Swelling Behavior of Hyaluronic Acid Gels

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SYNOPSIS

Hyaluronic acid is a naturally occurring mucopolysaccharide. It is an important component of the intercellular matrix. It controls the permeability of tissues by means of the highly entangled network which can be formed because of its high molecular weight. To be able to use hyaluronic acid for several biomedical applications it is necessary to have an understanding of its swelling properties. Equilibrium swelling properties of hyaluronic acid gels were investigated by varying the temperature, pH, ionic strength, and composition of the surrounding solution. Swelling was found to depend on pH and ionic strength and to be reversible. The gel was highly swollen at high pH, but it shrank continuously as the pH was lowered to 10% of its maximum volume. The gel was comparatively insensitive to temperature. Drying affected the swelling capacity of the gel significantly. Gel swelling was also affected by a high concentration of acetone or propyl alcohol in the aqueous swelling solutions.

INTRODUCTION

In the last decade study of the swelling properties of hydrogels has expanded rapidly. As a result numerous applications of hydrogels have emerged. Hydrogels have been used as extraction solvents,[1] as controlled release systems,[2] and as mechanochemical devices,[3] and also have several other biomedical applications.[4] The success of these applications depends on the ability of the gels to adjust their volume while interacting with the surrounding medium. They swell or collapse depending upon various prevailing conditions such as temperature, pH, ionic strength, electric field, solvent composition, and pressure.[5]

Most of the research work on polymer gels is focused on synthetic polymeric gels thus neglecting the considerable variety of networks in natural polymers. Since the biological responses to polymer surfaces are complex, each polymer system should meet certain requirements for biomedical applications. Biocompatibility of the material is critical, and for some applications biodegradability is desirable. The natural gels are ideal candidates for these biomedical applications. For example, they could be used to encapsulate and cultivate cells inside the gel, where the network will act as a semipermeable membrane allowing only growth factors to enter to aid the growth of cells. The study of natural gels could be useful in the development of novel synthetic polymer networks that mimic natural gels,[6] and a knowledge of the response of natural gels to the changes in the environment could be invaluable for verifying the trends observed in the experimental behavior of synthetic polymer gels. The varying capacities for binding water and the underlying molecular structures may cause a natural network to behave differently to synthetic gels.

Here we report on the swelling properties of gels of hyaluronic acid, a naturally occurring polysaccharide. Hyaluronic acid is an important component of the intercellular matrix, the material filling the space between the cells of diverse tissues such as skin, muscles, and cartilage. It usually occurs as a sodium salt and is one of the large group of natural polyelectrolytes.[7] The molecular configuration and the structure of a repeating unit of hyaluronic acid are shown in Figure 1. The acidic pendant groups are positioned on both sides of the chain and allow for maximum access of water. Hyaluronic acid has a very high molecular weight and therefore forms a highly entangled network in the solution.[8] The hydration characteristics of hyaluronic acid in the aqueous solutions have been investigated by Davis et al.[9] Laurent[10] has demonstrated the superior exclusion properties of crosslinked gels of hyaluronic

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Journal of Applied Polymer Science, Vol. 45, 293–298 (1992)
CCC 0021-8995/92/020293-06$04.00

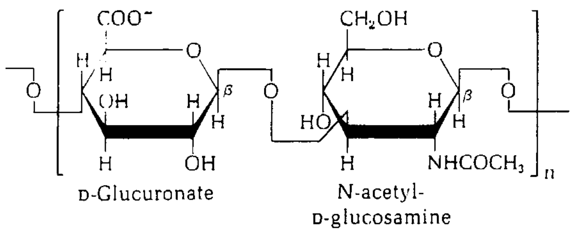

Figure 1 Repeating unit of hyaluronate. Hyaluronic acid is a linear polymer with alternating units of D-glucornic acid and *N*-acetyl-D-glucosamine connected by alternate β(1–3) and β(1–4) glucosidic bonds.

acid over those of dextran gels, using a gel filtration technique. These exclusion properties were found to be similar for hyaluronic acid gels and hyaluronic acid solutions.

