Chapter 3

Characterization and Tailoring the Properties of Hydrogels Using Spectroscopic Methods

Gabriela Ionita

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/62900

Abstract

Hydrogels represent heterogeneous systems that consist of a large amount of water retained by a three-dimensional network. The hydrogel network is the result of assembly through physical interactions or chemical cross-linking of polymers or small molecules. The applications of hydrogels (water purification, tissue regeneration, therapeutic delivery, bio-detection or bio-imaging, etc.) depend on their physicochemical properties and structural features. Although electron microscopy and viscoelastic measurements provide general information about a gel material, the spectroscopic methods complement these methods and also afford a deep insight into the gel structure. In this chapter, the applications of several spectroscopic methods for characterizing polymeric or supramolecular hydrogels are discussed. Thus, this review highlights the particular application of vibrational spectroscopy, circular dichroism, fluorescence (these providing information on assembly in the network), interactions that occur between network and solvent (water), pulsed-field gradient NMR (determination of mesh size) and EPR spectroscopy (a method that can provide extensive information regarding the assembly process, diffusion and release).

Keywords: hydrogels, IR, Raman, fluorescence, circular dichroism, PFG-NMR, EPR

1. Introduction

Gels represent a class of soft materials consisting of a 'solid-like' network that holds a large volume of solvent via surface tension or capillary effect [1]. 'Hydrogels' refer to the case in which the solvent retained in the frame of a gel network is water. The term hydrogel was first mentioned in the literature at the end of the nineteenth century describing a colloidal gel of inorganic salts

[2], but the current meaning referring to a water-swollen gel network became prevalent six decades ago with the developing research area of polymeric hydrogels.

Hydrogels are classified using different criteria, such as the nature of the gelators, the type of interactions that contribute to the building of the 'solid network' and physical properties of the gel network (**Figure 1**). The gel networks result by chemical or physical cross-linking of gelators, which can be either polymers or low molecular weight compounds able to generate fibrillary networks [1–3]. The best-known hydrogels are those obtained by physical or chemical assembly of natural and synthetic polymers.

Chemical hydrogels are the result of interconnection of polymer chains or molecular building blocks through the formation of covalent bonds, which obviously are non-reversible. Once the gel network is broken, the chemical hydrogels are unable to self-heal. The mechanical properties of these gels usually are easier to tune by varying the reaction conditions that finely influence the number of cross-links. Another particularity of chemical hydrogels is that their volume changes significantly during the transition from solution to gel state [1, 4, 5].

Physical hydrogels refer mainly to self-assembly of synthetic or natural polymers, but in recent years, there are more and more studies dedicated to designing new low molecular weight compounds able to hydrogelate [3]. The knowledge gained after studying numerous low molecular weight gelators (LMWG) is still not enough for predicting the ability of a new molecule to generate gel systems through non-covalent supramolecular interactions. Frequently, the LMWGs are characterized by amphiphilicity and the capacity to generate non-covalent interactions (π–π interactions, hydrogen-bonding and charge interactions among the molecules, host–guest interactions) allowing the formation of a three-dimensional fibrillary network.

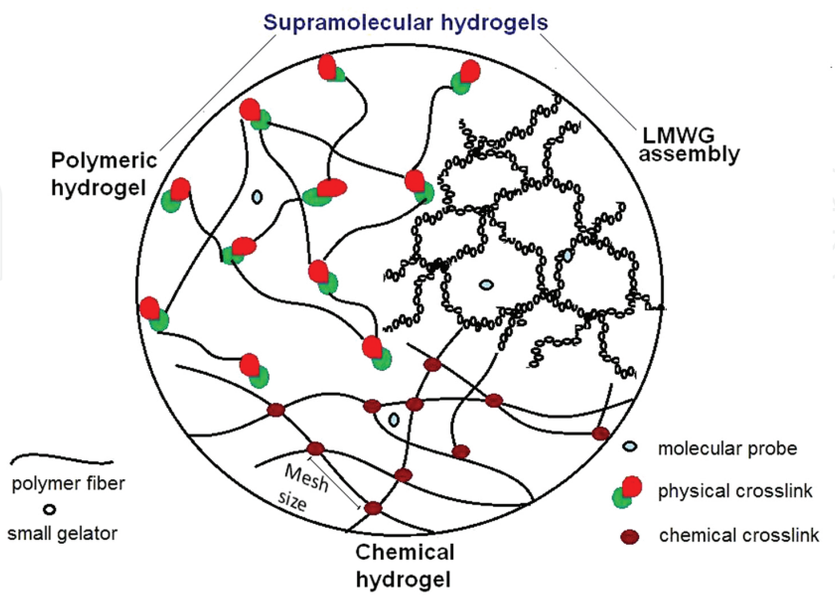

Figure 1. Classification of hydrogels function of the nature of interactions building the network.

http://dx.doi.org/10.5772/62900

It can be accepted that hydrogels formed through non-covalent interactions leading to assembly either of LMWG or polymers form a large family of supramolecular hydrogels [1, 3].

Research in the gels field is closely bound with their various applications. Gels find applications in daily life, being frequently used in cosmetics and food chemistry [3], but there are specific or high-interest domains that correlate structural gel characteristics with applications. Thus, whether their building blocks are biocompatible or are structural biomaterials, hydrogels are oriented towards regenerative medicine and tissue engineering, obtaining of enzyme-responsive hydrogels, enzyme–hydrogel hybrid materials, drug delivery and therapeutic agents with release control, separation processes or water purification [1, 2, 6]. As smart materials, gels may find applications in optoelectronic, responsive systems to pH, ions and light harvesting systems [7]. Supramolecular hydrogels resulting from assembly of LMWG can find application in catalysis generating either self-construction of the catalytic system or by generation of new catalytic properties arising from association modes [8, 9]. Metallic nanoparticles can be generated *in situ* in the chemical hydrogel matrices for further use as catalysts [8].

2. Spectroscopic methods used in hydrogels studies

Unravelling the molecular organization inside a gel represents a complex issue and involves a multitude of physicochemical methods. The majority of gel studies use electron microscopy to demonstrate the existence of gel fibrils, while mechanical properties expressed by rheological parameters reflect the density of cross-linking, thickness of gel fibril, all of these aspects being the result of molecular packing of gelators [10]. Hydrogels represent systems with a dynamic nature given the interactions between building blocks, the encapsulated species and interaction between solvent entrapped and gel fibres. Therefore, gel properties need to be investigated through a large variety of complementary physicochemical methods. In this chapter, the applications of several spectroscopic methods for characterizing polymeric or supramolecular hydrogels are discussed. **Figure 1** schematically represents different networks that can be investigated by the methods discussed in this review: vibrational spectroscopy, circular dichroism, fluorescence, pulsed-field gradient NMR and electron paramagnetic resonance (EPR) spectroscopy. Some methods can be involved in characterization of hydrogels irrespective of the driving forces that build the network, such as vibrational spectroscopy (IR and Raman). Circular dichroism can be relevant for assembly of LMWG, especially as preponderantly these molecules are chiral or in the particular case of polypeptides assembled into gel networks. Pulsed-field gradient NMR is a method applied to estimate the mesh size of the gel network, while other methods can be used to determine diffusion coefficients of solutes in gels or to monitor the release of encapsulated species (e.g. vibrational spectroscopy, fluorescence spectroscopy). Circular dichroism can also be used to estimate the transition from sol to gel in the case of supramolecular hydrogels following changes in ellipticity. Fluorescence and EPR spectroscopies are suitable methods to test the environment around the sensing groups (fluorescent and paramagnetic, respectively), and thus can be used to demonstrate the heterogeneity of the hydrogel systems, but also provide information on the dynamics of encapsulated species or the gel network.

2.1. IR spectroscopy

Vibrational spectroscopy or infrared (IR) spectroscopy is a suitable method to obtain substantial information on the self-assembly process leading to formation of supramolecular gels. IR spectroscopy deals with the infrared region of the electromagnetic spectrum. The photon energies (1–15 kcal/mol) corresponding to the IR region are not large enough to excite the electrons but may induce vibrational excitation. The active vibrations in IR cause a change in the dipole moment. Vibrations of bonds or a group of bonds include stretching, bending, scissoring, rocking and twisting [11–14]. The analysis of intensities or shifts of wavenumbers can probe involvement of specific bonds in gel network building.

Comparison of the IR spectrum in the gel state with the spectra of solution or solid states of gelators can provide information on the interactions driving the formation of a gel network. The formation of a supramolecular gel network involves different types of non-covalent interactions: hydrogen bonding, van der Waals and π–π stacking. These interactions can be identified in IR spectra by the appearance and disappearance of vibrational bands characteristic of associated and free groups. In many cases, the gelators have in their structure functional groups with characteristic vibration bands (carboxy, hydroxy, amino and amide groups) that are sensitive to formation of weak physical interactions. The hydrogen bonding involving, for example, the carbonyl or amide groups, which often contribute to the building of hydrogel network, is frequently hidden by the water hydrogen bonding. In such cases, the replacement of water with deuterated water is required [13, 14].

The following examples are selected from studies reported in the literature highlighting the applicability of IR spectroscopy in attempting to determine the assembly mode of gelators (polymers or LMWG) in a supramolecular network. Suzuki and co-workers [13] synthesized a lysine derivative, *Nα*-hexanoyl-*Nζ*-lauroyl-l-lysine and studied the organogelation and hydrogelation of the gelator in combination with corresponding alkali salts (Li^+, Na^+ and K^+). Deconvolution of the broadbands arising in the interval 1750–1500 cm^{-1} (which summarize the bands corresponding to the stretching vibration of the carboxylic acid, amide I and II and carboxylate groups) and analysis of the CH_2 stretching vibrations arising in the region 2850–2950 cm^{-1} demonstrated that the gel network is the result of hydrogen-bonding interactions between amide groups, interactions between carboxylic acid and alkyl carboxylate and hydrophobic interactions involving CH_2 groups.

Urea-based gelators represent a class of molecules suitable to be investigated by IR spectroscopy due to the presence of the ureido group, which is likely to be involved in hydrogen bonding. In general, a condition for obtaining supramolecular hydrogels resulting by assembly of urea-based gelators is the presence of other moieties able to establish hydrophobic bonds like π–π stacking or van der Waals interaction. The lack of a moiety able to interact through hydrophobic forces from the structure of a potential gelator can be compensated by other interactions. For instance, the study of Kleinsmann and co-workers [15] on the hydrogelation capability of *N*-[(uracil-5-yl)methyl]urea in different buffers revealed that phosphate ions can trigger the assembly in a gel network. IR spectra show that the characteristic N–H stretching band shifts from 3314 to 3146 cm^{-1} due to strong hydrogen bonding with C=O groups during the self-assembly, while the carbonyl stretching vibration band observed in solution at 1632

http://dx.doi.org/10.5772/62900

cm^{-1} shifts to 1675 cm^{-1} due to the hydrogen-bonding interactions with the uracil N–H protons. Moreover, the phosphate bands arising at 1070 and 982 cm^{-1} show intensity increase as a result of immobilization of the phosphate ion into the supramolecular assembly.

