Macromolecular Research, Vol. 11, No. 4, pp 291-295 (2003)

Notes

Swelling Behavior and Mechanical Strength of Crosslinked Dextran Hydrogel

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Received Mar. 19, 2003; Revised June 1, 2003

Abstract: Dextran as a candidate material for colon-specific drug delivery has been studied. Crosslinked dextran hydrogels were prepared by mixing dextran, $MgCl_2$, glutaraldehyde (GA) and polyethyleneglycol (PEG 400) in water. The dextran hydrogels were characterized by measuring equilibrium swelling ratios and mechanical strengths. Response surface methodology (Central Composite Design) was used to evaluate the swelling behaviors and mechanical strengths as functions of concentrations of $MgCl_2$, GA, and PEG 400, which was found to be useful for the evaluation. It showed that the swelling behavior and mechanical strengths were influenced significantly by PEG 400 and $MgCl_2$ concentrations.

Keywords: dextran, crosslinking, swelling, mechanical strength, response surface methodology.

Introduction

Among various drug carriers for drugs, polysaccharide hydrogels have attracted a lot of interest because they are natural, biodegradable, and nontoxic.[1] Dextran is a class of polysaccharides with a linear polymer backbone with mainly 1,6-α-D-glucopyranosidic linkages. It is obtained from bacterial cultures of *Leuconostoc mesenteroides* NRRL B-512. The glycosidic linkages are hydrolysed by moulds,[2] bacteria[3-5] and mammalian cells.[6]

The dextran is primarily utilized as a plasma extender,[7] although derivatives such as dextran sulfonate and dextran sulfate are used as anticoagulants.[8] Crosslinked dextran can be stable in the stomach and small intestine but degradable in the large intestine. Dextranase is an enzyme which hydrolyses glycosidic linkages in dextran. Dextranase activity in the colon is shown by anaerobic gram-negative intestinal bacteria especially *Bacteroides*.[9] Increasing interest is being focused on dextran prodrugs. First attempt was carried out by Harboe *et al.*[10] They conjugated naproxen to dextran by ester linkage. In another study, dextran with molecular weight 72,600 was used to deliver (dexamethasone) to the colon.[11] Apart from prodrug approach, biodegradable dextran hydrogels using diisocyanate as a crosslinking agent have been found to be fully degradable by dextranase in vitro and vivo in the rat cecum,[12-13] and glutaraldehyde-crosslinked dextran capsules were used for colon-specific drug delivery.[14]

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The objective of this study is to investigate swelling behaviors and mechanical strengths of dextran hydrogels composed of glutaraldehyde, polyethyleneglycol and $MgCl_2$.

Experimental

Materials. Dextran with the molecular weight of 513,000 (T-500), polyethyleneglycol with the molecular weight of 400 (PEG 400), $MgCl_2$, glutaraldehyde (GA), and dextranase (10 U/g) used as an enzyme were purchased from Sigma (USA). Phosphate bovine saline (PBS) was purchased from Gilcobrl (USA). All the chemicals were analytical grades.

Preparation of Dextran Hydrogel. Dextran was dissolved in deionized water and $MgCl_2$ and PEG 400 as a plasticizer were added to the solution. Crosslinking agent, GA, was added afterwards. The polymer mixture was poured into a Petri-dish (87 mm) and vacuum-dried for 6 hrs. After fabricating into disks of 10 mm diameter and 1 mm thickness, the dextran hydrogel disks were cured at 80 °C for 4 hrs and stored in a Temperature & Humidity control chamber(Sangwoo, Korea). Figure 1 shows schematics of preparation of dextran hydrogel.

