

# **New hydrogels based on the interpenetration of physical gels of agarose and chemical gels of polyacrylamide.**

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## **ABSTRACT**

In this paper we report a novel method for preparing interpenetrating polymer hydrogels of agarose and polyacrylamide (PAAm) in three steps. The procedure consists in (i) formation of physical hydrogels of agarose, (ii) diffusion of acrylamide, N, N'-methylene-bis-acrylamide and potassium persulfate (the initiator) from aqueous solutions inside the gel of agarose, and (iii) cross-linking copolymerization reaction of the aforementioned reactants to produce PAAm chemical gels interpenetrated with the agarose physical gels. Viscoelasticity measurements and thermal analysis have been performed in order to follow the kinetics of copolymerization. The viscoelastic, swelling and thermal properties of the resulting hydrogels confirm the formation of an interpenetrated system. Further evidence of interpenetration is obtained from inspection with Atomic Force Microscopy. The improvement of the agarose and PAAm gel properties in the resulting interpenetrated hydrogel is analyzed in view of the results.

## **KEYWORDS**

Interpenetrating hydrogels, agarose, Polyacrylamide, viscoelastic properties, thermal properties, AFM (atomic force microscopy)

## INTRODUCTION

Hydrogels are hydrophilic polymer networks that have a large water absorbing capacity and that are characterized by the presence of cross-links, entanglements, coexistence of crystalline and amorphous regions, and rearrangements of hydrophobic and hydrophilic domains [1-3].

The ability of polymer gels to undergo substantial swelling and collapsing as a function of their environment, up to 1000 times in volume[4], is one of the most notable properties of these materials [5]. Gel volume transitions can be induced by temperature, pH, or ionic strength, among other stimuli. The phenomenon has prompted researchers to investigate gels as potential actuators, artificial muscles, sensors, controllable separation membranes, and vehicles for drug delivery [6-11]. Gels are particularly appropriate for biomedical applications because of their ability to simulate biological tissues[12].

Agarose is one of the two major components of the polygalactoside agar, together with agaropectine. Agarose is the gelling fraction of agar. It consists of repeating units of alternating  $\beta$ -D-galactopyranosil and 3,6-anhydro- $\alpha$ -L-galactopyranosil groups (an individual unit is named agarobiose) [13]. Agarose forms thermoreversible gels when dissolved in water, and is normally insoluble in organic solvents, save a few exceptions such as DMF, DMSO,...In these latter solvents, it cannot form gels unless a certain amount of water is added. Apparently, the structure of water plays a decisive role to induce gelation [14,15].

A characteristic feature of agarose is that the gels show a large thermal hysteresis, attributed to the formation of large aggregates that remain stable at temperatures much higher than those at which the individual helices reform on cooling[16]. Gain of understanding in the gelation mechanism of agarose has been subject of many scientific

publications, justified by its importance as typical model for gelling materials, as texture modifier in the food industry, or as bacterial medium in the biomedical field, [17,18].

In recent years, polyacrylamide-based hydrogels have received considerable attention. These gels are also used in many applications: as specific sorbents, support carriers in biomedical engineering, aggregating agents, soil improvement agents, polymer processing or improving textiles, paper strengthening agents, adhesives, paints, oil salvaging agents, etc [19-22]. The kinetics of network formation in free-radical copolymerisation of acrylamide and N, N'-methylene-bis-acrylamide in aqueous solution has been extensively studied. It has been reported that the polyacrylamide network exhibits an inhomogeneous cross-link distribution [23]. Partially hydrolysed polyacrylamide gels experience volumetric phase transitions when changing the temperature, pH, and solvent composition[24]. Polyacrylamide gels are extensively used as matrix for electrophoresis, and to encapsulate drugs, enzymes and proteins for application in drug delivery systems and biosensors[25-26].

Combinations of agarose and PAAm polymers can be prepared in the form of blends, copolymers, and interpenetrating polymer networks or gels. Interpenetrating polymer hydrogels (IPHs) are combinations of two or more polymer hydrogels synthesized in juxtaposition[27]. They can also be described as polymer hydrogels held together by topological bonds due to permanent entanglements, essentially without covalent bonds between polymeric chains of different type. By definition, an IPH structure is obtained when at least one polymer gel is synthesized independently in the immediate presence of another. IPHs constitute an important class of materials. They are attracting broad interest both from fundamental and applications viewpoints [28,29].