Hyaluronic acid has been considered as a substance that has the potential to be safely used to coat artificial organs or to release drugs gradually into the body.[11] It can be engineered into any number of medically useful configurations, and can be utilized inside the spinal cord during surgery or to aid in eye surgery.[11] For the development of all of these applications, a knowledge of the swelling properties of crosslinked gels of hyaluronic acid is critical. Even though considerable research has been done on hyaluronic acid, the effects of various parameters such as temperature, pH, ionic strength, and solvent system on the swelling of the gels have not yet been reported.

EXPERIMENTAL

Materials and Methods

The sodium salt of hyaluronic acid (sodium hyaluronate) and divinyl sulfone (crosslinking agent) were purchased from Sigma Chemicals (St. Louis, MO) and were used as received. The reagents NaOH, HCl, NaCl, $CaCl_2$, acetone, and propanol were certified ACS grade, supplied by Fisher Scientific Co. (Fair Lawn, NJ). For all experiments Millipore-Q water system purified water (18 Megohm-Cm resistivity) was used.

Gel Preparation

Hyaluronic acid gels were prepared according to the technique developed by Balazs et al.[12] First a 4% solution of hyaluronic acid polymer was prepared in 0.2 *M* NaOH solution. Then divinyl sulfone was added to the solution to crosslink the polymer. After the addition of crosslinking agent the solution was mixed vigorously to homogenize it. The solution was allowed to cure in cylindrical glass molds of 2.5 mm i.d. The gels of different degrees of crosslinking were prepared using different amounts of divinyl sulfone. The degree of crosslinking is represented as crosslinking ratio, which is defined as the ratio of the amount of the polymer to the amount of divinyl sulfone. According to this definition, an increase in the crosslinking ratio means a decrease in the degree of crosslinking.

Kinetic Swelling Studies

The gel was removed from the mold and cut into small cylindrical rods. The radius of each rod was 2.5 mm while the length was 3 mm. To study the kinetics of swelling, gel samples, in triplicate, were immersed in 50 mL of water (pH = 5.5). At regular time intervals gel samples were taken out, wiped dry using fine weave filter paper, and weighed on an electronic balance. This was repeated until there was no further change in the weight of the gel sample. After equilibration swollen gel samples were placed into 0.1 *M* HCl solution, which caused gels to deswell. The deswelling was then followed by weighing the gel at various time. The reversibility of swelling and deswelling was determined using the same samples for consecutive swelling and deswelling experiments. The degree of swelling has been expressed as the swelling ratio, the ratio of final weight of the gel to initial weight of the gel.

Equilibrium Swelling Studies

Temperature Sensitivity Study

Several samples were immersed in 250-cm^3 Erlenmeyer flasks, each containing 50 cm^3 of water. The water temperature was adjusted and kept constant by means of a temperature controlled bath. After one day, each sample was taken out and weighed. The samples were then placed back into water which was then maintained at a temperature 5°C higher. This procedure was followed from 30°C to 60°C.

The effect of drying on the swelling of gel was determined by drying gel samples at 35°C for 12 h after their removal from the mold. These samples were then swollen in water. Similarly a few samples were first allowed to swell in water and were then dried as indicated before. These dried samples were then immersed in water to reswell.

pH and Ionic Strength Sensitivity Study

The pH sensitivity of the gel was studied in two different ways while increasing and decreasing the pH of the solution. In the first set, virgin gel samples, directly removed from the mold, were immersed in water and allowed to equilibrate. After several hours the weight of the sample and the final pH of the solution were measured. The pH was measured using an Orion pH meter (model SA 720) with gel combination electrode (91-05). The gel samples were then placed back in the water, the pH of which was adjusted by the addition of 0.01N HCl. In this way equilibrium data were obtained until the pH of the solution was sufficiently low. In the second set, the virgin samples were placed in a 0.1N HCl solution and then the pH of the solution was increased to determine an equilibrium value. During this experiment the effect of salt was tested by adding a known amount of NaCl (0.3 g/L) into the solution.

The effect of salt concentration was also studied by adding gel samples to solutions of uni-univalent (NaCl) and uni-bivalent ($CaCl_2$) salts.

