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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.

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.[1] 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.[2] 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.

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Maaike de Loos was born in Venray (The Netherlands) in 1975. During her undergraduate studies she worked in the laboratory of Prof. Ben Feringa in the University of Groningen, under the guidance of Dr. Jan van Esch on polymerizable Low Molecular Weight Gelators. In addition, she spent three months in the group of Prof. F. C. De Schryver, University of Leuven, performing STM studies on diacetylene bis-ureas. On the basis of these projects, she received her M.Sc. degree in organic chemistry with honors. Currently, she is finishing her Ph.D. thesis on hydrogen bonding Low Molecular Weight Gelators, with an emphasis on the relation between molecular structure and supramolecular performance. Her research interests include supramolecular chemistry and new organic materials, including both synthesis and function.

Ben L. Feringa obtained his PhD degree in 1978 at the University of Groningen in the Netherlands under the guidance of Professor Hans Wynberg. After working as a research scientist both at the Shell Research Center in Amsterdam and at the Shell Biosciences Laboratories in Sittingbourne, UK, he returned to his alma mater in 1984 as a lecturer and was appointed full professor at the University of Groningen in 1988. Dr. Feringa is currently director of the Stratingh Institute of Chemistry and Chemical Engineering of the University of Groningen, cofounder of the contract research company KIADIS/SelAct and serves as editor of the RSC journal Organic & Biomolecular Chemistry. In 2003 he was appointed the distinguished Jacobus H. van 't Hoff Professor of Molecular Sciences and received the Koerber European Science Award. In 2004 he was elected foreign honorary member of the American Academy of Arts and Sciences and was recipient of the Spinoza Award. His research interest include stereochemistry, organic synthesis, asymmetric catalysis, molecular switches and motors, self-assembly and nanosystems.

Jan van Esch studied chemistry at the University of Utrecht (1987, cum laude) and in 1993 he received his PhD from the University of Nijmegen with Prof. Roeland J.M. Nolte, on a thesis entitled "Studies on synthetic bilayer membranes: in search of supramolecular catalysts". He then was a post-doc with a Humboldt fellowship in the group of Prof. Helmut Ringsdorf at the University of Mainz, during which period he worked on recognition processes at interfaces. In 1995 he moved to the University of Groningen to work in the groups of Prof. Richard Kellogg and Prof. Ben Feringa on self-assembling small molecule gelators, first as a post-doc and from 1998 on with a fellowship of the Royal Dutch Academy of Science (KNAW). In 2003 he was appointed as a lecturer at the University of Groningen. His current research focuses on fundamental aspects of self-assembly phenomena by small molecules in solution and at interfaces, and the ability to exploit self-assembled objects in functional nanostructures. In 2004 he was a recipient of a VICI research grant from the Netherlands Research Foundation (NWO).

MICROREVIEWS: This feature introduces the readers to the authors' research through a concise overview of the selected topic. Reference to important work from others in the field is included.

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 water,[8] the development of self-assembling LMW hydrogelators attracted only considerable attention in recent years, arising from the progress in the field of LMW organogelators as well as the many applications that can be envisaged. Ever since, the finding of LMW hydrogelators progressed rapidly from serendipity to design and already numerous responsive hydrogels and applications of these systems have been reported offering great prospects for future developments. Recently, Hamilton presented an overview of LMW hydrogelators with the emphasis on structural aspects.[9] This Microreview will cover our efforts, as well as those of others, towards rational design of LMW hydrogelators and review recent developments on smart gels and applications of LMW hydrogels.

The Spectrum of LMW Hydrogelator Structures

Except for some early examples, most of the LMW hydrogelators have been reported only in recent years.[9] Their ability to gelate water was often discovered while studying their amphiphilic behaviour or their ability to gelate organic solvents, respectively. This led to a wide variety in structures, which frequently contain moieties based on natural products like amino acids, saccharides, nucleosides, nucleotides or bile acids. Despite this variety, all compounds exhibit the common feature that they are composed of both hydrophilic and hydrophobic units. The review of Hamilton especially emphasised the amphiphilic character of LMW hydrogelators.[9] However, many LMW hydrogelators do not exhibit a classic amphiphilic structure, and instead the spectrum of LMW hydrogelator structures varies between amphiphilic to more organogelator-like architectures.

Typical examples of LMW hydrogelators that possess an amphiphilic structure are represented by compounds **1**–**7** (Figure 1). Shinkai developed gelator **1**, in which a saccharide moiety is combined with a small aromatic group together with a long alkyl chain (Figure 1).[1] Compound **1** gelates water in the presence of trace amounts of methanol or ethanol as well as a large number of organic solvents at concentrations of 1–30 mg/mL. The gel consisted of occasionally twisted fibres, which were most likely built from interdigitated bilayer aggregates. These aggregates were formed due to a combination of π-π 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 π-π 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 linkage, 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

