NANOSTRUCTURED AND NANOCOMPOSITE HYDROGELS FOR BIOMEDICAL APPLICATIONS

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Abstract. Polymer hydrogels are interesting for biomedical applications due to their biocompatibility and good water sorption/diffusion properties. In previous work their mechanical stability could be improved by combination with a hydrophobic polymer. Instead, here we prepare poly(hydroxyethyl acrylate), PHEA/silica nanocomposites by sol-gel techniques as potential scaffold materials for tissue engineering. Their morphology and properties were investigated by scanning electron microscopy, thermogravimetry, dynamic mechanical analysis, water sorption/desorption, dielectric spectroscopy, and thermally stimulated depolarization currents techniques at various levels of relative humidity/water content. The results show that the nanoparticles are homogeneously distributed, form a continuous phase, and significantly reinforce the matrix, without affecting its hydrophilicity and, thus, biocompatibility. Water is more homogeneously distributed, as compared to pure PHEA matrix, forming smaller clusters. The glass transition temperature is controlled by water content, and less affected by the concentration of silica.

Keywords: hydrogels; poly(hydroxyethyl acrylate); nanocomposites; silica nanoparticles; water clustering

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1. Introduction

Polymer hydrogels are hydrophilic crosslinked polymers, which may absorb large amounts of water preserving their integrity. Of special interest are hydrogels based on biocompatible polymers for applications as biomaterials in drug delivery systems, implantation, preparation of scaffolds for tissue engineering, etc.[1,2] To optimize composition, preparation conditions and processing of such materials tailored to specific end-use requirements, a better understanding of structure - property relationships is essential. Issues related to the organization (state) of water, and the influence of water on the final properties of the hydrogel, are of fundamental interest in such considerations.

In previous work we employed broadband dielectric relaxation spectroscopy (DRS) and thermally stimulated depolarization currents (TSDC) techniques, in combination with other complementary techniques, to study water effects in biocompatible hydrogels based on poly(hydroxyethyl acrylate), PHEA.[3-7] In several cases PHEA was combined with a second, hydrophobic polymer in the form of blends, copolymers, and more successfully, interpenetrating polymer networks (IPNs), to improve mechanical stability.[6,7] Special attention has been paid to the investigation of the effects of water on polymer (chain) dynamics, and on the final properties of the hydrogel, including those of biotechnological significance. This work has been recently extended to include porous hydrogels, either homopolymers[8] or IPNS,[9] prepared by solution polymerization using water, methanol or ethanol as diluents, to improve water capacity. Pores are formed due to segregation of the solvent from the polymer during the polymerization process. They collapse during the drying process after polymerization, but reopen when the xerogel is immersed in water. The results show that volume fraction, size and morphology (connectivity) of the pores can be controlled by solvent type, composition of the mixture, and polymerization conditions.[8] Thus, it is possible to predict the hydrogel properties in terms of composition and preparation conditions.

An interesting result with respect to applications obtained with the IPN hydrogels is that these are two- phase systems (two glass transition temperatures), with the hydrophilic domains behaving essentially like the pure hydrophilic component.[6,7,9] Thus, the two basic functions of these IPN hydrogels with respect to applications, namely hydrophilicity and mechanical stability, are separately taken over by the two IPN components, the hydrophilic and hydrophobic domains, respectively. Figure 1 shows TSDC and DMA results for the water content dependence of the α relaxation (dynamic glass transition) of PHEA in sequential IPNS prepared from PHEA and poly(ethyl methacrylate) (PEMA) as the hydrophobic component.[9] In these IPNs a porous PEMA network was prepared first, and PHEA was then polymerized in the pores. In addition to the strong plasticization action of water, we observe in Figure 1 that practically a single reduced curve is obtained, demonstrating that all hydrophilic phases of the IPNs are equally plasticized. Water sorption isotherms show the same effect when the water content is referred to the PHEA weight fraction. Another interesting observation in Figure 1, is that water in excess of about 0.3 g of water per gram of PHEA, does not further contribute to plasticization of the PHEA chains. This indicates that water forms a separate phase at higher water contents, in agreement with the results of DSC measurements.[9]

