., ureas or amides, or by π - π -stacking, using benzene or pyridine rings. Another key aspect is solubility. If a compound is too soluble it will never form a gel and if it is too highly insoluble it will immediately precipitate or crystallize. Therefore, the gelators intramolecular interactions must be balanced with solvent molecule interactions. This process is mostly trial and error and typically requires synthesis of a series of structurally varied compounds.

The LMWHGs used in this thesis have a characteristic cyclohexane core or benzene core with three amide bonds at the 1-, 3-, and 5-positions in an all cis arrangement, and with amino acids attached to the amides (Figure 1). The cyclohexane core provides a hydrophobic rigid (due to conformational effects) core, which is an ideal starting point for stacking. The amides are hydrophilic and can potentially form multiple unidirectional hydrogen bonds. The R group of the amino acid can range from hydrophobic to hydrophilic thereby determines/influences the hydrogelator's (relative) solubility, precipitation and gelation properties. The peripheral carboxylic acid group is hydrophilic, aiding dissolution of the compound at high pH and triggering gelation or precipitation at low pH.

Cyclohexane based hydrogelators

In this section the previous work on cyclohexane based hydrogelators will be discussed and list previously found correlations between molecular structure and gelling properties. The key structural feature of the cyclohexane amide based gelators is the cyclohexane core that positions three amides to drive planar aggregation as opposed to linearly linked ureas or amides.¹³ The crystal structure of **1** (Figure 2) shows that all three amides can be involved in intermolecular hydrogen bonding in a linear fashion. Each pyridine hydrogen/proton is directed towards the π -face of its neighbour. Compounds **2-5** (Figure 2), which vary in alkyl chain lengths show are able to gel a wide range organic solvents, and are the first organogelators of this type.¹⁴ Compound **2** has the shortest alkyl tails and shows limited gelation properties, while **3** and **4** show gelation in a wide range of solvents, explained by an increased possibility for intermolecular hydrophobic interactions between gelators with larger alkyl chains. The hydrogen bonding of the amides is manifested in the shift in their respective bands by FTIR spectroscopy, and such bonding is noticeably absent for **5** in THF in which it does not form a gel.

These organogelators formed the basis for the design of hydrogelators in which amino acid groups replace the alkyl chains.¹⁵ Compounds **6-8** (Figure 4) can gelate water with high salt concentrations and show that when surfactants are added that both the gel and the surfactant self-assemble orthogonally.¹⁶ The surfactants used include the anionic sodium dodecyl sulfate (SDS), cationic cetyltrimethylammonium bromide (CTAB) and nonionic *n*-octyl- β -D-glucopyranoside (OG) both below and above their respective critical micelle concentrations (CMC) (Figure 3). The only combination where the gel was not formed was with **7** with CTAB where strong electrostatic interactions

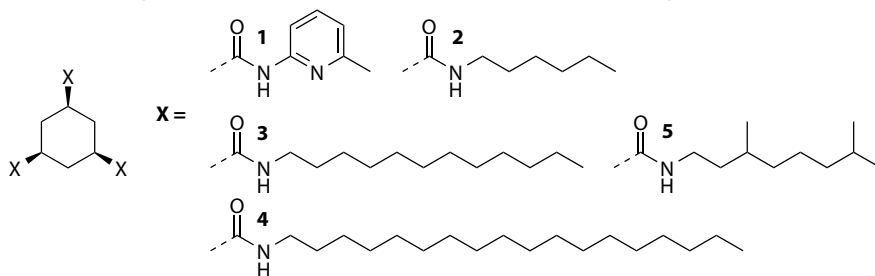


Figure 2. Structures of compounds 1-5.

anion present at low concentrations being surrounded by carboxylates, it can be concluded that the assignment was erroneous and it is in fact a potassium cation is present in the structure. The presence of the cation, as we will discuss in chapter 4, is of importance in understanding the effect of salts on gel stability. The phenol side groups of the tyrosine residue also show packing in a hexagonal manner providing an hydrophobic pocket for the side groups.

