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Design and Application of Self-Assembled Low Molecular Weight Hydrogels

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Over the past years, the gelation of aqueous solutions by low molecular weight (LMW) compounds has become an area of increasing interest, owing to developments in the field of LMW organogelators. Until recently, LMW hydrogelators were found only by serendipity, nowadays rational design as well as application of LMW hydrogelators has become feasible. As a consequence, an increasing number of responsive and functional LMW hydrogels are reported, offering great prospects for diverse applications including drug delivery and smart materials. (© Wiley-VCH Verlag GmbH & Co. KGaA, 69451 Weinheim, Germany, 2005)

Introduction

Gels are unique materials, which are well-known in daily life and have a broad range of applications in food, medicines, biomaterials, cosmetics, separation technology etc. Typical examples are for instance gelatin pudding, anti-insect gel, hair styling gel and detergent gels. At low stress values, these systems display solid-like behaviour, whereas the majority of the material consists of fluid and only a minority of solid is present. This behaviour arises from their unique structure comprising a dilute two-component system in which the minor (solid) and major (fluid) component form a separate, three-dimensional continuous phase.
As a result a large solid–liquid interfacial area is present within the gel, solutes can be entrapped in the pores formed by the solid component, and the fluid component can be used as reaction medium.

Gels of aqueous solutions (hydrogels) are of particular interest because of their wide use in personal care products and foods and their potential for new biomedical applications.[1] Most of these gels are based on polymeric gelators and a distinction between chemical and physical gels can be made. In chemical gels the solid component is linked covalently throughout the whole system, yielding an irreversible but very robust gel, applicable for instance in drug delivery.[1] Physical gels are formed when smaller polymer sub-units are linked noncovalently into a network structure. These gels benefit from their reversibility and the relative mild gelation conditions; properties which are highly desirable for a variety of applications.

For these polymer physical gels, the noncovalent interactions that hold the network together are often nonspecific, resulting in limited opportunities for the a priori design of new (smart) gels and the systematic tuning of their molecular interactions and control over the gel structure and other properties like reversibility, responsiveness and recognition. In this respect, the control that can be achieved by exploiting specific self-assembly processes offers interesting possibilities, yielding for instance self-assembling artificial proteins as reported by Tirrell.[3]

The formation of hydrogels by specific self-assembly is not limited to polymers, but is also well documented for small self-assembling molecules. Well-known examples are hydrogels of condensed vesicles (liposomes)[4,5] or entangled worm-like micelles.[5,6] Most of these surfactant systems can be characterised as weak gels due to the highly dynamic character of the network. Furthermore, they are very sensitive to additives like salts, and are only formed at relatively high concentrations of gelling agent. A different type of gel is produced by low molecular weight gelators (LMWGs), which form gels in which the molecules are self-assembled into a fixed three-dimensional network of fibers, solely held together by noncovalent interactions.[7] These gels exhibit some interesting features: i) within the fibers the molecules are assembled in well ordered arrays, ii) the formed gels are thermoreversible and strong, iii) low minimal gelation concentrations are found, and iv) they exhibit a high tolerance towards salts and other additives. The gelation of organic solvents by LMWGs is well known and has been extensively studied during the past decade. Although already in 1892 a LMWG was described to be capable of gelating pure water at concentrations as low as 0.5 mg/mL.[12,13] Most likely, hydrophobic interactions will be the driving force for gel formation, whereas $\pi\text{-}\pi$ stacking, hydrogen bonding and hydrophobic interactions. Recently, it has been shown that substitution of the amide group with an imine also yields a hydrogelator.[11]

As an alternative to the hydrocarbon chains larger, azobenzene-based, aromatic moieties were introduced as hydrophobic groups, resulting in the thermoreversible gelation of pure water at concentrations as low as 0.5 mg/mL.[12,13] Most likely, hydrophobic interactions will be the driving force for gel formation, whereas $\pi\text{-}\pi$ stacking of the azobenzene moieties provides the geometric organization of the molecules into H-type aggregates.

In addition to hydrocarbons, fluorocarbons have been used as the hydrophobic unit in amphiphilic LMW hydrogelators. An example is the semi-fluorinated fatty acid 2, which was reported to gelate pure water at the rather high concentration of 80 mg/mL (Figure 1).[14] The authors claim that self-assembly of the molecules is driven by the formation of hydrogen bonds between adjacent fluorocarbon chains using water molecules as linkages, however this peculiar assembly motif is not conclusively proven yet.

Besides the common amphiphiles, bolaamphiphilic LMW hydrogelators are reported. A recent example is the phospholipid 3, which forms transparent hydrogels at concentrations of 1–5 mg/mL.[15] The gels consist of fibrils with
a thickness of approximately one molecular length, which appear to be helical. The authors propose that 3 assembles through hydrophobic interactions into tapes of parallel stacked molecules, which become twisted due to the steric hindrance between the bulky head groups.