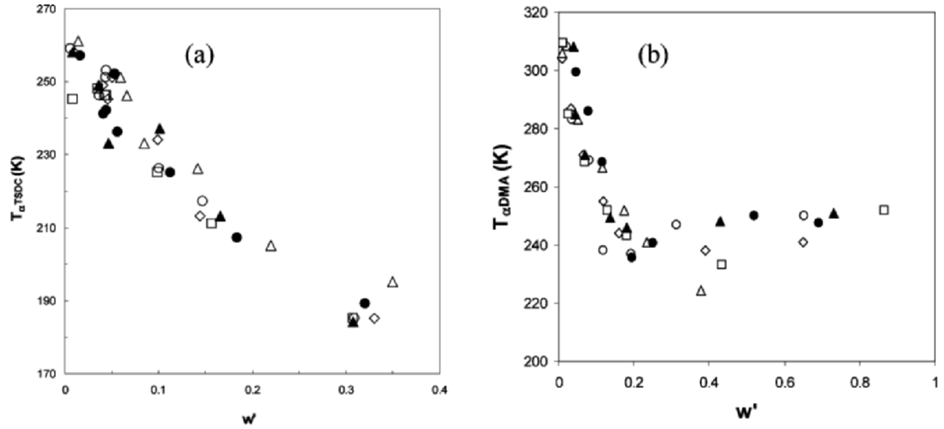

Figure 1. Temperature of the TSDC peak (a) and DMA loss tangent peak (b) corresponding to the main relaxation of PHEA, α, as a function of water content of the hydrogel referred to PHEA weight in the sample, w'. The samples are identified by their PHEA mass fraction equal to: 0.81 (□), 0.71 (◊), 0.64 (▲), 0.45 (O), 0.34 (•), 0.15 (Δ).

To further improve hydrogel mechanical stability, in particular for the preparation of scaffolds for tissue engineering, the concept of nanocomposites may be used. Polymeric nanocomposites, consisting of a polymeric matrix and an inorganic filler of typical size 1-100 nm, have been shown by several investigators to exhibit, for the same filler fraction, far better mechanical properties than conventional composites (micro- or macrocomposites).[10] The reasons for this improvement have not been yet fully understood. It is commonly accepted, however, that the large surface to volume ratio of the nanofiller plays an important role. Several other aspects of polymer nanocomposites are currently being intensively investigated, such as the effects of nanofiller on chain dynamics, and the dependence of the final properties on the degree of dispersion.[11]

In this paper we report results of our studies on PHEA hydrogels reinforced with silica nanoparticles. These nanocomposite hydrogels were prepared and investigated as potential materials for the development of scaffolds for tissue engineering. Other applications may also be envisaged, such as drug delivery systems and implantation materials. Sol-gel techniques in the presence of the polymeric matrix were employed for preparing the nanocomposite hydrogels. Owing to the combination of nanocomposites and hydrogel in one material several interesting fundamental questions arise. Issues such as the organization of water in relation with the hydrophilic/hydrophobic nature of the filler, the changes in free volume due to loosened molecular packing of the polymeric chains, interface and interphase effects etc. Several experimental techniques were employed to investigate the organization of water, and the effects of water and of the silica nanoparticles on the final properties of the nanocomposite hydrogels in relation to structure and morphology. These include, next to DRS and TSDC, electron microscopy, thermogravimetric analysis (TGA), water sorption/diffusion techniques, differential scanning calorimetry (DSC) and dynamic mechanical analysis (DMA). The results are discussed in terms of effects of the nanoparticles on chain dynamics, confinement of water in a small volume, specific polymer-water interactions, interfacial effects and filler-water interactions, all in relation to recently obtains results with other nanohydrogels.[12-14]

2. Experimental

2.1. MATERIALS

The nanocomposites were prepared in two steps. In the first step PHEA hydrogels were prepared following procedures described in previous work.[3-5] In the second step standard sol-gel techniques were employed to prepare silica nanoparticles in the presence of the PHEA hydrogel. Details of the preparation will be given elsewhere. The silica concentration in the dry nanocomposites was varied between 0 and 30 wt%.