The present study aims at the preparation of agarose-PAAm interpenetrating hydrogels with improved properties, in-between those of the agarose and PAAm systems. The

swelling and viscoelastic properties have been investigated to corroborate the formation of interpenetrating hydrogels. Further evidence of the formation of interpenetrating systems has been obtained by Atomic Force Microscopy (AFM).

## EXPERIMENTAL PART

### *Materials*

The agarose (D-1 LE) used in this study was kindly supplied by Hispanagar, Spain, and was stored under reduced pressure at room temperature prior to use. The molecular weight of an agarose sample was 102000 g/mol as determined by viscometry measurements. Sulphate percentage was 0.081%. Solutions were prepared using deionised water (milli-Q grade)..

The Acrylamide (AAm) monomer solution, the initiator potassium persulfate ( $K_2S_2O_8$ ) and the crosslinker N, N' methylene bisacrylamide (BMAAm), were supplied by Panreac, Fluka and Aldrich respectively, and used as received.

### *Preparation of agarose hydrogels*

Agarose solutions of different concentrations (0.5, 3, 5, and 8 %, g/mL) were prepared by dispersing the appropriate amount of polymer in 20 mL of distilled water at 100 °C while stirring until complete dissolution. Agarose hydrogels were prepared by pouring the prepared solutions into cylindrical moulds (20 mm in diameter, 2 mm thick), and allowing the gelation to proceed at room temperature.

### *Preparation of interpenetrating polymer hydrogels of agarose and PAAm*

Interpenetrating polymer hydrogels (IPH) of agarose and PAAm were prepared in a sequential process: In a first step, agarose hydrogels of different concentrations (0.5, 3, 5, and 8 %, g/mL) were prepared as mentioned above. After that, cylindrical specimens of agarose hydrogels (20mm in diameter, 2mm thick) were immersed in aqueous solutions of AAm, BMAAm and  $K_2S_2O_8$  at 0 °C, and the reactants were allowed to diffuse within the agarose hydrogels till equilibrium (around 24 h) (pre-IPH samples). The concentration of AAm and

BMAAm in the solutions was the appropriate to obtain hydrogels of AAm concentrations of 5, 10 and 25 %(g/mL) and crosslinking degrees of 1 and 8 %. The concentration of the initiator was fixed at 11,5 mM. The volume of the aqueous solution of reactants was chosen much higher than the volume of the agarose hydrogel specimens to assure that the concentration of reactants within the gels remains the same as in the starting solution .

In a third step, the pre-IPH samples were introduced in an oven at 50 °C for 24 h to obtain interpenetrating agarose-PAAm hydrogels. Some cylindrical specimens of the pre-IPHS samples were introduced between the plates of the rheometer in order to study the kinetics of copolymerization at 50 °C

Thin films of interpenetrating agarose-PAAm hydrogels for AFM experiments were prepared following the same sequential procedure. In this case, the samples were formed between glass slides, with a separation between slides of 500 µm, and dried in an oven at 60 °C before the AFM experiments were carried out.

#### *Swelling measurements*

The cylindrical specimens of the prepared gels were kept immersed in deionized water until equilibrium at room temperature. The relative degree of swelling was determined by weighting the specimens at different times until constant weight is obtained, according to the equation:

$$\frac{m - m_r}{m_r}$$

where  $m$  is the weight at different times and  $m_r$  is the weight of the starting specimen.

#### *Differential scanning calorimetry measurements*

A DSC-7 from Perkim-Elmer with 50  $\mu\text{L}$  aluminium measuring pans was used. DSC measurements were carried out on the pre-IPHs samples. Approximately 30-50 mg of gel material was transferred to the measuring pan. The gelation of AAm within the agarose hydrogel was monitored by scanning the temperature between 10 and 100  $^{\circ}\text{C}$ , at a heating rate of 10  $^{\circ}\text{C}/\text{min}$ .

#### *Rheological measurements.*

Dynamic viscoelastic measurements were performed in a TA Instruments AR1000 Rheometer, using the parallel plate shear mode to measure the storage modulus,  $G'$ , the loss modulus,  $G''$  and the loss tangent,  $\tan \delta$ . To study the kinetics of gelation of AAm within agarose hydrogels, cylindrical specimens of pre-IPHs were introduced between the parallel plates of the rheometer. Then, 90 min time sweeps were performed at 1Hz angular frequency and 50  $\mu\text{Nm}$  oscillation torque. The viscoelastic characterization of the IPHs was carried out in the following operating conditions: temperature sweeps between 10 and 100  $^{\circ}\text{C}$ , heating rate 2 and 10  $^{\circ}\text{C}/\text{min}$ , frequency 1 Hz, and torque 50  $\mu\text{Nm}$ . The linear viscoelastic region was located with the aid of a torque sweep. Frequency scans from 100 to 0.1 Hz in isothermal conditions and torque 50  $\mu\text{Nm}$  were also carried out. A solvent trap from TA Instruments was used to avoid the evaporation of solvent in the course of the rheological measurements,.