A different class of amphiphilic LMW hydrogelators is obtained by substituting the rather flexible long hydrocarbon chains with a hydrophobic, rigid tetracyclic steroid unit as present in bile acid-based hydrogelators (Figure 1). Bile acid-based hydrogelators belong to the earliest investigated LMW hydrogelators and are already known for several decades.\cite{16} The number of hydroxy groups present at the steroid unit has a pronounced effect on the gelation ability. In trihydroxy bile salts like cholic acid, the third hydroxy group prevents the formation of a gel.\cite{16e} However, in dihydroxy derivatives, like the sodium salts of deoxycholic acid 4 or lithocholic acid are able to form thixotropic gels at low concentrations (3 mg/mL) with aqueous solutions containing salts, phosphate, borate and acetate buffers, or acids. Gelation could be achieved in pH regions from 6.4 up to 12, depending on the gelator and its concentration.\cite{16a}

Recently, it was shown that a cationic or neutral group, providing the hydrogelators 5–7, could replace the carboxylic acid group of 4.\cite{16c,16f} Compounds 5 and 6 were able to gelate thermoreversibly aqueous salt solutions through the formation of fibrous networks. For compound 5 the thermal stability of the gel increased as usual with increasing gelator concentration but also with increasing salt concentration. In contrast, the thermal stability of the gels of 6 was found to be independent of these parameters above a NaCl concentration of 1 M. The neutral compound 7 is not water soluble, but in the presence of polar solvents like DMSO or ethanol, clear solutions were obtained at elevated temperatures and subsequent cooling resulted in the formation of stable hydrogels. As a consequence, the thermal stability of these gels decreased with increasing amounts of these polar solvents.

For a large group of LMW hydrogelators the structure exhibits less clear-cut amphiphilic architecture, however still separate hydrophobic and hydrophilic parts can be distinguished. Also for most of the typical LMW organogelators the amphiphilic architecture is absent.\cite{7}

For example, aromatic azo dye compounds like 8, which contain a large hydrophobic aromatic group combined with a small hydrophilic unit (Figure 1), were found to gelate aqueous solutions at concentrations of 3 mg/mL. VIS and NMR spectroscopy showed that at lower concentrations only dimers are present, whereas increasing the concentration results in n-mer formation and gelation.\cite{17}

2'-Deoxyuridine derivatives, like 9, are nucleoside-based hydrogelators, which consist of a small hydrophobic aromatic group and several hydrogen bonding moieties (Figure 1).\cite{16} The benzyl derivative 9, but also the methyl-, ethyl- or n-butylbenzyl derivatives, are reported to gelate pure water at a minimal concentration of 3 mg/mL. SEM showed that the hydrogels consisted of lamellar sheets or fibres, due to assembly of the molecules through hydrophobic interactions, \( \pi-\pi \) stacking and hydrogen bonding.

In structures, which feature strong and highly directional hydrogen bonding groups like amides or urea, the presence of relatively small hydrophobic units is sufficient to achieve hydrogelation. The resulting LMW hydrogelators possess structural elements, which are often also present in more versatile LMW organogelators. Most of these types of LMW hydrogelators are based on amino acids and contain amide groups, which are known for their hydrogen bonding properties. In addition small aliphatic or aromatic groups contribute to the aggregation ability through hydrophobic interactions and \( \pi-\pi \) stacking.

An example is the \( \text{N},\text{N}'-\text{bis(alkylamino)}\text{oxalamide} \) 10 prepared by the group of Žinić (Figure 1).\cite{19} The pure enantiomer of this compound gelates thermoreversibly pure water and water with co-solvent as well as some organic solvents. TEM micrographs revealed the presence of a network of highly intertwined fibres within the hydrogel. In view of results obtained for the related bis(amino acid)oxalamides\cite{20} it is most likely that aggregation is initially driven by \( \pi-\pi \) stacking of the phenyl groups and the formed aggregates then further assemble into fibers by lateral hydrogen bonding of the amide and hydroxy groups.
LMW Hydrogelators by Design

The majority of the compounds known to gelate water were found by serendipity rather than by design. Often the compounds were originally developed as amphiphiles or organogelators and their ability to gelate water was discovered accidentally. LMW hydrogelators are usually composed of a hydrophilic moiety and a hydrophobic aromatic group or long hydrocarbon chain. The hydrophilic moieties provide the water compatibility of the molecules, whereas the hydrophobic part is generally providing the main driving force for the self-assembly of the molecules by hydrophobic interactions. In addition, other noncovalent interactions such as \( \pi-\pi \) stacking, coulomb interactions and hydrogen bonding are important.

In a first approach towards the rational design of LMW hydrogelators, well-documented organogelators were converted into hydrogelators by means of simple structural modifications. Generally, organogelators were chosen containing long hydrocarbon chains, thereby enforcing aggregation in water through hydrophobic interactions. However, the lack of hydrophilic groups in these compounds results in very low water compatibility, making them unsuitable for hydrogelation.

Hamilton et al. were among the first to show that a typical organogelator could be transformed into a hydrogelator by the simple introduction of hydrophilic groups. A bis-urea organogelator was modified with hydrophilic carboxylic acids to obtain the class of bis-urea amino acid-based hydrogelators (Figure 2). These compounds were found to gelate phosphate buffers of \( \text{pH} = 5 \) and concentrated gel (\( n = 11 \)), both prepared in the presence of \( \text{CaCl}_2 \), a molecular model for the aggregate structure in the gel was proposed. According to this model, aggregation is driven by hydrogen bond formation between the urea groups, hydrophobic interactions of the alkyl chains, and \( \text{Ca}^{2+} \)-coordination of the carboxylates.

