

Networked polyuronic acid as a stimuli-responsive, mechanically stable, superabsorbent for controlled release of biologicals

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Introduction

Stimuli-responsive biomaterials swell, shrink or undergo phase or conformational transitions with environmental stimuli, such as pH, temperature, ionic strength, or presence of specific ions. So-called “smart polymers” have potential applications in targeted drug delivery, cosmetics, cell/protein encapsulation, tissue engineering, and as bioactuators¹. Alginate, a polyhexuronic acid glycoside, is one of the most abundant polysaccharides, and consists of (1→4)-O-glycosidic links of β-D-mannuronopyranosyl (M) and α-L-guluronopyranosyl (G) at varying sequential arrangements and proportions. Polyhexuronic acid can be covalently linked by di-aldehyde crosslinker into three-dimensional gel network via acetal bond formation. Owing to the nature of ionizable carboxylate moieties, polyhexuronic acid gel exhibits pH-responsive volume transition. The pH-sensitive hydrogels are of particular interest in the development of transmucosal pharmaceutical formulations for the delivery of therapeutic proteins. At acid environment, alginate gel contracts due to limited solubility of non-ionized acid moieties and protects protein from enzymatic and acid degradation in the gastric environment of the stomach (~ pH 1.2) while swells in alkaline environment as a result of increased hydrophilicity of ionized carboxylate moieties and releases active therapeutics in the intestinal lumen (~ pH 7.8).

The reaction between an alcohol and aldehyde to form an acetal and water is of considerable industrial interest and has variety of applications in areas such as perfumes, flavors, pharmaceuticals, plasticizers, rubbers and resins^{2,3}. According to IR spectra and X-ray diffractometry analysis, alginate can be crosslinked successfully with glutaraldehyde in the presence of acid catalyst⁴⁻⁷. This study is an attempt to provide the first available kinetic data for controlling the acetalization reaction and ultimately controlling the gel swelling and pore size.

Materials and Methods

Preparation of acid gel beads and covalent crosslinked polyhexuronic acid gel beads

Polyhexuronic acid solution of 4% (SF120, $F_G = 0.69$ and 308 kDa provided by FMC biopolymer, Brokeroya, Norway) was extruded dropwise via an automatic liquid dispenser into 0.05 M HCl, resulting in instantaneous acid gel beads. The sizes of polyhexuronic acid beads were controlled to 2.9 (± 0.1) mm by the inner diameter of the syringe needle, the coaxial airflow rate, and the distance between the tip of the needle and the capture solution. The gel beads were incubated in the supernatant solutions for 4 h prior to usage.

The resultant acid gel beads were suspended in reaction medium containing 2.54 M pentanediol (25% w/w EM grade packaged in 10 mL single dose ampoule and sealed under dry nitrogen, Sigma-Aldrich, Oakville, Canada) and 0.42 M HCl at the ratio of 70 % (w/v of polyhexuronic acid preformed acid gel beads to crosslinking reaction medium) at 40°C, unless otherwise specified. Chemical gel beads were removed from the reaction medium at specific time interval for the subsequent kinetic study whereby beads were dialysed against of 0.1 M, pH 7.8 NaCl solution for 72 hours and the swollen bead diameter were measured microscopically using Leica stereomicroscope (D3, Germany). The equilibrium swelling ratio, defined as the ratio of the equilibrium swollen gel volume to its initial dry state volume was calculated accordingly. Sample

mean and standard deviation were calculated based on 3 samples in each experiment. All data reported were the pooled mean and standard deviation of a minimum three repeated experiments, unless otherwise specified.

