SYNTHESIS AND NANOSTRUCTURAL CHARACTERIZATION OF POLYACRYLAMIDE HYDROGELS USED AS SIEVING MATRIX IN DNA ELECTROPHORESIS

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DEDICATION

To my parents and sisters for their love, encouragment and support.

To my relatives and friends.

Monita, Patico, mamita y papito

I am really proud of you

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ABSTRACT

TITLE: ***SYNTHESIS AND NANOSTRUCTURAL CHARACTERIZATION OF POLYACRYLAMIDE HYDROGELS USED AS SIEVING MATRIX IN DNA ELECTROPHORESIS****

Author: HUGO YESID ACOSTA RAMIREZ**

Keywords: In situ rheology, thermoporometry, solvent, mean pore size, pore size distribution.

DESCRIPTION:

Polymer hydrogels play a key role as sieving matrix materials for electrophoresis of charged biomolecules like DNA and proteins. In order to rationally select hydrogels that possess properties optimally suited for these applications, it is important to understand the nature of the gel pore structure and the parameters through which it can be manipulated. However, the complex polymerization reaction can exert a strong influence on the resulting gel structure and the current theories are still unable to predict their physical properties from the synthesis conditions. On the other hand, the large amount of water inside the network and mechanical fragility of the hydrogels are challenging the current porous characterization tools.

In situ rheology and thermopometry were used as characterization tools in order to measure the pore structure (mean pore size and pore size distribution) in polyacrylamide gels polymerized by chemical polymerization and photopolymerization in presence of deionized water, buffer solution 1x-TBE and buffer 1x-TBE-urea as polymerization solvent.

Hydrogels polymerized in presence of buffer 1x-TBE-urea were completely transparent in all gel formulations studied and exhibit smaller mean pore size than those polymerized in water and buffer solution 1x-TBE.

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* UNDERGRADUATE THESIS WORK

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RESUMEN

TÍTULO: ***SINTÉSIS Y CARACTERIZACIÓN NANOESTRUCTURAL DE HIDROGELES DE POLIACRILAMIDA EMPLEADOS COMO MATRICES POROSAS EN ELECTROFORESIS DE AND****

Autor: HUGO YESID ACOSTA RAMÍREZ**

Palabras clave: Reología *in-situ*, termoporometría, solvente, tamaño de poro promedio, distribución de tamaño de poro.

DESCRIPCIÓN:

Los hidrogeles poliméricos juegan un papel importante al ser empleados como matrices porosas o tamices en electrofóresis de biomoleculas cargadas como AND y proteínas. Con el fin de seleccionar hidrogeles que posean propiedades apropiadas para estas bioseparaciones, es importante conocer y entender la morfología del poro en las redes poliméricas y los parámetros a través de los cuales este puede ser modificado. Sin embargo, la compleja reacción de polimerización puede afectar fuertemente la estructura del gel resultante y por el momento no es posible predecir la nanoestructura final y las propiedades físicas del gel a partir de las condiciones de síntesis. De otro lado, la gran cantidad de agua que pueden absorber los hidrogeles y la fragilidad mecánica de estos materiales están retando las herramientas de caracterización disponibles.

Reología *in-situ* y termoporometría fueron empleadas como herramientas de caracterización para medir la estructura porosa (tamaño de poro promedio y distribución de poros) de hidrogeles de poliacrilamida polimerizados por polimerización química y fotopolimerización y empleando agua desionizada, solución buffer 1x-TBE y solución buffer 1x-TBE-urea como solventes de polimerización.

Los hidrogeles polimerizados en presencia de buffer 1x-TBE-urea fueron completamente transparentes en todas las formulaciones de gel estudiadas y exhibían menor tamaño promedio de poro que aquellos que fueron polimerizados en agua y solución buffer 1x-TBE.

***** TRABAJO DE GRADO

****** FACULTAD DE INGENIERIAS FISICOQUIMICAS, ESCUELA DE INGENIERIA QUÍMICA. DIRECTOR: Ph.D Victor M. Ugaz, Lector de texto: Ph.D Ramiro Martínez Rey.

1. INTRODUCTION

Separation is an important part of the biochemical process for obtaining bioactive compounds in pure/or concentrated form. In the past, distillation, liquid-liquid extraction, crystallization and filtration dominated conventional separation process. For separation of biological molecules (i,e., bioseparation) the conventional separation methods are not adequate.

Polyacrylamide gel electrophoresis (PAGE) has allowed carrying out genetic testing, analysis of forensic evidence and body identification. Due to the important role that the gel plays as sieving matrix, it is important to know its properties and select hydrogels that possess properties optimally suited for these applications.

In this research, polyacrylamide hydrogels (PA) were synthetized using chemical and photochemical polymerization; in both systems, acrylamide (AAm) was used as the monomer and bis-acrylamide (Bis) was used as the crosslinker agent. The hydrogels were polymerized in the presence of deionized water, buffer solution 1x-TBE and buffer solution 1x-TBE-urea. In the chemical polymerization, ammonium persulfate (APS) was used as an initiator and tetramethylethylenediamine (TEMED) as a catalyst. In addition to chemical initiation, polymerization can also be driven by a photo-induced process called *photopolymerization*, whereby the free radicals are generated by exposure to UV light. In order to characterize the polyacrylamide hydrogel nanostructure, we used in-situ rheology, a calorimetric method called thermoporometry and swelling studies.

We present a series of in situ dynamics small amplitude oscillatory shear studies *(Storage modulus (G'))* in photo and chemically crosslinked polyacrylamide gels in presence of water, buffer solution 1x-TBE and buffer solution 1x-TBE-urea.

Further, with this G' we estimate the mean pore size using the model proposed by Wang and Ugaz. These results are compared to thermoporometry studies, which allowed us to know the pore size distribution which is not currently reproduced by the in-situ rheology method. Swelling studies were carried out in photochemically crosslinked hydrogels with similar mean pore size in order to show the amount of water absorbed in the hydrogel related with the pore size and possibly with the pore size distribution.

We show: 1. The influence of water, buffer 1x-TBE and urea, %T-%C concentration and different polymerization conditions on the final hydrogel nanostructure. 2. In-situ rheology and thermoporometry experiments can serve as useful tools to identify optimal conditions for synthesis of polyacrylamide gels used in DNA electrophoresis. 3. Advantages and drawbacks between chemical polymerization and photopolymerization as polymerization methods to synthetize polyacrylamide hydrogels.

2. THEORICAL BACKGROUND

2.1. HYDROGEL DEFINITION

Hydrogels are important materials of both fundamental and technological interest. They are water-swollen networks (crosslinked structures) composed of hydrophilic homopolymers or copolymers. They are rendered insoluble due to the presence of chemical (covalent or ionic) or physical crosslinks. The latter can be entanglements, crystallites, or hydrogen-bonded structures. The crosslinks provide the network structure and physical integrity [15].

Hydrogels are usually obtained by free radical-polymerization of a monovinyl monomer with a divinyl monomer (crosslinker agent) in a homogeneous solution (Figure 1). However, the current theories are still unable to predict their physical properties from the synthesis conditions. This is due to the several non-idealities of the gel formation system such as the different and conversion dependent reactivities of the vinyl groups, cyclization and multiple crosslinking. Hydrogels formed in such non-ideal circunstances neccesarily include defects affecting their physical properties such as swelling, elasticity, transparency and permeability [19].

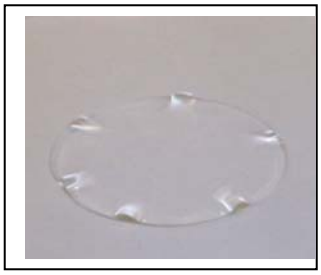


Figure 1. Polyacrylamide hydrogel

2.2. SYNTHESIS OF POLYACRYLAMIDE HYDROGELS

2.2.1. Mechanism of polymerization and polyacrylamide network formation

Polyacrylamide gels are formed by copolymerization of acrylamide (AAm) and bis-acrylamide (“Bis”, N,N’-methylene-bis-acrylamide). The reaction is a vinyl

addition-polymerization initiated by a free radical-generating system. *Chemical polymerization* is initiated by ammonium persulfate (APS) and tetramethylethylenediamine (TEMED), which accelerate the rate of formation of free radicals from (APS) and these in turn catalyze polymerization. In addition to chemical initiation, polymerization can also be driven by a photo induced process, *photopolymerization*, whereby the free radicals are generated by exposure to UV light. Figure 2 shows the polyacrylamide matrix formation.

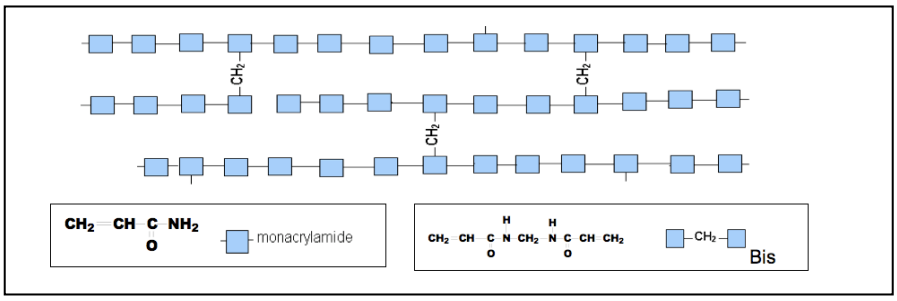


Figure 2. Formation of the polyacrylamide matrix

As soon as the free radicals are generated, they asociate with AAm monomers to produce a chain polymerization reaction, during which the growing polymer chains elongate and are randomly crosslinked by the Bis. This results in a polymer, which acts as a sieve with a defined mean pore size and pore size distribution. This sieve will be used to separate DNA molecules by electrophoresis as is shown in Figure 3.

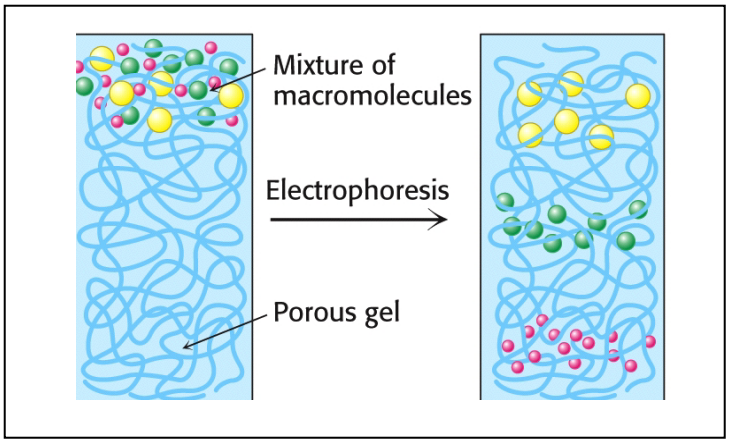


Figure 3. Polyacrylamide gel electrophoresis (PAGE)

The polymerization reaction is strongly inhibited by the presence of oxygen, therefore, a proper degassing is critical to polymerize reproducible gels.