Evaluation of Dextran Hydrogel. Equilibrium swelling of the dextran hydrogel disk was investigated under simulated intestine condition in pH 7.4 PBS. Dextran disks were immersed in the PBS solution at 37 °C. The initial disk weight (W_0) and the weight after 0.5, 1, 2, 3, 5, 7, 9 and 24 hrs of immersion in PBS solution (W) were measured with an analytical balance. Swelling ratio was calculated by $W_0/(W-W_0)\times 100\%$. Mechanical strength of the hydrogel was

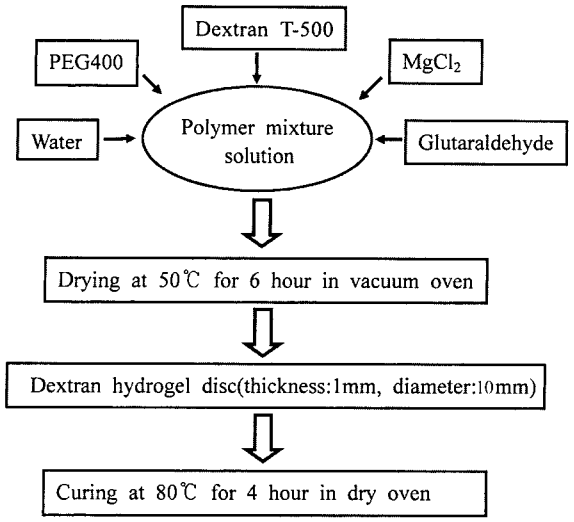

Figure 1. Schematics of preparation of dextran hydrogel.

evaluated using a Universal Test Machine (TIRA test 27025).

Experimental Design. Central Composition Design was established to investigate influence of the parameters such as GA, PEG 400, and $MgCl_2$ concentrations on the swelling behaviors and mechanical strengths of dextran hydrogels. This statistical analysis process basically involves three major steps: performing the statistically designed experiments, estimating the coefficients in a mathematical model and predicting the response, and checking the adequacy of the model. Suppose we code the levels in standardized units so that the values taken by each of the three variables X_1, X_2, and X_3 are -1, 0, and +1. Coded values were obtained by the following formula:

$$Z = (X - X^0) / \Delta X$$

where Z is the coded values, X is the corresponding natural value, X^0 is the natural value in the center of the domain, and ΔX is the increment of X corresponding to one unit of Z. For each factor, three levels were given and a second order was proposed. Data were analyzed by multiple regression to fit the following second order equation:

$$Y = B_0 + \sum_{i=1}^{k} B_i X_i + \sum_{i=j=1}^{k} B_{ij} X_i X_j$$

where Y = predicted response. It can be observed that three variables are involved and hence k takes the value 3. Thus, by substituting the value 3 for k, the equation becomes:

$$Y = B_0 + B_1X_1 + B_2X_2 + B_3X_3 + B_{12}X_1X_2 + B_{13}X_1X_3 + B_{23}X_2X_3 + B_{11}X_1^2 + B_{22}X_2^2 + B_{33}X_3^2,$$

where Y is the predicted response, X_1, X_2, X_3 are the input variables (GA, PEG 400, and $MgCl_2$ concentrations), B_0 is a constant, B_1, B_2, B_3 are linear coefficients, B_{12}, B_{13}, B_{23} are cross product coefficients, and B_{11}, B_{22}, B_{33} are quadratic coefficients.

The low, middle, and high levels of each variable, namely GA, PEG 400, and $MgCl_2$ concentration are coded as -1, 0, and +1 respectively as shown in Table I. Design Expert (Couresy: Stat-ease Inc., Statistics Made Easy, Minneapolis, USA) was used for regression analysis of the obtained data and estimation of the coefficients of the regression equation.

Table I. Central Composition Design Conditions

Run	Variable 1 Coded	Variable 2 Coded	Variable 3 Coded	X_1(GA(%))	X_2(PEG400(%))	X_3($MgCl_2$(%))
1	-1	-1	-1	35	2.5	2.8
2	1	-1	-1	45	2.5	2.8
3	-1	1	-1	35	9.6	2.8
4	1	1	-1	45	9.6	2.8
5	-1	-1	1	35	2.5	5.5
6	1	-1	1	45	2.5	5.5
7	-1	1	1	35	9.6	5.5
8	1	1	1	45	9.6	5.5
9	-1.682	0	0	30	6.1	4.2
10	1.682	0	0	48	6.1	4.2
11	0	-1.682	0	40	0	4.2
12	0	1.682	0	40	13.2	4.2
13	0	0	-1.682	40	6.1	1.3
14	0	0	1.682	40	6.1	6.3
15-20	0	0	0	40	6.1	4.2

Goodness of the model is evaluated based on the calculated coefficient of determination (R^2) by the program.