2.2. EXPERIMENTAL TECHNIQUES

Scanning electron micrographs were taken with an ISIDS-130 microscope. Thermogravimetric analysis (TGA) was performed with an SDT Q600 (TA Instruments). A Seiko DMS 210 dynamic-mechanical analyzer was used for DMA measurements. For water content determination samples were allowed to equilibrate over saturated salt solutions in sealed jars at controlled relative humidities. The water content h, defined as the ratio of the weight of water in the hydrogel to the weight of the dry sample (dry basis), was determined by weighing. Dry weights were determined by drying in vacuum (5×10^{-2} Torr) for 24 h at 60°C. DRS (10^{-2}-10^{6} Hz) and TSDC techniques (−180 to 30°C) have been described elsewhere.[3,4] The less familiar TSDC technique corresponds to measuring dielectric losses against temperature at low frequencies of 10^{-2}-10^{-4} Hz, i.e. in a range not easily available for DRS measurements. Moreover, the technique is characterized by high sensitivity and high peak resolving power[3]. A Novo control Alpha Analyzer was used for DRS measurements. The temperature was controlled to better than 0.1°C with a Novo control Quarto system. TSDC measurements were carried out using a Deathly 617 electrometer in combination with the Novocontrol Quatro cryosystem and a Novocontrol sample cell.

3. Results and Discussion

3.1. MORPHOLOGICAL, THERMAL AND MECHANICAL CHARACTERIZATION

SEM micrographs showed an excellent dispersion of nanoparticles in the matrix. Pyrolysis in TGA measurements revealed, in agreement with results of other measurements to be reported elsewhere, that the nanoparticles form a not fully-interconnected silica network in the samples with filler content below about 15%, and a fully interconnected network in the samples with higher filler content. Thus, the nanocomposites under investigation may be considered, at least at higher filler contents, as IPNs consisting of two networks, an organic (PHEA) and an inorganic network (silica).

Figure 2 shows results of DMA measurements: storage modulus as a function of temperature measured at 1 Hz for pure PHEA, and two nanocomposites equilibrated in air. We observe that the glass transition temperature T_g of the hydrogels is in the range of 10-20°C, slightly shifting to higher temperatures and becoming broader on addition of silica nanoparticles. The main result in Figure 2, however, is the significant and systematic increase of the storage modulus of the nanocomposites in the rubbery phase up to about three orders of magnitude on addition of 30 wt% silica, as compared to pure PHEA. This significant improvement, by far more than typically reported for silica nanocomposites, as well as for nanocomposite hydrogels,[12,13] is essential for scaffold applications. It may be suggested that the network structure of the silica phase, revealed by pyrolysis, is responsible for that. In that respect, it is interesting to note that the significant improvement of the storage modulus in the rubbery phase in Figure 2 is achieved for filler contents higher than about 15%.

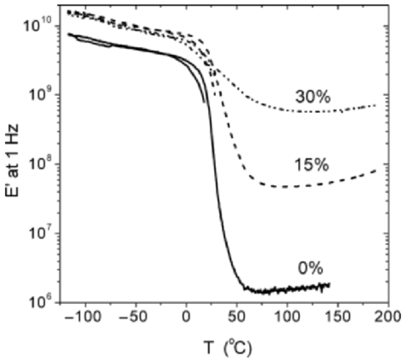

Figure 2. Temperature dependence of the storage modulus *E*' of the samples indicated on the plot at 1 Hz.