2.3. %T AND %C CONCENTRATION

The nature of the acrylamide polymer formed by the reaction shown previously is commonly expressed in terms of monomer and crosslinker concentration. %T equation (1) is the amount of AAm monomer (g) plus the amount of crosslinker agent Bis (g) divided by the total volume (ml). Thus, a 20%T gel has 20% w/v acrylamide plus crosslinker. %C (w/w) equation (2) is the amount of Bis (g) to the amount of total monomer acrylamide plus crosslinker (g). For example, a 5%C gel would have 5% of the total monomer weight made up by the crosslinker agent [25].

$$\%T = \frac{AAm(g) + Bis(g)}{V(ml)} \times 100 \qquad (1) \qquad \%C = \frac{Bis(g)}{AAm(g) + Bis(g)} \times 100 \qquad (2)$$

2.4. IN SITU RHEOLOGY AND THERMOPOROMETRY AS CHARACTERIZATION TOOLS

2.4.1. In situ rheology

It is an important gel characterization tool because in-situ experiments are relatively straightforward to perform during the polymerization process and because viscoelastic properties directly reflect the structure and morphology of the sample. The elastic or storage modulus (G') is a measure of the reversibly elastic storage energy, whereas the viscous or loss modulus (G'') represents a measure of irreversible energy dissipated during flow. G' and G'' can be measured using in situ small amplitude oscillary shear measurement with a rheometer. The higher the G' the harder the material and the more energy required to deform it. For a well-developed crosslinked polyacrylamide polymer G''<<G' [5].

(Calvet, Wong et al. 2004)[5] performed an extensive series of in situ small-amplitude oscillatory shear experiments during polymerization of polyacrylamide hydrogels using various crosslinker concentration and reaction temperatures. Rubber elasticity theory was then used to extract a measure of the average network cross-link density and strand molecular weight. In addition to observing gelation kinetics, it was possible to identify optimal crosslinker concentration and temperature conditions for synthesis of hydrogels incorporating desired microstructural properties.

(Wang and Ugaz 2006)[31] studied photopolymerized crosslinked polyacrylamide gels performing in situ small amplitude oscillatory shear measurements studying (G') under different UV intensities, %T-%C concentrations and reaction temperature. They developed a model to extract the mean pore size using the rubber elastic theory and compared their results to mobility electrophoresis experiments under the same polymerization conditions showing good agreement with their model as is shown in Table 1.

Gel Conc.	Mobility (nm)	Rheology (nm)
6 %T	9.6	12
9 %T	5.2	4.9
12 %T	3.7	3.4

Table 1. Mobility Vs in-situ rheology experiments.

(Loredana et al)[17] monitored the elastic G' and G'' during in situ polymerization of AAm with Bis in the presence of the redox initiation system potassium persulfate/ ascorbic acid to evaluate the gelation kinetics using oscillatory deformation tests at constant frecuency for different heating rates and shear stresses.They concluded that for low heating rates, up to 2ºC/min, fast decreases of the gelation time occur, whereas for higher heating rates, the gelation time remains relatively constant.

Under the same linear viscoelastic regime, the final viscoelastic properties and the final nanostructure of polyacrylamide gels are a function of *kinetics* (Polymerization time, catalyst nature and concentration, initiator nature and concentration, cyclization…), *reaction conditions* (initiation mode:photo-polymerization or chemical polymerization, temperature, %T-%C, solvent nature…).

2.4.1.1. Rubber elastic theory

The classical rubber elastic theory suggests [16]:

$$G' = nRT \tag{3}$$

Where:

G'=Elastic or storage modulus (Pa)

n=Active network links density (mol/m^3).

R=Universal gas constant (m^3.Pa/mol.K)

T=Reaction temperature (K)

Thus, at a given reaction temperature T, an increase in n is expected to be accompanied by a proportional increase in the steady-state value of G'.

2.4.1.2. Strand molecular weight

The molecular weight (W) of the polymer chain segments linking two microgel clusters can be estimated from n and the total acrylamide monomer concentration [AAm] as follows:

$$M = \frac{[AAm]}{n} \tag{4}$$

2.4.1.3. Mean pore size (L/2) in rheological experiments

With G' and T is possible to calculate the mean pore size in the polyacrylamide network using the model proposed by (Wang and Ugaz 2006) [31].

$$L = \left(\frac{1}{nN_A}\right)^{1/3} = \left(\frac{RT}{G'N_A}\right)^{1/3} \quad (5)$$

N_A = Avogadro's number.

Equation (5) shows that for a given T the more elastic hydrogels (high G') the smaller mean pore size. L/2 is used as the mean pore size in the polyacrylamide network as indicated in Figure 4.

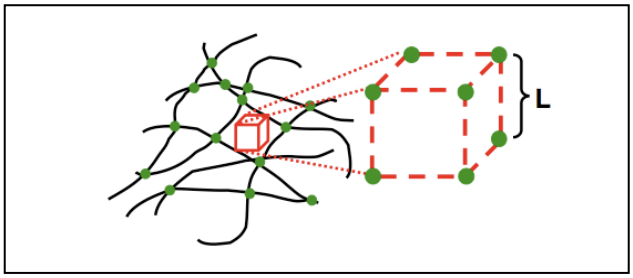


Figure 4. Mean pore size in in-situ rheology experiments.

2.4.2. Thermoporometry

The determination of porous structure of fragile materials from classical methods such as mercury porosimetry and nitrogen adsorption-desorption is often delicate and can be misleading. Thermoporometry is an alternative method and sometimes the only method available, allowing a better characterization of porous and fragile structures, soft materials and hydrogels.

2.4.2.1. Thermoporometry principle

The basic principle of thermoporometry is the freezing (or melting) point depression which is due to the strong curvature of the solid-liquid interface present within small pores. A full thermodynamic description of this phenomenon is given by (Brun, Lallemand et al. 1976) [4]. The physical basis for this shift is that the equilibrium temperature for a solid-liquid phase transition is determined by the radius of curvature of the interface between the solid and liquid phases. A liquid held inside a porous material is finely divided; therefore, the radius of curvature is closely related to the pore size.

A differential scanning calorimeter (DSC) is aptly suited to measure the temperature shifts because of its particular sensitivity to exothermic freezing and endothermic melt transitions. This technique is able to determine pore size distributions if the temperature dependence of physical parameters such as surface tension, contact angle, heat of fusion, and specific volume are known a priori. However, literature values for these parameters usually vary, leading to difficulty in the direct transformation of calorimetry curves into absolute pore size distributions [14].

Temperature difference (ΔT) can be determined in different ways (Figure 5) and will directly influence the measured value of mean pore size and pore size distribution. Three main methods: To, Tpk, Ton-pk are used to calculate the pore size using three different equations.

$$r_p(nm) = -\frac{32.33}{\Delta T(K)} + 0.68 \tag{6}$$

$$r_p(nm) = -\frac{33.3}{\Delta T(K)} + 0.32 \tag{7}$$

$$r_p(nm) = -\frac{19.08}{\Delta T + 0.1207(K)} + 1.12 \tag{8}$$

The mean pore size measured using the Ton-pk and equation (6) is very similar to the pores derived with in situ rheology and will be used in this research.

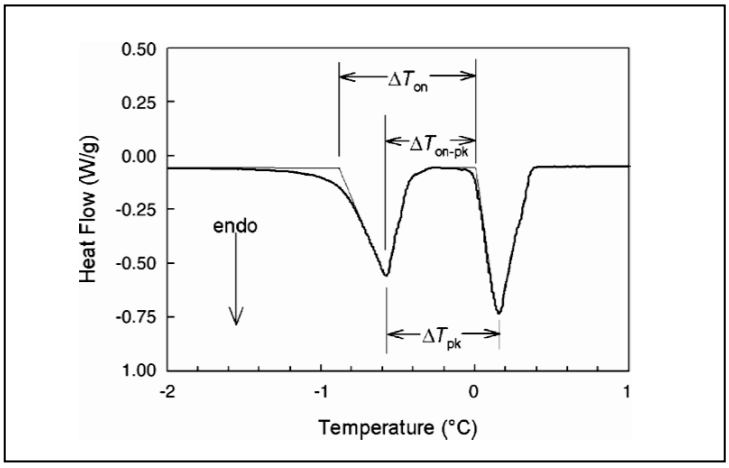


Figure 5. Temperature difference (ΔT) in thermoporometry

2.4.2.2. Pore Size Distribution (PSD)

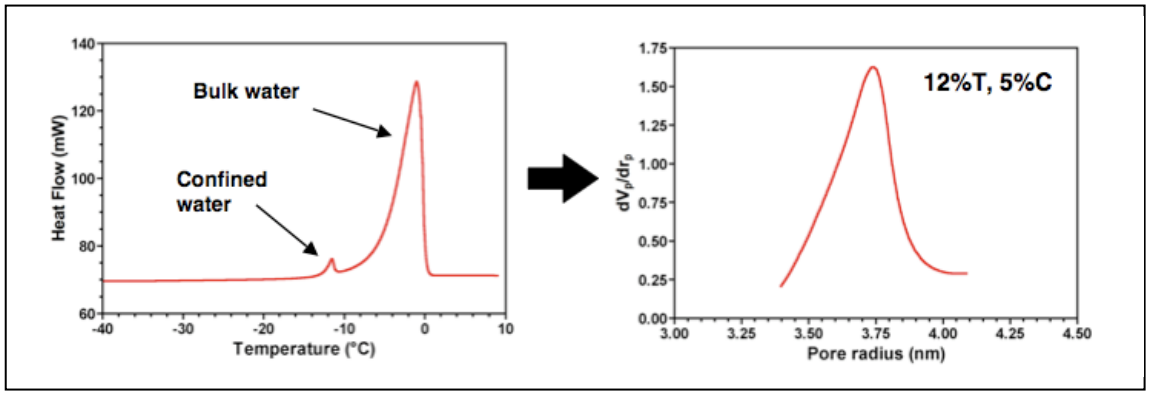
The calorimetric curve generated by the DSC (Figure 6a), the mass of the dry hydrogel (m), $\frac{dQ}{dt}$ (heat flow signal given by the DSC)(J/s), $\frac{d(\Delta T)}{dt}$ (the rate of heating or cooling)(ºC/s), $\Delta H_f(T)$(heat of fusion), $\rho_{sol}(T)$ (density for the probe fluid)(g/cm^3) and equation (9) allow us to find the PSD in the polyacrylamide network (Figure 6b). Further, the PSD is transformed to a normal PSD to obtain the mean pore size (R_p) and the variance of the distribution (σ^2).

$$\frac{dV_p}{dr_p} = \frac{dQ}{dt}\frac{dt}{d(\Delta T)}\frac{d(\Delta T)}{dr_p}\frac{1}{m\Delta H_f(T)\rho(T)} \tag{9}$$

Where:

$\frac{dV_p}{dr_p}$ = Differential pore volume (cm^3/g*nm) and from equation (6):

$$\frac{d(\Delta T)}{dr_p} = \frac{32.33}{(r_p - 0.68)^2} \tag{10}$$



a. **b.**

Figure 6. Thermoporometry experiments. a. Calorimetric curve given by the DSC. b. Pore Size Distribution.

The importance of having a quantitative description of a porous solid via its pore size distribution is that the shape of the distribution provides an additional perspective about how the pore sized are dispersed in the polyacrylamide network.

