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Stimuli responsive gels based on interpenetrating network of chitosan and poly(vinylpyrrolidone)

E. Marsano[a,*], E. Bianchi[a], S. Vicini[a], L. Compagnino[a], A. Sionkowska[b], J. Skopińska[b], M. Wiśniewski[b]

[a]*Dipartimento di Chimica e Chimica Industriale, Università di Genova, Via Dodecaneso 31, 16146 Genova, Italy*
[b]*Faculty of Chemistry, N. Copernicus University, Torun, Poland*

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Abstract

New spherically shaped crosslinked IPN hydrogels based on chitosan and poly(vinylpyrrolidone) were prepared. The IPN hydrogels were synthesized in two steps: (1) chitosan (CHT) crosslinked beads were obtained by reaction of chitosan with ethyleneglycol diglycidyl ether (EGDE); (2) absorption of a suitable amount of a solution of vinylpyrrolidone (VP) monomer from the porous CHT beads followed to VP polymerization and crosslinking inside the network. Sequential IPN's at different composition were obtained: they reversibly swell in water at various pH and show sensitive volumetric behaviour.

Keywords: Hydrogel; Chitosan; Poly(vinylpyrrolidone)

1. Introduction

Hydrogels (networks of hydrophilic polymers) have the capability of absorbing large amounts of water, without losing their three dimensional structure. When discontinuous volume variations upon changes of environmental parameters, like solvent composition, temperature, pH, etc., occur, hydrogels are named 'stimuli responsive gels' or 'intelligent materials' [1]. Their main applications concern the medical industry (contact lenses, artificial corneas, dressing and coating for sutures, electrode sensors) and systems for drug delivery. Most parts of these uses require biocompatible and biodegradable polymers.

Chitosan, [poly-β(1-4)-2-amino-2-deoxy-D-glucose], a polymer which derives from chitin deacetylation, has both these properties. Moreover, the presence of $-NH_2$ groups, whose amount is related to the chitin deacetylation degree, makes the polymer soluble in acid solutions. The solubilisation pH lowers as the degree of acetylation increases: as an example, chitosan, having acetylation degree about 15% (i.e. the original chitin without 85% of its acetyl groups) dissolves at pH 6,2 [2]. When the pH exceeds that value, the polymer forms a hydrated gel-like precipitate. Therefore, the lack of solubility at higher pH, requires how to improve the hydrophilicity of the system.

A first method consists in modifying the chitosan chain, for example by carboxymethylation before the crosslinking (these products captured noticeable interest due to their antibacterial activity [3,4]). A second way is to add a suitable amount of a hydrosoluble polymer. After crosslinking of chitosan, a semi-interpenetrate network (semi-IPN) can be obtained [3–6] with improved swelling behaviour in the complete pH range. Moreover, even the free polymer may be crosslinked giving a network interpenetrate with that of chitosan. Some examples are available in the literature [5–8]. Risbud et al [6] studied a semi-interpenetrate network chitosan–polyvinylpyrrolidone. Chitosan and polyvinylpyrrolidone dissolved in acetic acid 0.1 M and pure water, respectively, were mixed in suitable ratio (w/w about 1). Addition of gluteraldehyde causes the crosslinking of chitosan giving a semi-interpenetrate network, which may be used to prepare membranes having swelling properties in a wide pH range.

* Corresponding author. Tel.: +39 10 3538727; fax: +39 10 3536199.
E-mail address: marsano@unige.it (E. Marsano).

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Thermal and IR analysis performed on PVP–chitosan blends have recently demonstrated that the two polymers are miscible at the solid state [9], probably because hydrogen-bond between the biological polymer and the synthetic one easily occurs. However, when a solvent such as water is added, it becomes competitive with respect to the hydrogen bond formation causing immiscibility in aqueous solution.

This conclusion makes questionable the structural reproducibility of any process based on the transition solution–solid state of the polymer mixture.

Moreover, the uncrosslinked polymer (PVP) of the semi-interpenetrated gel is always subject to diffuse in the solvent, especially in swelled condition.

We believe that an interpenetrate gel may overcome the problem: so, we prepared sequential interpenetrate networks of varying chitosan/polyvinylpyrrolidone ratios and tested their behaviour.