In some cases, the formation of hydrogels through hydrogen-bonding interactions can be demonstrated by FT-IR analyses of xerogels. This was the case of the supramolecular hydrogel formed between 1,4-bi(phenylalanine-diglycol)-benzene (PDB) and sodium alginate (SA), a polysaccharide composed of (1–4)-linked β-D-mannuronic acid (M) and α-L-guluronic acid (G) residues [16]. In this hydrogel, nanofibres of PDB alternate with SA chains. The network represents a semi-interpenetrating polymer network (semi-IPN) through hydrogen bonds, which has been proven by analysis of the IR bands specific to carboxylate groups from SA and the amide group from the PDB structure.

There is a large literature dedicated to polymeric hydrogels with medical applicability that contain biocompatible polymers such as poly(*N*-isopropyl acrylamide) (PNIPAM) or natural polymers such as dextran. PNIPAM has the amido group in its structure, whose vibrational modes are sensitive to association through hydrogen bonding. Inclusion of PNIPAM polymer in the structure of a hydrogel network can be thus demonstrated by FT-IR [17].

Dextran is a polysaccharide consisting of chains of 1–6 linked α-D-glucopyranosyl units with 1–2, 1–3 and 1–4 ramifications. The presence of hydroxyl groups allows chemical modifications by grafting various functional groups. One of the most reported dextran derivatives refers to introduction of methacrylic groups, which further can be involved in realisation of gel networks that have been tested in drug delivery experiments. These systems are pH sensitive, and changes in IR spectra corresponding to vibration motion associated with the bonds formed between dextran groups and other reactive groups can be used to monitor synthesis or degradation of dextran-based hydrogels [18–20]. In recent years, there has been an increasing interest in studying self-healing polymer hydrogels. These hydrogels are characterized by their ability to autonomously repair themselves without external stimuli utilising a multitude of co-operative non-covalent interactions such as hydrogen bonds, hydrophobic interactions and host–guest interactions [21, 22]. IR spectroscopy shows that both hydrogen-bonding and hydrophobic interactions make significant contributions to obtaining self-healing materials [23–26].

IR investigations on hydrogels usually demonstrate the interactions involving gelator molecules in building the 'solid network'. The non-homogeneous character of a hydrogel system is not only given by the presence of two phases—the network and the solvent—but also by the different water organization in the proximity of the fibres and in the solvent pools. Okazaki and Satoh [27] have shown that FT-IR measurements can probe the hydration and water properties in a hydrogel formed by cross-linking of poly(4-vinyl phenol) with different amounts of ethylene glycol diglycidyl ether. The O–H stretching band around 3300 cm^{-1} was deconvoluted into four sub-bands. On the basis of the relative band area and the peak wavenumber, it was suggested that hydrogen bonding of water in the gel is the most stabilized when the acidic proton of the phenol residue is not involved in chemical cross-linking. The authors also studied the influence of salts in hydrophobic hydration of the polymer.

Hydrogels can incorporate inorganic materials (e.g. graphene or graphene oxide (GO), oxidic and metallic nanoparticles) resulting in hybrid materials with improved properties and potential applications in biomedical fields. IR spectroscopy can be a method for the investigation of these hybrid materials. In a systematic study on the poly(vinyl alcohol) (PVA)/graphene oxide composite hydrogels, Xue and co-workers [28] noticed that an increase of the PVA molecular weight favours the formation of hydrogels containing GO. GO contains various oxygen functional groups (e.g. hydroxyl, epoxide and carbonyl groups) on their basal planes and edges that assure the possibility to be dispersed in water and at the same time to be involved in supramolecular architectures. In such systems, both polar non-covalent interactions and π–π interactions are possible [28, 29]. For PVA–GO systems, the FT-IR spectra suggested that oxygen-containing groups on the GO surface possibly interact with the PVA molecules through hydrogen bonding involving the C=O group from the surface of GO and H–O bonds from PVA and water molecules.

Another example of hybrid material is represented by the composite chitosan/ZnO hydrogel, which can be used as a component for production of bandages for wound dressing due to the antibacterial action of ZnO and adhesion properties of chitosan [30]. FT-IR spectroscopy was among the various techniques involved in characterization of such material. IR spectra proved the interaction involving ZnO nanoparticles and chitosan through hydrogen bonding, which determined the broadening of O–H at 3400 cm^{-1}.

2.2. Raman spectroscopy

Raman spectroscopy also studies the vibrational energy of molecules, being a suitable method to investigate the interactions that generate the hydrogel network and also hybrid hydrogel/nanoparticles materials. Both IR and Raman methods provide fingerprints of a system, although the information is complementary and selection rules are different. In the case of Raman spectroscopy, a vibration is active if this causes a change in the polarizability of the molecule [31]. An advantage of using Raman spectroscopy compared with IR spectroscopy in studying hydrogels is the fact that Raman spectroscopy is insensitive to aqueous absorption bands, while IR spectra are dominated by the broad water absorbance band. Among various Raman techniques, surface-enhanced Raman scattering (SERS) is characterized by high sensitivity [32]. Herein will be illustrated the suitability of Raman investigation on structural organization or characterization of diffusion properties of solutes in hydrogels.

Raman measurements are useful in characterization of carbon materials like graphene or GO that are incorporated in hydrogels. The oxidation state of graphene sheet incorporated in hydrogel can be determined by the ratio of the Raman peaks of GO that appear at 1606 and 1341 cm^{-1}. Zu and Han [33] obtained stabilized solutions of GO in the presence of Pluronic block copolymers. These solutions in the presence of α-cyclodextrin led to formation of hydrogels. The authors of this study exploited simultaneously the property of poly(ethylene oxide) (PEO) chains to form pseudo-polyrotaxanes and the ability of Pluronic to disperse graphene in aqueous solution to obtain a hybrid supramolecular gel. They proved that the presence of GO favours the gelation process of poly(ethylene glycol) (PEG)/α-cyclodextrin systems.

http://dx.doi.org/10.5772/62900

The SERS method provides enhancement of a Raman signal arising from molecules adsorbed on a metal surface in an aqueous medium. This method has a high sensitivity, permitting detection of molecules in very dilute solutions [14, 16] and demonstrating the interactions between different species. Thus, SERS is a method of choice for studying gel formation or hybrid inorganic nanoparticles/hydrogel composites.

Miljanic and co-workers [14] investigated the self-assembly process of bis-(S-phenylalanine) in the presence of silver or gold nanoparticles. SERS measurements probe the existence of adsorbed gelator molecules on the nanoparticles entrapped in a gel. The authors found that the presence of nanoparticles perturbs the common fibrous gel structure, but their presence allows identification of intermolecular interactions responsible for gelation.

Ou-Yang and co-workers [32] obtained a PVA hydrogel decorated with silver nanoparticles (Ag NPs), which were obtained in situ using β-cyclodextrin (β-CD) acting as a reducing agent and a shape-control agent. This material was then tested as a facile SERS sensor for determining sulphonamides. Uniform dispersion of Ag NPs in hydrogels assures reproducibility of detection of this type of antibiotic, which has a weak affinity for nanoparticles in the absence of β-CD.

The study of IR and Raman vibrations in the spectra of hydrogels resulting from cross-linking of β-CD with a pyromellitic anhydride (PMA) in different ratios gave the possibility to explore the structural changes in the polymer network during the hydration process. Rossi and co-workers have studied intensively this type of nanosponge (NS) material, which can swell in water. First, a gel network was obtained by a cross-linking process between β-CD and PMA in anhydrous dimethyl sulfoxide (DMSO), followed by washing and drying to obtain a NS. The hydrogel was obtained by adding the NS to water [34–37]. Deconvolution of the spectral band arising from active vibrations either in IR or Raman demonstrated the type of interactions between water confined in gel and the functional groups from the network. The formation of hydrogen bonds was proved by comparing the spectra of hydrated networks with the corresponding dry networks. The analysis of IR and Raman spectra allowed estimation of the hydration level of the polymer network. Such analysis has an impact on the understanding of the contribution of the interactions determining the formation and stabilization of a hydrogel, which further allow a rational design of such systems.

Later, the same group of researchers used a combination of UV–Raman spectroscopy with IR spectroscopy and offered an insight into the molecular mechanism related to the thermal response of cyclodextrin-based hydrogels [34]. The results provided information on the degree of H-bond association of water molecules entrapped in the gel network and the extent of intermolecular interactions involving the hydrophobic/hydrophilic moieties of the polymer matrix, which determine the pH-dependent thermal activation of hydrogels. The authors [24] analysed the shift of the maximum of bands arising in the 1500–1800 cm^{-1} region corresponding to stretching motions of the C=C bonds of the aromatic moiety of PMA, towards lower wavenumber values. This effect was observed in the presence of Na_2CO_3, and it was explained by the formation of intermolecular hydrogen bonds C–H•••O–H involving C–H from the aromatic rings of PMA and O–H from water molecules.

Equally, Raman spectroscopy can be a valuable tool in investigation of structural changes of water and polymer networks during the dehydration process of a hydrogel. In a number of papers, Ikeda-Fukazawa and co-workers [38] investigated the dehydration of poly(*N*,*N*-dimethylacrylamide) (PDMAA), PVA [39] and polyacrylamide (PAA)-based hydrogels [40]. It is accepted that the water in hydrogels exists in three states, namely bound water, intermediate water and free water [41] and dehydration occurs in two stages, which implies structural changes in the polymer network and water. In the case of dehydration of PDMAA, the free water is evaporated in the first stage, while in the second stage, the intermediate and bound water to gel fibres evaporates [38]. The evolution of the dehydration process can be followed by changes in the vibrational bands corresponding to H–O and C=O bonds of the polymer, but also following the peaks corresponding to water molecules involved in various types of hydrogen bonding. The authors found that the polymer network shrinks with water evaporation and at the end in the gel the residual water forms a tetragonal structure [28]. Similar behaviour on dehydration has been noticed for all polymeric systems [38–40].

Diffusion characteristics in polymer hydrogels are relevant for possible applications in drug delivery and controlled release because this can predict the release rates and the transport properties. Raman spectroscopy is a method of choice to estimate such parameters. For instance, Kwak and Lafleur [42] measured the mutual diffusion coefficients of poly(ethylene glycol)s with different sizes in Ca-alginate gels, analysing the intensity of the C–H stretching band as a parameter to estimate the concentration of solute in gel.