3.2. WATER SORPTION

Figure 3 shows preliminary results of water sorption measurements of several nanocomposites at a few values of relative humidity at room temperature. The water content has been normalized to PHEA content. Several interesting results are observed in Figure 3. Similar to the IPNs in Figure 1, the normalized water content does not depend on composition, i.e. hydrophilicity and thus biocompatibility of the nanocomposite hydrogels are not affected by the presence of the nanoparticles. This result suggests that water sorption on the surface of silica nanoparticles is not significant. In addition, no hysteresis effects were observed in sorption/desorption measurements, indicating good performance of the hydrogels under investigation. Closer inspection of the data in Figure 3 suggests a higher water uptake for the samples with 10% silica. This is an interesting observation, since preliminary water uptake measurements by immersion in water indicate higher water uptakes for that composition, probably related to the connectivity of the silica network. Further immersion experiments are in progress to explore these results, and also for differences in the behavior of the hydrogels in sorption from the vapor phase and by immersion.[8,9] For comparison, the water uptake of poly(vinyl alcohol)/silica hydrogels immersed in water was found to be reduced with respect to the pure matrix , in particular at higher pH values, probably due to rigidity of the silica network restricting the extensibility of the composite.[12]

A second interesting result in Figure 3 is that the sorption isotherms can be classified as type III according to Brunauer,[15] indicating that the results should not be described in terms of free and bound water and of hydration layers. Instead, the water molecules are distributed randomly at the hydrophilic sites up to a critical water content h_c, above which clusters begin to form. The data were quantitatively analyzed by following the analysis proposed by Brown.[16] This analysis combines conventional solution theory (Flory-Huggins) and cluster theory (Zimm and Lundberg) and provides an interpretation for the sorption of water molecules in polar materials.[5] The data in Figure 3 were fitted to the equation

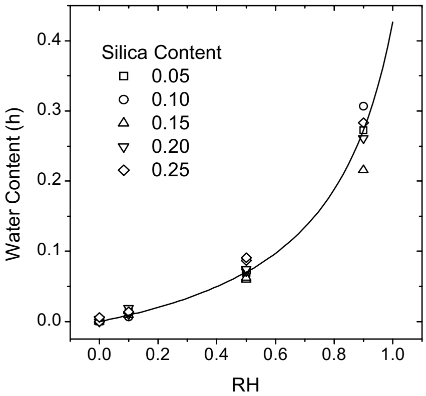

Figure 3. Water content *h*, normalized to PHEA content, against relative humidity RH of the samples indicated on the plot at 25°C. The line is a fit of Eq. (1) to the experimental data.

$$h = \frac{P_1 a_w}{P_2 + a_w} \tag{1}$$

where α_w is the water activity (equal here to relative humidity RH) and P a fit parameter, equal to the critical hydration layer h_c above which water clusters are formed, $h_c = P$. The inset to Figure 4 shows results for h_c of the nanocomposites. The mean number of water molecules in a cluster N_c at water content h can be calculated.[16]

Figure 4 shows results for N_c at $h = 0.9$. For comparison h_c was estimated to be 0.05 in pure PHEA hydrogels, and N_c about 4.5 at RH = 0.9 (Ref. 5). We observe that at higher silica contents h_c increases and N_c decreases. Thus, water is more homogeneously distributed in the presence of the silica nanoparticles. Water clusters are formed at higher water contents and are smaller, compared to pure PHEA, i.e. clustering is suppressed in the nanocomposites. Although quantitative analysis of the results suggests that water is sorbed only in the hydrophilic PHEA phase, it may be that the silica nanoparticles interact with and contribute to the good dispersion of water in the nanocomposites. This point, which is also of interest for silica nanocomposites prepared for other applications using various polymer matrices and preparation methods, in particular mixing of the silica nanoparticles with the polymer melt or solution, will be explored in future work by tailoring the hydrophilic/hydrophobic nature of silica used, and the type of chemical treatment.[10,11]

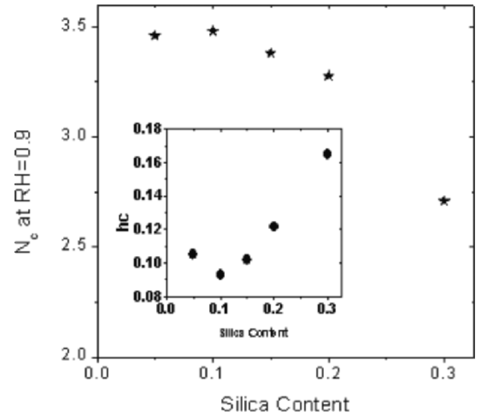

Figure 4. Number of water molecules in a cluster N_c at RH = 0.9 against silica content. The inset shows the critical water content h_c against silica content.