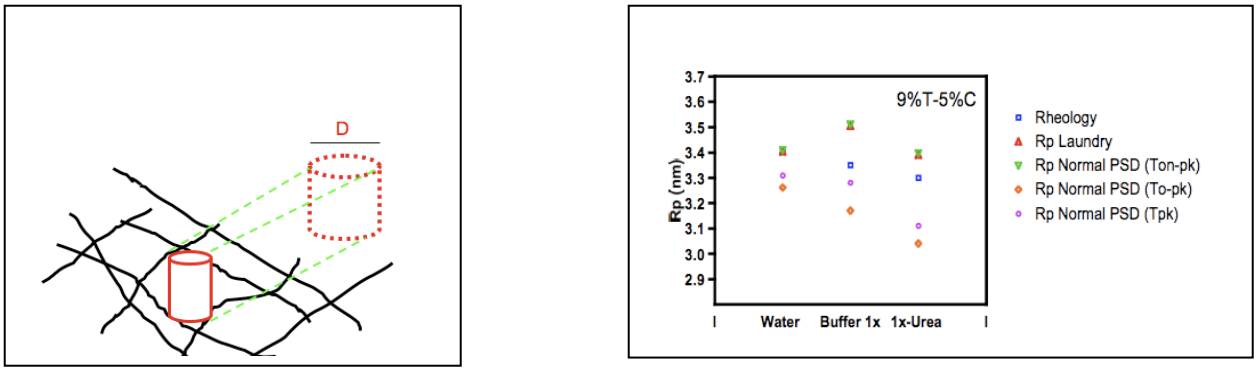
2.4.2.3. Mean pore size (R_p) in thermoporometry

Laundry [14] suggest that as soon as the pore size distribution is known, the total pore volume V_p, the internal surface area S_p and the average pore radius r_{ave} can be calculated as is indicated in equation (11), equation (12) and equation (13):

$$r_{ave} = \frac{2V_p}{S_p} \quad (11) \qquad V_p = \int_o^\infty \left(\frac{dVp}{dR_p}\right) dr_p \quad (12) \qquad S_p = \int_o^\infty \frac{2}{r_p}\left(\frac{dV_p}{dr_p}\right) dr_p \quad (13)$$

A cylindrical pore shape is assumed according with (Brun, Lallemand et al. 1976) [4] (Figure 7a).

A comparison between the mean pore size obtained using in situ rheology and thermoporometry experiments is shown in Figure 7b.



a. **b.**

Figure 7.Thermoporometry and rheology in situ experiments. a. Mean pore size in thermoporometry experiments. b. Comparison of the mean pore size obtained using in-situ rheology and thermoporometry experiments.

In our research, the mean pore size (R_p) is obtained from the normal pore size distribution (Figure 8) and equation (14).

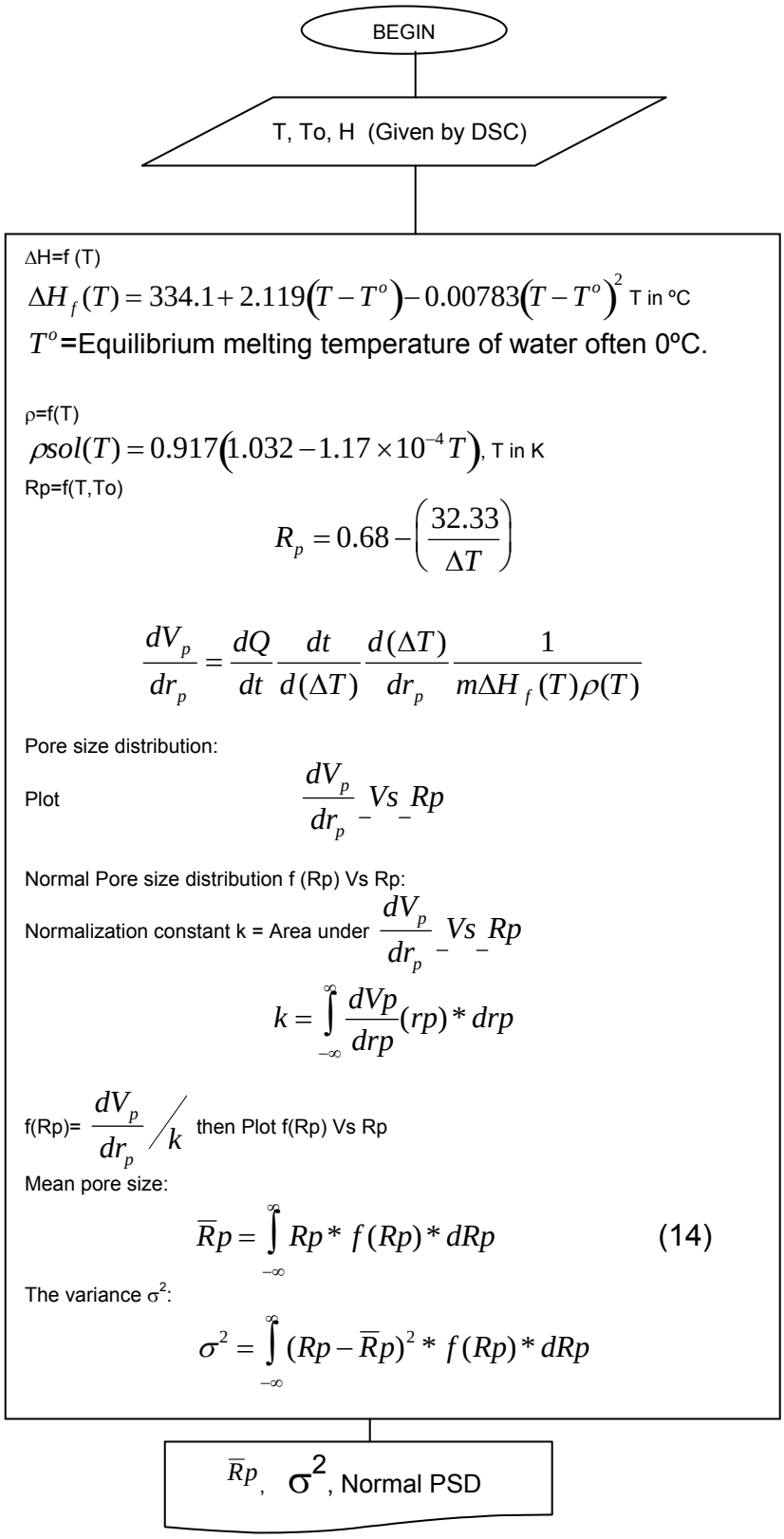


Figure 8. Flow chart: Normal Pore Size Distribution.

2.5 EFFECTS OF REACTION MEDIUM ON THE RADICAL POLYMERIZATION AND COPOLYMERIZATION OF ACRYLAMIDE.

It has been shown that the free radical polymerization and copolymerization of acrylamide and the properties of the resulting polymers depend considerably on the pH of the medium, the nature of solvent, the mode of initiation, concentration of monomer, addition of complexing agents and surfactants and other factors (Kurenkov and Myagchenkov 1979) [12].

(Kurenkov and Myagchenkov 1979)[12] summarized the data about peculiarities in the kinetic of the radical polymerization of AAm in various media. It is particularly necessary to note that, by varying some parameters (Temperature, nature of solvent, pH, complexing agents, surfactants, etc.), all stages of the radical polymerization (initiation, propagation, transfer and chain termination) can be affected. The effect of reaction medium shows change of the character and and intensity of interactions in the systems monomer- solvent and monomer-monomer.

(Gelfi and Righetti 1981)[24] studied the effect of temperature and solvent in polymerization kinetic of polyacrylamide gels. When they used water at low temperatures (1-4ºC), they found opaque hydrogels; however at 10ºC in presence of solution urea (8M) as polymerization solvent the gels were completely clear. They concluded: a) Opaque gels are faulty , i.e, the distribution of polyarylamide chains within the matrix is non-homogeneous. b) Opaque gels are highly porous as compared to transparent gels. c) Opaque gels have lower elasticity than transparent gels.

3. EXPERIMENTAL SECTION

Polyacrylamide gels (different %T-%C formulations) prepared in presence of water, buffer 1x-TBE and buffer 1x-TBE-urea were prepared under different polymerization conditions in order to rationally select hydrogels that possess properties optimally suited for DNA electrophoresis.

3.1. Materials

AAm, Bis, Urea, TEMED and 10x.Tris-Borato-EDTA (TBE) all electrophoreis grade were obtained from BioRad Laboratories (Hercules, CA). APS was obtained from Mallinckrodt Baker Inc (Paris, KY).

3.2. Buffer solution 1x-TBE

To prepare 100 ml of buffer 1x-TBE solution, 10 ml of 10x-Tris-Borato-EDTA (TBE) were placed in a flask, and then deionized water was added until the total volume reached 100 ml.

3.3. INFLUENCE OF THE %T-%C AND POLYMERIZATION SOLVENT IN THE POLYACRYLAMIDE MEAN PORE SIZE AND PSD

3.3.1. Chemical polymerization

3.3.2. Preparation of the polyacrylamide gels

Monomer solution (10ml) was prepared by dissolving AAm and Bis in the appropiate amount (Table 2), and according to the study the volume was then made up to 10 ml with: deionized water, buffer 1x-TBE and buffer 1x-TBE with 3.6036g urea (6M). The monomer solution was degassed by nitrogen injection (10 min) and set in a vacumm chamber (25 in Hg, 30 min) prior to addition of the initiator system composed of freshly made up 10%(w/v) APS and TEMED. In order to polymerize 1 ml of monomer solution, 5μl APS and 1μl TEMED were

added to the monomer solution. The polymerization was then allowed to proceed at 294.03 K in the rheometer plate prior rheological measurements were taken.

Table 2. Amount of acrylamide (AAm) and bis-acrylamide (Bis) to prepare 10 ml of monomer solution.

%T	**6**	**9**	**9**	**9**	**9**	**9**	**12**
%C	**5**	**1**	**3**	**5**	**7**	**9**	**5**
AAm (g)	0.5700	0.8910	0.8730	0.8550	0.8370	0.8190	1.1400
Bis (g)	0.0300	0.0090	0.0270	0.0450	0.0630	0.0810	0.0600

3.4. INFLUENCE OF THE %T-%C, POLYMERIZATION SOLVENT AND UV LIGHT INTENSITY IN THE POLYACRYLAMIDE MEAN PORE SIZE AND PSD

3.4.1. Photopolymerization

3.4.2. Preparation of the polyacrylamide gels

The photochemically crosslinked hydrogels were polymerized in the same way as described for chemically crosslinked hydrogels, the only difference was the initiator system. In order to polymerize 1 ml of solution 5μl 10% (w/v) APS were added to a bullet which contains the monomer solution. The polymerization was then allowed to proceed at 294.03 K in the rheometer plate under different UV intensities (Table 3) prior rheological measurements were taken.

Table 3. UV light intensities used to polymerize the monomer solution.

% UV	**10**	**25**	**40**	**55**
mW/cm^2	150	375	600	825

3.4.3. Swelling Index

The amount of water absorbed and the degree of swelling (SI) of the hydrogels with similar mean pore size in photopolymerization were measured. Once the polyacrylamide hydrogel was made, it was dried in a 50ºC oven for 24 h, weighed, and then immersed in 10 ml of water at ambient conditions.