The l-lysine-based bis-amide organogelators developed in the group of Hanabusa were modified with a cationic, heteroaromatic group or with an anionic carboxylate group to provide a series of hydrogelators, of which compounds 12–14 are representative examples (Figure 2). The compounds were found to gelate pure water at concentrations as low as 1 mg/mL, with a high tolerance towards inorganic salts and acids. For compound 12, the gelation ability decreased with increasing chain length (R1). For all compounds electron microscopy revealed the existence of a network of thin fibres in the gels. FT-IR studies on \( \text{D}_2\text{O} / \text{DMSO} \) solutions and gels showed that in the gels hydrogen bonds between the amides are present. Furthermore, low frequency shifts of the \( \text{CH}_2 \) stretching vibrations indicate that the alkyl chains become closely packed. Aggregation is proposed to be initially driven by hydrophobic interactions, after which the combination with hydrogen bonding interactions leads to gelation.

The results of Hamilton and Hanabusa indicate that in order to obtain effective LMW hydrogelators, fine-tuning of the balance between the hydrophilic (soluble) and hydrophobic (insoluble) parts is essential. This is also in agreement with detailed studies on existing types of hydrogelators, performed by Shinkai and Menger. The group of Shinkai extensively explored the possibilities of saccharides in combination with different types of hydrophobic groups, resulting in a large library of gelating and nongelating saccharide derivatives. The group of Menger studied the hydrogelation by aroyl-\( \text{L}-\text{cystine} \) derivatives like 15, a compound which was already known to form hydrogels in 1892 (Figure 3). In the twenties of the last century chemists had found that 15 formed transparent gels at 1 mg/mL, with a fibrillar structure. Furthermore, it was observed that gelation by 15 was thermoreversible and \( \text{pH} \) dependent, i.e. gelation occurred only at \( 3.4 \leq \text{pH} \leq 2.2 \), and that dyes could diffuse through the gel. Interestingly, they showed that replacement of the \( \text{S} \)-\( \text{S} \) bridge by a \( \text{CH}_2 \)-\( \text{CH}_2 \) or \( \text{CH} = \text{CH} \) bridge resulted in loss of gelation ability, as did the substitution of the aromatic groups by an aliphatic group. At this stage chemists lost interest until 50 years later the group of Menger continued the study of the gelation behaviour of 15. Structural variations showed that substitution of the carboxylic acid by a small amide resulted in an enhanced gelation ability. Furthermore, variations of the aromatic group were found to have a strong influence on the aggregation behaviour, indicating that this group is important to obtain gelation. X-ray crystallographic studies on tolouyl and nitrobenzoyl derivatives revealed two different packing modes: an aggregate structure in which the molecules are folded and packed together by hydrogen bonding and \( \pi-\pi \) stacking (Figure 3A) and a packing structure in which the molecule exhibits a linear conformation and the amides interact by intermolecular hy-
hydrogen bonding (Figure 3B). This suggests that within the gel several packing modes could be present.

From these and other studies it can be concluded that, in addition to the proper balance between the hydrophilic and hydrophobic parts of the compound, also the presence of aggregating units that provide anisotropic assembly is a primary prerequisite for LMW hydrogelators. Thus, as for organogelators,[7b,7c] the effective gelation of water by LMW compounds is based on the following factors: i) the control of fiber-solvent interfacial energy to tune solubility and prevent crystallization, ii) the presence of fiber-fiber interactions to achieve cross-linking and subsequently network formation and iii) the presence of multiple self-complementary and unidirectional interactions to achieve anisotropic self-assembly.

The concept of anisotropic self-assembly has already been used by our group in the development of bis-urea LMW organogelators.[7b–c,30] In a first approach to develop LMW hydrogelators the self-assembling properties of the well-studied and highly efficient cyclohexane bis-urea organogelators[30,31] were exploited (Figure 4). The cyclohexane bis-urea unit is designed to self-assemble into one-dimensional stacks, affording anisotropic fiber formation. The peripheral substituents can be varied without disturbing the ability of the molecules to self-assemble and it is thought that these substituents partly determine the scope of gelated solvents. It was observed that for 16 (pentyl spacer) only the enantiomeric pure form did form a hydrogel, whereas for 17 (hexyl spacer) only the racemate formed a gel. Apparently, the stereochemistry of these compounds together with the balance between the hydrophilicity of the hydroxy groups and the hydrophobicity of the alkyl spacers had a pronounced effect on their gelation behaviour.

These design guidelines resulted in the cyclohexane bis-urea hydrogelators 16–18, prepared both as racemate and in enantiomerically pure form (Figure 5).[32] The neutral dialkanol compounds 16 and 17 were found to gelate only pure water at a limited concentration of 10 mg/mL or 2–10 mg/mL, respectively, leading either to unstable gels or to gelation times of several weeks. Additionally, compound 17 (hexyl spacer) was capable of gelating several organic solvents. It was observed that for 16 (pentyl spacer) only the enantiomeric pure form did form a hydrogel, whereas for 17 (hexyl spacer) only the racemate formed a gel. Apparently, the stereochemistry of these compounds together with the balance between the hydrophilicity of the hydroxy groups and the hydrophobicity of the alkyl spacers had a pronounced effect on their gelation behaviour.