Compounds **6**, **8-12** all form hydrogels at mM concentrations following heating and cooling cycles. FTIR spectroscopy of powder and freeze dried gels led to the conclusion that all the C=O and N-H bonds were hydrogen bonded. TEM showed long fibrous structures or fibre networks for all six compound indicating that they all form long linear stacks when gelled. **8** and **9** were stable at up to at least 130 °C; the limit of the dropping ball method. A point of note regarding these gelators is that they were one of the first systems to show reversible pH switching. **6** and **9** are gels when in acidic media but dissolve when the pH is raised while **11** and **12** are gels at high pH but dissolve when the pH is decreased. They also show that there is a balance between the extent of deprotonation of acid groups and the attractive forces between gelator molecules, in the case of **6** only one carboxylic acid need be deprotonated for the gel structure to collapse while for **9** a more extensive deprotonation is required due to the extra amide hydrogen bonds.¹⁷

The gelators in this class all used L-amino acids as side groups, due to their ready availability. A series of compounds with phenyl alanine sided groups (**16**, Figure 5) showed that the pure LLL and DDD systems did not hydrogelate upon pH switching, instead they formed microcrystalline needles, however, the LLD and DDL systems showed hydrogelation under the same conditions.¹⁸ Breaking the symmetry of the compound changes its solubility and enforces different packing that inhibits crystallization or at least slows crystallization sufficiently for gels to form. Crystallization can be inhibited also by increasing the chain length by attaching hydrophilic moieties (**8-10**) or extra amino acids (**17-19**, Figure 5). For **17-19**, diminishing stereochemical purity had no effect on gelation properties. Gels based on **16** were studied by CD spectroscopy and the LLD and DDL gels show opposite CD. The intensity of the CD decreases as the temperature is raised but even at 90 °C a residual signal is present indicating that aggregates are still present.

Compound **20** (Figure 6) was designed so that the glycol tails would induce gelation and the phenylalanine side group was labile towards enzymatic cleavage by α -chymotrypsin releasing 6-aminoquinoline.¹⁹ 6-Aminoquinoline is used a model for certain drugs and is fluorescent. When attached to the gelator, it is non-fluorescent providing a switch-on mechanism to study its release from the gel. In the presence of α -chymotrypsin, 6-aminoquinoline is released over time and by increasing the temperature closer to the gel-sol transition the rate of cleavage increases due to the presence of a greater proportion of dissolved monomer. This indicates that the release has a two-step mechanism, first the release of monomer from the fibres and second the enzymatic cleavage. This observation is important as it indicates that gels of this type are not static but that there is dynamic exchange of gelator molecules between the gel fibres and solution.

It was already shown that these types of gelators were able to form gels in the presence of micelle forming surfactants and that the surfactants form micelles within the gel fibre matrix. Compounds

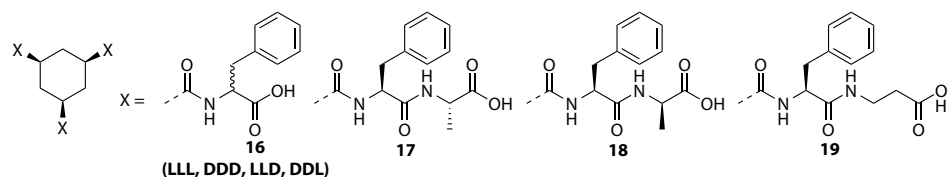


Figure 5. Structures of compounds **16-19** which are based on the cyclohexane core.

10, 20-22 (Figure 6) can also gelate in the presence of surfactants increasing the scope of these systems to seven different compounds.²⁰ Cryo-TEM micrographs show fibre formation inside micelles, forming so called gellosomes. The formed gel fibres then distort the shape of the liposomes from circular to elongated shapes, and as the properties of liposomes change upon deformation this system can be used to change the properties in, for example, cases where a switching function is built into the gel. The structures formed were likened to the cytoskeleton.