Every 20 min for 2 h, the gel was removed, blotted with paper to remove any excess surface water, weighed (swollen), and returned to the water. The swelling index (SI) was calculated using equation 15.

$$SI = \frac{weight(swollen) - weight(dried)}{weight(dried)} \quad (15)$$

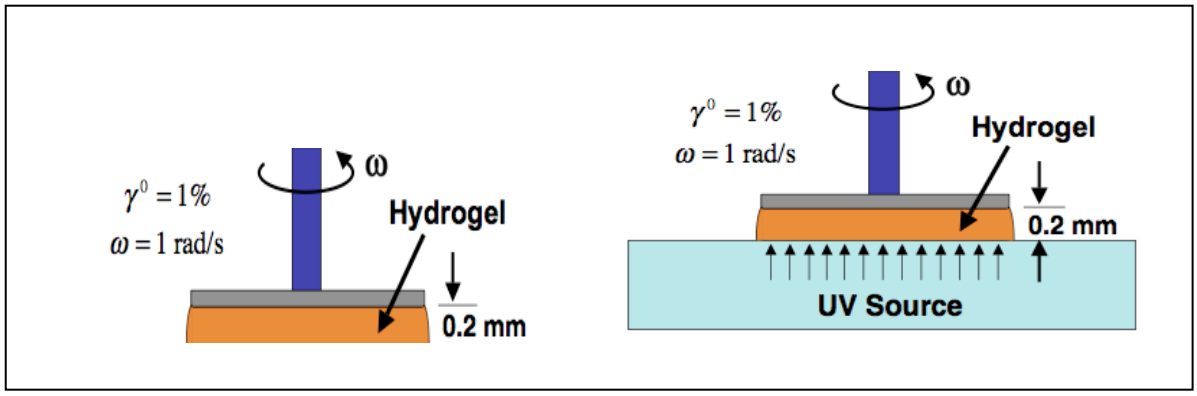
3.5. INSTRUMENTATION AND MEASUREMENTS

3.5.1. In situ rheology

Oscillatory frecuency sweep and strain amplitude sweep experiments were first performed in the range of 0.01-100 rad/s and γ^o = 0.1-50% respectively, in order to establish the extent of the linear viscoelastic regime. Based on these data, all subsequent experiments were performed at a strain amplitude γ^o = 1% and an angular frecuency of ω= 1 rad/s (Wang and Ugaz) [31].

Rheological experiments were performed using a Paar Physica MCR-300 (Anton Paar, Graz, Austria) equipped with a UV curing cell (photopolymerization experiments) incorporating a parallel plate geometry with a 25 mm-diameter upper stainless steel plate and an optically transparent lower base plate. An EXFO Omnicure Series 1000 UV source (EXFO, Vanier, Quebec, Canada) was used to control illumination intensity and exposure time. The maximun output of the UV source was 1.5 W/cm^2. A 0.2 mm gap setting was used to minimize the optical path length and ensure uniform UV penetration throughout the sample (0.17 ml loading volume). In chemical polymerization, the UV light source was turned off. The rheometer was equipped with a thermoelectric temperature control system.

Figure 9 shows the rheometer settings selected to polymerize the polyacrylamide hydrogels in chemical polymerization and photopolymerization.



a. **b.**

Figure 9. Rheometer Paar Physica MCR-300 settings. a. Chemical polymerization. b. Photopolymerization.

As soon as the polyacrylamide gels were synthetized, rheological measurements were taken to measure G'.

3.5.2. Thermoporometry experiments

Thermoporometry experiments were performed using a Perkin-Elmer Pyris1 Differential Scanning Calorimeter (DSC) equipped with a liquid nitrogen-cooling system. Once the polymer was synthesized in the rheometer and the rheological measurements (G') were taken, the gel was rinsed in water to eliminate residual reactants and allow the water to imbibe the nanopores. A 10-20 mg of sample was cut and placed in a pan with excess of water (2-5 μl bulk water). The pan was then sealed with a cover using a press. The samples were cooled to -40ºC and held for 10 min then heated from -40ºC to 15ºC at 2ºC/min. Once the sample was tested, it was placed in an oven at 50ºC overnight and then weighed to determine the dry sample weight.

4. RESULTS AND DISCUSSION

We have prepared different polyacrylamide hydrogel formulations (%T-%C) using water, buffer 1x-TBE and buffer 1x-TBE-urea as polymerization solvent to study their effect in the mean pore size and PSD. In situ rheology and thermoporometry were employed to characterize the nanostructure of photo and chemically crosslinked polyacrylamide gels to select those that possess properties optimally suited for DNA electrophoresis.

Gel compositions of 9%T with crosslinker concentrations ranging from X=1–9 %C were selected to be representative of typical formulations used in DNA electrophoresis. However, 6%T-5%C and 12%T-5%C polyacrylamide hydrogels were also studied to determine their effect in the mean pore size and PSD.

In photopolymerization and chemical polymerization, the evolution of the hydrogel network (viscoelastic properties) can be followed by observing the corresponding changes in the storage modulus G'. The G' was measured in real time during the gelation which took place directly between the parallel rheometer plates. G' was significantly larger than G'' characteristic of a well developed cross-linked polymer network [5].

In our research, we assumed complete conversion of the monomer to polymer. Factors such as the purity of the reagents used and ambiental conditions during the polymerization reaction will affect the accuracy of the results shown.

4.1 INFLUENCE OF THE %T-%C AND POLYMERIZATION SOLVENT IN THE POLYACRYLAMIDE MEAN PORE SIZE AND PSD

4.1.1 Chemical polymerization

In order to determine the dependence of %C for a given %T in the final nanostructure, gel compositions of 9%T with crosslinker concentrations ranging from X=1–9 %C were prepared and studied (Table 4 and Figure 10).

Table 4 shows the mean of three G' and the mean pore size obtained for different 9%T-X%C gel formulations polymerized in presence of water, buffer 1x-TBE and buffer 1x-TBE-urea. With G' data given in Table 4 and equation (5), the mean pore size (L/2) in the polyacrylamide network was calculated.

$$L = \left(\frac{1}{nN_A}\right)^{1/3} = \left(\frac{RT}{G'N_A}\right)^{1/3} \qquad (5)$$

Table 4. G' (Pa) and mean pore size L/2 (nm) evolution for different 9%T-X%C chemically crosslinked hydrogels polymerized in water, buffer 1x-TBE and buffer 1x-TBE-urea. Polymerization time 120 min.

Hydrogel 9%T-X%C	Water	Buffer 1X	Buffer 1X-Urea
9%T-1%C	6560 ± 398.80 L/2 = 4.26 ± 0.026	5373 ± 155.15 L/2 = 4.55 ± 0.028	6592 ± 195.3 L/2 = 4.25 ± 0.056
9%T-3%C	10100 ± 326.70 L/2 = 3.68 ± 0.017	9619 ± 190.00 L/2 = 3.74 ± 0.012	11208 ± 260.7 L/2 = 3.56 ± 0.027
9%T-5%C	12700 ± 278.90 L/2 = 3.41 ± 0.018	13443 ± 353.60 L/2 = 3.35 ± 0.012	13445 ± 251.7 L/2 = 3.30 ± 0.057
9%T-7%C	12600 ± 136.40* L/2 = 3.42 ± 0.020	10458 ± 243.00* L/2 = 3.64 ± 0.013	14142 ± 231.80 L/2 = 3.29 ± 0.009
9%T-9%C	11600 ± 170.11* L/2 = 3.52 ± 0.012	9767 ± 141.72* L/2 = 3.73 ± 0.010	16833 ± 187.46 L/2 = 3.11 ± 0.011

*** Cloudy hydrogels. Mean pore size data in Table 4 are shown in Figure 10.**

The dependence in the mean pore size in water and buffer 1x-TBE looks like a curve with a minimum in the 9%T-5%C hydrogel (Figure 10). For hydrogels polymerized in water and buffer 1x-TBE, the pore size decreases from 1-5%C and increases from 5-9%C. Nevertheless, in the presence of buffer 1x-TBE-urea the pore size decreases progressively with %C.

The 9%T-X%C hydrogels formulations studied allow selecting hydrogel in a range from 4.55 ± 0.028 nm to 3.29 ± 0.009 nm.

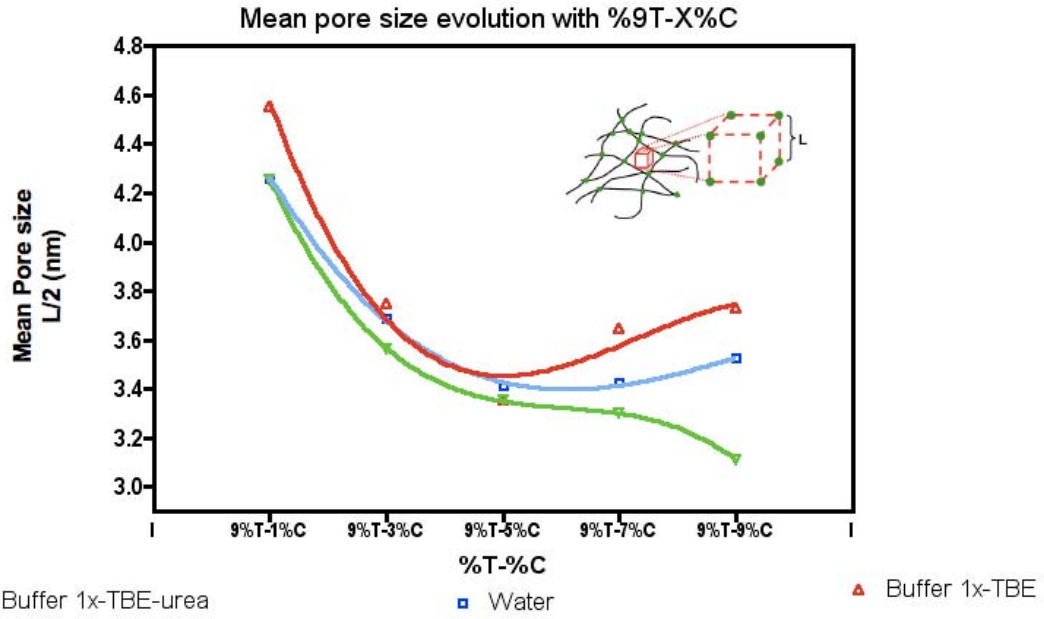


Figure 10. Mean pore size (L/2) for different 9%T-X%C chemically crosslinked polyacrylamide hydrogel. Polymerization time is 120 min.

The obtained results are in agreement with values presented by Kulicke and Nottelmann who observed in chemically crosslinked polyacrylamide hydrogels (polymerized in presence of water) a general behavior of G' as a function of %C. G' reached a maximum for %C ≈ 5, where the gel is considered as an "ideal" gel, i.e., exhibiting a maximal elasticity [5].

To investigate the dependence of %T with %C=cte, 6%T-5%C, 9%T-5%C and 12%T-5%C were polymerized and studied (Table 5).

Table 5. G' (Pa) and mean pore size L/2 (nm) evolution for 6%T-5%C, 9%T-5%C and 12%T-5%C hydrogels polymerized in presence of water, buffer 1x-TBE and buffer 1x-TBE-urea. Polymerization time 120 min.