2. Experimental

2.1. Materials

Commercial chitosan (CHT) Antartich Krill Euphasia Superba was produced by Riber Fisheries Central Board, Szcecin, Poland It was refined twice by dissolution in dilute acetic acid, filtration and precipitation with 70 vol % ethanol and 30 vol % ammonia aqueous solution (35% w/w) and finally freeze dried. CHT sample, used in this work, was characterized [10,11] using different techniques. Values indicating the degree of acetylation were perfectly coincident (42.4 from NMR [10] and 42 from IR [11]) and those of the molecular weight were also in good agreement (1200 kDa from light scattering [10] and 1380 kDa from viscometry [11]).

Vinylpyrrolidone (VP) (Fluka) was distilled under vacuum before use to eliminate the stabilizer.

2,2′-azo bis isobutyronitrile (AIBN), ethylene glycol dimethacrylate (EDMA), ethyleneglycol diglycidyl ether (EGDE), ethanol, ammonia, acetic acid were used as supplied by Aldrich. Deionised water was used throughout the work.

2.2. Preparation of chitosan crosslinked beads

Crosslinked beads of chitosan were previously obtained in our laboratory during the experimental work of a Master in Chemistry [11]; the procedure here described is based on those results with the exception of the crosslinking step. Moreover the dimensions of our beads are quite higher (3–5 mm). Three steps are involved in the process of preparation: chitosan dissolution, beads formation and chemical crosslinking.

One gram of chitosan was dispersed in 100 ml of water. After stirring the mixture for one hour, addition of 0.6 ml glacial acetic was performed and fast stirring pursued for 24 h. The crosslinker, EGDE, was directly added to the solution. Different ratios of cross-linker/chitosan were used, to modulate the crosslinking extent in the final product.

The above solution was poured into a syringe equipped with a micro-pipette tip and dropped into a 10 ml aqueous solution containing 70% ethanol and 30% vol/vol of an aqueous ammonia solution at 35 w/w %. The droplets give rise to a kind of beads suspended in the coagulant; they are left under very low stirring for 3–8 h, allowing the crosslinking reaction to complete.

The beads were filtered out, washed with deionised water to neutral pH, and stored in distilled water for use. The bead diameter was checked via the tips of the micro-pipette. In this way, we prepared wet beads with diameter ranging between 3 and 5 mm in water which become discs of about 2 mm diameter and 10 μm thickness when dried.

2.3. PVP crosslinking

A series of crosslinked PVP samples were prepared by dissolving VP in acetic acid (0.1 M) and adding different amounts of crosslinker (EDMA) and of initiator (AIBN). The solutions were heated in an oven at 35 °C for time varying from 8 to 24 h. PVP gels were washed several times with deionised water to eliminate the unreacted reagents before characterization.

2.4. Preparation of sequential interpenetrate chitosan–PVP beads

Crosslinked chitosan beads were swelled for different times in a solution of acetic acid (0.1 M), and VP monomer (volumetric ratio 60:40) besides EDMA and AIBN, both 2% wt/wt respect to VP. Times varying from 30 to 90 min at temperature of 5 °C were used to obtain different swelling degrees, before VP polymerization. After that, the swelled beads were filtered to eliminate the solution excess, weighed to evaluate the swelling degree and put in an oven at 35 °C for 8 h. During this period the polymerization and crosslinking of VP develop. The IPN's beads were washed several times with deionised water to eliminate the unreacted reagents and dried in a vacuum oven at 35 °C for 24 h.

The amount of PVP crosslinked inside the CHT beads was determined by measurement of the bead weight before and after the crosslinking reaction. Total polymer contents (CHT + PVP) of each IPN sample was obtained by weighing a known number of beads, at least ten (all of the same diameter), after a careful dehydration in vacuum oven. The same procedure was applied to an identical number of CHT beads. The difference of weight corresponds to the amount of PVP crosslinked in the IPN. The procedure was repeated at least for five set of measurements.

2.5. Gel swelling

The swelling degree, SW, is defined as the ratio between the weight W_g of the swollen gel, and of its weight W_p when dried ($SW = W_g/W_p$) In order to measure W_g a suitable number of crosslinked beads were placed in a stainless steel basket containing 0.1 M acetic acid when we dealt with chitosan samples, and various buffer solutions in the case of IPN's samples at 25 °C. The solvent wetting the surface was removed with filter paper, the samples were weighed, and put again in the previous solvent for different times. The procedure was repeated several times in order to determine W_g. Than the gels were dried in vacuum oven at 40 °C for 48 h to determine W_p. The ratio W_g/W_p generally increases to a plateau (SW_{eq} = swelling equilibrium value) in a period of time depending on the relative amount of the components. Measurements of SW_{eq} of IPN's at room temperature were performed in the range of pH 3–8 to determine the possible existence of a volumetric phase transition.