The more hydrophilic diaminoalkane 18[33] is found to be less efficient in the gelation of organic solvents compared to the dialkanols 16 and 17. However, the gelation ability for aqueous solutions has increased and became less dependent on the enantiomeric purity of the compounds. It was observed that compound 18 gelated water but also (buffered) basic solutions in a broad concentration range (5 to >20 mg/mL), with a gelation time of only a few minutes and which were stable for months. Interestingly, compound
18 is one of the very few examples where gelation of strong basic solutions like ammonia (25%), NaOH (1 N) or NaHCO$_3$ (1 N) is observed.$^{[41b]}$

For all gels, melting was found to be thermoreversible with a high $T_m$ of at least 70 °C, and in some cases even exceeding 120 °C. As commonly observed for other gela-


tors, the $T_m$ of the gels formed by 17 and 18 is con-


concentration-dependent, except for the gels of racemic 18, for which $T_m$ is almost independent of the concentration. TEM and FT-IR measurements showed that the hydrogels of 16–18 consisted of a network of fibers (Figure 5), in which all urea groups are involved in intermolecular hydrogen bonding. The latter indicates that water molecules do not interfere, presumably due to shielding of the urea groups from the water by the hydrophobic alkyl spacers. Most likely, gelation will be driven by hydrophobic interactions of the meth-

dylene units, whereas urea hydrogen bonding will provide the necessary anisotropy of the aggregation and the high thermal stability of the gels.$^{[34]}$ These results confirm the initial considerations in the design of the cyclohexane bis-urea or-

ganogelators as described above, i.e.: an anisotropic self-


assembling cyclohexane bis-urea unit combined with pe-


ripheral substituents that govern the solvent compatibility.

Another interesting example comprises the class of C$_3$-


ymmetric amino acid LMW (hydro)gelators, in which a cis,cis-1,3,5-cyclohexane tris-amide core is used as the gelat-


ing scaffold (Figure 6).$^{[35]}$ The choice of the core was based on the parallel orientation of the amide groups, which pro-


vides strong uni-axial intermolecular interactions affording 1D self-assembly perpendicular to the plane of the mole-


cule.$^{[36]}$ The core has been extended with amino acids or dipeptides, which provides the opportunity to introduce a broad scope of functionalities and tune the intermolecular interactions.

The range of gelated solvents could be controlled by the nature of the peripheral substituents. Application of amino acids (AA) with hydrophobic substituents (X) resulted in the development of novel organogelators,$^{[35a]}$ whereas application of hydrophobic amino acids (AA) to shield the hy-

drogen bonding amides from the surrounding water to-


gether with hydrophilic substituents (X) to achieve water solubility resulted in highly effective hydrogelators 19–22 (Figure 6A).$^{[35]}$ These compounds were able to gelate pure water but also physiological NaCl solutions at low concent-


rations. For instance, for 21 a remarkable low critical gela-


tion concentration of 0.33 mg/mL was observed, which is one of the lowest values found so far. For all compounds the gelation was found to be thermoreversible and the hy-


drogels proved to be stable up to temperatures above the boiling point of water. Within the gels a fibrous network is present and FT-IR spectroscopy revealed that the molecules aggregated through hydrogen bonds between the amides. X-


ray crystallography on a tyrosine-based nongelating deriva-


tive showed that the molecules formed 1D, hydrogen-


bonded stacks (Figure 6B), and that hydrogen bond formation between the amides is indeed most likely assisted by
shielding from the water by the hydrophobic groups (solid circle, Figure 6C).

In another approach, Boden exploited a well known self-assembly motif from nature to achieve anisotropic aggregation, i.e. the peptide β-sheet. Based on their work on longer oligopeptides, they designed short oligopeptide hydrogelators with the propensity to assemble into elongated tapes. For instance, compound 23 forms a thermostable hydrogel at neutral pH at concentrations of 15 mg/mL (Figure 9, vide infra). Other examples of oligopeptide hydrogelators forming β-sheets include compounds consisting of two oligopeptide strands connected by a 2,8-dibenzofuran derivative and oligopeptides with alternating polar and nonpolar amino acids.

Except for the finding of a rational for the development of new LMW hydrogelators, efforts have also been directed to the design of hydrogelators with controlled and defined fiber morphology. Generally, gels display ill-defined fiber dimensions with a large polydispersity. This is symptomatic for the instability of a kinetically trapped gel in which the gain of free energy from decreasing unfavourable interfacial energy together with increasing favourable attractive energy promotes the formation of thicker aggregates and eventually crystals. To prevent this instability, two approaches are reported in the literature.

In one approach, denoted the structure-shape concept, the structure of the LMW hydrogelator determines the shape of the aggregates formed. An example is the atypical packing observed for the bolaamphiphilic bisarborol hydrogelator 24 (Figure 7). The large head group of the dumbbell-shaped molecules prevents an effective monolayer packing, as usually observed for bolaamphiphiles, and instead the molecules aggregate by a crosswise stacking through hydrophobic interactions of the central alkyl chain (Figure 7). Electron microscopy revealed a monodisperse gel, consisting of fibrous rods instead of lamellae, with a diameter corresponding to the length of the molecules.