	HYDROGEL (5%C)		
Solvent	**6%T**	**9%T**	**12%T**
Buffer 1x-TBE	G' = 4580 ± 127.43 L/2 = 4.80 ± 0.030	G' = 13443 ± 353.60 L/2 = 3.35 ± 0.012	G' = 19917 ± 234.87 L/2 = 2.94 ± 0.0078
Water	G' = 4900 ± 186.63 L/2 = 4.69 ± 0.041	G' = 12700 ± 265.74 L/2 = 3.41 ± 0.018	G' = 22000 ± 154.57 L/2 = 2.85 ± 0.0048
Buffer 1x-TBE Urea	G' = 5270 ± 238.76 L/2 = 4.58 ± 0.048	G' = 13445 ± 234.44 L/2 = 3.30 ± 0.057	G' = 29575 ± 178.94 L/2 = 2.58 ± 0.0037

For the chemically crosslinked polyacrylamide hydrogels studied the smallest mean pore size corresponds to 2.58 ± 0.0037 nm (12%T-5%C) and the largest to 4.80 ± 0.030 nm (6%T-5%C).

It was found in chemically crosslinked hydrogels that for a given %C as %T increases the mean pore size in the polyacrylamide network decreases.

(Rockland 2000) [25] shows that %T and %C have a direct relation to pore size of the chemically crosslinked hydrogel polymerized in water. As %T increases, the pore size decreases. Alternatively, for any %T-5%C formulation, the final nanostructure presents the smallest pore size. Pore size progressively decreases from 2-5%C and progressively increases from 5-50%C. It is important to remember that these calculations assume complete conversion of the monomer to polymer.

The similarity of the trends in the chemically crossllinked polyacrylamide hydrogels studied suggests, that one of the effects of the use of 1x-TBE-urea solution as polymerization solvent is to decrease the mean pore size of the resultant network (Figure 10, Table 4 and Table 5) and improve the optical properties (Table 4). The more elastic the hydrogel the smaller the mean pore size.

In general in photopolymerization or chemical polymerization, the polymerization process can be decomposed in three regimes, first there is a brief incubation period in which the system behaves as a viscous liquid (G'<G'') **(pregel reaction)** until the gelation point is reached **(gelation)**. After the onset of gelation G' increases more gradually toward an equilibrium value **(postgel reaction)** and G'>>G''. Pregel reaction leads to the formation of highly crosslinked microgel clusters formed by preferential cyclization of pendant vinyl and cross-link between monomers not associate with growing chain. These clusters contain excess Bis owing to its higher reactivity relative to AAm. During gelation, the pregel particles are linked by polymerized AAm chains that are poorer in Bis than the microgels; as polymerization proceeds, less crosslinker is available for incorporating within individual clusters resulting in the formation of a global gel network. The postgel reaction would correspond to the formation of dangling AAm molecules since most of Bis molecules have previously been consumed [5].

The propagating radical and AAm and Bis reactivity can be influenced by the polymerization solvent because of the interactions between monomer-solvent and monomer-monomer can vary in presence of each media. It is generally accepted that the relative reactivity of Bis is higher than that of AAm partly because of its poor solubility [3]. When the reaction is allowed to proceed in presence of buffer 1x-TBE-urea (a classical bond breaking agent) the solubility of AAm and Bis improve in comparison to water and buffer 1x-TBE used as polymerization media. As result, all H-bonds presents in the monomer solution

are disrupted so that the Bis concentration in solution is homogeneous, thus leading to regular gel and fully transparent gels. This explains the recently observed phenomenon that all gels, when polymerized in presence of urea present smaller mean pore size and behave more elastic (high G') than those polymerized in water and buffer 1x-TBE.

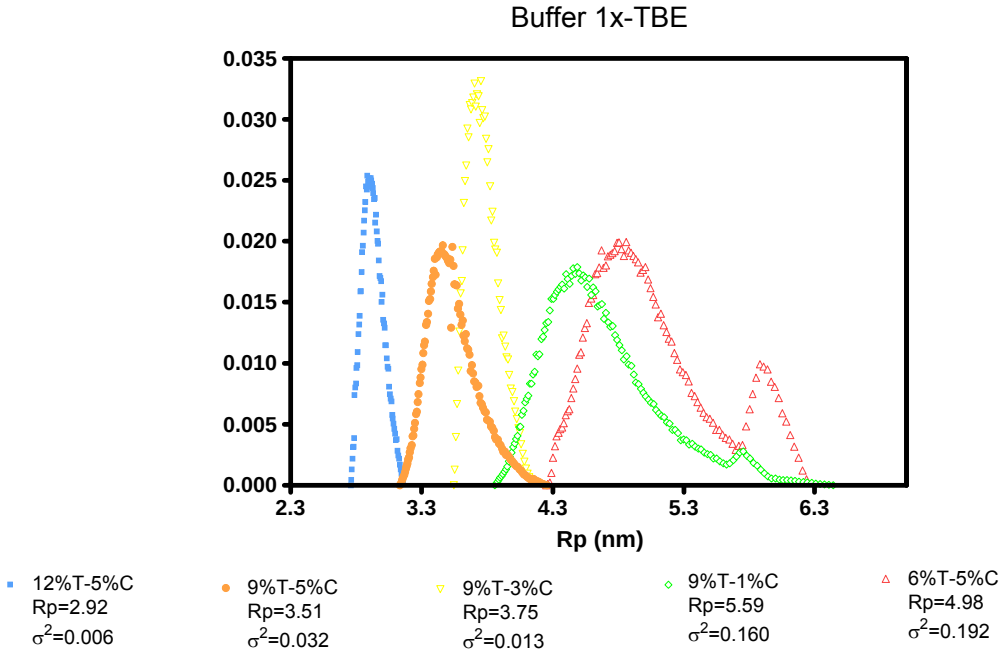
9%T-7%C, and 9%T-9%C polyacrylamide hydrogels were cloudy when polymerized in presence of water and buffer 1x-TBE, these inhomogeneities within polyacrylamide networks (opaque gels) are caused by the formation of highly hydrophobic and localized Bis sequences in the polymer chains. It is interesting to note that hydrogels prepared in presence of buffer 1x-TBE-urea (Table 3) are transparent, this suggest that the use of suitable buffer solution1x-TBE-urea as polymerization solvent results in improved solvatation of Bis during the polymerization process and consequently, the probability for the formation of highly concentrated Bis regions (cloudy gels) within the network is reduced.

In 6%T-5%C, 9%T-1%C and 9%T-3%C polyacrylamide hydrogels, both the Bis and AAm concentrations are relatively low. In consequence, not many microgel clusters are formed during the pregel reaction and the number of active network links decrease due to most of Bis and AAm is consumed in the cluster evolution and in the develop of the global polyacrylamide network. On the other hand, in a 12%T-5%C hydrogel a high number of microgel clusters and effective junction points are formed as a result of a high Bis and AAm concentration in consequence the degree of cross-linkage is elevated therefore a tighter network with lower mean pore size is obtained.

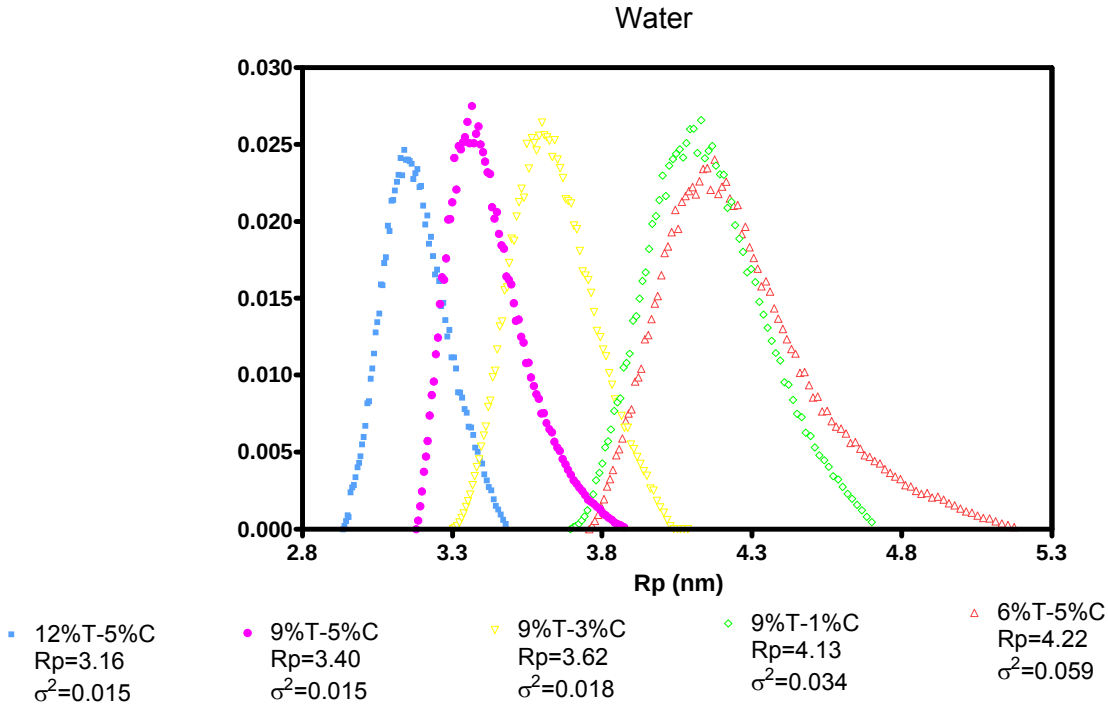
4.1.2. Thermopometry experiments

The normal pore size distribution and the mean pore size for different polyacrylamide formulations were obtained using the calorimetric curve given by the DSC and equation (14). The normal pore size distribution was then

transformed to obtain the pore size fraction distribution. The mean pore (R_p) size and the pore size fraction distribution for different polyacrylamide hydrogel formulations are shown in Figure 11.



a



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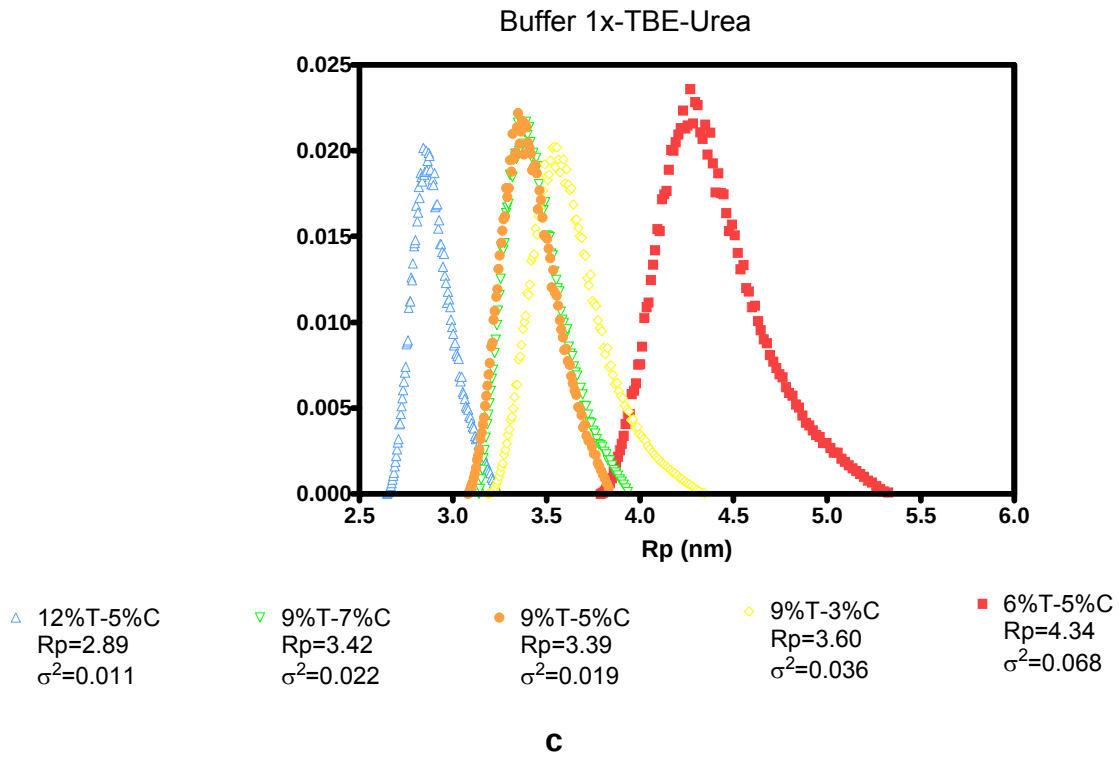


c

Figure 11. Pore fraction distribution for chemically crosslinked polyacrylamide hydrogels polymerized in: a. Buffer 1x-TBE. b. Water and c. Buffer 1x-TBE-Urea.