Results and Discussion

Correlation between crosslinking density, mesh pore size and equilibrium swelling ratio

A swelling model was developed to describe the deformation/swelling of a polyelectrolyte system in aqueous medium⁸. Since the aim of this study is to understand the kinetics of the network formation, a simple electrolyte swelling medium containing 0.1 M NaCl solution was used to reduce the degree of condensed ion effect. With a known external swelling condition, the crosslinking density and the resultant matrix pore size can be correlated from the equilibrium swelling data. The correlation between the equilibrium swelling ratio obtained experimentally and the calculated crosslinking density and matrix pore size is shown in figure 1. A 4% polyhexuronic acid bead had a maximum attainable equilibrium swelling ratio of 1000 at the critical gelling point, which corresponds to polyhexuronic acid concentration of approximately 1% w/v, demonstrating a superb water holding capacity. At this maximum swelling, the matrix pore size was estimated to be around 250 nm, and the minimum crosslinking density responsible for the network stability was about 0.014 mmole/cm³, corresponding to about 400 monomeric units between two crosslink points. If the reaction is allowed to proceed until the maximum crosslinking density, 1.75 mmole/cm³, corresponding to about 3 monomeric units between two crosslink points is achieved, the matrix pore size can expand to approximately 6 nm at equilibrium swelling gel volume in aqueous sodium chloride solution.

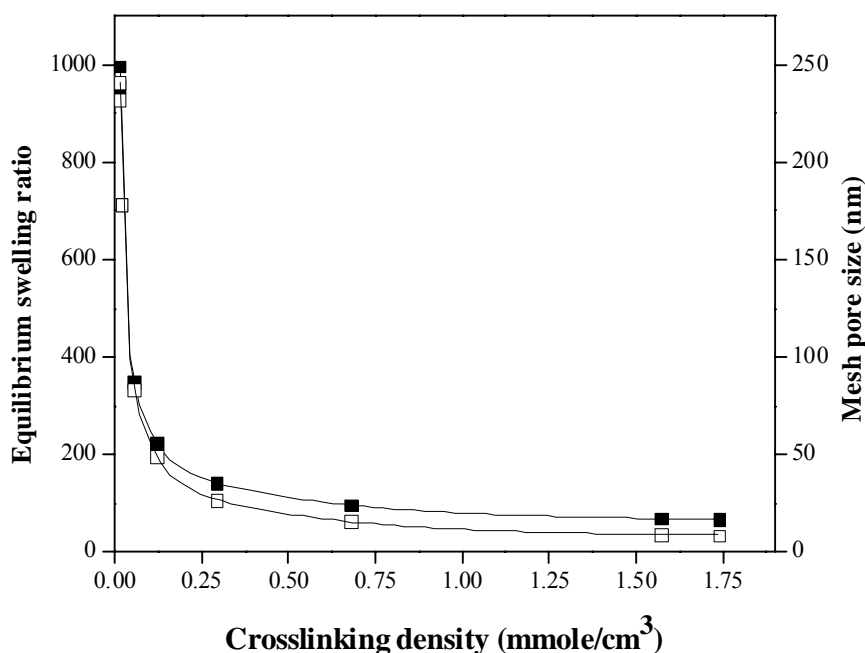


Figure 1: Correlation between the crosslinking density and equilibrium swelling ratio (left side ordinate, close symbols) and mesh pore size (right side ordinate, open symbols) of chemical hydrogel

Kinetics of the acetalization reaction and the rate equation

The acetalization kinetics can be described by the rate equation (equation 1):

$$\frac{\delta[R_2C(OR')_2]}{\delta t} = k[R-OH]^a [R-CHO]^b \quad \text{Equation 1}$$

where k is the rate constant and a and b are the reaction orders with respect to polyhexuronic acid and pentanedial concentrations and are determined experimentally.