Mixing **10** and cetyl-N,N,N-trimethylammonium tosylate (CTAT, Figure 6), a surfactant that forms cylindrical micelles, led to the formation of two interpenetrating fibre networks.²¹ Again self-aggregation is preferred over mixing. The viscoelastic properties can be tweaked to be between those of the pure **10** or CTAT by varying the ratio of the two components.

A variation on **21** with an added alkyl tail, **23** (Figure 6), was prepared to examine the difference between mixing **21** with **soap5** (Figure 6) or using a covalently linked analogue.²² Whereas a mixture of **21** and **Soap5** shows orthogonal assembly, it is not observed in the case **23**. The multi-segment amphiphile **23** showed higher thermal stability than its parent **21**. Cryo-TEM micrographs show that **23** forms 9 nm and 3 nm fibres at 0.25 mM while at higher concentrations 50-200 nm diameter tapes are formed consisting of 3 nm fibrils, that indicates that the gel part is spatially constrained by

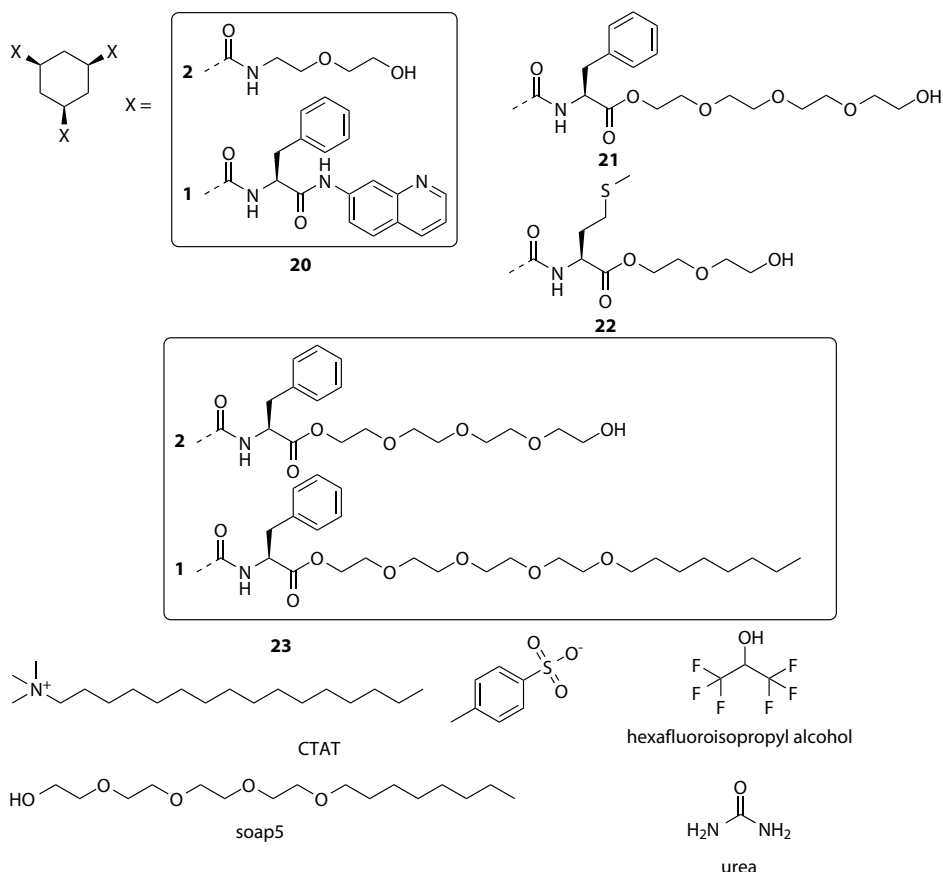


Figure 6. Structures of cyclohexane core based compounds **20-23**, micelle forming compounds CTAT, and soap5, and of HFIP and urea.

the covalent connection to the surfactant. The assembly can be disrupted or stabilized by mixing **23** with molecular chaperones.²³ The CGC of **23** decreases in the presence of CTAB and the gels formed turn clear compared to the opaque gels formed from **23** alone. This decrease in CGC indicates that the gelator co-aggregates with micelles of CTAB. The gels are weakened or do not form in the presence of urea or hexafluoroisopropyl alcohol (HFIP, Figure 6) due to hydrogen bond formation with the gelator disrupting stacking.