The number of studies regarding the applications of hydrogels in regenerative medicine is continuing to grow due to various synthetic routes and the possibility to investigate the final materials through various physicochemical methods. Among them, Raman spectroscopy is a suitable method for investigating the cross-link type and cross-linking density as well as conformation changes of polymers under various stimuli in a gel network. Moreover, this method has the advantage of hydrogels monitoring in cell cultures [31].

2.3. Fluorescence spectroscopy

Fluorescence spectroscopy can be used in characterizing gel systems that contain fluorophore species or moieties capable of absorbing energy allowing excitation from its ground electronic state to one of the vibrational states in an excited electronic state. Then, the excited state of the fluorophore reaches the lowest vibrational level of the excited state (singlet). This process involves conformational changes and interaction with the microenvironment. The third step involves transition from an excited electronic state to the ground electronic state and is accompanied by a photon emission characterized by a lower energy than that corresponding to the excitation photon.

To monitor the properties of gel formation using fluorescence methods, the presence of fluorescent moieties in the system is required. An impressive number of studies regarding investigation on gel systems that involve fluorescent methods have been reported. In most cases, fluorescence measurements are performed to demonstrate the application of gel materials as sorbents or as drug delivery agents, but can highlight as well the dynamics of

molecules inside the gel. To demonstrate the suitability of fluorescence spectroscopy in characterizing different aspects of gel systems, several examples are mentioned in this review.

Attachment of a fluorophore to polymeric hydrogels can find potential application in the detection and removal of toxic cations such as Cd^{2+}, Cu^{2+}, Pb^{2+} and Hg^{2+}. Chitosan has been proved to be a good absorbent for Hg^{2+}, and at the same time, this natural polysaccharide can be easily functionalized. Geng et al. [6] described the synthesis of a fluorescent three-dimensional chitosan-based hydrogel via cross-linking with glutaric aldehyde. This new hydrogel has been tested as a solid-phase fluorescent probe for the detection of Hg^{2+} in water. This cation determines the fluorescence quenching, and in the case of the chitosan-based hydrogel a remarkable selectivity and sensitivity determination of Hg^{2+} compared with other systems was noticed. The detection limit achieved with this material was 0.9 nM, a value at least one order of magnitude lower than with other fluorescent dye molecules.

An interesting example is represented by the study by Fraix and co-workers [43] reporting the formation of a supramolecular hydrogel based on assembly of four components: two polymeric structures—poly(β-CD) polymer and a hydrophobically modified dextran by attaching lauryl chains—a commercial zinc phthalocyanine and a tailored nitric oxide photodonor. The gel network resulted by inclusion complexes formed between cyclodextrin units and lauryl chains. The photoactive components were also held in the gel through host–guest interaction with the cyclodextrin cavity, this type of interaction avoiding their aggregation, which usually results in inactivation of photodynamic properties. Steady-state and time-resolved fluorescence methods showed that the photoactive components do not interfere when they are enclosed in a hydrogel assuring their simultaneous operation under the light stimuli. The Zn phthalocyanine complex is a well-known red photo emitter and an effective 1O2 photogenerator [44]. The structure of the other component, which generates NO under the light action, is a derivative of nitroaniline and a 4-amino-7-nitrobenzofurazan (known for its emission in the green region) [45]. The system described assures simultaneous generation of active species with therapeutic properties.

A similar example of hydrogel incorporating photoporphyrin units formed in this case by chemical cross-linking between a PEG-derivative porphyrin and sodium alginate has been reported by Dong et al. [46]. The system has been characterized by various methods: rheological analysis, thermogravimetric analysis and UV–visible and fluorescence spectrophotometry. This hydrogel with porphyrin as a sensing unit has been prepared giving biocompatibility of alginate and recognized bio-applications of porphyrin and tested for fluorescent imaging. The alginate acted as a spacer for the porphyrin units, preventing aggregation and preserving the fluorescent properties, which assure the tracking of hydrogel within organisms. Multispectral fluorescence imaging for drug delivery from the hydrogel shows high yields and excellent biocompatibilities have been demonstrated using doxorubicin, a fluorescent drug. The photoporphyrin and the drug used have different spectra, this technique offering the possibility of simultaneous tracking of two (or, in extension, more) probes.

Montalti and co-workers [47] demonstrated the organization of gel fibres in a supramolecular gel formed by assembly of 1,3,5-cyclohexyltricarboxamide-based gelator comprising two hydrophilic moieties and one hydrophobic substituent containing a naphthalene group, which

represents the fluorophore. This has been possible by analysing the energy transfer from the fluorescent supramolecular hydrogel to a hosted fluorophore. The organization of gelator molecules through naphthalene π–π stacking interactions has been demonstrated by introduction in the gel system of a fluorescent probe with similar structure, propyldansylamide, characterized by sensitivity to the polarity of the microenvironment. In general, the formation of one-dimensional assemblies of gelator represents a recognition process, but the possibility to insert probes with similar structures cannot be excluded. In this case, the dansyl derivative could be inferred in the gel fibres. Analysis of the fluorescence spectrum of dansyl derivative, excited state life-time measurements, steady-state and time-resolved fluorescence anisotropy measurements demonstrated the partition of the dansyl probe between the water environment and gel fibres. The measurements revealed a strong immobilization of the dansyl moiety in gel fibres. The authors also demonstrated that the percentage of photon transfer absorbed by the gelator through the dansyl probe depends on the ratio between the species; a higher concentration of probe increases the percentage. This system can be of particular interest in the field of technology and solar energy conversion.

2.4. Circular dichroism

Circular dichroism spectroscopy represents a valuable tool for investigating the assembly of gelators that include chiral centres in their structures [48, 49]. Circular dichroism (CD) refers to the differential absorption of left and right circularly polarized light by chiral molecules and is usually expressed as the ellipticity of the transmitted light [48]. In the case of achiral molecules, both polarized rays are equally absorbed and the result is a 'zero' spectrum. Formation of gel networks, in general, and hydrogel in particular, can be explored by CD spectroscopy in the cases of assemblies resulting from organization of chiral biomacromolecules, such as proteins or incorporating DNA, polypeptides or chiral LMWGs. A CD signal is observed where the molecules exhibit absorbing UV bands. In many cases, the CD band of chiral molecules is weak, but assembly in nanofibers often is translated into an increase of CD ellipticity [11, 50]. The possibility of recording CD spectra at variable temperature is useful to demonstrate the transition from sol phase to assemblies at the nanoscale level. CD measurements can be extended to the IR region [49], but in this case, there are only a limited number of studies compared with UV-CD. This new technique can provide information on the structural fragments of molecules involved in realization of gel networks, as in the case of self-assembled peptide [50], chiral bis-urea gelators [7] or guanosine-5′-hydrazide [51].

The vast number of studies on formation of gels through self-assembly of LMWGs stressed the observation that the presence of a chiral centre favours assembly into a gel network. In many cases, the urea unit appears in the structure of potential LMWG due to unidirectionality of the non-covalent interactions assuring formation of gel fibres and the ability to form hydrogen bonding. Rodriguez-Llansola et al. [7] reported studies on a self-assembly process of bis-urea molecules bearing aromatic hydrophobic moieties that allow π–π stacking arrangements and chiral centres. They found that similar compounds without the chiral centres do not form gels. The molecular solutions of a chiral molecule either in the R or S enantiomer did not exhibit the dichroic band. Once the gel was formed, the CD spectra showed the positive

and negative dichroic bands for the R and S enantiomers, respectively. Surprisingly, the presence of an achiral molecule with similar structure added to a solution of a chiral gelator results in a gradual increase of chiral bands attributed to assemblies of R enantiomer up to a certain concentration, while a further increase produces precipitation of the mixture and disappearance of the CD bands.

CD measurements can demonstrate the preservation of the natural state of proteins encapsulated in some hydrogels. For instance, Song et al. [52] reported that the formation of hydrogelator resulting from self-assembly of amylopectin grafted with lauryl chains. Bovine serum albumin (BSA) can be entrapped in this hydrogel without affecting its secondary structure. Moreover, recording the CD spectra at different temperatures, it was observed that the BSA is protected in the gel against thermal denaturation.

Polypeptides are often used as building blocks in hydrogelator structure. These fragments exhibit CD bands depending on the chirality of the amino acid used and analyses of the CD spectra can monitor changes in the secondary structure of polypeptide induced by the formation of various self-assemblies. For example, poly(L-alanine) grafted to PEG with different chain lengths changes its β-sheet structure observed in water to α-helical structure in gel fibres. The change depends gradually on the PEG chain length. This effect of PEG in peptide assembly results in a steric hindrance of polypeptide packing side-by-side characteristic of the β-sheet, favouring α-helix assembly [53].

CD measurements offer useful information on the assembly of hydrogelators with a common backbone highlighting the differences induced by specific groups. Vilaca et al. [54] characterized by various methods the dipeptide-type hydrogelators containing tryptophan N-capped with the non-steroidal anti-inflammatory drug naproxen and C-terminal dehydroamino acids, dehydrophenylalanine (ΔPhe), dehydroaminobutyric acid and dehydroalanine (ΔAla). The assembly into gel fibres of these gelators is assured by the capacity to generate hydrogen bonding, hydrophobic and aromatic π–π interactions. CD measurements revealed that assembly is similar for these gelators, all spectra exhibiting a broad positive cotton effect, assigned to the π–π^* short-axis polarized transitions of naphthalene (with maximum around 287 nm). The presence of positive and negative bands at 219 and 233 nm, respectively, suggests left-handed helical naphthalene arrangements through chiral stacking.

There is a trend to use chirality to tune the self-assembly, which determines later the applications of the resulting gels in the biomedical area [55, 56]. Many chiral hydrogels result by self-assembly of biomolecules, such as amino acids, peptides, cholesterol and saccharides [57–61], and they have potential applications, which include chiral adsorption and release and chiral catalysis [57, 62–64]. For example, hydrogels obtained by free-radical co-polymerization using N-acryloyl-L-alanine as chiral hydrophilic monomer and octadecyl acrylate as hydrophobic monomer are pH sensitive and exhibit enantio-differentiating release ability to chiral drugs such as ibuprofen [57]. This aspect is logical knowing that many biological processes are defined as chiral-recognition types. Marchesan et al. [55] obtained a series of eight tripeptides with the sequence Phe–Phe–Val having a combination of D and L enantiomers of amino acids. The authors investigated in what manner the self-assembly is influenced by the chirality of gelator and found that chirality of the CD signal is determined by the chirality of the central

amino acid. The peptides exhibit either a positive maximum or a negative minimum in the region 225–235 nm due to π–π stacking of phenylalanine with either L or D chirality, respectively.