In order to determine the reaction order with respect to hydroxyl concentration in polyhexuronic acid, the isolation method was used. In this method, the concentration of reactant of interest is made much smaller than concentrations of the other reactants. From creating a series of characteristic kinetic plots, a linear curve can be obtained by plotting the cumulative product concentration versus time, suggesting a zero-order rate reaction ($a=0$). A zero-order reaction has a constant rate which is independent of the concentration of the reactants and the reaction stops when the limiting reactants are completely consumed as observed in figure 2 where the product concentration or crosslinking density of 2 and 4% polyhexuronic acid beads increased linearly as the reaction proceeds. Both hydrogels exhibited high water holding capacity with a maximum attainable swelling ratio of 900 and 1000 for 2 and 4% polyhexuronic acid beads, respectively. It is apparent that under the same gel volume prior to the chemical reaction, the maximum attainable swelling volume can be increased by using gel beads containing a greater polymer fraction, thus a higher polyhexuronic acid concentration within the gel.

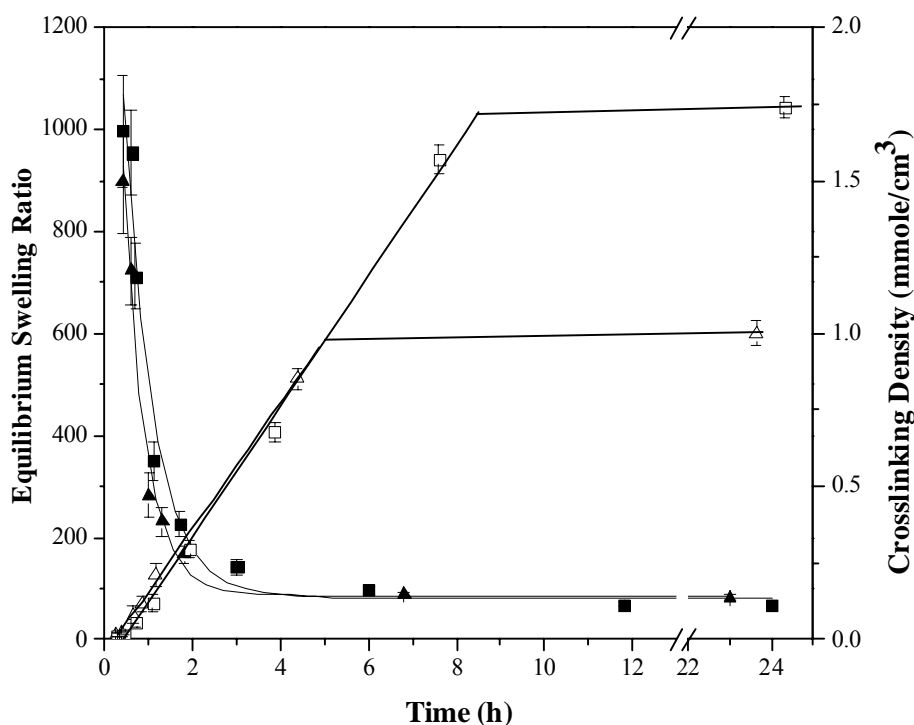


Figure 2: Zero-order reaction kinetics of polyhexuronic acid concentration with respect to network formation.

Polyhexuronic acid acid gel of 2 (triangle) and 4% (square) were suspended in reaction medium at 5% v/v in which di-aldehyde concentration is about 50 times greater than total hexuronic acid concentration.

The kinetics with respect to pentanedial concentration was studied by the method of initial rates which involved measuring the instantaneous rate of reaction before any significant changes in the reactant concentrations with various starting concentrations. A linear relationship can be obtained by plotting the square of initial effective pentanedial concentration, which is the normalized concentration accounting for the water content inside the gel beads, versus the inversed critical time required to form a stable network which is directly proportional to the rate of reaction (figure 3), suggesting a second order reaction with respect to pentanedial concentration ($b=2$). Both sets of data from experiments conducted using either 35 or 70% w/v beads to reaction medium lie on the same curve, indicating the bound water inside the gel beads diluted the pentanedial concentration initially present in the reaction medium, analogous to dilution with free water. A slope change at around 0.7 M is observed in figure 3 which may suggest a change of reaction

mechanism, possibly due to the occurrence of the side reaction of aldehyde degradation parallel to the acetalization reaction.