20 can be cleaved by α -chymotrypsin to release a fluorescent dye (vide supra).¹⁹ This release can be triggered by encapsulation of the α -chemotrypsin in micelles formed from phospholipids, as **20** has been shown to be compatible with such micelles.^{20,24} The enzymes loaded in the micelles can be released by heating the samples to 42 °C for 5 min. After this trigger, release of 6-AQ is manifested in an increase in fluorescence intensity. This combination of orthogonal selfassembly and catalytic cleavage of gels modified with drug like molecules could be of interest for making implants that release drugs upon a certain trigger.

26 is a gelator which forms by mixing the precursor **24** and **25** with a catalytic amount of acid or aniline (Figure 7).²⁵ This in situ formation of gelator can be used to make patterns by using a photo switchable catalyst or diffusion of the two solutions both containing one reagent.^{26,27} The strength of the gels can be increased by combining this system with small amounts of cross-linked aldehydes, glycol chains, or DNA.²⁸ In the case of the crosslinking, the system first forms spherical aggregates which decrease in size as the fibre network grows. Another system with in situ triggering of gel formation is based on a system that is a non gelator when it bears a carboxylate, **27**, and gels when the acid is methylated, **28** (Figure 7).²⁹ The system uses a strong methylating agent, dimethylsulfate, as a fuel for forming the gels in an alkaline solution. The alkaline solution continuously demethylates the acid, and when the fuel runs out the equilibrium shifts back to the fully dissolved state. In this system the growth of fibres and their concomitant destruction was observed by optical microscopy and represents an out of equilibrium dynamic system.

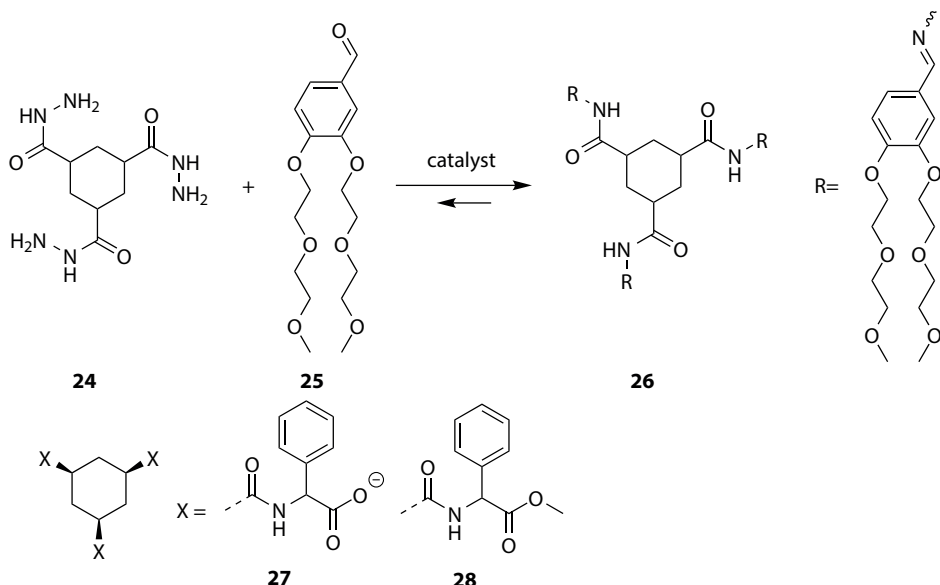


Figure 7. Reaction scheme for the formation of **26** and the structures of cyclohexane based compounds **27** and **28**.