Ma and co-workers [65] obtained new pentapeptides bearing aromatic fluorophore units (fluorenyl, pyrenyl or naphthyl groups) as potential hydrogelators and characterized the systems using CD and fluorescence spectroscopy combined with electron microscopy and rheological measurements. In the absence of these fluorescent aromatic units, they did not observe formation of a gel. Introducing proline in the peptide sequence, it was possible to control the supramolecular assembly avoiding β-sheet arrangements [66]. This has been proven by CD measurements, which demonstrated formation of α-helix assemblies.

CD measurements can also monitor the transition that occurs in the assembly of a peptide amphiphile solution induced by the presence of salt additive on supramolecular assembly. It was considered that the salt facilitated the α-helix to β-sheet transition and induced gelation in amphiphilic peptide due to the electrostatic screening effect [67].

Huang et al. [68] investigated the thermohydrogelation of PEG and an oligo(tyrosine) block copolymers with various PEG and tyrosine (Tyr) chain lengths. These copolymers self-assemble due to β-sheet organization of Tyr blocks and gradual dehydration of PEG by increasing temperature favouring entanglement of the PEG chains. The β-sheet conformation adopted in the hydrogel state has been proven by CD spectra, which exhibit an increased negative band at temperatures corresponding to the gel state [68].

A library of naphthalene–dipeptide hydrogelators has been investigated by CD, X-ray and electron microscopy to demonstrate structural aspects that govern the assembly in gel fibres [69]. The CD spectra demonstrated that fibres are the result of the π–π stacking of the naphthalene moieties as they exhibit bands corresponding to exciton couplets of the naphthalene groups. At the same time, the sign of the naphthalene exciton is determined by the dipeptide chiral properties, indicating that the handedness of the chiral arrangements in the fibres is directed by the dipeptide chain.

These few examples chosen from the very broad types of studies demonstrate that CD spectroscopy complements the electron microscopy methods, offering valuable information on the gel organization.

2.5. Estimation of mesh-size distribution by pulsed-field gradient NMR spectroscopy

Mesh size (**Figure 1**) of a hydrogel or correlation length, ξ, represents the average distance between consecutive cross-links [70]. Mesh-size distribution of a hydrogel is a characteristic relevant for the ability of the entrapped species (ions, drugs and biomolecules) to diffuse through the gel network [71, 72].

This parameter indicates the maximum size of solutes that can pass through the gel network. To characterize the mesh size of hydrogels, different methods can be used that rationally offer an average value [70, 73]. Methods such as solute exclusion, mercury porosimetry, nitrogen adsorption/capillary condensation and microscopy were used in the beginning [74]. Solute

http://dx.doi.org/10.5772/62900

exclusion is a method suitable to estimate the mesh size of polymeric networks that was introduced more than 50 years ago to investigate the penetration of polymer molecules in cellulose fibres [75]. The methods can be applied for wet samples. The other three methods mentioned above require dry samples of polymeric structures. The drying process can induce changes in network organization and distances between fibres. Other methods that can be applied to determine the mesh size for both polymeric and supramolecular hydrogels are based on determination of diffusivity of probes that have no specific interaction with gel fibres. Pulsed-field gradient NMR (PFG-NMR) is one of the methods based on investigation of the chaotic motion of molecules determined by molecular collisions. This method allows estimation of the average pore size in a hydrogel network from the ratio of the diffusion coefficient of the probe molecules in the gel network (D), to their diffusion coefficient in the solvent (D0) [75, 76].

Matsukawa [77] analysed in a review article the diffusional behaviour of molecular probes in polymer networks. Two parameters, the spin–lattice relaxation time and spin–spin relaxation time, reflect the solvent and polymeric probes' behaviours in the gel network. The strength of the field gradient has to be chosen as a function of diffusion coefficient. Thus, for molecules with a diffusion coefficient of the order of 10^{-5} cm^2 s^{-1} the strength of the field gradient is about 100 G cm^{-1}. A larger magnetic field gradient, increased up to 2000 G cm^{-1}, is necessary for probes with small diffusional coefficients. The scheme for pulsed-field gradient pulse sequence for measuring the diffusion coefficient D is represented in **Figure 2**:

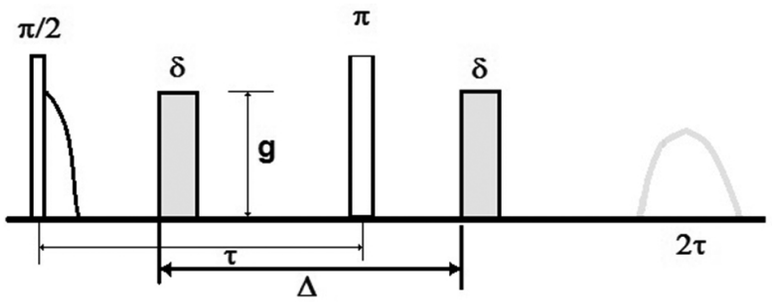

Figure 2. Pulsed field gradient NMR sequence for determination of diffusion coefficient.

The diffusion coefficient is extracted from Eq. (1):

$$ln\left[A(\delta)/A(0)\right]) = -\gamma 2g2D\delta 2(\Delta - \delta/3), \quad (1)$$

in which A(δ) and A(0) are echo signal intensities at 2τ with and without the magnetic field gradient pulse of length δ, respectively, τ is the pulse interval, γ the gyromagnetic ratio of the proton, g the field gradient strength, D the self-diffusion coefficient and Δ the gradient pulse interval [77, 78]. The diffusion coefficient is related to the hydrodynamic radius of the probe. Usually, NMR gives values of the hydrodynamic size, which is larger than the pore size of the network estimated from thermoporometry. Scherer estimated a relation between the values

provided by NMR and thermoporometry [79]. Estimation of the differences provided by different methods is relevant for studies aiming to predict whether the network is capable of encapsulation of large molecules.

Using this method, Matsukawa et al. [77, 78] analysed the diffusion behaviour in various polymeric networks such as polypeptide gels, starch gel, gellan gum gels, polyacrylamide gel or swollen polyisoprene.

Pescosolido and co-workers [72] investigated the properties of interpenetrating polymer network hydrogels based on calcium alginate and a dextran methacrylate derivative, due attention being given to determination of mesh size. The authors found significantly different values for pore size provided by the two methods used in their study: 44.5 nm from cryoporometry and 22.5 nm from low-field NMR (using a pulse sequence). The differences have been explained by the effect of water solidification, which occurs in the cryoporometry measurement. Release experiments of a model protein (myoglobin) from the interpenetrating polymer alginate/dextran methacrylate network hydrogel confirmed the value for pore size provided by NMR experiments.

Another example of application of the PFG-NMR technique refers to the microstructural analysis of the calcium alginate gels resulting from introducing $CaCO_3$ as an insoluble salt, which is dissolved gradually upon acidification [80]. In the presence of glucono-δ-lactone (GDL) as a proton donor, the calcium salt is dissolved. To tailor the microstructural properties of the gel, the ratio between alginate, Ca^{2+} and the co-solvent methanol were varied. Hydrophilic dendrimers with PEG surface groups have been used as probes. Image analysis of transmission electron microscopy (TEM) micrographs has revealed that the strand radii are in a narrow range of 2–2.3 nm and are not influenced by Ca^{2+} concentration or the presence of methanol. Dendrimer diffusion into these gels studied by PFG-NMR provided, as expected, larger values for hydrodynamic radii, and demonstrated that the presence of methanol affects the diffusion of the dendrimers into the gel networks. Walther et al. [81] studied the self-diffusion of PEG into ***K***-carrageenan gels with different microstructures tailored by adding potassium or sodium chloride using nuclear magnetic resonance (NMR) diffusometry and TEM. As expected, small voids observed in the case of potassium-induced gels reduced diffusion coefficients and gave a strong dependence of the self-diffusion coefficient, while in the case of sodium-induced gelation, the voids increase and the ratio D/D0 is close to 1.

Using PFG-NMR spectroscopy, Wallace et al. [82] determined the network's mesh size in a supramolecular hydrogel formed upon addition of Ca^{2+} to solutions of naphthalene diphenylalanine (2FF). A series of dextran probes with molecular weights in the range 6–2000 kDa and hydrodynamic diameters in the range 2–60 nm was used. These probes are soluble in water and have no specific interaction with naphthalene diphenylalanine.

The models applied in analysing the variation of the decrease in the diffusion coefficients relative to those measured in dilute solutions suggested a value of mesh size for supramolecular hydrogel of approximately 40 nm. It was found that probes with hydrodynamic diameter <40 nm move freely through the network, while a dextran probe with molecular weight 2000 kDa has a restricted diffusion.

Jowkarderis and Van de Ven [83] prepared a hydrogel by cross-linking of cellulose nanofibrils with diamines, and mesh-size analysis was performed by solute exclusion and PFG-NMR spectroscopy using dextran-type probes. Diffusion coefficients have been determined both in hydrogels (D) and in dilute solutions of dextrans (D0). The pore sizes of the hydrogel network were determined by analyses of the ratio D/D0. It was found in this case that the average mesh size was around 15 nm. They also found a group of large pores accessible to all dextran probes.

The spectroscopic methods referred to above are well recognized in studying the complex and diverse hydrogel systems. Compared with these, EPR spectroscopy is a less-used method. Therefore, the next part of this review is dedicated to general aspects of EPR spectroscopy and will summarize the applicability of this method in studying formation and properties of hydrogels.

2.6. EPR spectroscopy

Electron paramagnetic resonance (EPR) is a spectroscopic technique that detects transitions induced by electromagnetic radiation between the energy levels of electron spins, in the presence of a static magnetic field. EPR spectral features (e.g. resonance frequencies, splitting, line shapes and line widths) are sensitive to the electron distribution, molecular orientation, molecular motion and the local environment. Numerous books and review articles cover the theoretical and experimental EPR methods, targeting various fields of applications: materials and polymer sciences, physical chemistry, biochemistry and medicine, catalysis, environmental sciences, radiation dosimetry and geological dating [84–90]. In this section, the application of X-band spectroscopy in providing structural and dynamics (in the time range of 10^{-10}–10^{-7} s) information on the hydrogels will be mentioned.

Hydrogels are usually EPR-silent systems, therefore, to have access to the type of information that EPR spectroscopy can provide, it is necessary to introduce paramagnetic reporters, spin labels and probes. Spin probes are stable free radicals or in some cases paramagnetic transition metal ions introduced in the system of interest, while spin labels are stable radicals covalently bound to a constituent of the studied system. Spin labelling involves modification of the system or material studied by introducing a new moiety (with a paramagnetic property) [84–88].