#### *Atomic force microscopy*

AFM micrographs were obtained using a Multimode AFM with a Nanoscope III controller from Digital Instruments, and a NANOTECH AFM instrument. Measurements were done in tapping mode, at a scanning rate of 1Hz, at ambient temperature.



## RESULTS AND DISCUSSION

Interpenetrating hydrogels based on a physical gel of agarose and a chemical gel of PAAm were prepared by the sequential procedure describe in the experimental section. First, agarose hydrogels were prepared by quenching agarose aqueous solutions obtained at high temperature. Secondly, pieces of these gels were immersed in aqueous solutions of AAm, BMAAm and initiator, and the diffusion of reactants within the agarose hydrogels was allowed to proceed to obtain pre-IPHs. Finally, the cross-linking copolymerization reaction of AAm and BMAAm of the pre-IPHs was carried out.

The study of the kinetics of gelation of AAm within the agarose hydrogel was monitored by viscoelasticity and Differential Scanning Calorimetry (d.s.c.) measurements in order to confirm the diffusion of reactants (AAm, BMAAm and initiator) within the gel of agarose, and the formation of IPHs . The evolution of the storage modulus as a function of time is depicted in Figure 1, (i) for a pure agarose hydrogel of 0,5% (w/v) concentration, and for two pre-IPHs of 0,5% (w/v) agarose concentration and (ii) 10 and (iii) 25% (w/v) AAm concentrations (8% crosslinking degree in both samples). The three curves show a decrease in the storage modulus at short times, which can be explained by the increase in temperature from 0°C, temperature at which samples are introduced in the rheometer, to 50 °C, temperature at which the formation of the IPHs takes place. Thereafter, the elastic modulus of the pure agarose sample does not show any further variations. In contrast,  $G'$  in the pre-IPH samples experiences an important increase with time. This increase of  $G'$  is a consequence of the copolymerization reaction taking place within the agarose hydrogel that increases the density of cross-links in the system. Finally, at longer times, the

storage modulus levels off, due to the end of the copolymerization process. Some differences can be observed between the IPH samples depending on the AAm concentration. (i) The onset of the elastic modulus increase diminishes with increasing concentration. That means that the copolymerization reactions begins at shorter times for higher concentrations. (ii) The value of the elastic modulus in the upper plateau increases with increasing concentration. A variable can be defined,  $\Delta G'$ , as the difference between the final value of the elastic modulus (the elastic modulus of the IPH) and the starting value of  $G'$  (the elastic modulus of the pre-IPH). The existence of this increment of modulus confirms both the diffusion of the AAm, the BMAAm and the initiator within the agarose hydrogel, and the copolymerization reaction of AAm and BAAm inside the agarose hydrogel giving rise to the formation of an interpenetrated polymer hydrogel of agarose and PAAm.

The effects of AAm concentration, cross-linking degree and concentration in the initial agarose hydrogel on the increment of the elastic modulus as a consequence of the formation of the IPH is further analyzed in Figure 2. Figure 2a-d shows the variation of  $\Delta G'$  as a function of the cross-linking degree in the PAAm gel, for different concentrations of the starting agarose hydrogel (0,5 Fig. 2(a); 3 Fig. 2(b); 5 Fig. 2(c), and 8 % (w/v) Fig. 2 (d), for different AAm concentrations in each case. From Fig. 2, it is noticeable that in all cases,  $\Delta G'$  increases with AAm concentration. This effect is more pronounced as the cross-linking degree increases. The concentration of the initial agarose hydrogel also plays an important role to determine the behaviour of the elastic modulus. The greater increase in the elastic modulus relative to the initial value, i.e. the more pronounced difference in  $G'$  due to the cross-linking copolymerization of

AAM inside the agarose hydrogel, is obtained for pre-IPhs of 0,5 % (w/v) agarose concentration.