Table 1. The critical gelation concentration all variants of cyclohexane triamide gelators in mM and w/v%.

Compound	CGC mM	CGC w/v %
1	crystal	
2¹⁴	Organogelator	
3¹⁴	Organogelator	
4¹⁴	Organogelator	
5¹⁴	Organogelator	
6^{6,17}	0.98	0.06
7¹⁶	3.00	0.23
8¹⁶⁻¹⁸	0.76	0.07
9^{17,18}	0.97	0.08
10^{17,18}	0.36	0.03
11¹⁷	11.75	1.25
12¹⁷	4.72	0.42
13¹⁷	Crystal, Non gelator	
14¹⁷	Non gelator	
15¹⁷	Non gelator	
16¹⁸		
LLL/DDD	non gelator	
LLD/DDL	0.5	0.03
17¹⁸	1	0.09
18¹⁸	0.75	0.07
19¹⁸	1	0.09
20²⁰	1.18	0.1
21²⁰	0.84	0.1
22²⁰	1.14	0.1
23²²	5	0.65
26²⁵⁻²⁸	Dynamic system	
27²⁹	Non gelator	
28²⁹	Dynamic system	

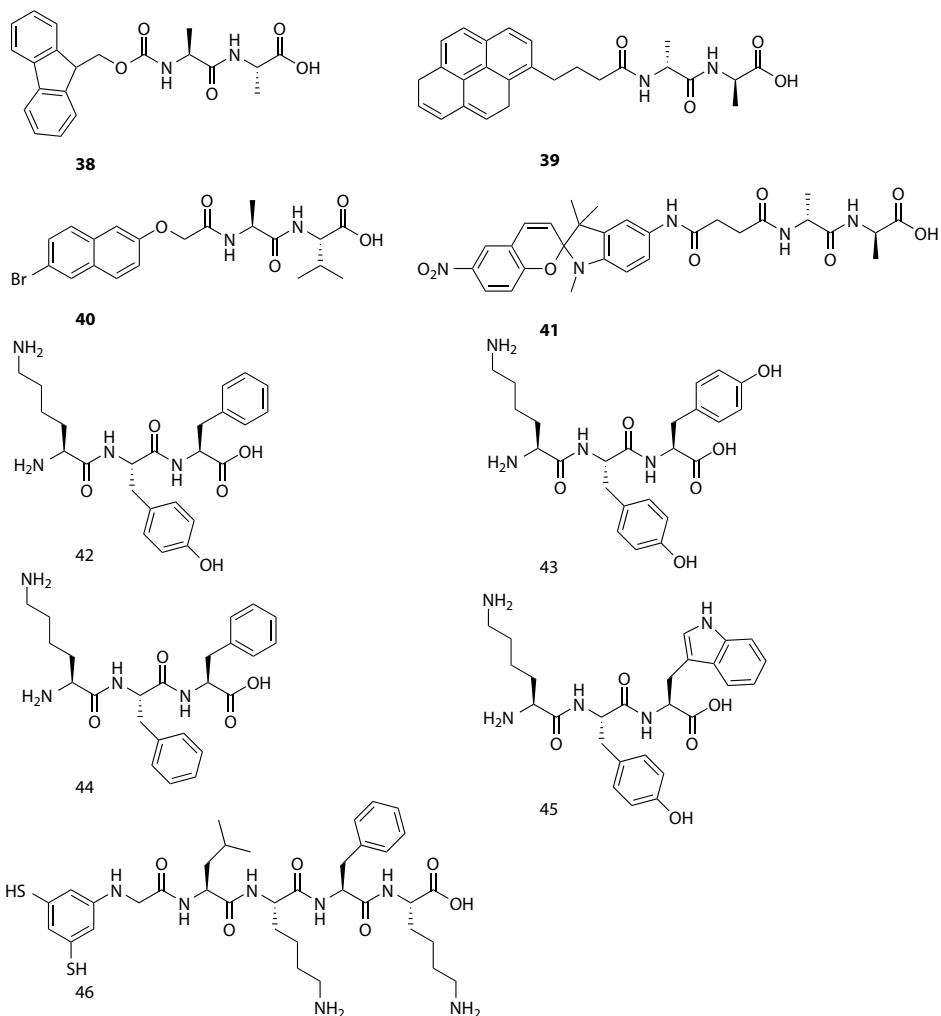


Figure 10. Structures of other amino acid based hydrogelators 38-46.

where the G' drops below the G'' which corresponds to the force needed to break a gel.