Also the group of Zhang applied the structure-shape concept to direct the morphology of peptide materials (Figure 8). He showed that by using different amino acids a set of self-assembling peptides could be constructed with completely different aggregate shapes. For instance, the use of alternating polar and nonpolar amino acids results in an ionic self-complementary peptide that forms nanofibers and subsequently a hydrogel (Figure 8A). Construction of a peptide that has a distinct charged head group and a non-polar tail consisting of hydrophobic amino acids yields a surfactant-type of peptide, which forms nanotubes and vesicles (Figure 8B). Dynamic Light Scattering studies showed that these structures were very monodisperse. However, the size distribution of the structures becomes broader in time. Application of three distinctive amino acid segments: a segment that interacts with proteins or cells, a linker segment and an anchor for attachment to the surface.
Figure 9. (top) Oligopeptide LMW hydrogelator. (bottom) Model for the hierarchical self-assembly of the rod-like molecules into finite fibrils and fibers (reprinted from ref. [37b] with permission; Copyright 2001, National Academy of Sciences, USA).

Figure 10. Structure of the gemini surfactant and cryo-TEM images of the formed twisted ribbons at an ee of a) 0% (racemate), b) 50% and c) 100% (pure l-tartrate) (reprinted from Nature (http://www.nature.com) with permission of the authors of ref. [44b]; Copyright 1999, Macmillan Magazines Ltd.).
yields a surface nanocoating peptide, which can be used as peptide ink (Figure 8C).

In another approach, the fiber morphology is controlled by stress arising from the introduction of chirality. In this regard, the peptide hydrogelator 23 developed by Boden[37b] is of particular interest (Figure 9). Due to the molecular chirality of 23 supramolecular structures are formed with an exclusively left-handed twist, which gives rise to a helical distortion energy. Upon increasing the width of these structures, this unfavourable helical distortion energy increases and compensates the gain in favourable attractive energy. This leads to a maximum in net free energy gain at a defined fiber and fibril width, yielding finite, well-defined and monodisperse structures. Hence, the chirality of the molecules frustrates the process of the formation of larger aggregates that leads to gel instability. It is important to note that the monodisperse nature enables the hierarchical assembly of the rod-like molecules into β-sheet tapes, ribbons (double tapes) and subsequently into fibrils (twisted stacks of ribbons), and finally fibers (entwined fibrils).[37b]

Chirality has also been exploited as design element by Huc and co-workers to control the width and helical twisting of gel fibers formed by the gemini surfactant 25 (Figure 10).[44b] These compounds were found to gelate both pure water and organic solvents. Electron microscopy showed that the hydrogels consists of a network of helical ribbons, which handedness depended on the chirality of the tartrate counterion. Interestingly, the pitch and width of these twisted ribbons could be tuned by changing the enantiomeric excess (ee) of the tartrate counterion (Figure 10).[44b] Upon increasing the ee from 0% (racemate) to 100% (pure L-tartrate) the pitch of the ribbons changes from infinite (flat ribbons) to 200 nm (right-handed helical ribbons). Simultaneously, the width of the ribbons decreases from 400 nm to 40 nm and becomes more regular. Apparently, the introduction of chirality does not only result in a reduction of the pitch and width of the fibers but also to the formation of monodisperse fibers, a phenomenon that has also been described by Boden.[37b]

**Smart Self-Assembling LMW Hydrogels**

Of particular interest in materials science are “smart gels”, i.e. gels which properties can be triggered by an external stimulus like pH, light, chemicals, etc.[45] Such responsive systems are highly desirable in sensors or applications like drug delivery or catalysis.[31,46] LMW hydrogels exhibit some special features which makes them highly attractive for the development of such “smart gels”: (i) the gelation process is completely reversible due to the noncovalent nature of the gels, (ii) the molecular structure and thus the gel properties can easily be tuned by synthetic methods, and (iii) within the gel the molecules are assembled in well ordered arrays. Several groups exploited these features for the development of “smart” LMW hydrogels. Examples will be discussed below.
Figure 11. A) Concentration dependent pH\textsubscript{gs} values for 19 (□) and 20 (○). B) Schematic representation of a stack of gelator molecules (reprinted from ref.\textsuperscript{[35c]} with permission; Copyright 2004, Wiley-VCH).

quent hydrogel formation by this peptide was also responsive to an increase in ionic strength of the solution, i.e. the addition of NaCl triggered gel formation.\textsuperscript{[49]}

Figure 12. Hydrogelation triggered by pH-dependent intramolecular folding of an oligopeptide (reprinted from ref.\textsuperscript{[48]} with permission; Copyright 2002, American Chemical Society).

In addition to changes in pH also other external stimuli have been used to trigger gel-sol phase transitions. The use of light to direct gelation was elegantly demonstrated by the group of Žižić (Figure 13).\textsuperscript{[50]} Due to the \textit{cis}-configuration of the double bond, the maleic amide 26 was not able to form a hydrogel but remained in solution. In contrast, the \textit{trans}-fumaric amide 27 was found to gelate water. Irradiation of a concentrated solution of 26 in the presence of traces of bromine resulted in the irreversible photoisomerisation of \textit{cis}-26 into \textit{trans}-27 and subsequent gel formation.

NMR measurements of a melted gel revealed the presence of a large excess of the fumaric amide 27 in the hydrogel.

Figure 13. Hydrogelation triggered by the irreversible photoisomerisation of 26 into 27 (reprinted from ref.\textsuperscript{[50]} with permission; Copyright 2003, American Chemical Society).