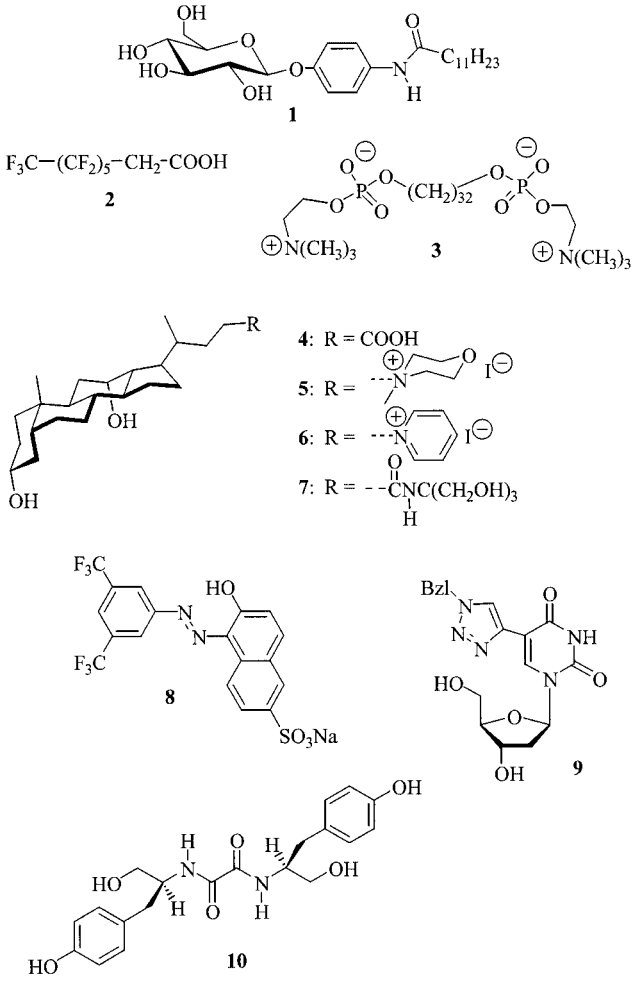


Figure 1. Variety in LMW hydrogelator structures.

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.[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.[16e] However, 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.[16a]

Recently, it was shown that a cationic or neutral group, providing the hydrogelators **5–7**, could replace the carboxylic acid group of **4**.[16e,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 clear amphiphilic architecture is absent.[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.[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).[18] 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, π-π 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 π-π stacking.

An example is the *N*,*N*′-bis(alkylamino)oxalamide **10** prepared by the group of Žinić (Figure 1).[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,[20] it is most likely that aggregation is initially driven by π-π 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 π-π 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.[22,24] 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[21] was modified with hydrophilic carboxylic acids to obtain the class of bis-urea amino acid-based hydrogelators **11** (Figure 2).[22] These compounds were found to gelate phosphate buffers of $5.9 \leq pH \leq 7.9$ with high ionic strength. The exact pH and ionic strength required to obtain a gel was dependent on the total number of methylene groups or the linker length, respectively. Cryo-TEM revealed the existence of twisted ribbons in the hydrogel. In combination with X-ray diffraction results from a dried precipitate ($n = 5$) and concentrated gel ($n = 11$), both prepared in the presence of $CaCl_2$, 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 Ca^{2+}-coordination of the carboxylates.

The L-lysine-based bis-amide organogelators developed in the group of Hanabusa[23] 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).[24] 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 gelating ability decreased with increasing chain length (R^1). For all compounds electron microscopy revealed the existence of a network of thin fibres in the gels. FT-IR studies on D_2O/DMSO solutions and gels showed that in the gels hydrogen bonds between the amides are present.[25] Furthermore, low frequency shifts of the CH_2 stretching vibrations indicate that the alkyl chains become closely packed. Aggregation is proposed to be initially driven by hydrophobic interactions,

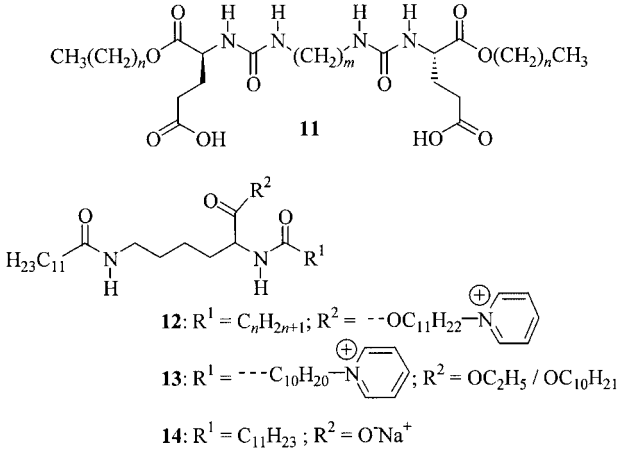


Figure 2. LMW hydrogelators obtained by conversion of amino acid-based organogelators.

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[10,12,13,26] and Menger.[27] 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.[10,12,13,26] The group of Menger studied the hydrogelation by aroyl-L-cystine derivatives like **15**,[27] a compound which was already known to form hydrogels in 1892 (Figure 3).[8] In the twenties of the last century chemists had found that **15** formed transparent gels at 1 mg/mL, with a fibrillar structure.[28] Furthermore, it was observed that gelation by **15** was thermoreversible and pH dependent, i.e. gelation occurred only at $3.4 \leq pH \leq 2.2$, and that dyes could diffuse through the gel.[29] Interestingly, they showed that replacement of the S–S bridge by a CH_2–CH_2 or CH=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**.[27] Structural variations showed that substitution of the carboxylic acid by a small amide resulted in an enhanced gelation ability.[27c] 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 toluoyl and nitrobenzoyl derivatives revealed two different packing modes: an aggregate structure in which the molecules are folded and packed together by hydrogen bonding and π-π stacking (Figure 3A) and a packing structure in which the molecule exhibits a linear conformation and the amides interact by intermolecular hy-

drogen bonding (Figure 3B). This suggests that within the gel several packing modes could be present.

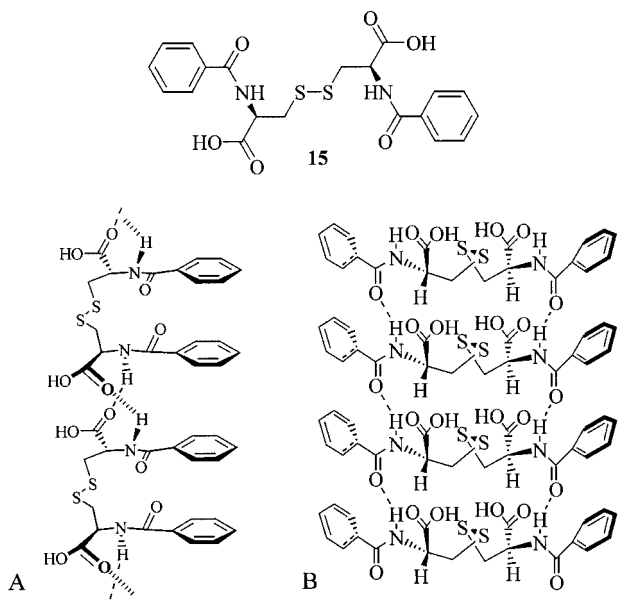


Figure 3. Hydrogelator **15** and packing modes observed for derivatives of **15**.