Results and Discussion

A disk made of pure dextran was very brittle and highly soluble in water. Plasticizer, PEG 400, was added to make it soft. GA was added with $MgCl_2$ to make it insoluble. GA, which has been used as a crosslinking agent of PVA for a long time, can crosslink dextran molecules through the reaction between its aldehyde groups on both ends and hydroxyl groups in dextran.[15] $MgCl_2$ is known to act as a catalyst for the crosslinking reaction.[14]

Swelling Behavior and Response Surface Analysis. Swelling behavior of dextran hydrogel was influenced by GA, PEG 400, and $MgCl_2$ concentrations. Figure 2 shows

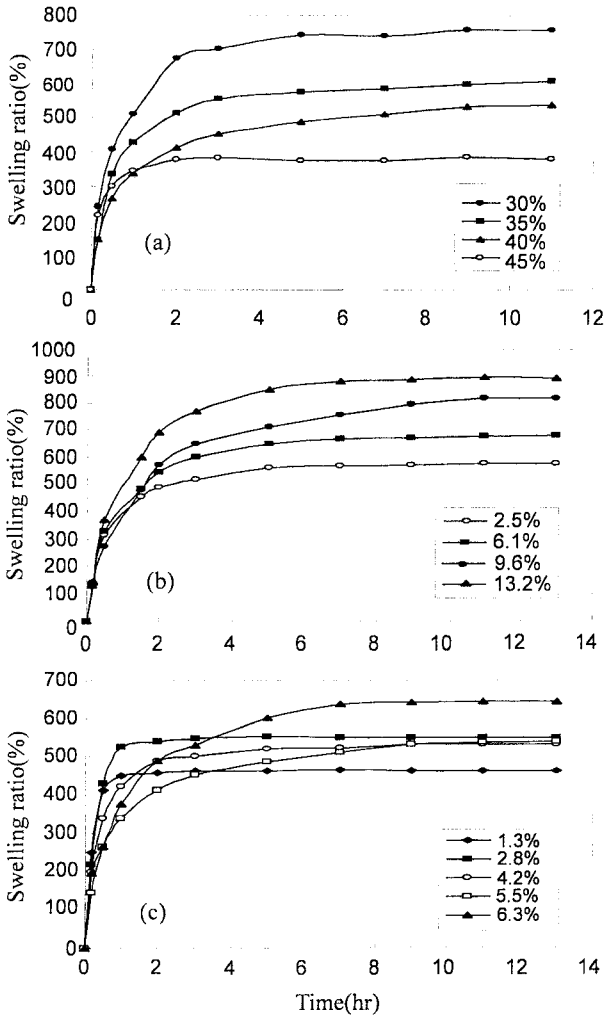

Figure 2. Swelling ratios of dextran hydrogels as a function of time (a) for various GA concentrations (PEG 400: 6.1%, $MgCl_2$: 5.5%), (b) for various PEG 400 concentrations (GA: 35%, $MgCl_2$: 5.5%), and (c) for various $MgCl_2$ concentrations (GA: 35%, PEG 400: 6.1%).