A more complex photochemically triggered irreversible hydrogelating system was reported by the group of Messersmith.\textsuperscript{[51]} They developed a system based on a 16-amino acid oligopeptide, which gelates water in the presence of chloride salts (NaCl, KCl or CaCl\textsubscript{2}). An aqueous solution of this peptide was mixed with photoresponsive liposomes in which these salts are encapsulated. Irradiation of this solution with near-infrared light causes the liposomes to release the salts, which subsequently triggers the peptide to assemble into a hydrogel.

Also specific intermolecular interactions have been used to trigger gel-sol phase transitions. Recently, Xu and co-workers developed a series of Fmoc-dipeptides, which gel water at pH's dependent on the amino acids employed.\textsuperscript{[52]} Furthermore, the gelation of water by alanylalanine and glycyglycine derivatives was found to be pH-responsive in a reversible manner. Most interestingly, the gel-to-sol transition could chemically be triggered by the addition of vancomycin, due to the binding of vancomycin to the dipeptide derivative, which results in the loss of gelation ability (Figure 14). Interestingly, the binding of vancomycin to the alanylalanine derivative is enantioselective, as only the \textit{D} enantiomer did undergo the vancomycin induced gel-to-sol transition.

Recently, the same authors reported the enzymatically triggered gelation of water.\textsuperscript{[53]} They prepared Fmoc-protected tyrosine phosphate, which formed solutions in aqueous phosphate buffer (pH, 9.6) to which Na\textsubscript{2}CO\textsubscript{3} was added. Addition of the enzyme alkaline phosphatase to this solution results in cleavage of the phosphate group from the Fmoc-protected tyrosine and subsequently gel formation by the protected amino acid.

Whereas most of the "smart" LMW hydrogels refer to responsiveness of the gel-sol phase transition, Hamachi et al. reported a system in which a thermally induced volume transition of a LWM hydrogel was observed.\textsuperscript{[54]} They found that the hydrogel formed by the glycosylated amino acid derivative 28 (Figure 15) did not melt by increasing the temperature, but instead shrank while expelling water. Subse-
Figure 14. Proposed binding of vancomycin to the Fmoc-dipeptides, inducing the gel-to-sol transition (reprinted from ref.[52] with permission; Copyright 2003, American Chemical Society).

Figure 15. Structure of the glycosylated amino acid 28 and the carboxylic acid amino acid 29 together with a schematic representation of the pH-responsive gelation behaviour of the mixed hydrogel formed by 28 and 29 (reprinted from ref.[55] with permission; Copyright 2003, Taylor and Francis Ltd. (http://www.tandf.co.uk/journals)).

Recent experiments showed that entrapped DNA could effectively be released from the gel by the thermally induced shrinking of the gel, indicating that this gel could find application as drug-delivery material.[54] Recently, the same group reported that upon mixing compound 29 into the hydrogels formed by compound 28, the thermally induced phase behaviour became dependent on pH (Figure 15).[55] At pH 4, the carboxylate groups of compound 29 are protonated and neutral, yielding a closely packed structure in the fibers that resembles the packing in the hydrogel formed by pure compound 28 (Figure 15a). As a consequence, raising the temperature leads to shrinking of the gel while expelling the water. However, at pH 7 the carboxylate groups are deprotonated and due to the resulting charge, the close packing of the molecules is dis-
turbed (Figure 15b). As a result, the gel does not shrink upon heating, but instead displays a “normal” gel-to-sol phase transition.

Applications of LMW Hydrogels

Hydrogels find use in numerous applications like separation technology, sensors, food, cosmetics, and pharmaceuticals. Most of these commercial hydrogels are based on polymers. However, compared to polymeric hydrogels, in the use of LMW hydrogelators advantage is taken of their special features (vide supra). Based on these properties, together with their relative easy preparation, it is evident that LMW hydrogels are excellent candidates for various applications. In addition, due to the presence of structural units derived from natural products, like saccharides and amino acids, many of the hydrogelators are expected to be biocompatible; an important prerequisite for their use in for instance pharmaceutical or personal care products.

The possible application of LMW hydrogels for drug delivery has recently been discussed by Tiller following the first report of a bioactive hydrogelator by Xu. Xu extended the antibiotic vancomycin with a hydrophobic pyrene group to obtain compound 30 (Figure 16), which gels thermoreversibly pure water at concentrations of 3.6 mg/mL. CD and fluorescence spectroscopy together with electron microscopy demonstrated that within the gel the molecules are assembled through π-π stacking and hydrogen bonding into helical fibres. Interestingly, compound 30 was found to exhibit an antibiotic activity against different bacteria, which was even 11-fold higher than the parent vancomycin. This result suggests that the increased aggregation ability of 30 has a positive contribution to its antibiotic activity. The authors speculated that 30 might form fiber-like aggregates at the bacterial cell surface, resulting in an increased local concentration of the drug and thus higher activity.

Another example of a LMW hydrogel that can act as a drug was recently reported by the same group. They showed that a mixture of the two FMOC-protected amino acids 31 and 32 was able to form a hydrogel in the presence of Na2CO3 (Figure 17). The hydrogels consisted of a network of fibers, in which the fluorenyl groups are linked through π-π-interactions. Interestingly, these compounds belong to a novel class of anti-inflammatory agents, offering the possibility to use this gel as a drug. In addition, other therapeutic agents might be incorporated to yield a multipurpose drug delivery system.