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 should be possible to convert this typical organogelator into a hydrogelator by simply modifying the substituents with hydrophilic functionalities X, like hydroxy groups, carboxylic acids or amines (Figure 4). A short hydrophobic spacer will remain between the urea and the hydrophilic groups, which might facilitate the formation of intermolecular urea hydrogen bonds by shielding the urea from the aqueous phase.

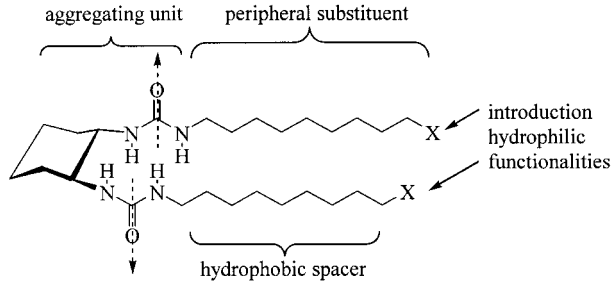


Figure 4. Molecular structure of the cyclohexane bis-urea organogelators, showing the directionality of multiple hydrogen bonding by the urea groups and the design guidelines for the conversion of these compounds into hydrogelators (reproduced from ref.[32] by permission of The Royal Society of Chemistry).

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.

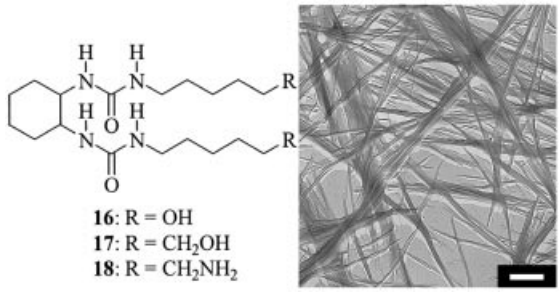


Figure 5. Cyclohexane bis-urea-based hydrogelators with a TEM-micrograph of a hydrogel of racemic **17** (c = 5 mM; Pt-shadowed; bar represents 1 μm) (reproduced from ref.[32] by permission of The Royal Society of Chemistry).

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 gelators, the T_m of the gels formed by **17** and **18** is 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 methylene 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 organogelators as described above, i.e.: an anisotropic self-assembling cyclohexane bis-urea unit combined with peripheral substituents that govern the solvent compatibility.

Another interesting example comprises the class of C_3-symmetric amino acid LMW (hydro)gelators, in which a *cis*,*cis*-1,3,5-cyclohexane tris-amide core is used as the gelating scaffold (Figure 6).[35] The choice of the core was based on the parallel orientation of the amide groups, which provides strong uni-axial intermolecular interactions affording 1D self-assembly perpendicular to the plane of the molecule.[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 hydrogen bonding amides from the surrounding water together 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 concentrations. For instance, for **21** a remarkable low critical gelation 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 hydrogels 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 derivative 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

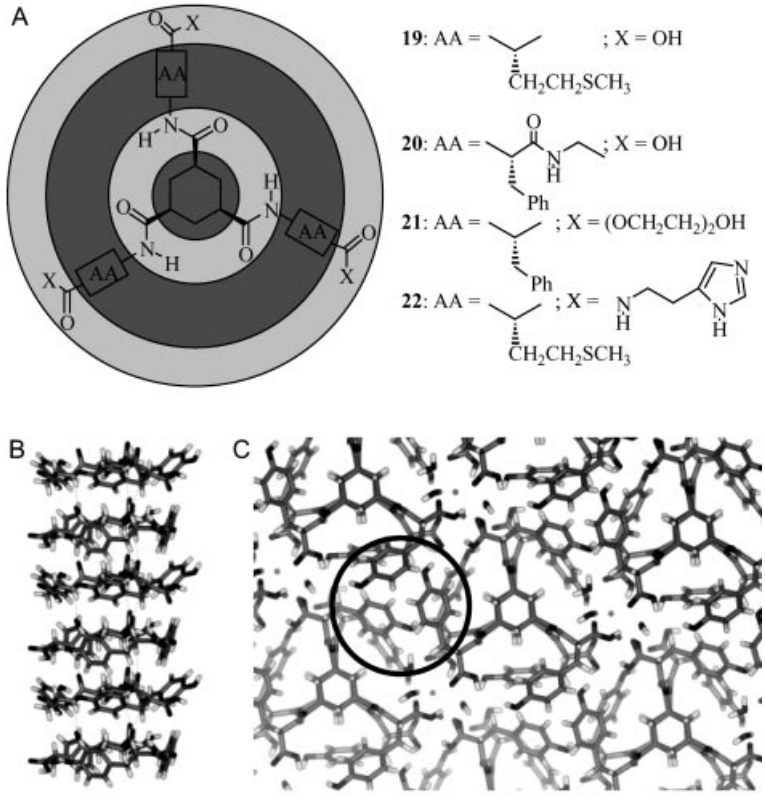


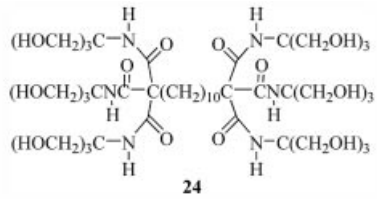
Figure 6. A) Structure of the C_3-symmetric hydrogelators. Light regions are hydrophilic; dark regions are hydrophobic. B) Side view of the stacked tyrosine derivative [AA = $CH(CH_2$–*p*-PhOH); X = OH]. C) Top view of the X-ray structure of the tyrosine derivative (reprinted from ref.[35c] with permission; Copyright 2004, Wiley-VCH).