The dropping ball experiment is used to measure the temperature stability of a gel. In the measurement, gels are prepared in vials and when fully formed a small metal ball is placed on top of the gel. If the gel is strong enough the ball remains on top of the gel. The vial is heated slowly and once the temperature required to melt the gel is reached the ball drops through the solution. The point at which the ball reaches the bottom of the vial is recorded as the temperature for gel collapse.

Gels generally consist of large fibrous networks, the thickness of these fibres can range between a couple of nanometers (single molecule stacks) to micrometers (an ensemble of molecules stacking). The fibres can be imaged by a number of techniques. (Cryogenic) Transmission Electron Microscopy, (cryo) TEM, can image samples in dried and vitrified states giving a good overview of the structures formed in the gel down to the nanometer range. It should be noted, however, that these images are static and almost no insight can be gained as to the formation of the fibres.

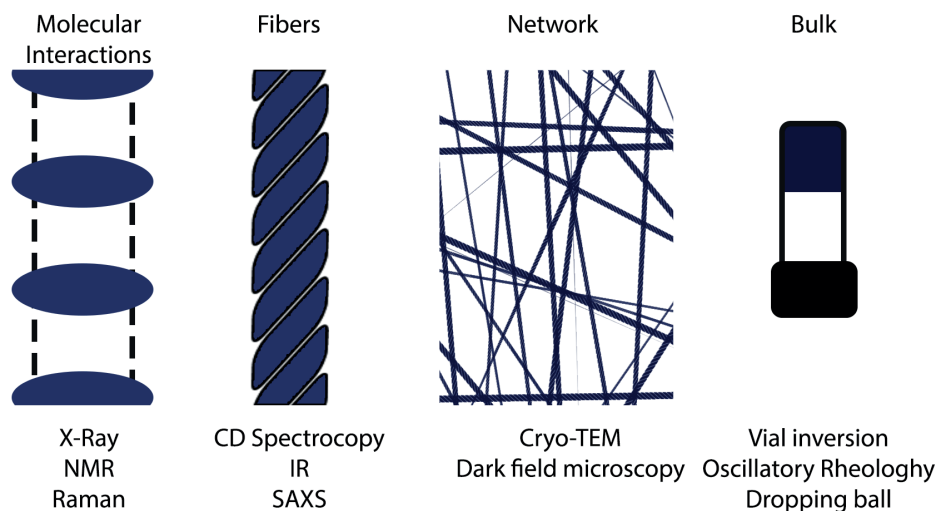


Figure 11. Analytical techniques applied to investigating gelation at different scales.

Optical microscopy, either bright field or fluorescence, are used to image systems on a micrometer scale, and the resolution is limited by the diffraction limit (Abbe's limit). In contrast to electron microscopy, optical microscopes operate under ambient conditions and can be used to study changes in samples over time giving insight into the dynamics of the gel. Microscopy measurements often require a reporter molecule as the system under study does not give high enough contrast or is non fluorescent. The introduction of these reporter compounds is not necessarily an innocent process and can potentially influence the gel properties.³⁸

In this thesis dark field microscopy is employed to study gel formation. Dark field microscopy is a technique that uses illumination under an acute angle such that the light does not enter the optical path (i.e. reach the camera). If the samples scatters light, this light is observed by the camera yielding bright object on a dark background. A caveat is that the refractive index of an object should be different from that of the surrounding solvent for it to scatter and hence be observed.