Solvent Sensitivity Study

To determine the response of the gel to aqueous solutions of other solvents several experiments were done using aqueous solutions of acetone and n-propyl alcohol of various concentrations. The final equilibrium weight of all samples was obtained.

RESULTS AND DISCUSSIONS

The response of the gel in adjusting to its environment, or surrounding solution, is shown in Figure 2 for one cycle of swelling and deswelling. We can see that the gel reaches an equilibrium condition within one and one-half hours. The deswelling rate of the gel appears to be faster than the swelling rate. It is observed that the two different diffusion mechanisms are followed during the swelling and the deswelling of the gel.[13] During the collapse, the H^+ counter ions diffuse from the solution and associate strongly with fixed ionic groups on the gel network. Due to such interaction the fixed charges are neutralized and a nonionic shell forms, surrounding the ionic core. The neutralization reaction enhances the diffusion of the H^+ counter ions, thus accelerating the collapsing rate.

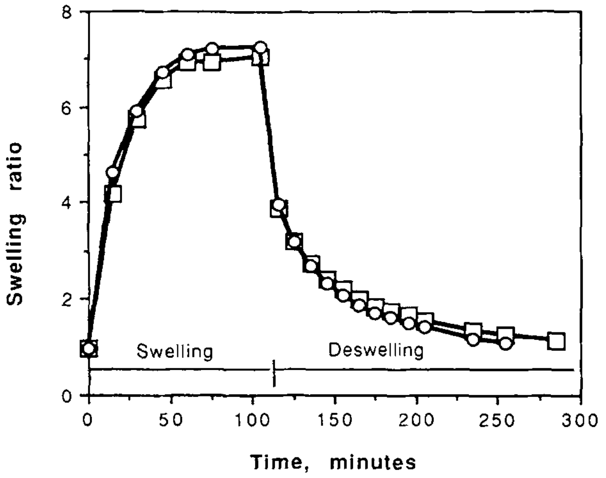

Figure 2 Swelling-deswelling cycle of hyaluronic acid gels (crosslinking ratio = 1).

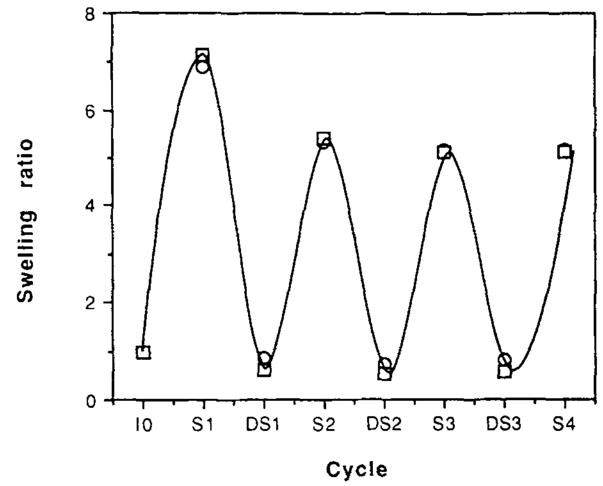

Figure 3 Reversibility of hyaluronic acid gel swelling: IO indicates initial condition; S_i = swelling cycle equilibrium condition; and DS_i = deswelling cycle equilibrium condition.

The ability of hyaluronic acid gel to undergo several cycles of swelling and deswelling is shown in Figure 3. We can see that after the first cycle the gel did not achieve the original swollen state but that in all of the following cycles it swelled back to its previous swollen state. Since the virgin gel was used without any prior washing, some salt might have been present which could have leached out upon deswelling, reducing the degree of successive swelling.