Hydrogels synthetized in buffer 1x-TBE seemed mechanically more fragile than those polymerized in water and urea possibly due to the sample preparation or pore water freezing leading to modification of the nanostructure. Figure 11a shows the presence of two peaks in 6%T-5%C and 9%T-1%C hydrogels perhaps due to the modification in the nanopores morphology.

In general, in all the polymerization solvents studied, the pore size distribution grew narrower as pore size decreases. The pore fraction distribution suggest that narrower distributions are obtained as the %T increases for a given %C or when %C ≤ 5% for a given %T likewise that the pore size decreases as was shown previously. As the PSD becomes narrow the variance (σ^2) decreases as well.

A polyacrylamide hydrogel with a small mean pore size implies the formation of a high number of microgel clusters rich in Bis during the pregel reaction and in the

same way the formation of a high number of active network links during the gelation and postgel reaction (high degree of cross-linkage), it means all the pores in the matrix will be closed firmly. A narrow distribution hints that all the pores obtained as result of assemble the clusters using polymer chains rich in AAm are gather orderly and the pore sizes are not as dispersed as in a broad distribution.

We postulate: 1. That an elevated cluster formation and high effective active network links originate polyacrylamide matrixes with small pore sizes and narrow PSD. 2. The pore size distribution will influence the properties of the gel and the electrophoresis performance.

Figure 12 shows a comparison between in-situ rheology and thermoporometry experiments. Possible deformation of the pore structure during the solidification process (freezing) can be produced during thermoporometry experiments. However, the mean pore size obtained using thermoporometry is consistent with rheology in-situ data obtained previously.

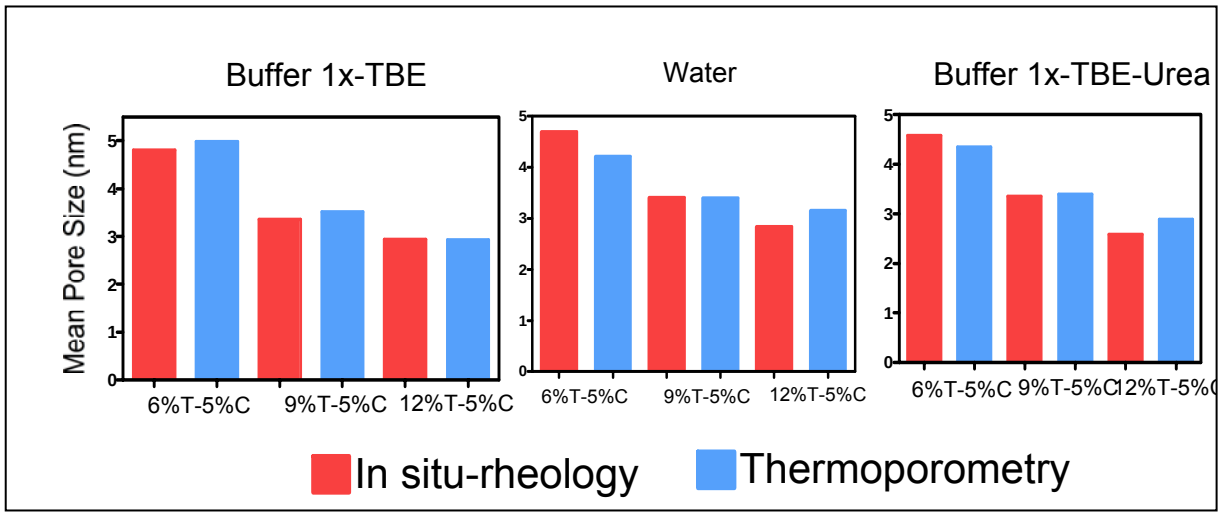


Figure 12. Comparison between the mean pore size obtained using in-situ rheology and thermoporometry experiments.

4.2 INFLUENCE OF THE %T-%C, POLYMERIZATION SOLVENT AND UV LIGHT INTENSITY IN THE POLYACRYLAMIDE MEAN PORE SIZE AND PSD

Photopolymerized crosslinked polyacrylamide hydrogels are atractive sieving matrix formulations for DNA electrophoresis owing to their rapid polymerization times compared to chemical polymerization and the potential to locally tailor the gel pore structure through spatial variation of illumination intensity. This capability is especially important in microfluid systems, where photopolymerization allows gel matrices to be precisely positioned within complex microchannel networks. Separation performance is also directly related to the nanoscale and PSD, which is in turn strongly influenced by polymerization kinetics [30].

The UV light intensities used to polymerize the hydrogels are shown in Table 3 and were selected for being representative of typical formulations used in DNA electrophoresis and in microdevices applications.

Table 3. UV light intensities used to polymerize the monomer solution

% UV	**10**	**25**	**40**	**55**
mW/cm^2	150	375	600	825

4.2.1 Photopolymerization

Table 6 shows the mean of three G' and the mean pore size (L/2) for a 9%T-5%C gel polymerized in presence of water, buffer 1x-TBE and buffer 1x-TBE-urea under different UV light intensities. In the same way that in chemical polymerization with G' data the in Table 6 and equation (5) the mean pore size in the polyacrylamide matrix was calculated.

Table 6. G'(Pa) and mean pore size L/2(nm) evolution for a 9%T-5%C gel photochemically crosslinked under different UV light intensities. Polymerization time 50 min.

Solvent	UV 10%	UV 25%	UV 40%	UV 55%
Buffer 1x-TBE	5320 ± 98.34 L/2 = 4.32 ± 0.008	10100 ± 105.72 L/2 = 3.72 ± 0.012	10800 ± 126.65 L/2 = 3.52 ± 0.021	13800 ± 189.19 L/2 = 3.36 ± 0.031
Water	7023 ± 101.28 L/2 = 4.16 ± 0.016	10376 ± 203.11 L/2 = 3.65 ± 0.012	11523 ± 97.34 L/2 = 3.45 ± 0.018	14323 ± 164.23 L/2 = 3.28 ± 0.009
Buffer 1x-TBE Urea	8410 ± 102.43 L/2 = 3.92 ± 0.021	11300 ± 117.44 L/2 = 3.55 ± 0.031	13800 ± 99.71 L/2 = 3.32 ± 0.006	15100 ± 98.33 Rp = 3.22 ± 0.004

Mean pore size data in Table 6 are shown in Figure 14.

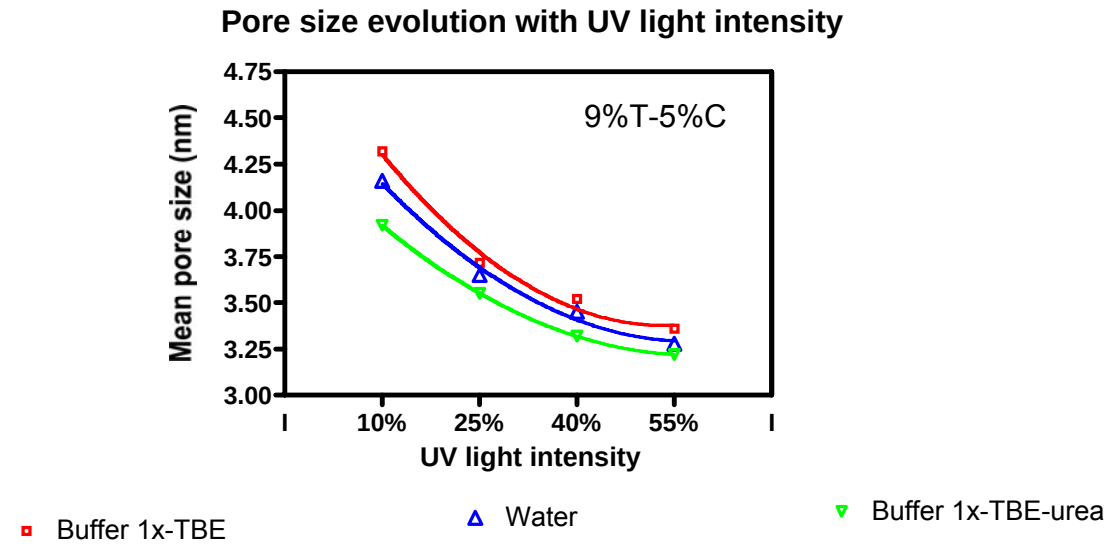


Figure 13. Mean pore size (L/2) in a 9%T-5%C photochemically crosslinked under different UV light intensities and polymerization solvents. Polymerization time 50 min.

A hydrogel with 9%T-5%C photochemically crosslinked under 10, 25, 40 and 55% UV light intensity allow selecting hydrogel ranging from 3.22 ± 0.004 nm to 4.32 ± 0.008 nm.

It was found that in each polymerization solvent, the increasing of UV light intensity leads to the increasing of gel elasticity and a decrease in the mean pore size. In each polymerization solvent and under the same %T-%C formulation, the pore size progressively decreases with a raise in the UV intensity. However, under the same polymerization conditions (%T-%C, % UV light intensity and polymerization time), gels polymerized in buffer 1x-TBE-urea present smaller pore size than those obtained in water and buffer 1x-TBE.

UV illumination strenght strongly influences the rate of polymerization throught its effect on the initiator decomposition rate. As the UV light intensity is raised the initiator decomposition rate increases and the free radical formation as well. AAm and Bis will be consumed during the microgel cluster formation therefore a raise in the UV light intensity will promote a high cluster formation and improve the polyacrylamide network elasticity (G') due to high and effective entanglement points are formed.

In general, hydrogels prepared using 1x-TBE-urea as polymerization solvent under high UV light intensities present lower mean pore size than those polymerized in water and buffer 1x-TBE at low UV light intensity.

In order to study the solvent effect and %T-%C dependence in the final nanostructure, different photochemically crosslinked hydrogels were synthetized under the same UV light intensity.