2.6. Scanning electron microscopy

The surface morphology of the hydrogels was determined using a scanning electron microscope, Cambridge Stereoscan model 440 at 20 kV accelerate voltage Small pieces of swelled gels were freeze-dried to avoid the collapse of porous structure. Then they were plunged in liquid nitrogen and the vitrified samples were cut with a cold knife. The samples were mounted on SEM stub using silver glue and sputter-coated with gold (20 μm).

3. Results and discussion

Before discussing the chitosan–PVP IPN behaviour we need to know the properties of the networks of single polymers. Concerning chitosan we deal with a sample having a relatively high molecular weight and less than an half of acetylated amino groups: as a consequence, polyelectrolyte properties are expected and hence a strict control of pH is necessary when swelling measurements are performed.

3.1. Chitosan crosslinked beads

Crosslinked chitosan beads were prepared using different amounts of EGDE whose molar ratio with respect to $-NH_2$ groups (RR) ranged between 10 and 30 No crosslinking reaction happened in acidic solutions, whilst in basic condition the crosslinking reaction between the $-NH_2$ of the chitosan and the oxirane group of the EGDE happened in about 3–8 h at 35 °C.

Fig. 1a shows the SW_{eq} in 0.1 M acetic acid solution at room temperature as a function of the crosslinking time for samples obtained using different crosslinker amount (RR).

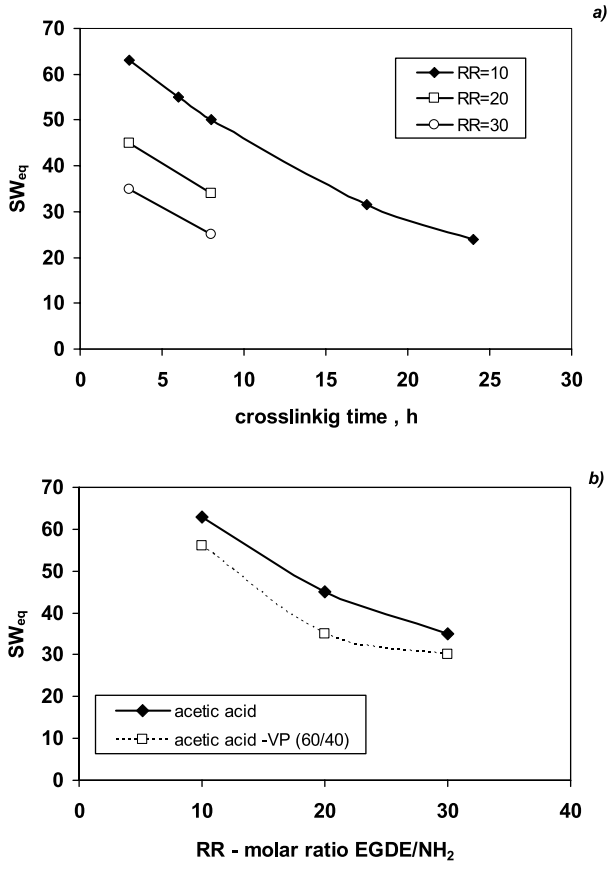

Fig. 1. (a) Equilibrium swelling degree SW_{eq} vs. crosslink time for CHT samples using different crosslinker/CHT ratio (RR); (b) SW_{eq} vs RR measured in different solvent system.

The decreases of SW_{eq} when time increases or, at constant time, when RR increases is in line with the progressive predictable increase of crosslinking. Finally we observe that for crosslinking time lower than 3 h or ratio RR lower than 10 gels are no more self supporting. Therefore, the best conditions to have highly swellable and stable beads are crosslinking time equal 3 h and RR = 10. The diameter of beads, as prepared, is 3 mm, in order to shorten as much as possible the swelling and deswelling times.