Due to its reversibility and rapidity of swelling, the gel could be considered as a mechanochemical system in which chemical ionization energy could be transformed directly into mechanical energy.[14]

During swelling the shape of the gel sample followed a repeatable pattern. Initially a swelling front moved inward separating the swollen surface layer and the unswollen inside core. As a result the sample assumed a dumbbell shape. Observation through an optical microscope showed the presence of stresses. The surface of the gel was full of cracks which disappeared after a while, yet the dumbbell shape was maintained, although it gradually changed back to a cylindrical shape. This behavior was observed during the swelling of all samples including dried samples. Similar observations are reported by Tanaka et al.[15] as mechanical instabilities during the swelling of polyacrylamide gel beads. It is concluded that the thin swollen layer would be in mechanical constraint due to the free outer surface and fixed inner surface. Hence, two opposing forces, one forcing the gel to swell and the other to remain unswollen would be resolved depending on the osmotic pressure developed.

The equilibrium swelling of gels with various degrees of crosslinking is shown in Table I. The swelling ratio shown is the mean value of the swelling of at least 20 samples. A range of swelling ratios for various samples is also shown in the brackets. The reason for the differences in swelling could be different degrees of crosslinking, which could have arisen due to polydispersity of the polymer, or to possible nonhomogeneous mixing of the crosslinking agent with the polymer solution. Hence, all the experiments were done with at least 10 gel samples. The equilibrium swelling increased with the increase in the crosslinking ratio. There was a relation between the degree of crosslinking (i.e., crosslinking ratio) and the equilibrium degree of swelling and mechanical integrity of the gel. With an increase in the crosslinking ratio, the extent of equilibrium swelling increased significantly but the integrity of the gel was much reduced. It would be necessary to use some grafting or coating to improve the mechanical strength at such a high degree of swelling.

In Figure 4 the temperature sensitivity of the gel is shown. This shows that the gel swells the most at 25°C, and that it shrinks continuously with an increase in temperature. Up to 60°C, the shrinkage is only 30% of its original swelled level. Similar temperature sensitive swelling behavior is reported by Huang et al.[16] for polyvinyl methyl ether gels, and by Freitas et al.[17] for poly(*N*-isopropyl acrylamide) gels. The study on the temperature dependence of hydration of hyaluronic acid in solution, has established the fact that in water, the hyaluronic acid has a negative temperature coefficient of hydration.[9] Hence, an increase in the temperature is accompanied by the loss of oriented or bound water. In aqueous solution at 25°C hyaluronic acid is closely associated with not less than nine molecules of water of hydration.[9] This result indicates that hyaluronic acid in solution or as a crosslinked gel shows similar behavior.

Table I Equilibrium Swelling of Hyaluronic Acid Gel at 25°C, in Water

Crosslinking Ratio	Swelling Ratio (range)
1	8 (6–9)
1.271	14 (12–18)
2.1	28 (25–30)
Higher	120

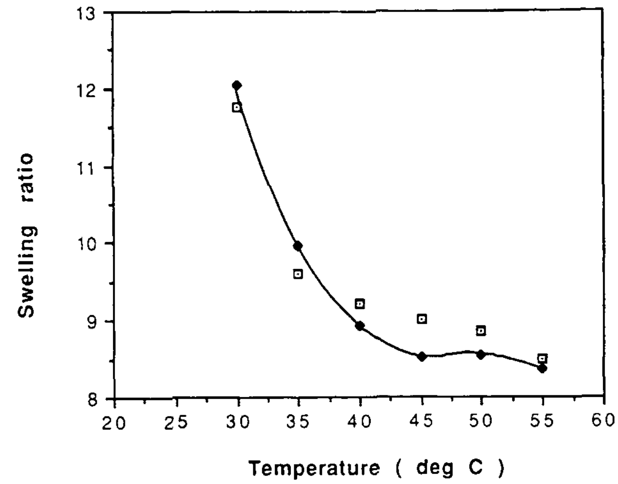

Figure 4 Equilibrium swelling of hyaluronic acid gels (crosslinking ratio = 1.271) at different temperatures.

The effect of drying on the swelling of the gel is reported in Figure 5. If the gel, after removal from the mold, is dried and then allowed to swell, the swelling is only 10% of the swelling observed if the gel is swollen directly. If the gel is allowed to swell first and then dried, it swells significantly yet is still affected by drying to some extent. This effect could be due to the introduction of some irreversible changes in the gel structure and in its water binding capacity by the drying process.