Table 7 shows the mean of three G' and the mean pore size for different %T-%C photochemically crosslinked hydrogels polymerized in presence of water, buffer 1x-TBE and buffer 1x-TBE-urea under 55% UV light intensity. In bold, the study of the increase in %T for a given -5%C in the gel elasticity G' and mean pore size.

Table 7. G' (Pa) evolution for different %T-%C photochemically crosslinked hydrogels polymerized under 55% UV light intensity in water, buffer 1x-TBE and buffer 1x-TBE-urea.

Hydrogel	Buffer 1X	Water	Buffer 1X-Urea
6%T-5%C	**5350 ± 201.23 L/2 = 4.56 ± 0.034**	**5394 ± 198.32 L/2 = 4.54 ± 0.012**	**5420 ± 188.4 L/2 = 4.54 ± 0.021**
9%T-1%C	5990 ± 121.32 L/2 = 4.39 ± 0.022	6024 ± 232.32 L/2 = 4.38 ± 0.03	6170 ± 234.87 L/2 = 4.34 ± 0.027
9%T-3%C	10500 ± 98.03 L/2 = 3.64 ± 0.011	10525 ± 103.23 L/2 = 3.63 ± 0.098	11000 ± 93.94 L/2 = 3.58 ± 0.01
9%T-5%C	**13800 ± 189.19 L/2 = 3.32 ± 0.02**	**14323 ± 164.23 L/2 = 3.28 ± 0.021**	**15100 ± 98.33 L/2 = 3.22 ± 0.015**
9%T-7%C	17900 ± 177.77* L/2 = 3.05 ± 0.023	18327 ± 129.43* L/2 = 3.02 ± 0.014	19000 ± 188.73 L/2 = 2.98 ± 0.018
9%T-9%C	21400 ± 223.19* L/2 = 2.87 ± 0.031	22378 ± 98.23* L/2 = 2.82 ± 0.003	24100 ± 200.22 L/2 = 2.76 ± 0.022
12%T-5%C	**22200 ± 101.13 L/2 = 2.84 ± 0.02**	**23276 ± 132.77 L/2 = 2.79 ± 0.018**	**24735 ± 88.92 L/2 = 2.73 ± 0.002**

* **Cloudy hydrogels.**

In each polymerization solvent and under the same UV light intensity, G' increases with %T for a given %C concentration but also increases as the concentration of %C increases for a given %T concentration.

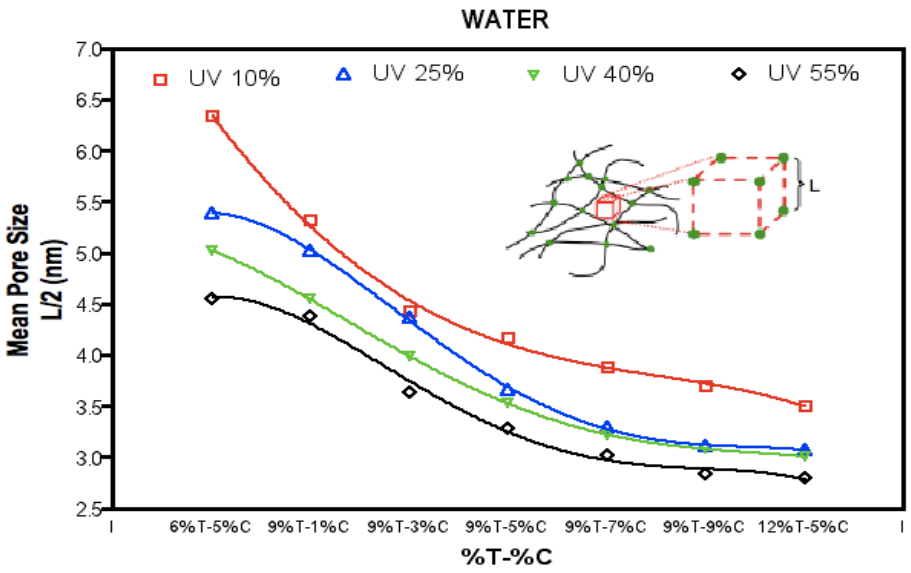
As soon as the UV light lamp is turn on, the initiator rate decomposition is initiated and likewise as UV light increases the free radical formed from APS does as well.

As in chemically crosslinked 9%T-7%C, and 9%T-9%C photochemically crosslinked polyacrylamide hydrogels were cloudy for all of the UV light intensities studied when polymerized in presence of water and buffer 1x-TBE (Table 7), these inhomogeneities within polyacrylamide networks (cloudy gels) are caused by the formation of highly hydrophobic and localized Bis sequences in the polymer chains. It is interesting to note that hydrogels prepared in presence of buffer 1x-TBE-urea are transparent, this suggest that the use of suitable buffer solution1x-TBE-urea as polymerization solvents results in improved solvatation of Bis during the polymerization process and consequently, the probability for the formation of highly concentrated Bis regions (cloudy gels) within the network is reduced.

In general, hydrogels with high AAm and Bis concentration (12%T-5%) polymerized at high UV ligh intensities will present small mean pore that those with low AAm and Bis (6%T-5%C, 9%T-1%C) polymerized because of the high cluster formation and their link with effective juntion points within the polyacrylamide network.

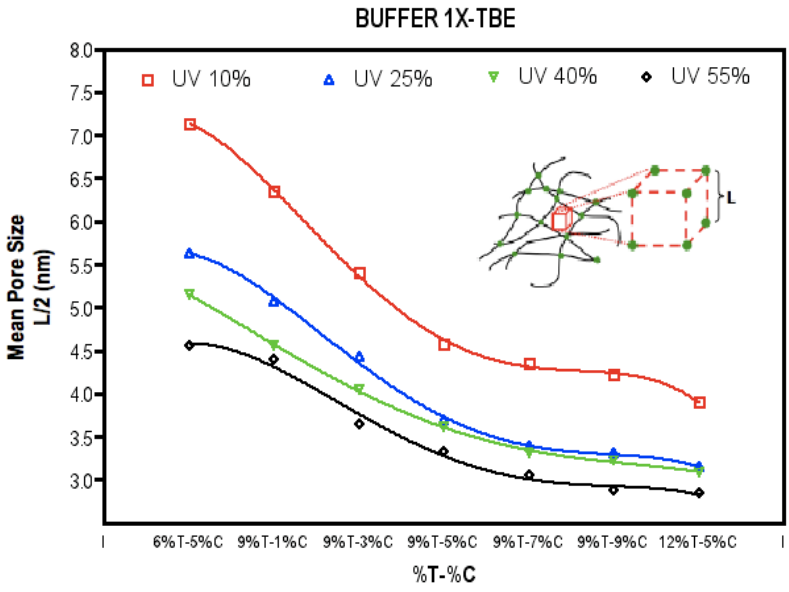
Figure 14 shows photochemically crosslinked hydrogels under different UV light intensities in: a. Water, b. Buffer solution 1x-TBE and c. Buffer solution 1x-TBE-Urea. Hydrogels with similar mean pore size can be produced under different gel formulations as is shown in the table below each graph in Figure 14.

Under low UV light intensity (below 25%), gels seemed mechanically weak. The similarity of the trends in the photochemically crossllinked polyacrylamide hydrogels studied suggests, that one of the effects of the use of buffer solution 1x-TBE-urea as polymerization solvent is to decrease the mean pore size of the resultant network.



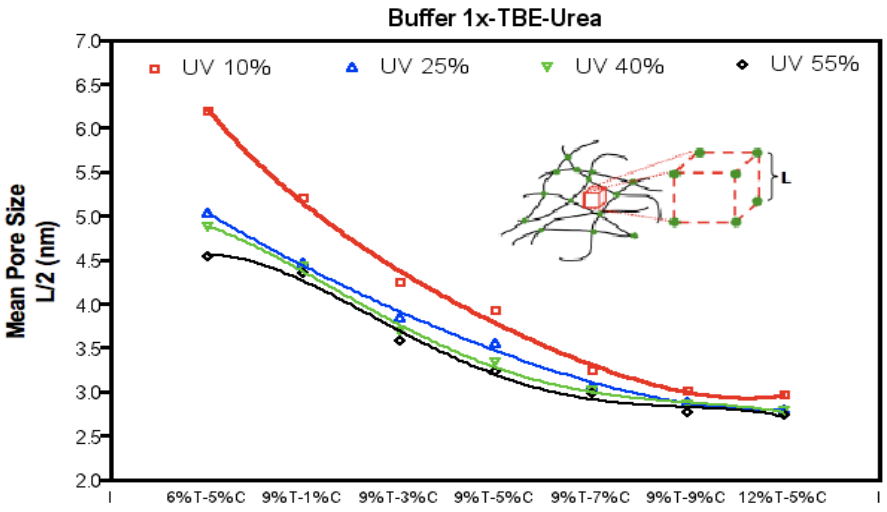
Hydrogel	UV %	L/2 (nm)
9%T-3%C	55	3.56
9%T-5%C	25	3.53

a



Hydrogel	UV %	L/2 (nm)
9%T-1%C	55	4.39
9%T-7%C	10	4.36

b



Hydrogel	UV %	L/2 (nm)
9%T-9%C	55	2.76
12%T-5%C	25	2.79

c

Figure 14. Photochemically crosslinked hydrogels under different UV light intensities in: a. Water, b. Buffer solution 1x-TBE and c. Buffer solution 1x-TBE-Urea. Polymerization time 50 min.

According with Figure 14, for the UV light intensities studied, there is no minimal in the pore size for a 9%T-5%C using water and buffer 1x-TBE as in chemical polymerization. At present, we are not able to offer a complete explanation, although it is possible that for both high UV light intensities and high polymerization time we obtain similar results to those exhibited in chemically crosslinked polyacrylamide hydrogels.

4.2.2. Swelling experiments

Figure 15 shows the obtained swelling index for photochemically crosslinked polyacrylamide hydrogels polymerized in presence of water, buffer 1x-TBE and buffer 1x-TBE-urea. Hydrogels with similar mean pore size were selected to investigate their behavior in swelling experiments.

