

EFFECT OF IONIC STRENGTH ON SWELLING OF GELATIN HYDROGELS IN MARGINAL SOLVENTS

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ABSTRACT

Swelling behavior of gelatin hydrogels prepared in different concentration of NaCl suspended in ethanol-water marginal solvent was studied at room temperature (20⁰C). The swelling ratio was measured by measuring the change in the mass of the gel to the original gel mass. It was found that the gel containing 0.01M NaCl shows more swelling than the normal gel without salt which infer that the salt concentration gives strength to the gels which swells more than normal gels. The laser light scattering technique was used to estimate the mesh size of swollen gel in marginal solvent and effect of ionic strengths were analyzed. It was also observed that electrostatic interaction helps in swelling (at low concentration of salt) as well as collapsing (at high concentration of salt) of gel network.

Keywords: Gelatin, Hydrogels, Swelling, Dynamic Light Scattering

I. INTRODUCTION

A typical gel is a mixture of a cross-linked polymer network and a solvent having non zero shear modulus [1]. In a gel, the liquid (solvent) prevents the polymer network from collapsing into a compact mass and the network in turn retains the liquid. Gels are of two types based on the formation, (1) physical gels [2-4]: The gels are formed and stabilized in the solvent by physical interaction mostly through hydrogen bonding, van der Waal forces, hydrophobic and ionic interaction for example gelatin, agarose etc, (2) chemical gels [4]: the gels formed by a reaction for example copolymerization, polycondensation, vulcanization etc leading to formation of a branched or a cross-linked network attached by covalent bonds and surrounded by large quantity of solvent. The gel can change its volume by taking in or expelling out the solvent or by some external forces. Such volume phase transitions occurring in both physical and chemical gels [3-4] have been observed in a variety of thermodynamical situations [5]. A small change in the environmental condition of supporting medium like temperature, pH, ionic strength, quality of solvent etc. leads to swelling or de-swelling of gel network. A detailed study of swelling and syneresis of gelatin gel at 5 ⁰C with various concentration of gelatin at acetate buffer at pH 4.7 were done in past [6].

A typical polyelectrolyte gel consists of a charged network of macroscopic size and having an abundance of counterions. The trapped counterions present inside the gel generate positive internal osmotic pressure in order to establish Donnan equilibrium with the surrounding solvent. Counterions are free in the solvent whilst they are trapped in the equilibrium gel to ensure macroscopic electrical neutrality. The osmotic pressure of these trapped

counterions with no counterpart in solution can be identified to be the main reason for giving rise to swelling. Effect of monovalent and divalent salt on osmotic properties of DNA gels was studied by Horkay and Bassar[7], which revealed that gels can maintain higher ion and polymer concentration without causing macroscopic phase transitions.

We have investigated previously the effect of swelling in marginal solvent at different pH. In the present work, we investigate the swelling and de-swelling kinetics of a gelatin hydrogel prepared in different ionic strength of NaCl suspended in water-ethanol binary solvent at slightly above than pI, since the degree of ionization is not known with the pH. Volume changes were monitored as function of time until equilibrium was observed, and mesh size was also estimated for swollen/shrunken gel using laser light scattering technique. The effect of swelling is presented in terms of scattered intensity correlation function, how the correlation function changes with swelling/de-swelling?