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.[37] 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).[37a,37b] Other examples of oligopeptide hydrogelators forming β-sheets include compounds consisting of two oligopeptide strands connected by a 2,8-dibenzofuran derivative[38] and oligopeptides with alternating polar and nonpolar amino acids.[39]

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,[40] 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).[41] The large head group of the dumb-bell-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.



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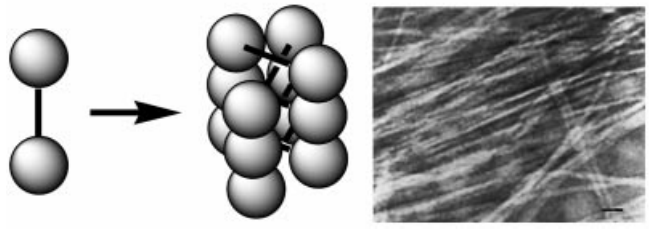

Figure 7. The dumb-bell shape of bisarborol **24** leads to a crosswise stacking, as schematically shown here, resulting in fibrous rods (reproduced from ref.[41a] by permission of The Royal Society of Chemistry).

Also the group of Zhang applied the structure-shape concept to direct the morphology of peptide materials (Figure 8).[42] 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 nonpolar 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.[43] 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,

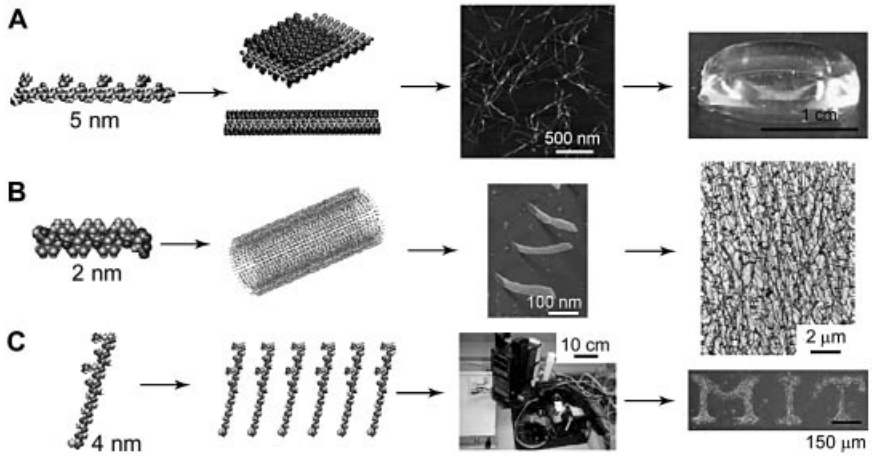

Figure 8. Various peptide materials, for which the applied amino acids determine the shape of the aggregates. A) Ionic self-complementary peptide. B) Surfactant-type peptide. C) Surface nanocoating peptide (peptide ink) (reprinted from ref.[42b] with permission; Copyright 2004, Elsevier).

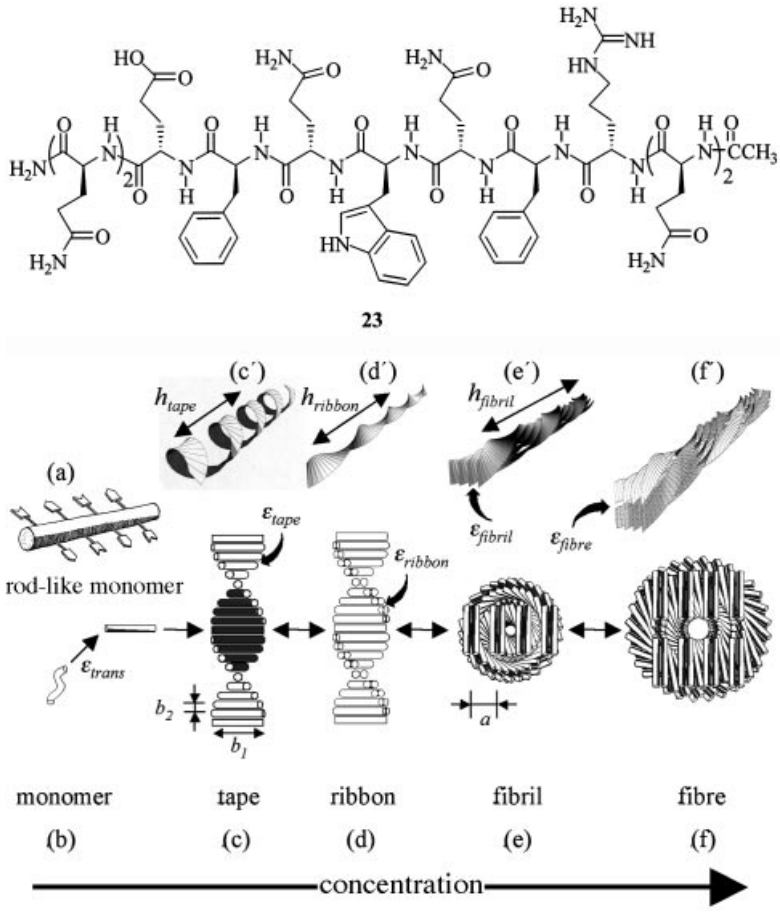

Figure 9. (top) Oligopeptide LMW hydrogelator. (bottom) Model for the hierarchical self-assembly of the rod-like molecules into finite fibrils and fibers (reprinted from ref.[37b] with permission; Copyright 2001, National Academy of Sciences, USA).