In order to confirm that the cross-linking copolymerization of AAm inside the agarose hydrogel takes place without any interference of the agarose network, the reaction process was studied by d.s.c. both in pure water and in the agarose hydrogel. Figure 3 shows thermograms corresponding to the cross-linking copolymerization reaction of AAm and BMAAm in pure water (continuous line) and inside an agarose hydrogel of 0,5% (w/v) concentration (dotted line). In both systems, the concentration of reactants is the same. As noticeable from Fig. 3, there are practically no differences between both systems. A slight displacement in the peak position is observed, but the enthalpy of reaction has the same value. Agarose gels melt at about 80-90 °C. Nevertheless, the melting enthalpy of an agarose hydrogel of 0,5 % (w/v) concentration is around 1,5 J/g, much lower than the reaction enthalpy of AAm (around 76 J/g for the concentration of AAm studied). Therefore, the agarose melting endotherm is negligible and cannot be observed.

Additional evidence of the gelation process of AAm inside the agarose hydrogel was obtained from swelling experiments. It is well known that agarose hydrogels do not swell in pure water [14]. Figure 4 shows the kinetics of swelling of a pure agarose hydrogel of 0,5% (w/v) concentration. As can be observed, the system deswells at short times to evolve to the starting degree of swelling at longer times. In Figure 4 also the kinetics of swelling of different IPhs of 0,5% (w/v) agarose concentration, 5; 10; 16 and 25% (w/v) AAm concentrations and 1% cross-linking degree are represented. From the Figure, the differences between the swelling behavior of the pure agarose hydrogel, on

the one hand, and the IPHs on the other hand become evident. IPHs do swell in water, being the relative degree of swelling in the equilibrium dependent on the AAm concentration. The degree of swelling in the equilibrium increases as the AAm concentration increases. This result is very interesting as it allows us to obtain IPHs from agarose hydrogels with both improved elastic modulus and improved swelling capability. It should be noted that a large swelling automatically entails a destruction of the agarose gels into microgels that remain trapped within the PAAm network.

Different aspects influencing the swelling capability of the IPHs are analyzed in Figure 5. Figure 5(a) presents the effect of the AAm concentration. The relative swelling equilibrium degree ( $Q_{r,eq}$ ) increases with concentration up to around 15 % (w/v). Then  $Q_{r,eq}$  reaches a plateau in the region of 25 %. The effect of the concentration of agarose on the magnitude of  $Q_{r,eq}$  is the opposite (see Figure 5-b). The relative swelling equilibrium degree decreases with the concentration of agarose. This result can be explained as due (i) to increasing impediments to the formation of a PAAm network as the density of the pre-IPH increases, and/or (ii) to a prevalence of the swelling properties of the agarose hydrogel over the swelling properties of the PAAm gel. Eventually, Fig. 5 (c) shows the effect of the cross-linking degree of the PAAm gel. As expected, the relative swelling degree in the equilibrium decreases as the cross-linking degree increases, irrespective of the concentrations of PAAm and agarose.

In order to deepen in the understanding of the viscoelastic behavior of IPHs, some standard experiments on PAAm-agarose IPHs prepared in an oven (out of the rheometer) were performed. Figure 6 shows the frequency sweeps obtained at different temperatures (from 10 to 60 °C) for an IPH of 5%

(w/v) agarose concentration, 10% (w/v) PAAm concentration and 1% cross-linking degree. The evolution of the elastic modulus with frequency is the characteristic of gelled systems (solid-like behavior, with  $G'$  being nearly independent of frequency). The same behavior is observed for pure agarose hydrogels[18]. Therefore, frequency sweeps do not supply any further proof for the interpenetration of agarose and PAAm.

Figure 7 shows the evolution of the elastic modulus as a function of temperature for four IPHs of concentration of 10% (w/v) AAm, 1% crosslinking degree and concentrations of agarose of 0,5, 3, and 8 % (w/v), in the equilibrium of swelling. The curves corresponding to pure agarose hydrogels of concentrations of 0,5; 3 and 8 % (w/v) have also been plotted for comparison. Interesting conclusions can be extracted from the observation of these figures: i) the elastic modulus of the IPHs increases with agarose concentration, as expected ii) the storage modulus of the IPHs as a function of temperature is nearly constant in a wide range of temperatures (from 10 up to around 80 °C), iii) in the vicinity of the melting point of the agarose hydrogels (around 80 °C, depending on the concentration of the gel), the storage modulus of the IPHs shows a sharp decrease but, the melting of the IPHs is never observed (the value of the elastic modulus always presents a finite value in contrast to the case in pure agarose hydrogels in which fusion makes the elastic modulus to become negligible). The latter statement was visually corroborated: the IPHs maintained their dimensional stability at temperatures above the melting point of the agarose hydrogels. Unfortunately, it was not possible to extend the range of analysis of the viscoelastic properties to higher temperatures due to problems of evaporation of water in the hydrogels.