II. MATERIALS AND METHODS

Gelatin (300 bloom, type A, nominal pI \approx 4.9) bought from Sigma-Aldrich, USA, was used to prepare the samples. The molecular weight of gelatin was (100 ± 10) kD. The solutions were prepared by dissolving gelatin powder (5% w/v) in pre-heated (at 50 °C) water with different concentration of NaCl which was further stirred for 1 hr to produce a homogeneous solution. A small quantity of Sodium Azide (NaN_3) was added to the samples to prevent bacterial contamination. The solution pH was titrated to 5.2 for the good solubility of the gelatin. These samples were stored at room temperature (20 °C) overnight, allowing these to cool slowly through Newtonian cooling and which form gels. The gel samples appeared transparent to the eye and did not contain air bubbles, sometime in the course of preparation of solution of gelatin if the speed of stirrer is high then it form air bubble inside the gel after cooling the solution. A series of 6 marginal solvents of water-ethanol binary mixture (0, 10, 30, 45, 60, and 100 % of ethanol) was prepared. Six cubes of gel were cut each measuring 1.0 cm X 1.0 cm X 1.0 cm approximately. These were weighed on a Mettler micro-balance and released into the beakers containing the marginal solvents. Thus, we had one gel-cube in each separate solvent beaker. The beakers were sealed and stored at 20°C. The gel-cubes were taken out carefully at repeated intervals; the minute amount of solvent on the gel surface was carefully wiped with tissue paper without touching the surface, and weighed again to ascertain gain or loss in weight. These were returned to the solvents immediately following weighing. This procedure was continued till swelling equilibrium was noticed as evidenced from observation of invariance of weight of gel-cubes with time. Typically, a gel-cube was weighed 18 times during this period. The reported degree of swelling is defined as $w = (w_2 - w_1)/w_1$, where w_2 and w_1 are the swollen (at time $t = t > 0$) and dry weight (at time $t = 0$) of the gel sample. The value of degree of swelling less than one it means there is a de-swelling and more than one means that there is a swelling i.e. solvent were entered into or came out of the gels. Dynamic light scattering (DLS) experiments were performed (scattering angle = 90°, laser wavelength = 632.8 nm) on a 256 channel Photocor-FC (Photocor Inc., USA) that was operated in the multi-tau mode (logarithmically spaced channels). Small cubical shape gels were placed in different marginal solvent into the quartz cell and intensity correlation was measured. The data was analyzed both in the CONTIN regularization and discrete distribution modes (multi-exponential). The CONTIN software generates the average relaxation time of the intensity correlation function, which is solely related to Brownian dynamics of the diffusing

networks inside the gels. This yielded the apparent translational diffusion coefficient values. Correspondingly, the apparent mesh size of the network, ζ , at room temperature (20°C) was determined from the knowledge of translational diffusion coefficient, D_T which was obtained by fitting the intensity correlation function. These values were used in Stoke-Einstein equation, $D = k_B T / f$ with the translational friction coefficient, $f=6\pi\eta_0\zeta$ where k_B is Boltzmann constant, and η_0 is solvent viscosity.

III. RESULTS AND DISCUSSION

The nominal pI of gelatin is 4.9. Since the solubility of gelatin at this pH is very low we conducted swelling experiments slightly above pI (at pH= 5.2). At this pH there is ought to be an exact balance of positive and negatively charged sites on gelatin network. Typically elastic free energy and volume mixing energy is involved in the deformation of gel in a solvent. This would also imply that the co-ion and counter-ion concentration inside the gel is identical, which will be higher than the same in the surrounding solvent. Thus with respect to each of these two types of ions, there would be an osmotic pressure arising from Donnan effect. All the swelling of gel in marginal solvent data were presented in Figure 1 to Figure 2 corresponding to different concentration of the salt. Swelling and de-swelling behavior in different ethanol-water mixture for different ionic strength gel are summarized in table 1.

Interestingly near to the concentration of 45% ethanol concentration there was minimal volume change of the gel. Maximum swelling was observed in the solvent containing 0% ethanol. Swelling behavior was observed for spherical shaped gel where the swelling ratio increases/decreases exponentially [8], $r=r_0 (1-\exp(-t/ \tau))$, where ‘ τ ’ is characteristic time, and r_0 is the initial size of the gel. A similar behavior was observed for all cases of swelling, and also very similar behavior for de-swelling gel with different characteristic time constant.