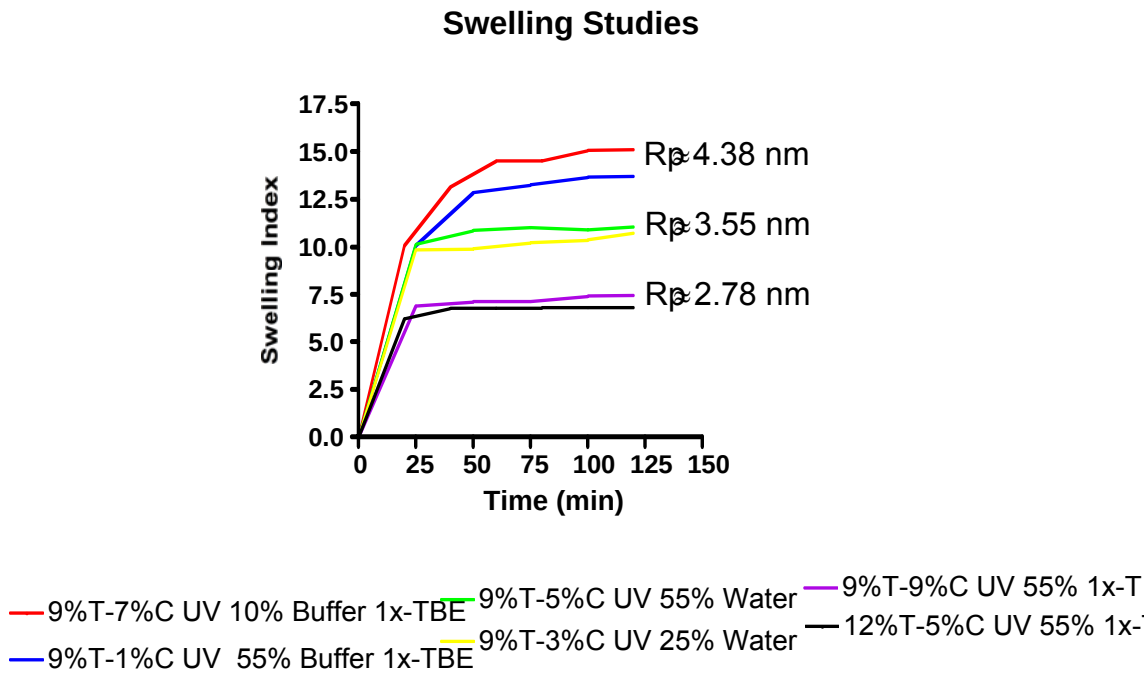


Figure 15. Swelling index for different photochemically crosslinked hydrogels that present similar mean pore size under different polymerization conditions.

Hydrogels with high swelling index present large mean pore sizes, likewise hydrogels with low swelling index present small pore sizes. It goes to show that the degree of swelling strongly depends on the degree of cross-linkage. The lower the degree of cross-linkage (large mean pore size), the more the gels swells. It is apparent that the observed swelling index cannot solely attributed to the mean pore size in the network, swelling experiments suggest that hydrogels with similar mean pore size can present different PSD (Figure 16).

a. **b.**

Figure 16. Hydrogels with similar mean pore size but different PSD. a. Broad PSD. b. Narrow PSD.

4.2.3. Thermoporometry experiments

The pore size fraction distribution and the mean pore size (R_p) for a 12%T-5%C hydrogel photochemically crosslinked hydrogel under different UV light intensities is shown in Figure 17.

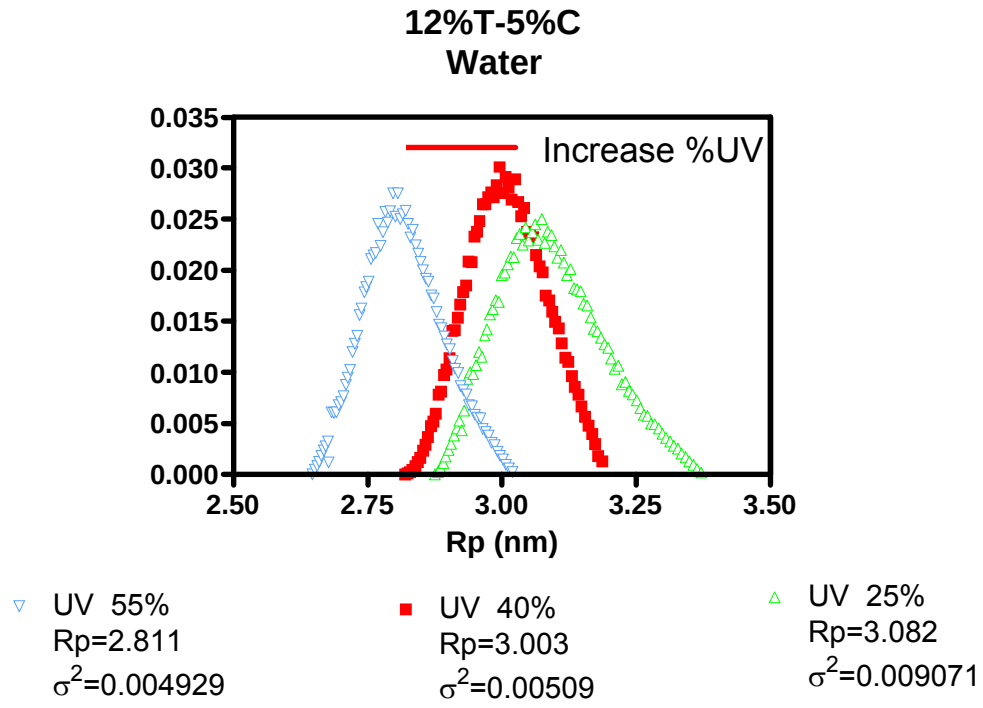
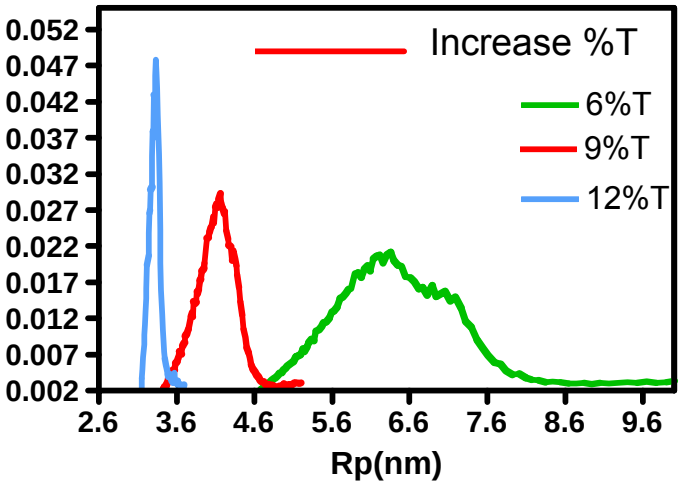


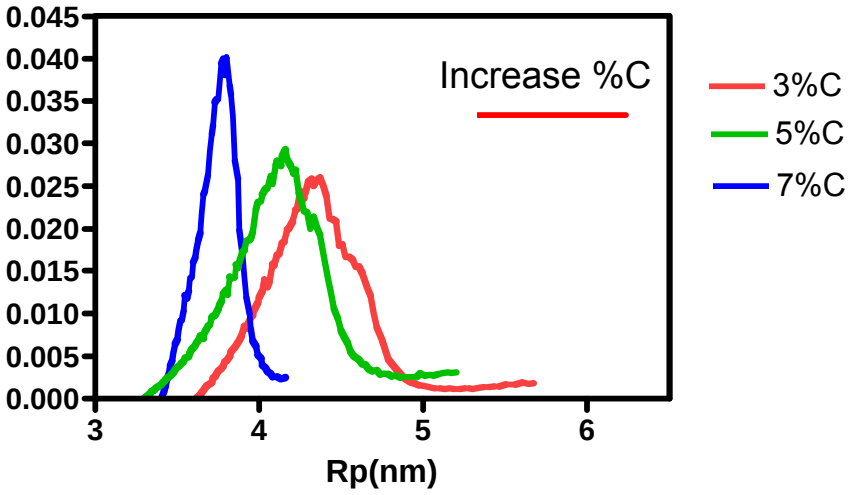
Figure 17. Pore size fraction distribution for a 12%T-5%C hydrogel polymerized in water under different UV light intensities. Polymerization time 50 min.

In general, the pore size distribution grew narrower as pore size decreases and the UV ligh intensity increases.

Figure 18 shows the pore size evolution for hydrogels polymerized under the same UV light intensity with an increase of %T and %C for a given %C and %T respectively.



a



b

Figure 18. Pore size fraction distribution for hydrogels polymerized under the same UV light intensity. a. Study of %T. b. Study of %C.

The pore size fraction distribution suggest that narrower distributions are obtained as the %T increases for a given %C or when %C increases for a given %T likewise that the pore size decreases in photochemically crosslinked hydrogels as was shown previously.

A polyacrylamide hydrogel with a small mean pore size implies the formation of a high number of microgel clusters rich in Bis during the pregel reaction and in the same way the formation of a high number of active network links (high degree of crosslinkage) during the gelation and postgel reaction. A narrow distribution hints that all the pores obtained as result of assemble the clusters using polymer chains rich in AAm are gather orderly and the pore sizes are not dispersed as in a broad distribution.

We postulate: 1. That an elevated cluster formation and high effective active network links originate polyacrylamide matrixes with small pore sizes and narrow PSD (High %T and %C and high UV light intensities). 2. The pore size distribution will influence the properties of the gel and the electrophoresis performance.

4.3. PHOTOPOLYMERIZATION Vs CHEMICAL POLYMERIZATION

Table 8 shows a summary of the advantages and drawbacks of using chemical polymerization and photopolymerization.

Table 8. Advantages and drawbacks between chemical polymerization and photopolymerization in DNA electrophoresis.

	Chemical Polymerization	**Photopolymerization**
Polymerization time	Hours	Minutes
Control of reaction rate	No	Yes
Applications	Not easy to manipulate in miniaturized systems	Suitable for miniaturized electrophoresis systems

Due to the UV light lamp can be turned off anytime (it means stop the initiator decomposition rate and so break the free radical formation) photopolymerization allows a direct control of the polymerization process. As a result, it is possible to

control the polyacrylamide network formation and select the suitable hydrogel according with the DNA separation. This is an important advantage compared to chemical polymerization where once the reaction has started there is no direct control in the initiator decomposition rate.

As we can see in Table 8, photopolymerized crosslinked polyacrylamide hydrogels are more atractive than chemically crosslinked polyacrylamide formulations owing to their rapid polymerization times and the potential to locally tailor the gel pore structure through spatial variation of illumination intensity. This capability is especially important in microfluid systems, where photopolymerization allows gel matrices to be precisely positioned within complex microchannel networks. In the last ten years there has been an explosion of advances in the fields of structured and intelligent materials science and nanomaterials properties.

The variety of chemical structures, together with the precise control of the molecular architecture and morphology, rationalize the numerous uses of polymer in high-technology and biological applications. Clearly, the future of bioseparation will be significantly influenced by microfabrication technologies and nanotechnology [23].

5. CONCLUSIONS

In situ rheology and thermoporometry are powerful characterization tools to determine the best polymerization conditions leading to select the desired structure and properties in polyacrylamide hydrogels.

In all gel fomulation studied, the use of buffer 1x-TBE-urea as polymerization solvent improve the elasticity (G') and the optical properties in the polyacrylamide network compared to water and buffer 1x-TBE as polymerization media.

%T-%C strongly affects the elastic properties (G') and structure (mean pore size and pore size distribution) in polyacrylamide hydrogels. Under the same polymerization conditions and polyacrylamide hydrogel formulation the solvent studied influence slightly the mean pore size and pore size distribution.

For the polyacrylamide hydrogel formulations studied it is possible to select gels for DNA electrophoresis ranging from 2.58 to 7.13 nm.

Different hydrogel formulations can present similar mean pore size but different pore size distribution.

RECOMMENDATION

In the Escuela de Ingeniería Química of Universidad Industrial de Santander there is a differential scanning calorimeter (DSC) which could be used to carry out thermoporometry experiments which allow us to characterize different materials such as biomaterials, asphalts, catalysts, polymers, ceramics, etc., besides, it is possible to study their behavior under low temperatures. However, it is necessary to assemble a cooling system to the DSC, which could be obtained if different research groups show interest on it and if they attempt to get it.

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