The most widely used spin probes or labels are nitroxides, which are stable paramagnetic molecules with a paramagnetic N–O• fragment bearing an unpaired electron. This fragment is surrounded by shielding substituents (usually methyl groups) [84]. Nitroxides have the advantage that by varying their chemical structure, the paramagnetic properties do not suffer significant changes. TEMPO- and PROXYL-type nitroxides (**Figure 3**) are commercially available, offering the possibility to be covalently attached to other molecules of interest through reactive groups R (carboxy, amino, oxo and hydroxy). Depending on the system analysed and the information needed to be obtained, they can be used directly as spin probes or labels.

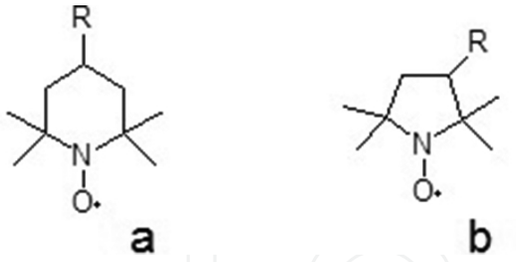

Figure 3. Structures of the most common classes of nitroxide spin probes and labels. (a) TEMPO-type nitroxides, (b) PROXYL-type nitroxides.

The EPR parameters of nitroxides, the rotational correlation time and nitrogen hyperfine splitting constant (a_N), are usually correlated with the properties of the microenvironment sensed by the paramagnetic probes, like local viscosity or polarity. Although the information provided by EPR spectroscopy is local and not global as in the case of CD, IR or Raman spectroscopy, these can be further correlated with macroscopic properties of a system, in particular, a hydrogel. Sol-to-gel transition can be easily demonstrated involving methods providing global information on the system (vide supra), but the macroscopic observed changes are the result of reorganization at microscopic or nanoscopic levels. Thus, a method-like EPR spectroscopy can be used to demonstrate such a transformation.

Compared with other spectroscopic techniques, EPR spectroscopy has been very rarely used for studying gel properties and dynamic aspects of these systems. The sol-to-gel transition can be demonstrated in particular cases by other spectroscopic methods, such as CD if the gelators are chiral or assembly induces a chiral conformation, vibrational spectroscopy, following vibrational band intensities of groups involved in the assembly process generating the gel network. For dynamic studies, EPR spectroscopy can be used only if such changes take place in the range of 10^{-6}–10^{-9} s. However, a hydrogel represents a complex system and a good strategy for such investigation needs to involve different techniques. The literature indicates hundreds or thousands of studies involving the spectroscopic methods described above, while the number of EPR spectroscopy studies is very small. Most of the EPR studies reported in the literature regard polymeric gels [89, 91, 92]. For instance, the EPR spectroscopy with its spin probe method had been proved a valuable method for studying thermoresponsive systems and in particular thermoresponsive hydrogels. Using an appropriate spin probe, it is possible to demonstrate the inhomogeneities inside a gel system [89, 93–95]. EPR spectroscopy and EPR imaging were used to study the translational diffusion process of spin probes in PVA hydrogels, with vast applications in biotechnological field, to get information on the polymer dynamics. The diffusion of 4-*N*-butylamino-TEMPO and 4-amino-TEMPO (4-NH_2T) in PVA hydrogel and in polymeric blends of PVA with other polymers has been determined as a function of various parameters: molecular weight and concentration of polymer [91].

Natural polysaccharides are often part of hydrogel networks and the literature indicates a number of EPR studies in studying the hydrogels resulting from assembly of polysaccharides. A series of EPR studies has been reported on investigation of polysaccharide hydrogels

http://dx.doi.org/10.5772/62900

resulting from complexation of polymeric chains with various cations or dynamics of synthetic spin probes in polysaccharide hydrogels [92, 96–101].

Alginate or pectin form hydrogels in the presence of numerous divalent cations, most of them being paramagnetic. Kajsheva [92] analysed the EPR spectra of metals in alginates and pectinates to get information about the configuration of the corresponding complexes with Cr(III), Cu(II), Ni(II), Mn(II) and Co(II).

EPR spectroscopy has been applied to study the binding of paramagnetic metal ions to extracellular polysaccharide surfaces produced by cyanobacteria *Anabaena spiroides* [98]. The results underline the potential application of this complexation process in water purification. Kanesaka and co-workers [100] investigated the effect of Cu^{2+} on gelation of gellan solution using EPR, CD and viscoelasticity measurements. The CD and EPR experiments revealed valuable information regarding the sol-to-gel transition of gellan in the presence of this cation, which can be considered as a two-step process. In the first phase Cu^{2+} binds to the gellan chain determining a coil-to-helix transition. In the second stage, the hydrogen bonds formed between gellan chains determined a further coil–helix transition.

Kempe [96] used spin-labelled insulin as a spin probe to analyse its behaviour in chitosan/glycerol-2-phosphate (beta-GP) gel in terms of dynamics and pH sensitivity. In addition, the release of this drug from the chitosan gel has been monitored. Bertholon and co-workers [97] prepared a series of spin-labelled polysaccharides (dextran, dextran sulfate or chitosan), which were incorporated into poly(isobutylcyanacrylate) nanoparticles aiming to study mobility of the nanoparticle surface groups. The EPR spectra showed a two-component feature, the ratio between them depending on the ability of polysaccharide to fold on the nanoparticle surface. Numerous cations of transitional metals are paramagnetic and form complexes with various starches. Such complexes were investigated by EPR [99].

The formation of alginate gel in the presence of different divalent cations has been investigated by EPR spectroscopy using spin-labelled alginate (ALG-L) obtained by reaction of alginate

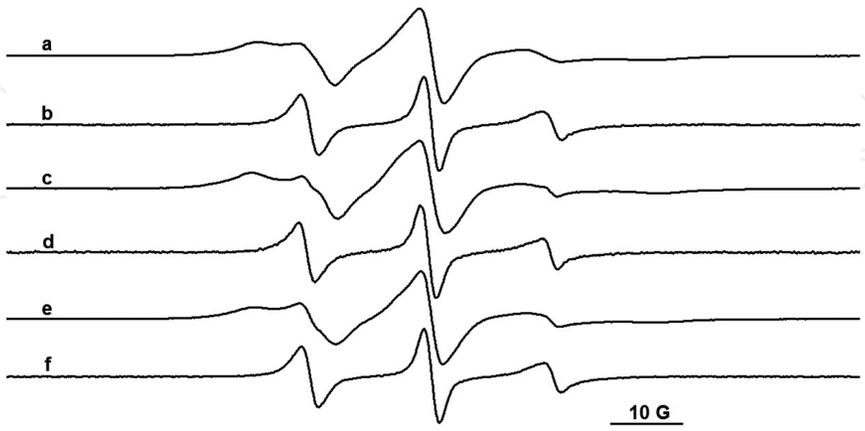

Figure 4. The EPR spectra of 4-NH_2-T-ALG-L in: Zn^{2+}/alginic acid gel (a), alginic acid/Zn^{2+}/EDTA mixture (b), Ba^{2+}/alginic acid gel (c), alginic acid/Ba^{2+}/EDTA mixture (d), Ca^{2+}/alginic acid gel (e), alginic acid/Ca^{2+}/EDTA mixture (reproduced from [90]).

with amino-TEMPO [101]. Sol-to-gel transition induced by the presence of dications was demonstrated by change of EPR features of spin-labelled alginate reflecting different dynamics. Thus, in the presence of dications (Ca^{2+}, Zn^{2+} and Ba^{2+}) spin-labelled alginate became immobilized (**Figure 4**, spectra a, c and e). It was noticed that the distance between the outer lines in the EPR spectra of complexes can be correlated with the strength of the gel.

The complexes of divalent cations are dissolved in the presence of a stronger ligand for cations, like EDTA. This is expressed in the EPR spectra by the change in the dynamics of spin-labelled alginate, which became more mobile (**Figure 4**, spectra b, d and f).

Diffusion experiments revealed that both the cation and alginate polyanion in the gel fibres can exchange with cations and molecules, respectively, in solution [101].

As EPR spectroscopy is sensitive to environmental changes around a paramagnetic probe and its dynamics, Ionita and co-workers investigated by EPR spectroscopy the synthesis [102] and properties [103] of covalent hydrogels that resulted via cross-linking of diisocyanate-terminated PEG with β-CD [104, 105]. In fact, these hydrogels were obtained by replacing the reaction solvent (anhydrous DMF) with water. The synthesis has been monitored following the dynamics of various spin probes (like commercially available TEMPO derivatives or synthesized spin probes: spin-labelled cyclodextrins [106, 107], spin-labelled oligo(ethylene glycol)s [108]). Spin-labelled cyclodextrins (SL-CD) introduced in the reactions showed progressive immobilization as the gel network was formed (**Figure 5**).

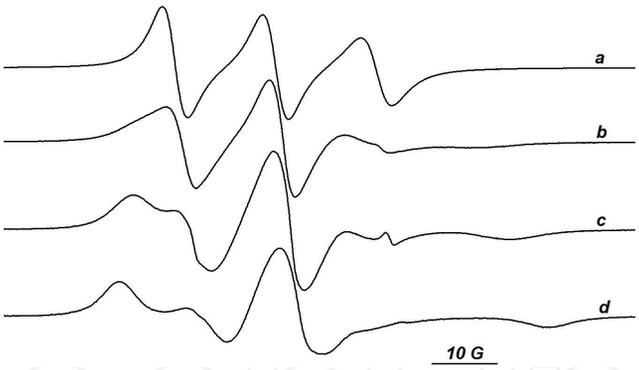

Figure 5. EPR spectra of SL-CD: (a) in DMF, initial solution, (b) during gel formation in DMF, (c) after gel formation in DMF and (d) after replacing the DMF form gel network with water (reproduced from [102]).

Using both spin probes and spin-labelling methods, it was possible to explore by EPR spectroscopy the changes in the gel network as a function of temperature, solvent, polymer chain length and initial ratio between reactants [103].

The EPR spectra of spin-labelled PEG/β-CD gels are dominated by two components, showing strongly immobilized and relatively mobile spin labels (**Figure 6**). These components were assigned to two different positions occupied by the β-CD units in the network. The immobilized component is associated with the β-CD units located at the cross-linking points of the gel network, while the mobile component corresponds to the spin labels at the ends of the PEG chains. **Figure 6** shows EPR spectra of spin-labelled gels with different PEG/β-CD ratios and

http://dx.doi.org/10.5772/62900

different PEG chain lengths at 293 K. These demonstrate that mobility of spin labels attached to cyclodextrins representing the cross-links in the hydrogel network is due to the flexibility of PEG chains connecting the spin label to the β-CD. In this sense, longer PEG chains determine higher mobility.