De-swelling of gels was observed at above 45% ethanol concentration in our studies. For these gels the volumes shrunk by almost 50%. In the past, volume collapse in polyelectrolyte gels has been attributed to ion-pair formation with their aggregation to multiplets. In contrast to a polar medium where counterions are mobile inside the gel volume in solvents of low polarity, ion-pairing is facilitated between complementary ions by the low dielectric constant of the partially polar medium due to dipole-dipole interactions.

Table 1. Swelling /Deswelling of Gelatin Gel of Different Salt Concentration with Different Marginal Solvent

Ethanol NaCl	0%	10%	30%	45%	60%	100%
0M	S	S	S	NC	DS	DS
0.01M	S	S	S	NC	DS	DS
0.1M	S	S	S	NC	DS	DS
1.0M	S	S	DS	DS	DS	DS

S= swelling, NC= No Change, DS= Deswelling.

Table 2 Mesh Size of Gelatin Gel Measured by Dynamic Light Scattering in Marginal Solvent

NaCl conc.	0%Ethanol	100%Ethanol
	Mesh size(nm)	Mesh size(nm)
0M	187, 212,360	67,67,63

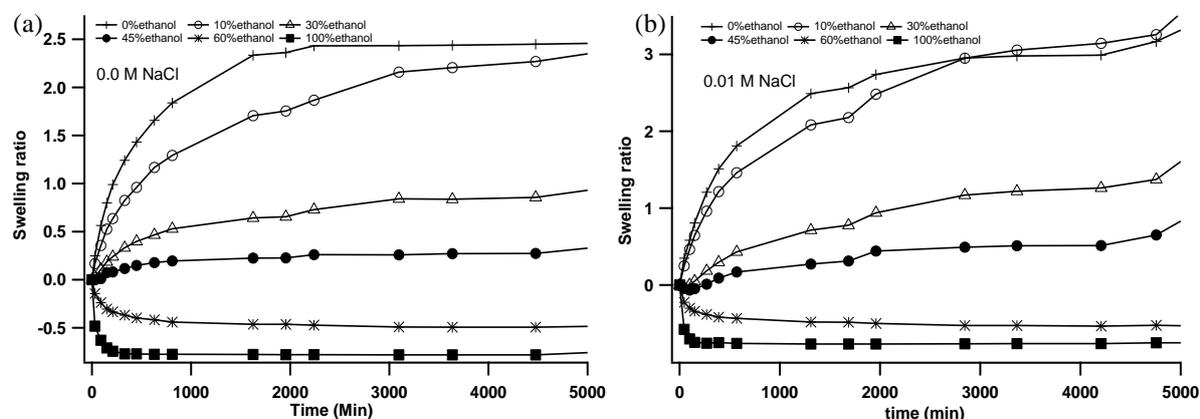


Figure 1 Swelling Ratio Versus Time (in min) for (a) 0.0 M NaCl and (b) 0.01 M NaCl