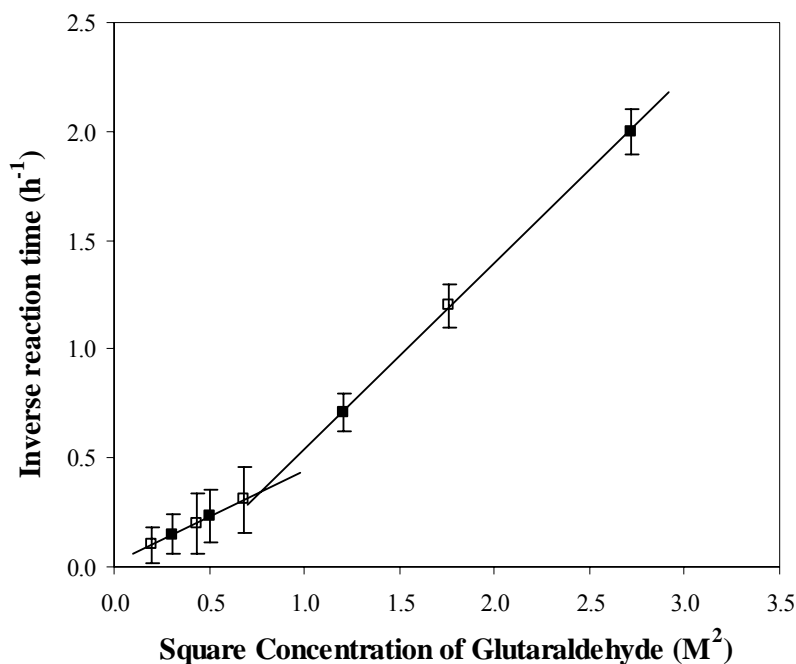


Figure 3: Second-order reaction kinetics with respect to pentanediol concentration in the formation of acetal linkage. The ratio of the reaction bead mass to reaction medium volume was 35 (close square) and 70% (open symbol) and HCl concentration was 0.42 M for all crosslinking condition. The effective pentanediol concentration was calculated based on the total amount of water present during the reaction which accounts for the diluent present in the hydrogel.

Conclusion

Network alginate hydrogel exhibiting pH-sensitive swelling characteristic was synthesized by acid catalyzed acetalization reaction between alcohols and aldehydes. The resultant hydrogel has a maximum equilibrium swelling ratio of over 1000, corresponding to about 1% of alginate solution. From the kinetic study, the acetalization reaction is an overall second order reaction of which reaction is independent of alginate concentration and is second order with respect to glutaraldehyde concentration. The rate constant determined using the proposed rate equation is $6.82 \text{ L}\cdot\text{mol}^{-1}\cdot\text{sec}$ for reaction carried out at 40°C . With the knowledge of the kinetic parameters, the gel swelling and pore size can be controlled by the extent of acetalization or crosslinking density, a potential encapsulation matrix for a wide range of molecular size biologicals in the development of transmucosal pharmaceutical formulations.

References

1. Kashyap, N. et al.(2005). *Critical Reviews in Therapeutic Drug Carrier Systems* (22)107-149
2. Aumo, J. et al. (2002). *Catalysis Letters* (84)219-224
3. Chopade, S.P. & Sharma, M.M. (1997). *Reactive & Functional Polymers* (32)53-65
4. Kulkarni, A.R. et al. (2002) *Journal of Applied Polymer Science* (85) 911-918
5. Kulkarni, A.R. et al. (2000) *Journal of Controlled Release* (63) 97-105
6. Park, J.K. et.al (1999) *Biotechnology and Bioengineering* (63)116-121
7. Yeom, C.K. & Lee, K.H. (1998) *Journal of Applied Polymer Science* (67)209-219
8. Chan, A.W.J. & Neufeld, R.J. (2007) *In submission*