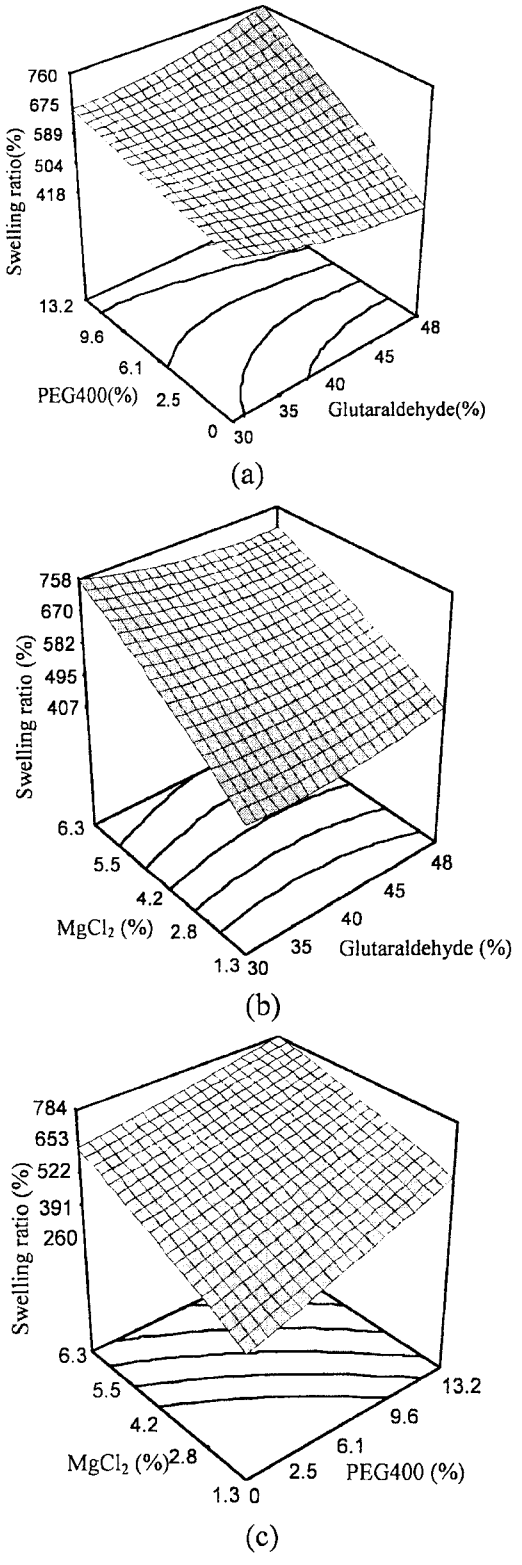

Figure 3. 3D response surface plot of swelling ratios of dextran hydrogel after 9 hrs immersion in PBS solution as functions of (a) GA and PEG 400 concentrations ($MgCl_2$: 5.5%), (b) GA and $MgCl_2$ (PEG 400: 6.1%), and (c) PEG 400 and $MgCl_2$ (GA: 35%).

swelling ratios of dextran hydrogels as a function of time for various GA, PEG 400, and $MgCl_2$ concentrations. It is shown that swelling of the hydrogels reaches the equilibrium in 0.5 to 3 hrs depending on the composition. As GA concentration increases, time for the equilibrium is getting shorter with reduced swelling ratio. This indicates that the degree of crosslinking increases as GA concentration increases. As PEG 400 concentration increases, equilibrium swelling ratio increases due to the increased plasticizing effect. The equilibrium swelling ratio also increases as $MgCl_2$ concentration increases, which may be explained by chelation effect of $MgCl_2$.[14] This means that too much $MgCl_2$ may interfere with the crosslinking reaction although it may help the reaction. Without $MgCl_2$, dextran hydrogel disk was too brittle to handle or measure the swelling ratio.

The equilibrium swelling ratios (the values obtained after 9 hrs immersion in PBS solution) were regressed with respect to preparation conditions using the Design Expert®. The multiple regression equation is as follows:

$$Y = 571.58 - 6.33X_1 + 114.92X_2 + 147.24X_3 + 56.08X_1X_2 - 23.8X_1X_3 - 37.37X_2X_3 + 22.33X_1^2 + 2.06X_2^2 - 13.64X_3^2$$

The results are plotted in Figure 3. These results show that the swelling behavior of dextran hydrogel was influenced more significantly by the first order term of $MgCl_2$ and PEG 400 concentrations than GA concentration.

Statistical testing was performed in the form of analysis of variance (ANOVA) to evaluate the significance and adequacy of the model. The test results revealed that swelling ratios were influenced significantly (90.5% level) by GA, PEG400, and $MgCl_2$ concentrations. The determination coefficient R^2 for swelling ratios was 0.9047, which indicates that the model gives a high degree of correlation between observed and predicted values.