Information on the stacking at single fibre level has been measured using a wide range of techniques. CD spectroscopy can be used to show supramolecular helicity and give insight in the packing. This technique generally requires low concentrations, often below the gel formation point. IR spectroscopy can show changes in vibrational bands due to hydrogen bonding and conformational changes, however measuring in the gel state is limited by the low concentration and the overlap with absorption by water. Small Angle X-Ray scattering can be used to analyse the size of fibres and the packing, this technique suffers from the fact that the largest structure will be sampled and that small crystals can dominate the data sets.

Molecular interactions can be analysed by NMR spectroscopy showing exchange between certain parts of the gel or between solution and fibres.

Single crystals of gelators or analogues can be used as a start for modelling molecular packing, however, proving that the molecular arrangements in the crystals are relevant to the gel fibres is challenging. The orientation of bonds with respect to fibre axes can be inferred from polarized Raman spectra. In Raman spectroscopy the vibrational modes of a molecule that undergo a change in polarizability during the vibration induce inelastic (Raman) scattering. The polarizability is often not

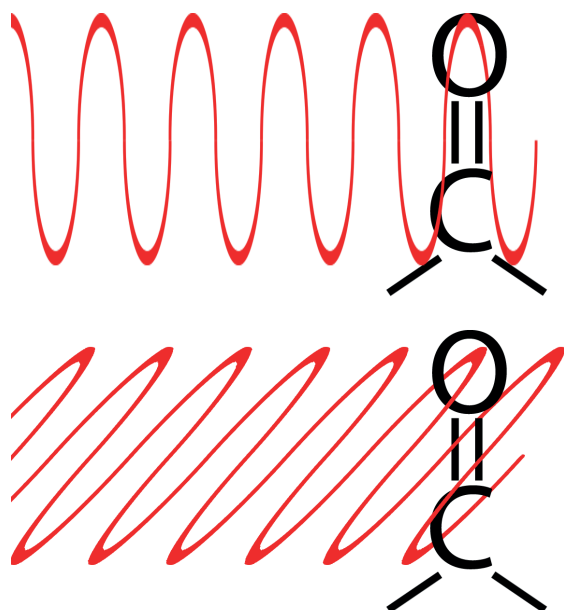


Figure 12. Illustration of Polarized Raman excitation parallel (top) and orthogonal (bottom) to the bond axis.

isotropic and hence the magnitude to scattering depends on the correspondence of the polarization of the excitation laser and the alignment of the bond (Figure 12). By either rotating the sample or the laser polarization (using a half wave plate) a correlation between angle and signal intensity can be made. In highly aligned samples, such as crystals or fibres, this allows for linking the measured orientation to the orientation in the sample. The underlying mathematics, detailed explanations and examples can be found elsewhere.^{39–45}

Challenges and Chapters

The series of cyclohexane triamide hydrogelators has been investigated for decades and insights on the effect of substituents and additives have been gained. Despite that, of the simple modifications of the hydrogels that can be made with the 26 canonical amino acids only 5 have been synthesized so far: Met, Phe, Gly, Ser and Tyr. From the many reports made on these gels some information can be gained on how the gel forms and looks like when fully formed, however insight into the steps leading up to gel formation is lacking. By understanding the mechanism involved in the gel formation new gels and directed assembly can possibly be reached.

In Chapter 2 we describe the synthesis of new variations on the cyclohexane triamide gelators. Then we focus on the effect of salts and D_2O on the gel stability.

In Chapter 3 we will continue with these gelators and describe the steps in the self-assembly of the hydrogelators that lead to gel formation.

In Chapter 4 we investigated a tyrosine based variant on the cyclohexane triamide structure as a possible reporter compound and its gel properties.

In Chapter 5 we describe how to use these gelators as a scaffold for gold and silver nanoparticles, to

use the gel as a substrate for surface enhanced Raman scattering (SERS) spectroscopy.

In Chapter 6 the knowledge gained is applied to the rational design of a new gelator based on the cyclohexane triamides but with a benzene triamide core instead.

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