3.3. MOLECULAR MOBILITY

The effects of silica nanoparticles and sorbed water molecules on polymer (chain) dynamics investigated by dielectric techniques are now discussed. Figure 5 shows results of DRS measurements: real, ε′(a), and imaginary dielectric permittivity (dielectric loss), ε″(b), at room temperature for samples equilibrated in air (RH around 0.5, i.e. with water contents between 0.05 and 0.10 (Figure 3)). At the measurement temperature the PHEA polymer is in the rubbery phase.[3-5] This fact is reflected in the high values, and the frequency dependence of dielectric loss at low frequencies, related with dc conductivity. A shoulder observed in ε″(f) and, less clear, a drop in ε′(f) in the kHz frequency region are related with the α relaxation, associated with the glass transition (dynamic glass transition[17]) of the PHEA matrix. The increase of ε″at higher frequencies in Figure 5 arises from the secondary γ and β_{sw} relaxations, which are intimately related with polymer-water interactions.[4] The secondary relaxations will be studied in detail by both DRS and TSDC in future work. ε′ and ε″ in Figure 5 do not change systematically with composition, even at high frequencies where space charge and conductivity effects are significantly suppressed. Two explanations may account for that behavior: (1) the water content at ambient conditions is different for different samples, in correlation with the well established strong dependence of dielectric properties on water content;[4] and (2) real differences exist between the various compositions with respect to the degree of dispersion of the nanoparticles, related with details of preparation which should be better controlled in the future. We will come back to this point later.

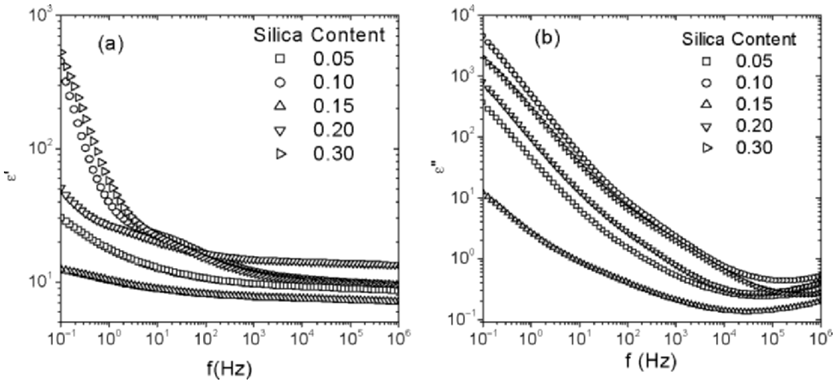

Figure 5. Real ε′(a) and imaginary part ε″(b) of complex dielectric permittivity against frequency *f* of the nanocomposites indicated on the plot at ambient temperature and relative humidity conditions.

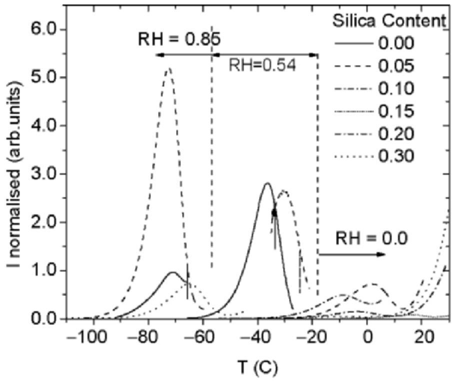

Figure 6. TSDC thermograms (normalized depolarization current *I* against temperature *T*) in the glass transition region of the samples and the relative humidity conditions indicated on the plot.