Figure 17. Anti-inflammatory agents 31 and 32 and their gelation process (reproduced from ref.[58] by permission of The Royal Society of Chemistry).

Whereas compounds 30–32 represent LMW hydrogelators that itself can act as an antibiotic or anti-inflammatory drug other pharmaceutical applications often involve the use of the hydrogel as drug carrier system for entrapped drugs. For instance, Valenta et al. studied hydrogels based on sodium deoxycholate 4 (Figure 1) for pharmaceutical and cosmetic use. Hydrogels of 4 containing mannitol and the model drug rutin were prepared. The addition of mannitol increased the viscous modulus and was expected to have a positive effect on dry skin. Investigation of the diffusion of rutin from hydrogels of 4 through an artificial membrane or excised rat skin revealed an increased release rate compared to established polymer hydrogels. Thus the gel of 4 not only acts as drug carrier but additionally 4 increases the membrane and skin permeability. Furthermore, its microbial stability was comparable to that of the polymer gels. These results together with the observed thixotropy and the fact that no detectable residue was observed at the application area suggest that hydrogels of 4 are promising as drug carrier systems for both topical pharmaceutical and cosmetic use.

In another example, the incorporation of linear calf thymus DNA into aqueous cavities present in hydrogels formed by a uridine phosphocholine amphiphile was reported. This offers the possibility to use these gels as gene delivery agents.

Whereas in general compounds incorporated in a LMW hydrogel are present in an aqueous environment, the group of Maitra reported entrapment in hydrophobic pockets, offering the possibility to incorporate compounds with low water solubility. A tripodal cholic acid-based hydrogelator was used, which was able to gel thermoreversibly aqueous acids at a very low concentration of 0.4 mg/mL. In the gel hydrophobic pockets are present, most likely due to association of the lipophilic β-faces of the cholic acid groups. These hydrophobic pockets are capable to specifically rec-
ognise and entrap the blue, ionised form of bromophenol blue and not its yellow, neutral form. The binding of the guest was observed as a colour change upon gelation from yellow to green.

The hydrophobic regions present in a hydrogel have also been exploited by Shinkai and Hamachi in the context of pharmaceutical applications.[62] It was shown that glycosylated amino acid LMWGs, like compound 28 (Figure 15), formed hydrogels consisting of entangled fibers that contain a hydrophobic core and a hydrophilic surface. Within the aqueous cavities present in the hydrogel, active, native state proteins could be entrapped, as first shown for the oxygen storage protein myoglobin.[62a] These results suggested that these hydrogels could be used as a slow release system of proteins. More interestingly, the authors showed that a semi-wet peptide array could be constructed using hydrogels in which the peptide was entrapped (Figure 18). The aqueous cavities in the hydrogel provided a reaction medium for sensing enzymatic activity, here illustrated by the addition of an enzyme and subsequent enzymatic cleavage of a fluorophore from the entrapped peptide substrate. After cleavage, the hydrophobic fluorophore moves to the hydrophobic fiber core, resulting in an increase of its fluorescence intensity together with a shift of the emission maximum. Addition of an inactive enzyme did not induce such a fluorescence change. These results enable monitoring of this reaction by fluorometry.[62b] Alternatively, this system can be used for the screening of inhibitors of the active enzyme. Interestingly, this example can be considered as a classic case of the employment of both the fluid properties (reaction medium) and the solid character (array of separate gel parts) of a LMW gel.

Xu et al. showed that next to fluorescence also gel formation itself could be used in a simple visual assay for the screening of enzyme inhibitors.[63] They showed that the enzymatically triggered gel formation by an Fmoc-protected amino acid derivative (vide supra)[53] could be prevented by the addition of inhibitors of the used enzyme. This allows the detection of enzyme inhibitors by simply monitoring whether gel formation is taking place or not. The present system is only suitable for the detection of inhibitors for phosphatase, but the same principle might be applied to other enzymatically triggered gel forming systems.

Our group is currently developing hydrogels based on compound 19 (Figure 6A) for application in drug delivery systems.[35] Preliminary in vitro and in vivo experiments show that the gelator molecules are not cytotoxic and do not have a negative influence on the health of rats.[35a] In addition, it was shown that concurrent self-assembly of these hydrogelators and various surfactants resulted in the formation of a fibrous gel network with encapsulated micelles.[35b] Fluorescent probe techniques showed that both supramolecular structures still exhibit their own characteristics but co-exist in a single system. These findings offer the possibility to place these hydrogels as cytoskeleton mimics inside liposomes, affording a system, which can be used as a drug carrier.[64] Furthermore, they could be applicable in the controlled release of liposomes or in surfactant formulations.