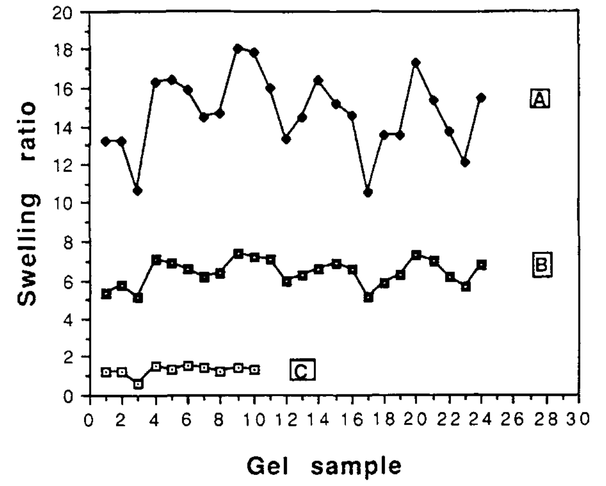

Figure 5 Effect of drying on the equilibrium swelling: A indicates swelling of the gel in water; B shows the reswelling of gel after drying; C indicates swelling when the gel was dried without any prior swelling.

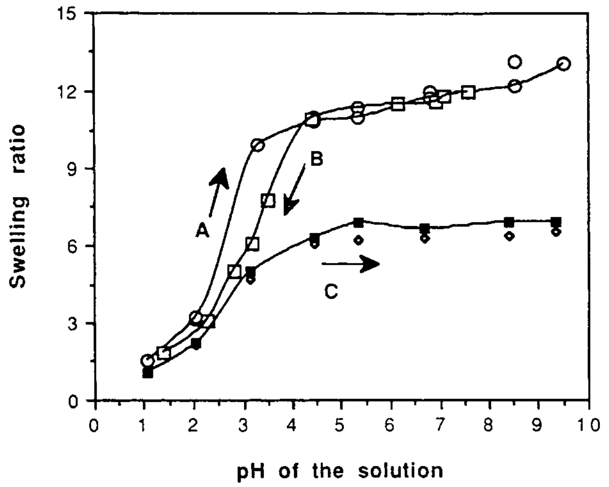

Figure 6 Equilibrium swelling of hyaluronic acid gels at different pH: curve A represents swelling when initial pH of the solution was lower; curve B represents swelling when pH was lowered from the initial higher pH; curve C indicates the swelling when some salt was present in the solution.

In Figure 6 the results of the swelling experiments with respect to changes in the pH of the solution are shown. Hyaluronic acid gel is fully swollen at pH 6 and above, while it is collapsed at lower pH. The largest part of the change in swelling occurs between pH 2 and 4 as shown. Similar pH sensitivity is observed for polyacrylamide and dextran gels by Cussler et al.[1] Polyacrylamide gels show maximum changes at pH 5–6, and dextran gels shift to higher volume at pH 2–3. In the figure equilibrium conditions of the gel with increasing and decreasing pH are shown. These experiments confirm the reversible nature of swelling and collapsing. The observed hysteresis is within experimental error.

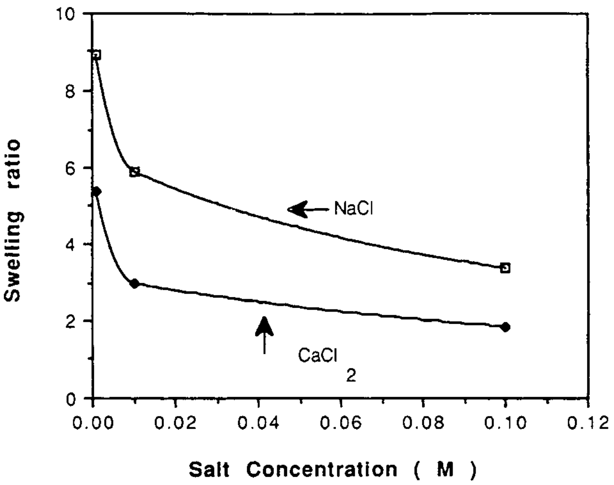

Figure 7 Equilibrium swelling in solutions of NaCl and $CaCl_2$ of different concentrations.