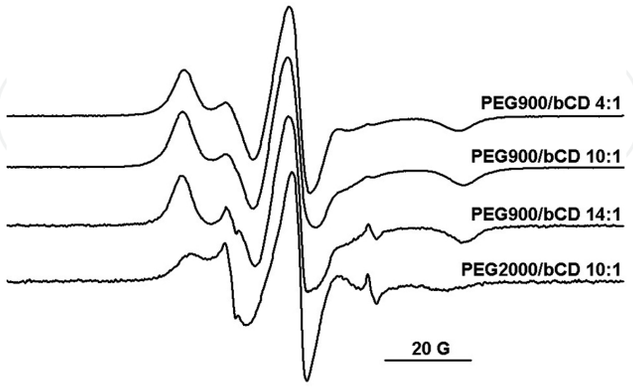

Figure 6. EPR spectra of spin-labelled gels PEG900/β-CD and PEG2000/β-CD recorded at 293 K in water (reproduced from [92]).

Hydrogels can encapsulate in the solvent pools various species as long as their size is smaller than the mesh size of the network. In this particular case of chemical hydrogel resulting from reaction of β-CD with PEG, the ability of cyclodextrin to act as a host for hydrophobic compounds enhanced the encapsulation properties of the hydrogel. To probe the host properties of the β-CD groups in the gel, a spin probe with high affinity for β-CD was used adamantane–TEMPO (AT) 92. Diffusion into PEG/glycerol (glycerol replacing β-CD) and PEG900/β-CD gels of this spin probe was followed using water or dichloromethane as solvents (**Figure 7**). In the case of PEG900/glycerol gel, the EPR spectra of AT (**Figure 7**, spectrum A) showed only one rapidly tumbling component consistent with the spin label dissolved in the solvent pool. The same feature of EPR spectra was observed for the PEG900/β-CD gel, when DCM was used as a solvent. This behaviour is normal as host–guest interactions of cyclodextrin are demonstrated in water solutions. However, when AT was uploaded into the PEG900/β-

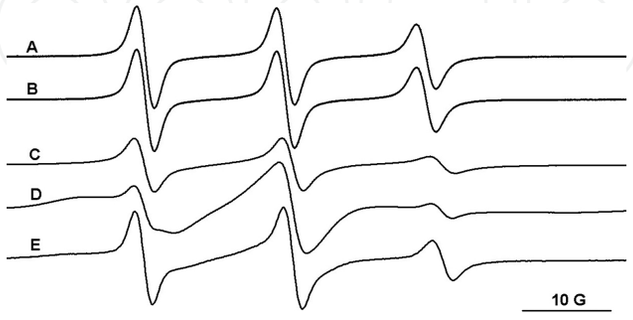

Figure 7. EPR spectra of AT in PEG900/β-CD gel in DCM (A); PEG/glycerol gel in DCM (B); PEG/glycerol gel in water (C); PEG900/β-CD gel in water (D), and PEG900/β-CD gel in water in the presence of a large excess of amino-adamantane (E) (reproduced from [92]).

CD hydrogel (**Figure 7**, spectrum D), the EPR spectrum showed two components, a rapidly tumbling AT situated in water pools of the gel network and a slowly tumbling AT complexed with the β-CD units at the cross-linking points of the gel network.

Complexation of AT to the β-CD cavity was further confirmed by a competition experiment with 1-adamantylamine. The sample of PEG900/β-CD gel loaded with AT was placed in a 10^{-1} M solution of 1-adamantylamine. The EPR solution spectra recorded after equilibrium was reached indicated that AT had partially diffused from the gel to the solution. The EPR spectra of the gel (**Figure 7**, spectrum E) showed that the rapidly tumbling component became dominant. This is a consequence of the replacement of AT from the β-CD cavities inside the gel structure by 1-adamantylamine. This result proves that the β-CD cavities in the gel retain their host–guest properties. Analyses of the EPR spectra of the TEMPO spin probe corresponding to frozen hydrogel samples showed that gel fibres prevent ice formation.

Few EPR studies reported in the literature regard the supramolecular gels resulting from assembly of LMWG [109–112]. One explanation is probably that the self-assembly process represents a reorganization and thus selective process and insertion of a different molecule in the fibrillar assembly is, in general, less probable. Some studies involving EPR measurements in studying formation of supramolecular hydrogels through assembly LMWG have appeared recently.

Takemoto et al. [110] reported the synthesis of an amphiphilic optically active compound that contains a paramagnetic (2S,5S)-2,5-dimethyl-2,5-diphenylpyrrolidine-N-oxyl radical fixed in the inner position with a hydrophobic long chain and a hydrophilic R-alanine residue in the opposite terminal position. They found that this compound acted as a LMWG in water, resulting in a spin-labelled hydrogel. From the variable temperature EPR spectra recorded, it was possible to determine the gel-to-sol transition temperature by analysing the EPR spectral line-width of the central line from peak-to-peak (Hpp). This parameter increased at the sol-to-gel transition due to spin–spin interactions and decreased in the heating run. This study demonstrated the suitability of EPR measurements in evaluation of the temperature for the sol-to-gel transition, which depends on various factors such as gelator structure or solvent nature.

Hydrogelation of three isomers of carboxy-dibenzylidene sorbitol induced by lowering the pH from 13 to 4 has been investigated by rheological measurements, SEM and EPR spectroscopy. Introducing in the system the corresponding spin-labelled carboxy-dibenzylidene sorbitol, it was concluded that these are not included in the gel fibres, as gelation was not accompanied by a change in the probe's dynamics. Surprisingly, in the case of the spin probe 5-doxyl stearic acid, its aggregation was noticed once the gel was formed from water solution. The behaviour of spin probes in gel systems depends on the solvent used. Thus, it was observed that the spin probe 5-doxyl stearic acid is entrapped in the gel fibres when the solvent composition water/DMSO was 1:1 (v/v) [111].

These few examples presented above demonstrate that EPR spectroscopy is a valuable tool for characterizing hydrogel properties, which can accompany other 'classical' well-established physicochemical methods for investigating complex hydrogel systems.

3. Conclusion

In summary, the aim of the review was to demonstrate the usefulness of different spectroscopic techniques in physical characterization of the complex systems represented by hydrogels. The results of these investigations orient each material towards a specific application. Nowadays, there is a trend in development of multifunctional nanocomposites based on different types of hydrogels acting as a matrix for various nanomaterials. Various physicochemical methods, including those presented in the review, can be used to characterize new materials. Only a limited number of spectroscopic techniques have been considered, most of them being classically considered for characterization of hydrogels. Some of them provide global information on the hydrogel system (vibrational spectroscopy, circular dichroism and PFG-NMR spectroscopy), others provide local information, like fluorescence or EPR spectroscopy. Although less involved in characterization of hydrogels, EPR spectroscopy can bring an important contribution not only in demonstrating the heterogeneity of hydrogel-based materials by using spin-labelling and spin-probe methods mentioned here, but also to monitor radical processes taking place in hydrogels. The choice of spectroscopic methods that can be combined with microscopic and rheological methods depends on structural characteristics of the investigated hydrogels.

Acknowledgements

This work was supported by a grant from the Romanian **National Authority for Scientific Research, CNCS – UEFISCDI, Project Number PN-II-ID-**PCE-2011-3-0328.

Author details

Gabriela Ionita

Address all correspondence to: gabi2ionita@yahoo.com

Institute of Physical Chemistry 'Ilie Murgulescu' of the Romanian Academy, Splaiul Independentei, Bucharest, Romania

References

[1] Appel EA, del Bario J, Loh XJ, Scherman OA. Supramolecular polymeric hydrogels. Chem Soc Rev. 2012;41:6195–6214.

[2] Buwalda SJ, Boere KW, Dijkstra PJ, Feijen J, Vermonden T, Hennink WE. Hydrogels in a historical perspective: from simple networks to smart materials. J Control Release. 2014;190:254–273.

[3] Du X, Zhou J, Shi J, Xu B. Supramolecular hydrogelators and hydrogels: from soft matter to molecular biomaterials. Chem Rev. 2015;115(24):13165–13307.

[4] Peppas NA, Huang Y, Torres-Lugo M, Ward JH, Zhang J. Physicochemical foundations and structural design of hydrogels in medicine and biology. Annu Rev Biomed Eng. 2000;2:9–29.

[5] Jonker AM, Löwik DWPM, van Hest JCM. Peptide- and protein-based hydrogels. Chem Mater. 2012;24(5):759–777.

[6] Geng Z, Zhang H, Xiong Q, Zhang Y, Zhao H, Wang G. A fluorescent chitosan hydrogel detection platform for sensitive and selective determination of trace mercury (II) in water. J Mater Chem A. 2015;3:19205–19217.

[7] Rodriguez-Llansola F, Hermida-Merino D, Nieto-Ortega B, Ramirez FJ, Navarrete JTL, Juan Casado J, Hamley IW, Escuder B, Hayes W, Miravet JF. Self-assembly studies of a chiral bisurea-based superhydrogelator. Chem Eur J. 2012;18(46):14725–14731.

[8] Escuder B, Rodrıguez-Llansola F, Miravet JF. Supramolecular gels as active media for organic reactions and catalysis. New J Chem. 2010;34:1044–1054.

[9] Hapiot F, Menuel S, Monflier E. Thermoresponsive hydrogels in catalysis. ACS Catal. 2013;3(5):1006–1010.

[10] Raeburn J, Cardoso AZ, Adams DJ. The importance of self-assembly process to control mechanical properties of low molecular weight hydrogels. Chem Soc Rev. 2013;42(12): 5143–5156.

[11] Yu G, Yan X, Hana C, Huang F. Characterization of supramolecular gels. Chem Soc Rev. 2013;42:6697–6722.

[12] Silverstein RM, Webste FX, Kiemle DJ. Infrared spectroscopy in: spectrometric identification of organic compounds. John Wiley & Sons, Ltd: New Jersey. 2005.

[13] Suzuki M, Yumoto M, Shirai H, Hanabusa K. Supramolecular gels formed by amphiphilic low-molecular-weight gelators of Nα,N$\circledcirc$-diacyl-l-lysine derivatives. Chem Eur J. 2008;14:2133–2144.

[14] Miljanic S, Frkanec L, Biljan T, Meic B, Zinic M. Surface-enhanced Raman scattering on colloid gels originated from low molecular weight gelator. J Raman Spectrosc. 2008;39:1799–1804.

[15] Kleinsmann AJ, Weckenmann NM, Nachtsheim BJ. Phosphate-triggered self-sssembly of N-[(Uracil-5-yl)methyl]urea: a minimalistic urea-derived hydrogelator. Chem Eur J. 2014;20:9753–9761.

[16] Li P, Dou XQ, Tang YT, Zhu S, Gu J, Feng CL, Zhang D. Gelator-polysaccharide hybrid hydrogel for selective and controllable dye release. J Colloid Interface Sci. 2012;387:115–122.