However, the factors that influence the entrapment of drug molecules in a LMW hydrogel and their subsequent release are still unclear. Therefore, in an approach to gain more insight, it was decided to study these factors by first using a well-known LMW hydrogelator, i.e. dibenzoyl-L-cystine (15) (Figure 3). The entrapment and release of two small antimalarial and antileishmanial drug molecules was studied: 8-Aminoquinoline (AQ; strong interactions with LMWG 15 due to amine group) and 2-hydroxyquinoline (HQ; weaker interactions with LMWG 15).[65] It was found that the incorporation of AQ slightly improved the thermal stability of the gel up to an equimolar amount, whereas the incorporation of HQ did not have an effect. Furthermore, the release of HQ from the hydrogel was about seven times faster than the release of AQ and the initial release of the latter follows the kinetics of gel degradation (Figure 19). These differences are most likely a result of the differences in interactions between the drug molecules and the gelator molecules. This study shows that depending on the structure and thus intermolecular interactions of the drug and gelator molecules, drug molecules can have a significant influence on the gel properties. Additionally, the interactions between the drug and gelator molecules influence release rates, which offers the possibility to fine-tune release profiles. This suggests that a careful choice of both the drug and gelator molecules is necessary to obtain an efficient drug delivery hydrogel.

Figure 18. Semi-wet peptide/protein chip using a LMWG hydrogel (reprinted from Nature Materials (http://www.nature.com) with permission of the authors of ref. [62b]; Copyright 2004; Macmillan Magazines Ltd.).
Recently, an enzymatic cleavable LMWG-(model) drug conjugate gel system was developed, which could act as a two-step enzyme induced drug release system. When active enzymes are incorporated into the hydrogel, enzymatic cleavage is not observed and the LMWG-drug molecules appear to be protected by the incorporation in gel fibers. However, upon increasing the temperature of the system, the gel fibers dissociate and molecules become available for enzymatic cleavage, leading to release of the drug.

Another interesting application is the use of LMWG hydrogels as biocompatible scaffolds for tissue repair and tissue engineering. Zhang designed oligopeptide hydrogel scaffolds, which were found to support neuronal cell attachment and differentiation as well as neurite outgrowth and functional synapse formation between the attached neurons. The hydrogel scaffolds could be prepared in various geometries and were readily transportable to different environments after cell attachment. Interestingly, injection of the oligopeptide into animals did not result in detectable immune responses or inflammation, indicating that the scaffolds are tolerated in vivo and might readily be used for tissue repair and engineering. Comparable results are obtained by Shinkai, who prepared mesoporous, fibrillar silica by sol-gel transcription of LMW organogels. Recently, this technique was applied to water/pyridine gels formed by compound 25 (Figure 10). Sol-gel transcription of the twisted ribbons formed by the gemini surfactants resulted in the formation of double helical silica fibers (Figure 21). Interestingly, by first tuning the helical pitch of the gel fibers (see Figure 10 and text), the pitch of the resulting silica fibers could be tuned (Figure 21). The group of Stupp designed an amphiphilic oligopeptide which hydrogel could be used as a scaffold for the mineralisation of hydroxyapatite (bone mineral). During mineralisation the hydroxyapatite crystals grow with their c-axis in alignment with the gel fibres. The resulting mineralised nanofibres resemble the lowest level of hierarchical organisation of bone. Hamilton used the hydrogel of 11 (m = 8, n = 11; Figure 2) as a matrix for the growth of calcite crystals. During growth of these crystals, gelator molecules become

resulting in an increased material stiffness. These results indicate that these systems might be suitable as scaffolds in the preparation of implants for cartilage tissue repair.

Related is the use of hydrogels as templates for biomineralisation for hard tissue repair. Pioneering work was done by Shinkai, who prepared mesoporous, fibrillar silica by sol-gel transcription of LMW organogels. Recently, this technique was applied to water/pyridine gels formed by compound 25 (Figure 10). Sol-gel transcription of the twisted ribbons formed by the gemini surfactants resulted in the formation of double helical silica fibers (Figure 21). Interestingly, by first tuning the helical pitch of the gel fibers (see Figure 10 and text), the pitch of the resulting silica fibers could be tuned (Figure 21). The group of Stupp designed an amphiphilic oligopeptide which hydrogel could be used as a scaffold for the mineralisation of hydroxyapatite (bone mineral). During mineralisation the hydroxyapatite crystals grow with their c-axis in alignment with the gel fibres. The resulting mineralised nanofibres resemble the lowest level of hierarchical organisation of bone. Hamilton used the hydrogel of 11 (m = 8, n = 11; Figure 2) as a matrix for the growth of calcite crystals. During growth of these crystals, gelator molecules become

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Design and Application of Self-Assembled Low Molecular Weight Hydrogels

MICROREVIEW

Conclusions

For a long period the gelation of water by LMW compounds did not attract the attention of chemists despite the fact that already more than a century ago a LMW compound was mentioned to gelate water. However, benefiting from the developments in the field of LMW organogelators, the field of LMW hydrogelators has seen a tremendous progress and the rational design of new hydrogelators with tailor-made properties is now feasible. For instance, several “smart” LMW hydrogels, responsive to pH, light or additives have already been developed, and the first applications involving LMW hydrogels, mainly in the biomedical area, were reported.

From the recent progress in this rapidly developing field, it is evident that LMW hydrogelators offer fascinating prospects, in particular towards pharmaceutical applications and smart materials. Compared to the commonly used polymeric hydrogels, LMW hydrogels benefit from their biocompatibility, their intrinsic reversibility, their synthetic accessibility, the ability to tune their properties and their high level of molecular organisation.

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By using D$_2$O instead of H$_2$O, hydrogen bonding between the amide I band (around 1635 or 1610 cm$^{-1}$) changes, which can be observed spectrally. The amide II band is not affected by the solvent change. H stretching bands are not observed.

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