The α relaxation in Figure 5 is masked by conductivity, in particular at higher water contents, similar to many other polymers.[17] TSDC, which is less influenced by dc conductivity, offers an attractive alternative to investigate the α relaxation. Figure 6 shows results of TSDC measurements on the nanocomposites in the region of the α relaxation at three values of relative humidity. The depolarization current *I*, normalized to the same polarizing field, sample surface area and heating rate, is a measure of $\varepsilon''(T)$ at a frequency range of 10^{-2}-10^{-4} Hz (equivalent to TSDC measurements[3]). The TSDC peak temperature is a good measure of the calorimetric glass transition temperature T_g. Detailed TSDC studies of PHEA hydrogels have indicated that dc conductivity makes a significant contribution to the TSDC α peak without significantly affecting the temperature position of the dipolar contribution.[3] We observe in Figure 6 that the α peak (and thus T_g) shifts significantly to lower temperatures on addition of water. This shift reflects the strong plasticizing action of water on the glass transition, and the corresponding α relaxation studied in detail in previous work.[3-8]

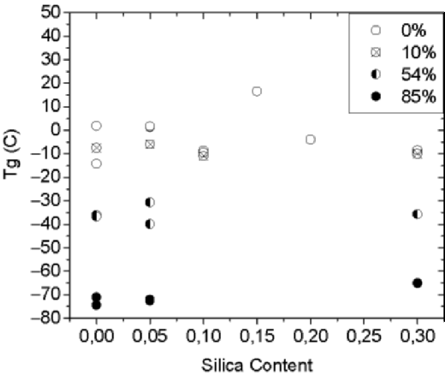

Figure 7. Glass transition temperature T_g against silica content at four levels of relative humidity indicated on the plot

The composition and relative humidity dependence of T_g determined by TSDC is shown in Figure 7. The silica content dependence is rather weak compared to the relative humidity dependence, in particular at higher levels of relative humidity/water content. A possible explanation is that any differences between the dry nanocomposites, associated with the presence of various amounts of silica nanoparticles and their effects on chain dynamics, are masked by the strong plasticization induced by water at higher water contents. It may also be that drying of the samples was incomplete, resulting in different amounts of residual water in the samples considered completely dry in Figure 7. Detailed studies of the water content dependence of T_g in pure PHEA by DSC and TSDC have revealed that this dependence is particularly pronounced at the initial levels of hydration, in agreement with phenomenological descriptions by Fox and Couchman-Karasz.[5]

TSDC measurements in pure PHEA hydrogels have shown that, under the conditions adopted for drying in this work, T_g is around −10°C, (Ref. 7), i.e. in the same range as in the nanocomposites in Figure 7. Compared to DMA, the values of T_g by TSDC are shifted to lower temperatures. The higher frequency of DMA measurements can explain this shift. Much work has been devoted in recent years to the investigation of the composition dependence of chain dynamics and glass transition in organic-inorganic nanocomposites.[18-20] The shift to higher temperatures due to constraints imposed on the motion of polymeric chains by rigid nanoparticles, in particular in the case of covalent bonds between the two components,[18] is often cancelled out, or even overcompensated, by an increase in molecular mobility. A shift of T_g to lower temperatures, due to loosened molecular packing of the polymeric chains, results in an increase in free volume. This point will be studied in future work by systematic DSC, DMA and TSDC studies on the nanocomposites under investigation here, at defined levels of relative humidity / water content.

4. Conclusion

PHEA/silica nanocomposites were prepared by sol-gel techniques. Morphology, water sorption, and chain dynamics were investigated by various techniques. Results indicate that the PHEA/silica nanocomposites exhibit enhanced properties compared to pure PHEA regarding their application as scaffold materials for tissue engineering. DMA measurements show that the polymer matrix is significantly reinforced by the nanoparticles. SEM and pyrolysis studies show that the nanoparticles are homogeneously distributed, forming a not fully-interconnected silica network for filler contents below about 15% and a fully interconnected network at higher filler contents. Water sorption/desorption measurements from the vapor phase show that the hydrophilicity, and with that the biocompatibility of PHEA, is preserved in the nanocomposites. No hysteresis effects exist. Water molecules are more homogeneously distributed in the nanocomposites, forming smaller water clusters at the same relative humidity. Water sorption/desorption measurements by immersion in water to check the effects of the silica network on swelling are in progress. The dynamic properties, investigated by dielectric techniques, are governed by water content, similar to the pure PHEA matrix, and to a lesser extent by the concentration of nanoparticles.

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