Considering that the IPN synthesis is based on the initial adsorption of VP from the crosslinked CHT beads, the behaviour of those gels in presence of VP was examined. A first result is that neat liquid VP does not swell the beads: nevertheless, in a solution of VP in acetic acid 0.1 M [ratio 40/60 (vol/vol)] a significant swelling occurs. Fig. 1b shows the variation of SW_{eq} respect to the ratio RR for a sample crosslinked for 3 h and swelled in 0.1 M acetic acid or in VP–acetic acid solution.This solvent, after addition of AIBN and EDGE, was used for IPN syntheses as explained in Section 2. Finally, we remark that the swellability of CHT gel depends on the initial conditions. If a total drying is performed, beads are no more swellable and their shape becomes analogous to a very thin disc, stabilized from hydrogen bonds among OH and NH_2 groups. Therefore, to

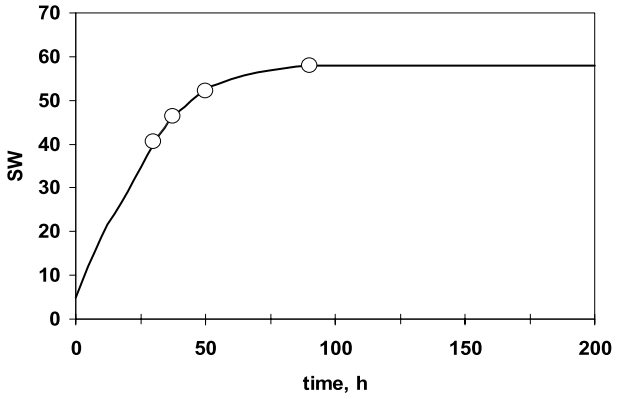

Fig. 2. Swelling of CHT beads vs time (sample: crosslinking time 3 h and RR = 10) Circle represent CHT samples partially swelled in VP which were used to prepare the IPN with different composition.

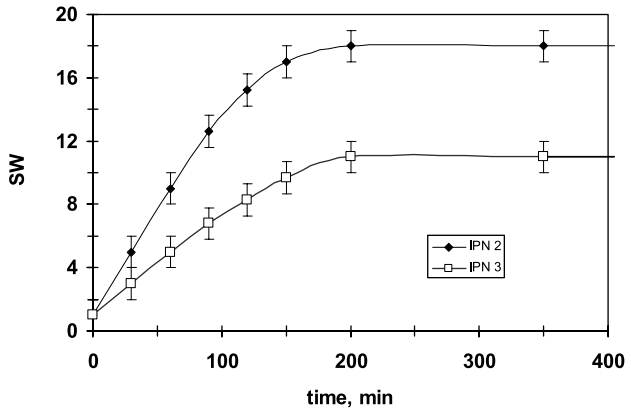

Fig. 3. Swelling variation with time for IPN samples.

obtain a good swelling in any solvent it is essential to start with not completely dried beads.

3.2. VP polymerization and PVP crosslinking

Nine solutions of different composition, columns 2–4 of Table 1, were used to polymerize VP and crosslink the polymer All the solutions are stable at low temperature and only when temperature was increased up to 35 °C polymerization and crosslink develop giving jellified systems. The gels were characterized by measuring the equilibrium swelling degree in water and the SW_{eq} values are are reported in column 6. It may be observed that low amount of crosslinker gives gels no more self supporting (compare samples 1–7 and 2–8) and for low VP concentration is necessary to overcome a critical AIBN concentration to obtain a crosslinked system (see samples 1 and 4). On the basis of these data IPN were prepared according to the synthesis condition of sample 1.

3.3. Chitosan–PVP: IPN synthesis

IPN samples made of CHT and PVP were synthesised according the procedure explained in Experimental Absorption of VP solution from CHT beads (sample: crosslinking time 3 h and RR = 10) was interrupted at different swelling times as indicated in Fig. 2 where the swelling curve of CHT beads vs time is reported. The VP, EDMA and AIBN concentration used to polymerize and crosslink VP were the same used for sample 1 of Table 1.

As shown in Table 2, SW_{eq} in water at room temperature and pH = 7 of sample IPN1 was not detectable as gel breaks during the swelling, moreover in this case inhomogeneous beads are formed showing an external shell of PVP.