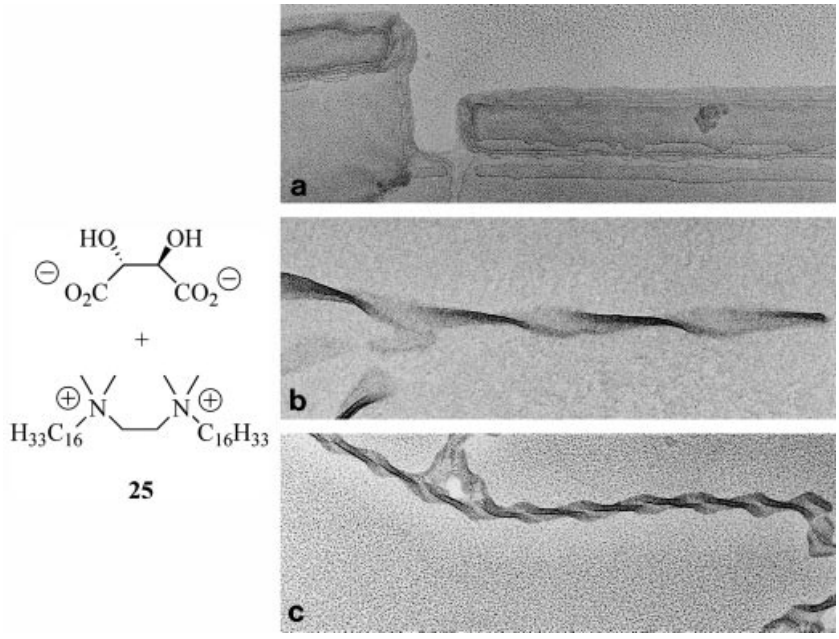

Figure 10. Structure of the gemini surfactant and cryo-TEM images of the formed twisted ribbons at an *ee* of a) 0% (racemate), b) 50% and c) 100% (pure L-tartrate) (reprinted from Nature (http://www.nature.com) with permission of the authors of ref.[44b]; Copyright 1999, Macmillan Magazines Ltd.).

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).[44] 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.[1,46] 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.

pH-Responsive gelation, i.e. reversible gel formation upon changes in pH, is of particular interest in the context of biological applications, like drug delivery, since many biological systems exhibit a distinct pH. However, whereas gelation at a specific pH range, due to the presence of basic (e.g. amines) or acidic (e.g. carboxylic acids) groups is observed for many of the LMW hydrogelators, so far only for a few systems *pH-responsiveness* of the gels has been observed. For example, the group of Boden prepared oligopeptides rich in anionic glutamine residues [–$(CH_2)_2$-COOH]) or cationic ornithine residues [–$(CH_2)_3NH_2$], whose sol-to-gel transitions were found to be pH responsive under acidic or basic conditions, respectively.[37] Interestingly, mixing of aqueous solutions of these anionic and cationic oligopeptides resulted in the formation of gels that cover a broad pH range (ca. 1–12).[37d] Stupp and coworkers reported the pH-responsive hydrogelation by amphiphilic oligopeptides but also hydrogelation triggered by the addition of Ca^{2+} ions.[47]

The hydrogelators developed in our group also displayed pH-responsive sol-gel transitions. The gel-to-sol transition of diamine derivative **18** (Figure 5) could be triggered under highly basic conditions (pH > 10) by the addition of HCl to the hydrogel, resulting in protonation of the amines and subsequently loss of gelation ability.[32] The C_3-symmetric hydrogelators (Figure 6) were found to exhibit a reversible pH-responsive gel-to-sol transition as well, in a pH-range depending on the used amino acid derivatives and the nature of the substituents.[35c] For instance the acidic compounds **19** and **20** formed gels at pH < 4 and pH < 6, respectively (Figure 11A), and the basic compound **22** formed gels at pH > 6. The pH range of gelation was not only related to the different pK_a's of the amino acids but also to differences in strength of the molecular interactions, as demonstrated by compound **19** and **20**. Both feature carboxylic acid groups (Figure 6A) with a similar pK_a, resulting in a similar increase of repulsive charges upon the addition of base (Figure 11B). However, **20** has additional amide groups and π-π-stacking phenyl moieties, which increase the attractive interactions compared to compound **19** (Figure 11B). These stronger attractive forces can compensate for the accumulation of the repulsive forces up to a higher pH, resulting in higher pH values at which the aggregates remain stable and, as a consequence, the gel can survive.

A particular interesting example pertains to the pH-responsive hydrogelation through pH-dependent intramolecular folding of a β-hairpin peptide (Figure 12).[48] Under basic conditions part of the lysines in this peptide is neutral, and the peptide can fold into β-sheets as shown by FT-IR and CD measurements. As a result, the oligopeptides are able to self-assemble into a hydrogel. In acidic medium the lysine residues are charged and the resulting charge repulsion between the lysines causes the oligopeptide to unfold into an unstructured form, affording an aqueous solution. Switching between the gel and the solution phase can be achieved by the addition of base (NaOH) or acid (HCl). Recently, the authors showed that the folding and subse-

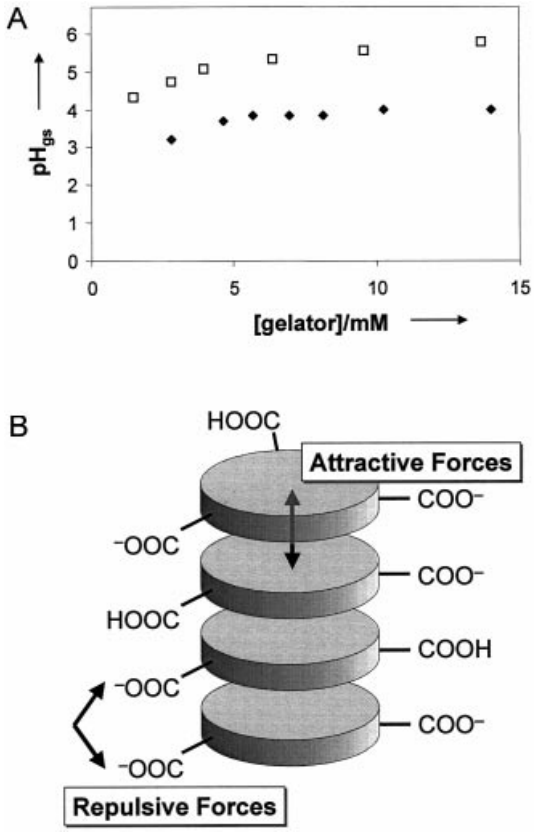

Figure 11. A) Concentration dependent pH_{gs} values for **19** (♦) and **20** (□). B) Schematic representation of a stack of gelator molecules (reprinted from ref.[35c] with permission; Copyright 2004, Wiley-VCH).