[17] Sun T, Zhu J, Wang M, Lu M, Ding J, Lv Z, Huab P, Zhang Y. A glucosyl triblock copolymer: synthesis and its injectable thermo-and pH-responsive behaviours. RSC Adv. 2015;5:24231–24238.

[18] Zhao Z, Zhang Z, Li Chen, Cao Y, He C, Chen X. Biodegradable stereocomplex micelles based on dextran-blockpolylactide as efficient drug deliveries. Langmuir. 2013;29:13072–13080.

[19] Vlugt-Wensink KDF, Jiang X, Schotman G, Kruijtzer G, Vredenberg A, Chung JT, Zhang Z, Versluis C, Ramos D, Verrijk R, Jiskoot W, Crommelin DJA, Hennink WE. In vitro degradation behavior of microspheres based on cross-linked dextran. Biomacromolecules. 2006;7:2983–2990.

[20] Giannuzzo M, Corrente F, Feeney M, Paoletti L, Paolicelli P, Tita B, Vitali F, Casadei MA. pH-Sensitive hydrogels of dextran: synthesis, characterization and in vivo studies. J Drug Target. 2008;16(9):649–659.

[21] Ding F, Wu S, Wang S, Xiong Y, Yan Y, Li B, Deng H, Du Y, Xiao L, Shi X. A dynamic and self-crosslinked polysaccharide hydrogel with autonomous self-healing ability. Soft Matter. 2015;11:3971–3976.

[22] Yang X, Yu H, Wang L, Tong R, Akram M, Chen Y, Zhai X. Self-healing polymer materials constructed by macrocycle-based host–guest interactions. Soft Matter. 2015;11:1242–1252.

[23] Cui Y, Tan M, Zhu A, Guo M. Non-covalent interaction cooperatively induced stretchy, tough and stimuli-responsive polyurethane–urea supramolecular (PUUS) hydrogels. J Mater Chem B. 2015;3:2834–2841.

[24] Maiti B, Ruidas B, De P. Dynamic covalent cross-linked polymer gels through the reaction between side-chain β-keto ester and primary amine groups. React Funct Polym. 2015;93:148–155.

[25] Vivek B, Prasad E. Reusable self-healing hydrogels realized via in situ polymerization. J Phys Chem B. 2015;119:4881–4887.

[26] Sharma M, Mondal D, Mukesh C, Prasad K. Self-healing guar gum and guar gum-multiwalled carbon nanotubes nanocomposite gels prepared in an ionic liquid. Carbohydr Polym. 2013;98(1):1025–1030.

[27] Okazaki E, Satoh M. Hydrations and water properties in the super salt-resistive gel probed by FT-IR. Polymer. 2010;51:4362–4367.

[28] Xue R, Xin X, Wang L, Shen J, Ji F, Li W, Jia C, Xu G. Systematic study of the effect of molecular weights of polyvinyl alcohol on polyvinyl alcohol/graphene oxide composite hydrogels. Phys Chem Chem Phys. 2015;17:5431–5440.

[29] Xu YX, Wu Q, Sun YQ, Bai H, Shi GQ. Three-dimensional self-assembly of graphene oxide and DNA into multifunctional hydrogels. ACS Nano. 2010;4:7358–7362.

[30] Kumar PTS, Lakshmanan VK, Anilkumar TV, Ramya C, Reshmi P, Unnikrishnan AG, Nair SV, Jayakumar R. Flexible and microporous chitosan hydrogel/nano ZnO composite bandages for wound dressing: in vitro and in vivo evaluation. ACS Appl Mater Interfaces. 2012, 4, 2618−–2629.

[31] Mather ML, Tomlins PE. Hydrogels in regenerative medicine: towards understanding structure–function relationships. Regen Med. 2010;5(5):809–821.

[32] Ou-Yang L, Zhu L, Ruan Y, Tang H. Preparation of native β-cyclodextrin modified plasmonic hydrogel substrate and its use as a surface-enhanced Raman scattering scaffold for antibiotics identification. J Mater Chem C. 2015;3:7575–7582.

[33] Zu SZ, Han BH. Aqueous dispersion of graphene sheets stabilized by pluronic copolymers: formation of supramolecular sydrogel. J Phys Chem C. 2009;113:13651–13657.

[34] Rossi B, Venutic V, D'Amicoa F, Gessinia A, Mele A, Puntad C, Meloned L, Crupic V, Majolinoc D, Trotta F, Masciovecchioa C. Toward an understanding of the thermosensitive behaviour of pH responsivehydrogel based on cyclodextrins. Soft Matter. 2015;11:5862–5871.

[35] Crupi V, Majolino D, Mele A, Rossi B, Trotta F, Venutia V. Modelling the interplay between covalent and physical interactions in cyclodextrin-based hydrogel: effect of water confinement. Soft Matter. 2013;9:6457–6464.

[36] Rossi B, Venuti V, D'Amico F, Gessini A, Castiglione F, Mele A, Punta C, Melone L, Crupi V, Majolino D, Trotta F, Masciovecchio C. Water and polymer dynamics in a model polysaccharide hydrogel: the role of hydrophobic/hydrophilic balance. Phys Chem Chem Phys. 2015;17:963—971.

[37] Rossi B, Venuti V, Mele A, Punta C, Melone L, Crupi V, Majolino D, Trotta F, D'Amico F, Gessini A, Masciovecchio C. Probing the molecular connectivity of water confined in polymer hydrogels. J Chem Phys. 2015;142:014901.

[38] Sekine Y, Ikeda-Fukazawa T. Structural changes of water in a hydrogel during dehydration. J Chem Phys. 2009;130:034501.

[39] Kudo K, Ishida J, Syuu G, Sekine Y, Ikeda-Fukazawa T. Structural changes of water in poly(vinyl alcohol) hydrogel during dehydration. J Chem Phys. 2014;140:044909.

[40] Sekine Y, Takagi H, Sudo S, Kajiwara Y, Fukazawa H, Ikeda-Fukazaw T. Dependence of structure of polymer side chain on water structure in hydrogels. Polymer. 2014;55:6320–6324.

[41] John MS, Andrade JD. Water and hydrogels. J Biomed Mater Res. 1973;7:509–522.

[42] Kwak S, Lafleur M. Raman spectroscopy as a tool for measuring mutual diffusion coefficients in hydrogels. Appl Spectrosc. 2003:57(7);768–773.

[43] Fraix A, Gref R, Sortino S. A multi-photoresponsive supramolecular hydrogel with dual-color fluorescence and dual-modal photodynamic action. J Mater Chem B. 2014;2:3443–3449.

[44] Snow AW. Phthalocyanine aggregation. In: The Porphyrin Handbook. Phthalocyanines: Properties and Materials. Kadish KM, Smith KM, Guilard R, editors. Academic Press: San Diego, CA, USA 2003, vol. 17, 129-176.

[45] Uchiyama S, Santa T, Imai K. Fluorescence characteristics of six 4,7-disubstituted benzofurazan compounds: an experimental and semi-empirical MO study. J Chem Soc Perkin Trans. 2. 1999;11:2525–2532.

[46] Dong X, Wei C, Liu T, Lv F. Protoporphyrin Incorporated alginate hydrogel: preparation, characterization and fluorescence imaging in vivo. RSC Adv. 2015;5:96336–96344.

[47] Montalti M, Dolci LS, Prodi L, Zaccheroni N, Stuart MCA, Bommel KFC, Friggeri A. Energy transfer from a fluorescent hydrogel to a hosted fluorophore. Langmuir. 2006;22:2299–2303.

[48] Smith DK. Lost in translation? Chirality effects in the self-assembly of nanostructured gel-phase materials. Chem Soc Rev. 2009;38:684–694.

[49] Gotarrelli G, Lena S, Masiero S, Pierracini S, Spada GP. The use of circular dichroism spectroscopy for studying the chiral molecular self-assembly: an overview. Chirality. 2008;20:471–485.

[50] Schweitzer-Stenner R, Measey TJ. Simulation of IR, Raman and VCD amide I band profiles of self-assembled peptides. Spectroscopy. 2010;24:25–36.

[51] Setnicka V, Urbanova M, Volka K, Nampally S, Lehn JM. Investigation of guanosine-quartet assemblies by vibrational and electronic circular dichroism spectroscopy, a novel approach for studying supramolecular entities. Chem Eur J. 2006;12:8735–8743.

[52] Song F, Zhang LM, Li NN, Shi JF. In situ crosslinkable hydrogel formed from a polysaccharide-based hydrogelator. Biomacromolecules. 2009;10:959–965.

[53] Choi YY, Jang JH, Park MH, Choi BG, Chi B, Jeong B. Block length affects secondary structure, nanoassembly and thermosensitivity of poly(ethylene glycol)-poly(L-alanine) block copolymers. J Mater Chem. 2010;20:3416–3421.

[54] Vilaça H, Hortelão ACL, Castanheira EMS, Queiroz MJRP, Hilliou L, Hamley IW, Martins JA, Paula MT, Ferreira PMT. Dehydrodipeptide hydrogelators containing

naproxen N-capped tryptophan: self-assembly, hydrogel characterization, and evaluation as potential drug nanocarriers. Biomacromolecules. 2015;16:3562–3573.

[55] Marchesan S, Easton CD, Styan KE, Waddington LJ, Kushkaki F, Goodall L, McLean KM, Forsythec JS, Hartleya PG. Chirality effects at each amino acid position on tripeptide self-assembly into hydrogel biomaterials. Nanoscale. 2014;6:5172–5180.

[56] Zhang M, Qinga G, Sun T. Chiral biointerface materials. Chem Soc Rev. 2012;41:1972–1984.

[57] Cheng R, Liu J, Xie P, Wu Y, Deng J. Chiral, pH-sensitive polyacrylamide hydrogels: preparation and enantio-differentiating release ability. Polymer. 2015;68:246–252.

[58] Marchesan S, Easton CD, Styan KE, Waddington LJ, Kushkaki F, Goodall L, McLean KM, Forsythec JS, Hartley PJ. Chirality effects at each amino acid position on tripeptide self-assembly into hydrogel biomaterials. Nanoscale. 2014;6:5172–5180.

[59] Xu FM, Wang HN, Zhao J, Liu XS, Li DD, Chen CJ, Ji J. Synthesis of photoresponsive cholesterol-based azobenzene organogels: dependence on different spacer lengths. Macromolecules. 2013;46:4235–4246.

[60] Ogawa Y, Yoshiyama C, Kitaoka T. Helical assembly of azobenzene-conjugated carbohydrate hydrogelators with specific affinity for lectins. Langmuir. 2012;28:4404–4412.

[61] Clemente MJ, Tejedor RM, Romero P, Fitremann J, Oriol L. Maltose-based gelators having azobenzene as light-sensitive unit. RSC Adv. 2012;2:11419–11431.