The time-dependent swelling behaviours of IPN2 and IPN3 in buffer solution at pH = 7, at 25 °C is shown in Fig. 3. All swelling behaviour are plotted on the average of three trials. The curves are reproducible, independently on the initial state of the gels, the dry state included. Therefore PVP present in the beads partially hinders the hydrogen links formation responsible for the inability to swell shown from the dry beads of crosslinked chitosan. The hydrogels swell rapidly and reach equilibrium within 200 min, while the time necessary to reach the half of the equilibrium swelling is about 50 min.

To investigate swelling behaviour at various pH levels, the IPN 2 sample was swollen in several buffer solutions having pH between 3 and 8 at 25 °C. Fig. 4 shows the equilibrium swelling dependence on pH values. The hydrogel shows a lower SW_{eq} at pH 6–8 as compared with that at pH 2–4 and an abrupt jump around pH 5.

Table 1
Composition of the solutions subjected to VP crosslink reaction and swelling behaviour in H_2O

Samples	% VP, vol	% Acetic acid 0.1 M, vol.	% EDMA, w/w	AIBN, w/w	SW_{eq}, wt/wt
1	40	60	2	2	38
2	50	50	2	2	14
3	60	40	2	2	19
4	40	60	2	1	n.d.
5	50	50	2	1	22
6	60	40	2	1	22
7	40	60	1	2	n.d.
8	50	50	1	2	n.d.
9	60	40	1	2	29

n.d.: swelling not detectable because the hydrogels were not self-supporting.

Table 2
IPN's composition and swelling behaviour in pH = 7 buffer solution

Samples	CHT %, w/w	PVP %, w/w	SW_{eq}, buffer pH 7, w/w
IPN1	19.8	80.2	n.d
IPN2	38.4	61.2	18
IPN3	45.2	54.8	11
IPN4	60.1	39.9	n.d

n.d.: swelling not detectable because the hydrogels were not self-supporting.

This behaviour is typical of sensitive hydrogels and particularly of ionic polymeric hydrogels: the high level of swelling at low pH is due to to the charge repulsion that in our case is correlated to an high concentration of charged $–NH_2$ groups. When the degree of ionization of $–NH_2$ groups is decreased the swelling too decreases.

Since the IPN's hydrogel beads swell differently in different pH media, we have investigated their pH dependent swelling reversibility; sample IPN2 put in contact with buffer solution at pH = 4 swells, when put in a buffer solution at pH 9 shrinks. It takes about 60 min. both for swelling until SW ~ 30, and shrinking until SW ~ 18. Typical pulsate reversible swelling concerning this sample is reported in Fig. 5. This result demonstrates the IPN-beads capability to absorb and deabsorb water upon changing the pH from acid to basic conditions and viceversa. It is also observed that the time of swelling is about the same as that of shrinkage. This process may be repeated many times, with excellent reproducibility and with a fast response. Moreover, it is similar to that observed by us for interpenetrating networks based on Hydroxypropylcellulose and poly(N-isopropylacrylamide) [12], even if in this case the temperature is the variable determining the swelling – shrinking process.

3.4. Morphology

Cross sectional morphology of dry hydrogel of crosslinked CTH and IPN2 are shown in Fig. 6. Pure CHT (Fig. 6a) shows macro-pores of a few micrometers, and a fibrous texture. The morphology of IPN, obtained from PVP synthesised and crosslinked inside the CHT (sample in Fig. 6a) is shown in Fig. 6b. The original structure of crosslinked CHT partially disappears and a more complicated network of two polymers is observed with the appearance of extended area of thin sheets. No phase separation is evident. This could be due to some positive interaction between CHT and PVP as reported in a previous paper [9]. The structure is still porous and this agrees with the actual swelling behaviour.

4. Conclusions

Sequential IPNs synthesized in this work show some peculiar properties: the first one is the capability of reversible swelling in water at room temperature at pH 7, unlike neat CHT gels which swell only in acidic environments. Moreover, IPN's swell quickly even starting from the dry state and this could be connected to the thermodynamics interaction between the two polymers. In fact, previous studies [9] demonstrated that CHT and PVP are miscible at the solid state and not in aqueous solutions. This behaviour was attributed to the competition of the polymer–water hydrogen bonds with respect to those between the polymers. When CHT beads are put in contact with a VP–acetic acid aqueous solution, VP is absorbed in the voids of gel and amongst the CHT charged molecules. As the polymerization and crosslinking take place, PVP chains are entrapped in the phase where VP was absorbed and assure the possibility of reversible swelling in a wide pH range. Moreover PVP crosslinking hampers the release of the polymer in any swelling condition giving stability to the IPN in the time.