The effect of AAm and agarose concentration on the viscoelastic properties of the IPHs was analyzed at a temperature of 50 °C in order to compare the results with the values obtained from the study of the kinetics of gelation. Figure 8 shows the elastic modulus of IPHs at 50 °C as a function of the AAm concentration for different concentrations of agarose and for two different degrees of cross-linking of the PAAm gel 1% (Figure 8-a) and 8% (Figure 8-b). The following observations can be drawn from these figures: i) at low agarose concentrations (0,5% (w/v)), the variation of the elastic modulus of the IPHs with AAm concentration is the expected, i.e. a significant increase of  $G'$  with AAm concentration takes place for both degrees of cross-linking, and ii) at concentrations of agarose higher than 3%(w/v), the variation of the elastic modulus with AAm concentration exhibits an anomalous behavior:  $G'$  slightly decreases with AAm concentration, being the effect more pronounced at higher degrees of cross-linking. This anomalous behavior, which was already observed in the study of the kinetic of gelation, can be explained assuming that at high agarose concentrations, the densification of the agarose hydrogel induces phase separation, hindering the formation of the PAAm network.

The latter statement is justified from AFM micrographs.

Figure 9 shows a series of AFM micrographs obtained from xero-IPHs of different concentrations of agarose, AAm and cross-linking degrees. The micrograph in Figure 9-a corresponds to a pure agarose xero-gel of 1 % (w/v) concentration. The observed morphology corresponds to a network of intertwined fibrils, as reported elsewhere[17,32]. Figure 9b shows the micrograph of a xero-IPH of 1% (w/v) agarose concentration, 10 % (w/v) AAm concentration and 10% cross-linking degree . The observed morphology is

consistent with a less dense fibrillar network dispersed within a non-fibrillar homogeneous matrix of PAAm. An increase in the agarose concentration while keeping constant the rest of variables, produces an increase in the density of fibrils, but the presence of the non-fibrillar PAAm matrix is still observed (see Figure 9c). At concentrations of agarose higher than 8% (w/v) we can only observe the fibrillar phase corresponding to the agarose(see Figure 9d). The effect of the increase of PAAm concentration on the morphology of the IPHs can be inferred from the comparison of Figures 9c and 9e: A dilution of fibrils is observed as a result of the increasing content of the PAA phase. The analysis of the AFM results allows us to extract the following conclusions: i) The two step procedure used in this study to produce interpenetrating polymer hydrogels does work well under certain concentrations of the interpenetrating polymers, ii) the density of fibers of the xero-IPHs increases with the concentration of agarose, and hence the modulus of the IPHs increases, ii) for higher agarose concentrations the density of fibrils is too high to permit the interpenetration of the PAAm network, and phase separation occurs.

## **CONCLUSIONS**

A sequential method has been developed to obtain new hydrogels based on the interpenetration of a physical gel of agarose and a chemical gel of polyacrylamide.

The method allows us to prepare controlled and interpenetrating hydrogels of agarose and PAAm with defined concentrations and cross-linking degrees.

IPHs of agarose and PAAm provide materials with intermediate properties between those of the separate systems. In this way, it is possible to obtain a material with the good elastic properties of agarose hydrogels, and the swelling capacity and thermal stability of the PAAm hydrogels, as one passes from a thermoreversible system to a non-melting system.

### **ACKNOWLEDGMENTS**

Financial support from CICYT (MAT 2005-1179) is gratefully acknowledged. This work has been performed in the frame of NoE Nanofun-Poly EU contract No. NMP3-CT-2004-500361. E. Fernández is grateful to the Spanish Ministry of Science and Innovation for a grant in aid.