[62] Shankar BV, Patnaik A. Chiral discrimination of a gemini-type surfactant with rigid spacer at the air–water interface. J Phys Chem B. 2007;111:11419–27.

[63] Bag DS, Alam S. Chiral chemical absorption property of a cross-linked poly(N-isopropyl acrylamide-co-sodium acrylate) thermoresponsive smart gel. Chirality. 2012;24:506–511.

[64] Roy S, Javid N, Sefcik J, Halling PJ, Ulijn RV. Salt-induced control of supramolecular order in biocatalytic hydrogelation. Langmuir. 2012;28:16664–16670.

[65] Ma M, Kuang Y, Gao Y, Zhang Y, Gao P, Xu B. Aromatic-aromatic interactions induce the self-assembly of pentapeptidic derivatives in water to form nanofibers and supramolecular hydrogels. J Am Chem Soc. 2010;132:2719–2728.

[66] Wood SJ, Wetzel R. Martin JD, Hurle MR. Prolines and amyloidogenicity in fragments of the Alzheimer's peptide β/A4. Biochemistry. 1995;34:724–730.

[67] Otsuka T, Maeda T, Hotta A. Solutions on the sol–gel transitions, the gelation speed, and the gel characteristics. J Phys Chem B. 2014;118:11537–11545.

[68] Huang J, Hastings CL, Duffy GP, Kelly HM, Raeburn J, Adams DJ, Heise A. Supramolecular hydrogels with reverse thermal gelation properties from (oligo)tyrosine containing block copolymers. Biomacromolecules. 2013;14 (1):200–206.

[69] Morris Kl, Chen L, Rodger A, Dave J, Adam DJ, Serpell LC. Structural determinants in a library of low molecular weight gelators†. Soft Matter. 2015;11:1174–1181.

[70] Kirchhof S, Abrami M, Messmann V, Hammer N, Achim M, Goepferich, Grassi M, Brandl FP. Diels–Alder hydrogels for controlled antibody release: correlation between mesh size and release rate. Mol Pharm. 2015;12 (9):3358–3368.

[71] Sutton S, Campbell NL, Cooper AI, Kirkland M, Frith WJ, Adams DJ. Controlled release from modified amino acid hydrogels governed by molecular size or network dynamics. Langmuir. 2009;25:10285–10291.

[72] Pescosolido L, Feruglio L, Farra R, Fiorentino S, Colombo I, Coviello T, Matricardi P, Hennink WE, Vermonden T, Grassi M. Mesh size distribution determination of interpenetrating polymer network hydrogels. Soft Matter. 2012;8:7708–7715.

[73] Canal T, Peppas NA. Correlation between mesh size and equilibrium degree of swelling of polymeric networks. J Biomed Mater Res. 1989;23:1183–1193.

[74] Gallegos DP, Munn K, Smith DS, Stermer DL. A NMR technique for the analysis of pore structure: application to materials with well-defined pore structure. J Colloid Interface Sci. 1987;119(1):127–140.

[75] Aggerbrandt LJ, Samuelson O. Penetration of water-soluble polymers into cellulose fibers. J Appl Polym Sci. 1964;8:2801–2812.

[76] Jowkarderis L, van de Ven TGM. Mesh size analysis of cellulose nanofibril hydrogels using solute exclusion and PFG-NMR spectroscopy. Soft Matter. 2015;11:9201–9210.

[77] Matsukawa S, Yasunaga H, Zhao C, Kuroki S, Kurosu H, Ando I. Diffusion processes in polymer gels as studied by pulsed field-gradient spin-echo NMR spectroscopy. Prog Polym Sci. 1999;24:995–1044.

[78] Matsukawa S, Ando I. A study of self-diffusion of molecules in polymer gel by pulsed-gradient spin-echo 1H NMR. Macromolecules. 1996;29:7136–7140.

[79] Scherer GW. Hydraulic radius and mesh size of gels. J Sol-Gel Sci Technol. 1994;1:285–291.

[80] Bernin D, Goudappel G-J, van Ruijven M, Altskar A, Strom A, Mats Rudemo M, Hermansson AM, Nyden M. Microstructure of polymer hydrogels studied by pulsed field gradient NMR diffusion and TEM methods. Soft Matter. 2011;7:5711–5716.

[81] Walther B, Loren N, Nyden M, Hermansson AM. Influence of K-carrageenan gel structures on the diffusion of probe molecules determined by transmission electron microscopy and NMR diffusometry. Langmuir. 2006;22:8221–8228.

[82] Wallace M, Adams DJ, Iggo JA. Analysis of the mesh size in a supramolecular hydrogel by PFG-NMR spectroscopy. Soft Matter. 2013;9:5483–5490.

[83] Jowkarderis L, van de Ven TGM. Mesh size analysis of cellulose nanofibril hydrogels using solute exclusion and PFG-NMR spectroscopy. Soft Matter. 2015;11:9201–9210.

[84] Lund A, Shiotani M, Shimada S. Principles and Applications of ESR Spectroscopy. Springer: Dordrecht Heidelberg London New York, 2011.

[85] Schlick S, Ed. Advanced ESR Methods in Polymer Research, Wiley Interscience: New York, 2006.

[86] Gerson F, Huber W. Electron Spin Resonance Spectroscopy of Organic Radicals. Wiley-VCH: Weinheim, 2003.

[87] Poole CP. Electron Spin Resonance, 2nd ed.; Dover Publications: New York, 1983.

[88] Eaton GR, Eaton SS, Salikhov KM., Eds. Foundations of Modern EPR, Singapore, World Scientific, 1998.

[89] Hinderberger D. EPR spectroscopy in polymer science. Top Curr Chem. 2011;321:67–90.

[90] Lucarini M, Mezzina E. EPR investigations of organic non-covalent assemblies with spin labels and spin probes. Electron Paramagn Reson. 2011;22:41–70.

[91] Pitt CG, Wang J, Shah SS, Sik R, Chignell CF. ESR Spectroscopy as a probe of the porphology of hydrogels and polymer-polymer blends. Macromolecules. 1993;26:2159–2164.

[92] Kajsheva NS. Configuration of central metals in polyuronates studied by EPR spectroscopy. Pharm Chem J. 2012;46(8):51–53.

[93] Kurzbach D, Reh MN, Hinderberger D. Nanoscale inhomogeneities in thermoresponsive triblock copolymers. ChemPhysChem. 2011;12:3566–3572.

[94] Junk MJN, Jonas U, Hinderberger D. EPR spectroscopy reveals nanoinhomogeneities in the structure and reactivity of thermoresponsive hydrogels. Small. 2008;4:1485–1493.

[95] Wasserman AM, Yasina LL, Motyakin MV, Aliev II, Churochkina NA, Rogovina LZ, Lysenko EA, Baranovsky VY. EPR spin probe study of polymer associative systems. Spectrochim Acta Part A. 2008;69:1344–1353.

[96] Kempe S, Metz H, Bastrop M, Hvilsom A, Contri RV, Mader K. Characterization of thermosensitive chitosan based hydrogels by rheology and electron paramagnetic resonance spectroscopy. Eur J Pharm Biopharm. 2008;68:26–33.

[97] Bertholon I, Hommel H, Labarre D, Vauthier C. Properties of polysaccharides grafted on nanoparticles investigated by EPR. Langmuir. 2006;22:5485–5490.

[98] Freire-Nordi CS, Vieira AAA, Nascimento OR. The metal binding capacity of Anabaena spiroides extracellular polysaccharide: an EPR study. Process Biochem. 2005;40:2215–2224.

[99] Ciesielski W, Lii CY, Yen MT, Tamasik P. Internation of starch with salt of metals from the transition groups. Carbohydr Polym. 2003;51:47–56.

[100] Kanesaka S, Watanabe T, Matsukawa S. Binding effect of Cu(2+) as a trigger on the sol-to-gel and the coil-to-helix transition processes of polysaccharide, gellan gum. Biomacromolecules. 2004;5:863–868.

[101] Ionita G, Ariciu AM, Smith DK, Chechik V. Ion exchange in alginate gels—dynamic behaviour revealed by electron paramagnetic resonance. Soft Matter. 2015;11(46):8968–8974.

[102] Ionita G, Chechik V. Exploring polyethylene glycol/cyclodextrin hydrogels with spin probes and EPR spectroscopy. Chem Commun. 2010;46:8255–8257.

[103] Ionita G, Ariciu AM, Turcu IM, Chechik V. Properties of polyethylene glycol/cyclodextrin hydrogels revealed by spin probes and spin labelling methods. Soft Matter. 2014;10:1778–1783.

[104] Cesteros LC, Ramirez CA, Pecina A, Katime I. Poly(ethylene glycol-β-cyclodextrin) gels: synthesis and properties. J Appl Polym Sci. 2006;102:1162–1166.

[105] Cesteros LC, Ramirez CA, Pecina A, Katime I. Synthesis and properties of hydrophilic networks based on poly(ethylene glycol) and β-cyclodextrin. Macromol Chem Phys. 2007;208:1764–1772.

[106] Ionita G, Chechik V. Spin-labelled cyclodextrins as hosts for large supramolecular assemblies. Org Biol Chem. 2005;3:3096–3098.

[107] Chechik V, Ionita G. Bis spin-labelled cyclodextrins. New J Chem. 2007;31:1726–1729.

[108] Ionita G, Meltzer V, Pincu E, Chechik V. Inclusion complexes of cyclodextrins with biradicals linked by a polyether chain—an EPR study. Org Biomol Chem. 2007;5:1910–1914.

[109] Caragheorgheopol A, Edwards W, Hardy JG, Smith DK, Chechik V. Using EPR spectroscopy as a unique probe of molecular-scale reorganization and solvation in self-assembled gel-phase materials. Langmuir. 2014;30:9210–9218.

[110] Takemoto Y, Yamamoto T, Ikuma N, Uchida Y, Suzuki K, Shimono S, Takahashi H, Sato N, Oba Y, Inoue R, Sugiyama M, Tsue H, Kato T, Yamauchi J, R. Tamura. Preparation, characterization and magnetic behavior of a spin-labelled physical hydrogel containing a chiral cyclic nitroxide radical unit fixed inside the gelator molecule. Soft Matter. 2015;11:5563–5570.

[111] Ariciu AM, Staicu T, Marin Micutz, Neacsu MV, Ionita P, Tecuceanu V, Munteanu C, Ionita G. Investigations on carboxy dibenzylidene sorbitol hydrogels using EPR apectroscopy. Appl Magn Reson. 2015;46:1395–1407.

[112] Kveder M, Andreis M, Makarević J, Jokić M, Rakvin B. The EPR study of low molecular weight organogels by means of a nitroxide spin probe. Chem Phys Lett. 2006:420;443–447.

IntechOpen