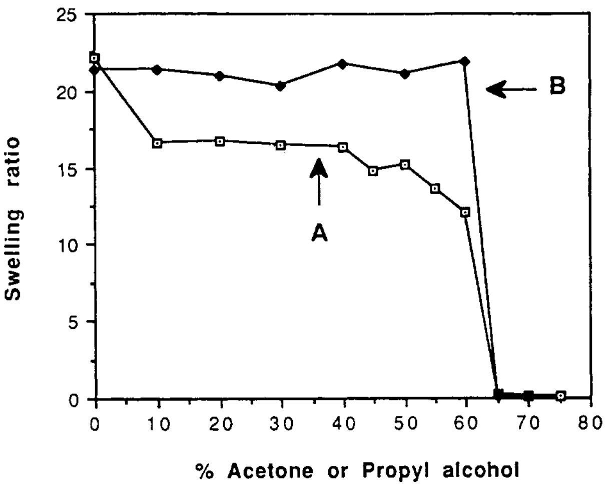

Figure 8 Equilibrium swelling of hyaluronic acid gels for different concentrations of acetone or *n*-propyl alcohol: A and B curves represent swelling for aqueous solutions of acetone and alcohol respectively.

The effect of salt on the swelling is also shown in Figure 6. It is observed that the presence of salt causes a 42% reduction in equilibrium swelling at pH 7. Hyaluronic acid gel has acidic carboxyl groups and it behaves as a hydrophilic gel while swelling significantly at neutral pH. Most of the carboxyl groups are ionized at higher pH (near 7) while at lower pH they become protonated. The intrinsic pK value of the carboxyl group of the hyaluronic acid has been found to be 3.21.[18] The counter ion concentration inside the gel network increases upon ionizaton of the gel. Due to higher concentration of the counter ions in the solution inside the gel, an osmotic pressure difference is generated between the solutions, internal and external to the gel network. This osmotic pressure is balanced by the swelling of the gel. The presence of salt ions causes a hydration sheath surrounding the polymer, with a consequent reduction in the degree of ionization and the equilibrium swelling.[19] The contraction of the hyaluronate chain on addition of a simple electrolyte also decreases the potential hydrogen bonding sites.[9] As shown in Figure 7 the swelling is affected more by uni-bivalent ($CaCl_2$) salt ions than by uni-univalent (NaCl) salt ions as only half number of cations are needed to maintain electroneutrality in the ionized gel network.[19] The ionic strength dependence of the swelling of the gel is found to be reversible.

The response of the gel sample in aqueous solutions of acetone and alcohol is shown in Figure 8.

The gel does not swell if the acetone or alcohol concentration in the solution is above 65%. This swelling appears to be irreversible, however, the swelling of other natural polymers like DNA (polynucleotide), gelatin (polypeptide) and agarose (polysaccharide) gels has been observed to be reversible.[20] The irreversible nature of the swelling indicates that acetone or propyl alcohol at high concentrations produces some irreversible changes in the gel structure.

CONCLUSIONS

The swelling of hyaluronic acid gel is reversible and dependent on pH and ionic strength. The gel is not much affected by temperature changes. For the size of the samples used in the experiments, swelling-deswelling is very rapid. Higher concentrations of acetone and alcohol produce the same major changes in the structure as are produced by drying. A gel with a low degree of crosslinking swells profoundly in water but its mechanical integrity is reduced with higher swelling. These observations are consistent with the observed behavior of hydrophilic synthetic hydrogels. The nature of the swelling of hyaluronic acid either in solution or in a crosslinked system appears to be similar. The pH and ionic strength dependence along with the ability to undergo rapid and reversible transitions could be very useful in the development of artificial muscle or physiologically sensitive drug delivery systems. The reversible nature of swelling along with the high degree of swelling of lightly crosslinked gels and their proven exclusion abilities are characteristics which would be useful in the extractions of biocomponents from dilute solutions by a modified gel filtration technique.

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Received January 21, 1991
Accepted July 24, 1991