A second behaviour concerns the pH influence on SW_{eq} values: as shown in Fig. 4 an increase of pH from acid to basic conditions causes a jump of SW_{eq} around pH = 5. This sensitive behaviour is correlated to the ionization of $–NH_2$ groups present in CHT.

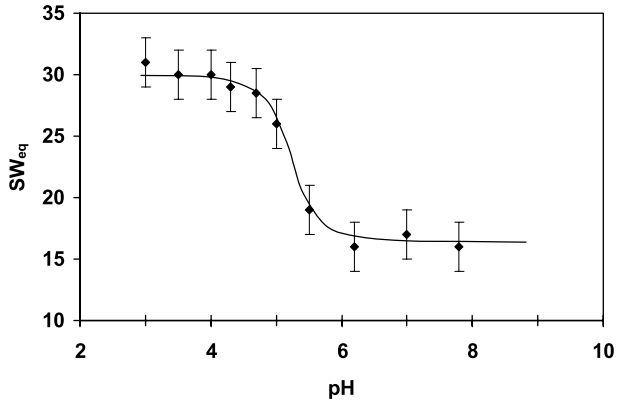

Fig. 4. Equilibrium swelling degree vs pH of IPN (sample 2) at room temperature.

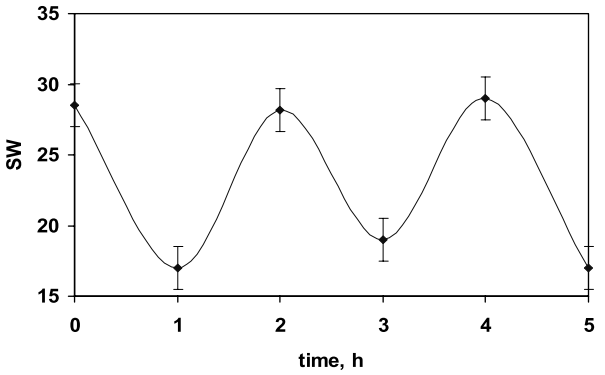

Fig. 5. Trend of SW as a function of time by varying pH between 4 and 9 every 60 min.

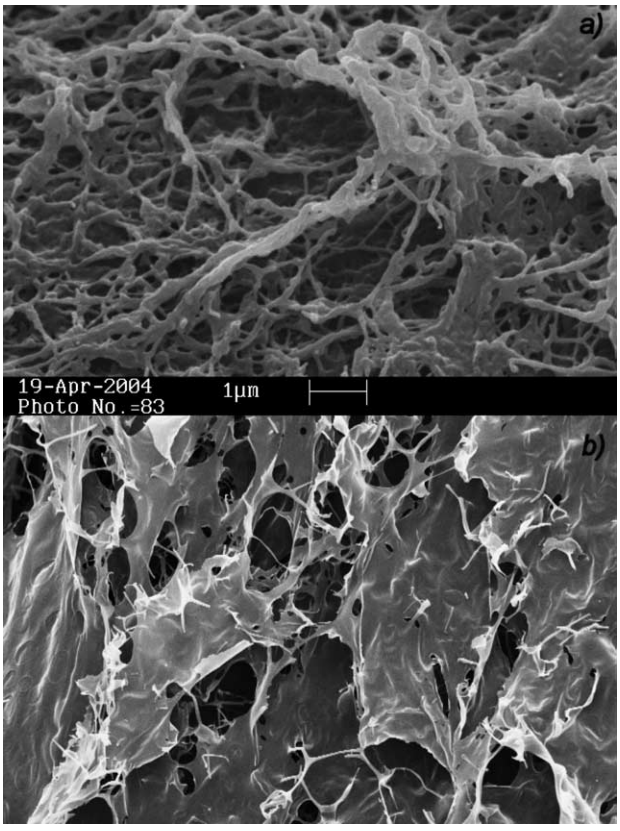

Fig. 6. SEM micrographs of freeze dried gels. (a) CHT sample; (b) IPN sample 2.

Finally, it must be underlined that the degree of CHT acetylation strongly influence the specific condition of IPN synthesis of beads with suitable porosity and swelling behaviour.

Acknowledgements

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