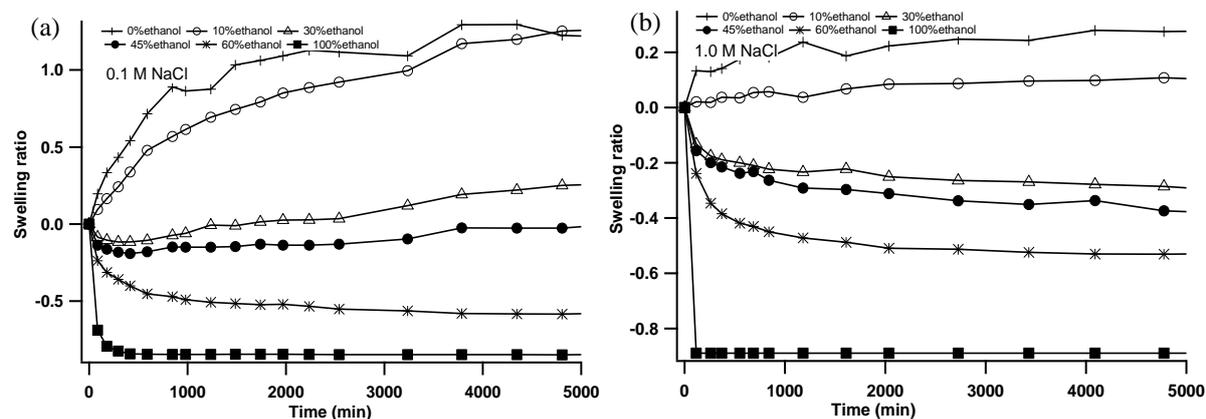


Figure 2 Swelling Ratio Versus Time (in min) for (a) 0.1 M NaCl and (b) 1.0 M NaCl

It is very clear from the graph (Figure 2b) for 1M NaCl solution that the de-swelling takes place after 30% ethanol concentration. It means that NaCl present in the solution screen the total charge present on the polymer chain. Thus the osmotic pressure due to the ionic part increases as the concentration of the ethanol increases from 30% onwards. It also means that the hydrophobicity due to the ethanol increases after this concentration. The dielectric constant of the ethanol-water solvent is less than the pure water solvent; this may also be the cause for the de-swelling of the gels from this concentration of salt as well as ethanol.

In our experiments, volume reduction in gel samples was observed for ethanol concentrations $> 45\%$ (v/v). The dielectric constant of water is 78.5 while that for ethanol is 24.3. Swelling behaviors was clearly observed in solvents containing ethanol concentration up to $\approx 45\%$ (v) that refers to solvent dielectric constant ≈ 60 . When the dielectric constant value of the solvents was less than 60, we started observing gel collapse regardless of the salt concentration. It can be argued that the critical degree of ionization of gel network is reached at ethanol concentration $\approx 45\%$. Thus, this ethanol concentration defines the boundary between swelling and collapse behavior of physical gels [9]. This type of volume phase transition [10-13] observed by our previous work in the course of simple coacervation.

Swelling/Deswelling of gel was quantitatively verified with the Dynamic Light Scattering experiment by measuring the network size during swelling/de-swelling time and in different marginal solvent. As the gel swells/de-swells the size of the network increases/decreases respectively. It is very difficult to measure the scattering from the gel in course of swelling or de-swelling because the dynamics of bound solvent so the system is not in ergodic equilibrium. So an average network size was estimated from correlation function at different time. The network sizes of the gel were estimated only for pure water (increasing size) and pure ethanol solvent (decreasing size). The pure ethanol is poor solvent for the all prepared gel in different salt approaches an equilibrium collapsed network size within an hour and no more change in the network size was observed. The intensity correlation function from gel in the pure water solvent is very much fluctuating compared to the pure ethanol solvent which describes the collapse of the network in poor solvent (see Figure 3 to Figure 4), also it was observed fluctuating correlation function for smaller salt concentration compared to higher salt content gels.