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.[49]

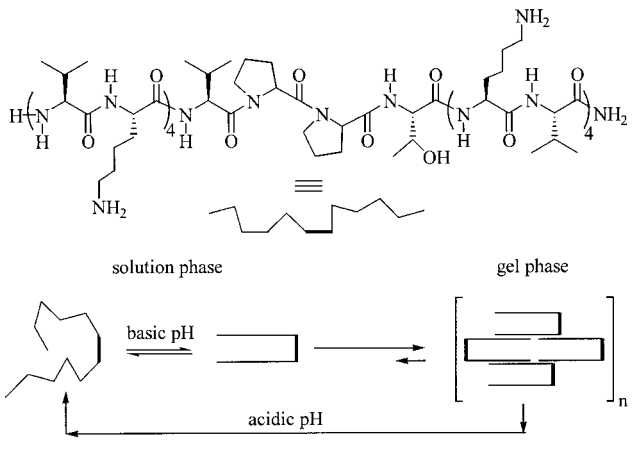

Figure 12. Hydrogelation triggered by pH-dependent intramolecular folding of an oligopeptide (reprinted from ref.[48] with permission; Copyright 2002, American Chemical Society).

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 Žinić (Figure 13).[50] Due to the *cis*-configuration of the double bond, the maleic amide **26** was not able to form a hydrogel but remained in solution. In contrast, the *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 *cis*-**26** into *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.

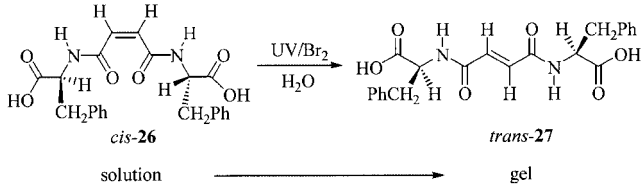

Figure 13. Hydrogelation triggered by the irreversible photoisomerisation of **26** into **27** (reprinted from ref.[50] with permission; Copyright 2003, American Chemical Society).

A more complex photochemically triggered irreversible hydrogelating system was reported by the group of Messersmith.[51] They developed a system based on a 16-amino acid oligopeptide, which gelates water in the presence of chloride salts (NaCl, KCl or $CaCl_2$). 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 coworkers developed a series of Fmoc-dipeptides, which gel water at pH's dependent on the amino acids employed.[52] Furthermore, the gelation of water by alanylalanine and glycylglycine 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 DD enantiomer did undergo the vancomycin induced gel-to-sol transition.

Recently, the same authors reported the enzymatically triggered gelation of water.[53] They prepared Fmoc-protected tyrosine phosphate, which formed solutions in aqueous phosphate buffer (pH, 9.6) to which Na_2CO_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.[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-

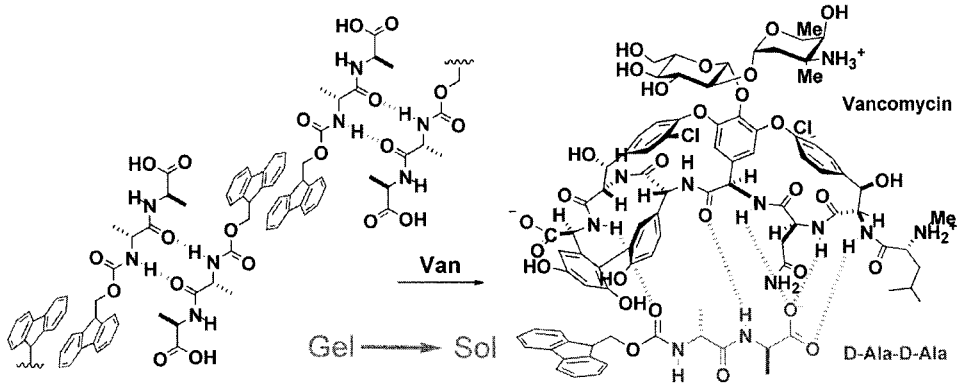

Figure 14. Proposed binding of vancomycin to the Fmoc-dipeptides, inducing the gel-to-sol transition (reprinted from ref.[52] with permission; Copyright 2003, American Chemical Society).