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## FIGURE CAPTIONS

Figure 1. Storage modulus as a function of time for: a pure agarose hydrogel of 0.5 %,g/mL polymer concentration ( $\Delta$ ); an agarose hydrogel of 0.5%,g/mL polymer concentration swollen to equilibrium in an AAm / BMAAm aqueous solution of appropriate concentrations to produce an AAm hydrogel of 10 %,g/mL polymer concentration and crosslinking degree 8 % ( $\square$ ); and an agarose hydrogel of 0.5%,g/mL polymer concentration swollen to equilibrium in an AAm / BMAAm aqueous solution of appropriate concentrations to produce an AAm hydrogel of 25 %,g/mL polymer concentration and 8 %crosslinking degree ( $\circ$ )

Figure 2. Increment of the storage modulus as a consequence of the crosslinking copolymerization of AAm and BMAAm within agarose hydrogels as a function of crosslinking degree for different AAm concentrations: 5 ( $\blacksquare$ ); 10 ( $\bullet$ ); 16 ( $\blacktriangledown$ ) and 25 ( $\blacktriangle$ ) %,g/mL: (a)  $C_{\text{agarose}} = 0.5$  %,g/mL; (b)  $C_{\text{agarose}} = 3$ %,g/mL; (c)  $C_{\text{agarose}} = 5$  %,g/mL; and (d)  $C_{\text{agarose}} = 8$  %,g/mL.

Figure 3. d.s.c. thermograms corresponding to the crosslinking copolymerization of AAm (10 %concentration,g/mL) and BMAAm (8%) crosslinking degree in pure water (continuous line) and within an agarose hydrogel of polymer 0.5 %,g/mL concentration (dotted line).

Figure 4. Swelling degree as a function of time for interpenetrated polymer hydrogels of 0.5 %g/mL agarose concentration, 1% AAm crosslinking degree and different AAm concentrations: 0 ( $\nabla$ ); 5 ( $\square$ ); 10 ( $\diamond$ ); 16 ( $\triangle$ ); and 25 %,g/mL ( $\circ$ ).

Figure 5. Relative swelling degree at equilibrium: (a) as a function of AAm concentration for IPHs of Agarose concentration 0.5 %,g/mL and AAm crosslinking degree 1 %; (b) as a function of agarose concentration for IPHs of AAm concentration 10 %,g/mL and crosslinking degree 1%; and (c) as a function of crosslinking degree for IPHs of different compositions: ( $\blacksquare$ ) Cagarose=0.5 and CAAm=10; ( $\blacktriangle$ )Cagarose=0.5 and CAAm=16; ( $\bullet$ ) Cagarose=0.5 and CAAm=25; and ( $\blacktriangledown$ ) Cagarose=5 %,g/mL and CAAm=10 %,g/mL.

Figure 6. Storage and loss shear modulus as a function of oscillation frequency for an IPH of agarose concentration 5 %,g/mL, AAm concentration 10 %,g/mL and crosslinking degree 1 % at 10 °C ( $\blacksquare$ ); 25 °C ( $\bullet$ ); 50 °C ( $\blacktriangle$ ); and 60 °C ( $\blacktriangledown$ ).

Figure 7. Storage shear modulus as a function of temperature for pure agarose hydrogels (polymer concentrations 0.5 ( $\square$ ); 3( $\circ$ ); and 8 %,g/mL( $\triangle$ )) and IPHs (AAm concentration 10 %,g/mL, crosslinking degree 1% and agarose concentrations 0.5 ( $\blacksquare$ ); 3 ( $\bullet$ ) and 8 %,g/mL ( $\blacktriangledown$ )) in the equilibrium of swelling.

Figure 8. Equilibrium shear modulus as a function of AAm concentration for IPHS of agarose concentration: 0.5 (■); 3 (●); 5 (▲) and 8 %g/mL (▼). Crosslinking degree of the AAm gel: 1 % (a) and 8 % (b).

Figure 9. AFM micrographs of different xerogels obtained from IPHS of compositions: a)  $C_{\text{agarose}} = 1 \text{ \%}, \text{g/mL}$ ,  $C_{\text{AAm}} = 0 \text{ \%}, \text{g/mL}$  and C.D. = 0%; b)  $C_{\text{agarose}} = 1 \text{ \%}, \text{g/mL}$ ,  $C_{\text{AAm}} = 10 \text{ \%}, \text{g/mL}$  and C.D. = 8%; c)  $C_{\text{agarose}} = 3 \text{ \%}, \text{g/mL}$ ,  $C_{\text{AAm}} = 10 \text{ \%}, \text{g/mL}$  and C.D. = 8%; d)  $C_{\text{agarose}} = 8 \text{ \%}, \text{g/mL}$ ,  $C_{\text{AAm}} = 10 \text{ \%}, \text{g/mL}$  and C.D. = 1%; e)  $C_{\text{agarose}} = 3 \text{ \%}, \text{g/mL}$ ,  $C_{\text{AAm}} = 25 \text{ \%}, \text{g/mL}$  and C.D. = 1%.