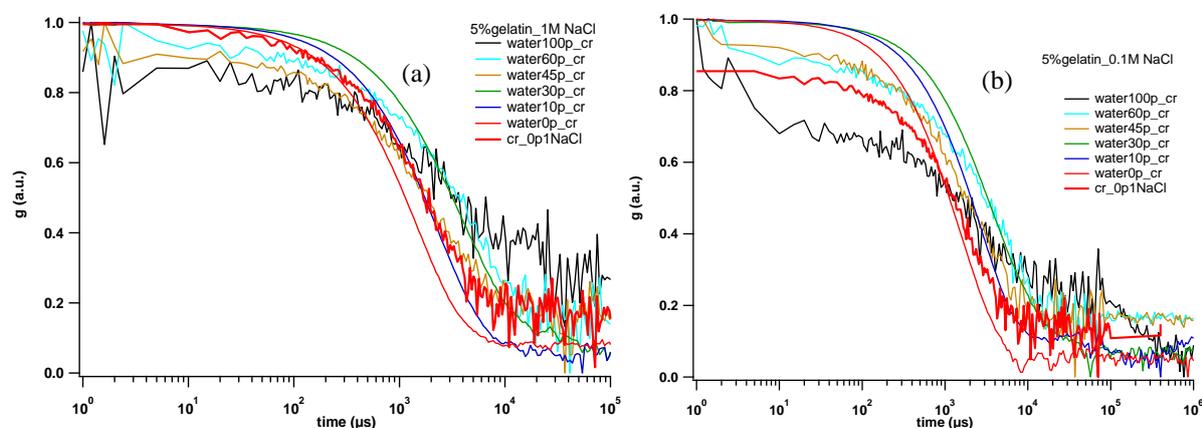


Figure 3. Intensity Correlation Functions from Gel Containing (a) 1 M NaCl and (b) 0.1 M NaCl.

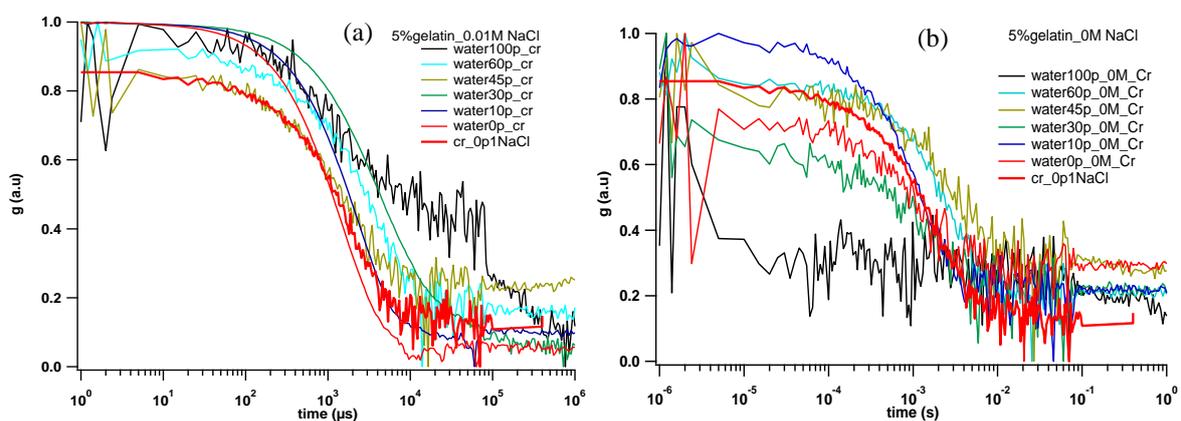


Figure 4 Intensity Correlation Functions from Gel Containing (a) 0.01 M NaCl and (b) 0.0 M NaCl

IV. CONCLUSION

Swelling and de-swelling equilibrium in gelatin hydrogels of different sodium chloride concentrations suspended in ethanol-water binary solvent was studied as function of varying ethanol concentration, which was a marginal solvent for gelatin. It was found that there was a normal swelling in gels in which there were no salt.

But in case of those gels which were prepared in salt solution shows very interesting results. As the concentration of salt was increased the swelling gets affected and it was found that for 0.01M NaCl gel which shows more swelling than the normal which infer that the salt concentration (0.01M NaCl) gives strength to the gels which swells more than normal gels in the same period of time. There may be a strong interaction between the gel biopolymer network and the salt, which tighten the network and last for longer time. Again we found that after the addition of more salt the trend is normal and there was regular collapse of gel network in case of 0.1M and 1M NaCl concentration in the same time domain. Gels prepared at normal pH exhibited swelling when ethanol concentration was less than $\approx 40\%$ (v/v). For ethanol concentrations greater than 40% (v/v), de-swelling or gel collapse was noticed in all gel samples. This behavior could be qualitatively understood through the formalism of ion-pair formation, and the consequent dipole-dipole interactions between the charged network and, counter and co-ions. Swelling/de-swelling of gel was quantitatively verified with the Dynamic Light Scattering experiment by measuring the network size with time and with different marginal solvent.

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