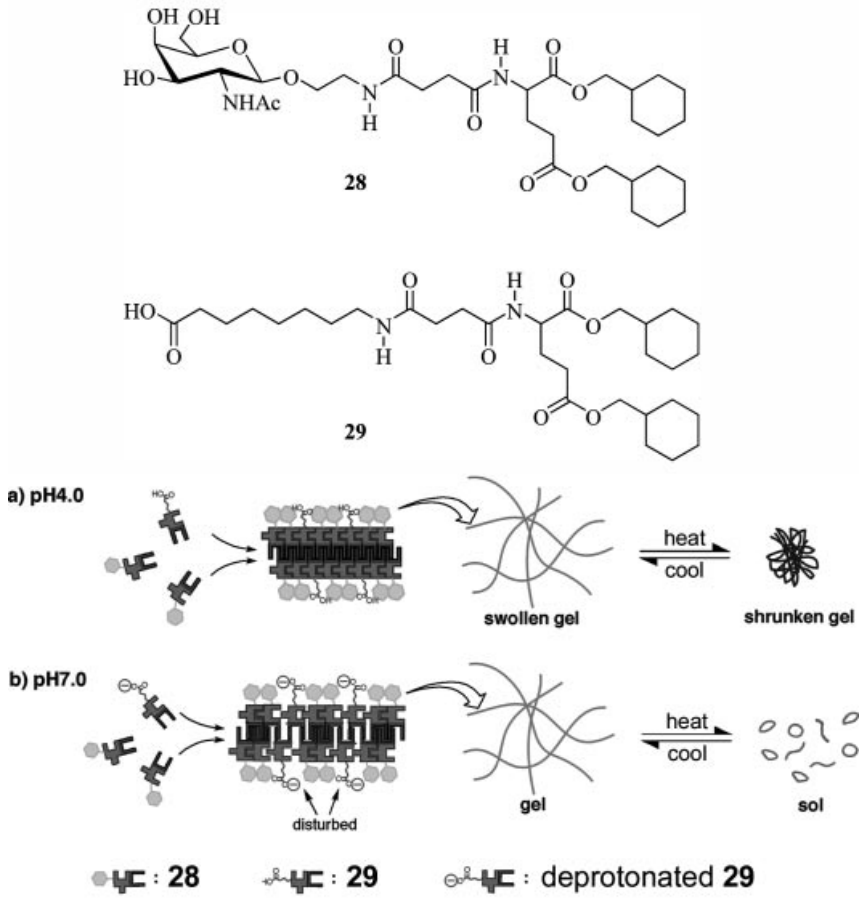

Figure 15. Structure of the glycosylated amino acid **28** and the carboxylic acid amino acid **29** together with a schematic representation of the pH-responsive gelation behaviour of the mixed hydrogel formed by **28** and **29** (reprinted from ref.[55] with permission; Copyright 2003, Taylor and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

quent cooling resulted in swelling of the gel in the water till the hydrogel was re-formed. This finding is very remarkable, because this behaviour has so far only been observed for polymeric systems.[45] 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.[1,46a] 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[56] following the first report of a bioactive hydrogelator by Xu.[57] Xu extended the antibiotic vancomycin with a hydrophobic pyrene group to obtain compound **30** (Figure 16), which gelates 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.

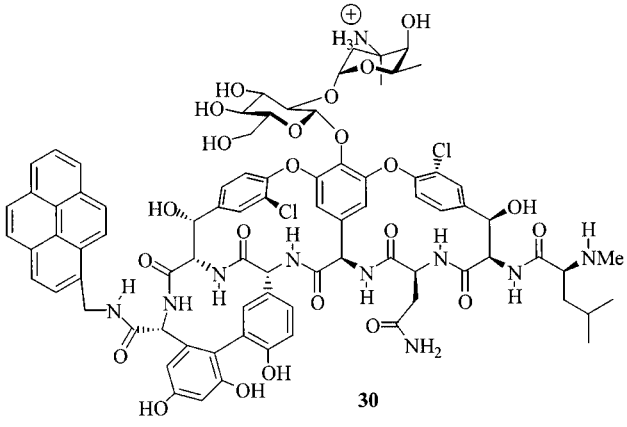

Figure 16. Antibiotic LMW hydrogelator obtained by the extension of vancomycin with pyrene.[57]

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 Na_2CO_3 (Figure 17).[58] 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.

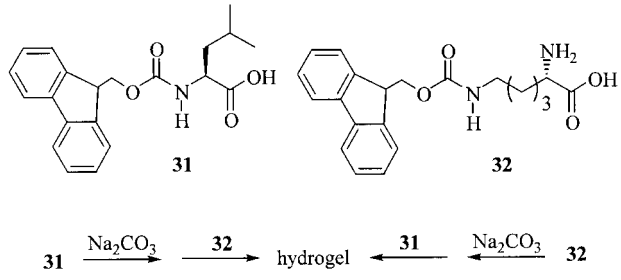

Figure 17. Anti-inflammatory agents **31** and **32** and their gelation process (reproduced from ref.[58] by permission of The Royal Society of Chemistry).

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.[59] 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.[60] 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.[61] 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.[35c] 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.

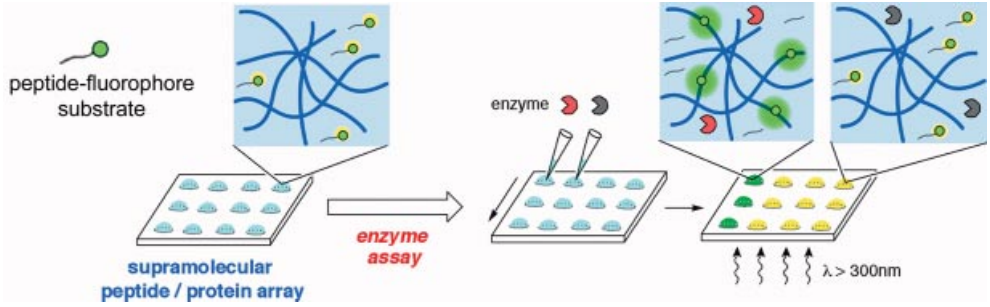

Figure 18. Semi-wet peptide/protein chip using a LMWG hydrogel (reprinted from Nature Materials (http://www.nature.com) with permission of the authors of ref.[62b]; Copyright 2004, Macmillan Magazines Ltd.).

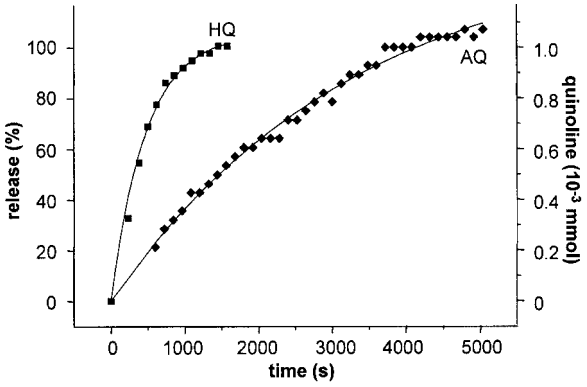

Figure 19. Released percentages and concentrations of AQ and HQ from 0.2 wt.-% gels of **15**, containing 1.0 mM of quinoline each (reprinted from ref.[65] with permission; Copyright 2004, Elsevier).

Recently, an enzymatic cleavable LMWG-(model) drug conjugate gel system was developed, which could act as a two-step enzyme induced drug release system.[66] 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.[67] 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.[68] 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 Stupp et al., who used a specially designed peptide amphiphile that was able to form hydrogels.[69] Instead of seeding the cells on top of the hydrogel scaffold, they incorporated neural progenitor cells inside the hydrogel by mixing prior to gelation. It was shown that the cells survived the gel forming process and readily differentiated into neurons, while suppressing astrocyte differentiation. Remarkably, the self-assembly of the hydrogel could be triggered by the injection of peptide solution into tissue or rat spinal cords, yielding a localized solid scaffold. Another designed oligopeptide was found to be able to form hydrogel scaffolds in which chondrocytes are encapsulated (Figure 20).[70] Within the hydrogels cell division was taking place, as well as extracellular matrix (ECM) production, rich in proteoglycans and type II collagen. The ECM accumulation was accompanied by an increase in mechanical stiffness, indicative of the deposition of mechanically functional neo-tissue. Recently, it was shown that the proteoglycan synthesis could be stimulated by dynamic compression, resulting in an increased material stiffness.[70b] These results indicate that these systems might be suitable as scaffolds in the preparation of implants for cartilage tissue repair.

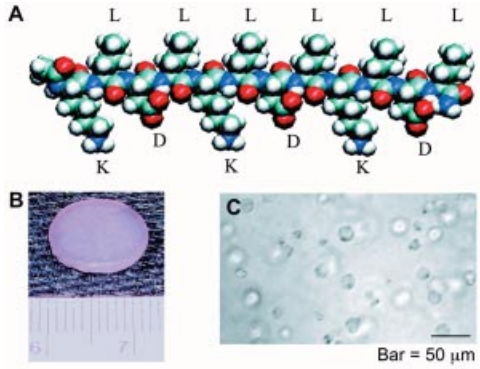

Figure 20. A) Molecular model of the designed oligopeptide (L = leucine; K = lysine; D = aspartic acid. B) A chondrocyte-seeded peptide hydrogel plug, punched from 1.6 mm-thick slabs. C) Light microscope image of chondrocyte cells encapsulated in the peptide hydrogel (reprinted from ref.[70a] with permission; Copyright 2002, National Academy of Sciences, USA).

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.[71] Recently, this technique was applied to water/pyridine gels formed by compound **25** (Figure 10).[44] Sol-gel transcription of the twisted ribbons formed by the gemini surfactants resulted in the formation of double helical silica fibers (Figure 21).[72] 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).[47a] 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.[73] During growth of these crystals, gelator molecules become

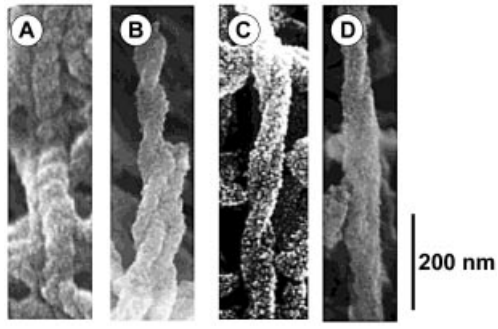

Figure 21. The double-stranded silica fibers obtained by sol-gel transcription of **25** gels at an *ee* of A) 100%, B) 50%, C) 33% and D) 20% (2.0 wt.-%, after calcination) (reproduced from ref.[72] by permission of The Royal Society of Chemistry).

included in the crystal lattice at sites of imperfection, resulting in different dissolution behaviour of the crystals.

The number of LMW hydrogel applications that is not related to pharmaceutical use is limited. An interesting example pertains to a LMW hydrogel-based sensor chip, in which use is made of both the fluid and solid character of the hydrogel.[74] An artificial receptor for phosphate derivatives was entrapped in a hydrogel formed by a glycosylated amino acid. Due to the fluid character, titration of the resulting system with phosphate results in an increase in fluorescence intensity, similar to that of an aqueous solution of this receptor. The solid character of the hydrogel enables the construction of an array of the gel system on a glass support, making high-throughput sensing of many analytes possible (Figure 22A). Interestingly, it was shown that an integrated sensor chip could readily be prepared by the use of various chemosensors in one array, in this case a phosphate probe (phos probe), a Zn^{2+} probe, a Ca^{2+} probe and a pH probe. Analysis of mixed solutions of distinct analytes, revealed an emission pattern corresponding to the composition of the solutions (Figure 22B).

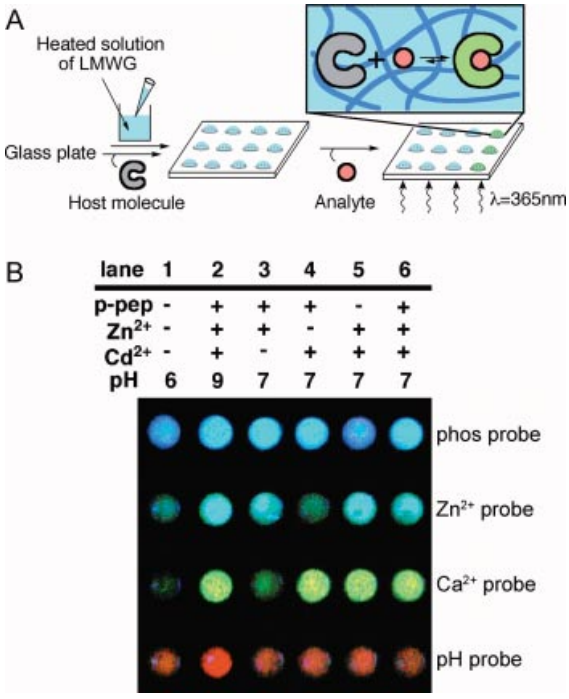

Figure 22. A) Construction of the semi-wet sensor array. B) Analysis of six mixed solutions varying in pH and containing a phosphorylated peptide (p-pep), Zn^{2+} or Ca^{2+} with a sensor chip having four different chemosensors [a phosphate probe (phos probe), a Zn^{2+} probe, a Ca^{2+} probe and a pH probe] (reprinted from ref.[74] with permission; Copyright 2004, American Chemical Society).

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 *organo*gelators, the field of LMW *hydro*gelators 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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