Mechanical Strength and Response Surface Analysis. Figure 4 shows ultimate tensile strengths and elongation at break of dextran hydrogels with various concentrations of GA, PEG 400, and $MgCl_2$. As GA concentration increases, mainly tensile strength increases due to the increased degree of crosslinking. On the contrary, only elongation increases as PEG 400 concentration increases. Theses results are consistent with the swelling behavior. Hydrogels with low $MgCl_2$ concentration shows high tensile strength but low elongation. As $MgCl_2$ concentration increases, tensile strength decreases dramatically but elongation increases. Hydrogels with $MgCl_2$ concentrations more than 7.5% became sticky while hydrogels without $MgCl_2$ was very brittle.

The multiple regression equation of the elongation (tested values after 24 hrs storage in temperature & humid chamber) is as follows:

$$Y = 247.41 - 31.31X_1 + 42.72X_2 + 74.57X_3 + 71.35X_1X_2 + 79.55X_1X_3 + 11.17X_2X_3 + 20.4X_1^2 - 16.6X_2^2 - 20.23X_3^2$$

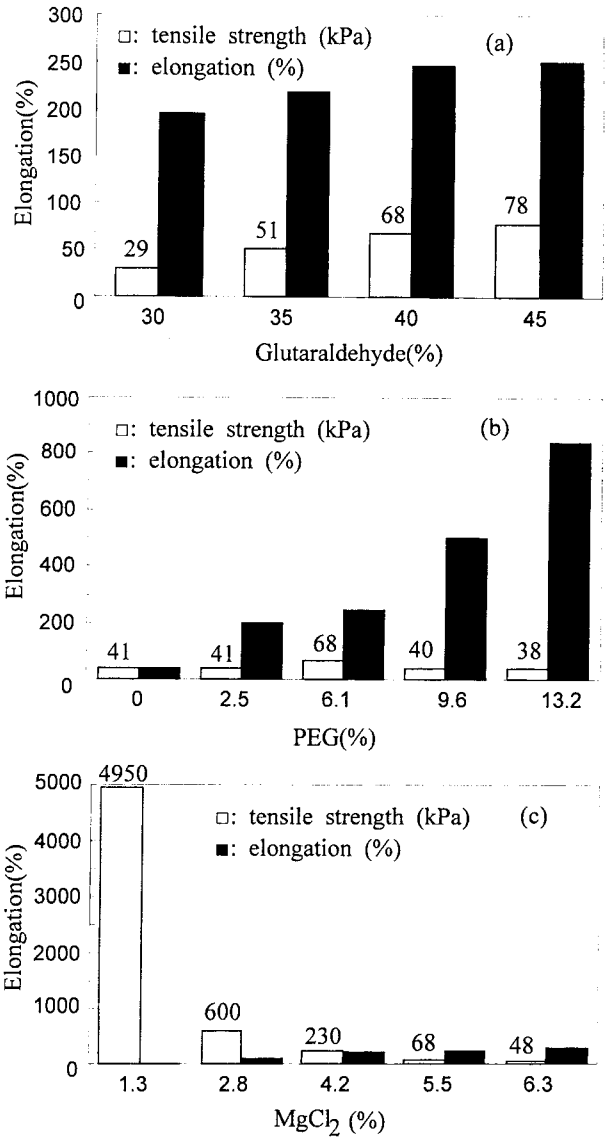

Figure 4. Ultimate tensile strengths and elongation at break of dextran hydrogel (a) with various GA concentrations (PEG 400: 6.1%, $MgCl_2$: 5.5%), (b) with various PEG 400 concentrations (GA: 35%, $MgCl_2$: 5.5%), and (c) with various $MgCl_2$ concentrations (GA: 35%, PEG 400: 6.1%). □ : tensile strength, ■ : elongation.

The results are plotted in Figure 5. The determination coefficient R^2 for the elongations was 0.8513. The regression results indicate that the elongation of dextran hydrogel was influenced significantly by the interaction term between GA and $MgCl_2$ concentrations and first order term of $MgCl_2$ concentration.

Conclusions

Swelling behavior and mechanical strength of dextran hydrogel were evaluated by response surface methodology.

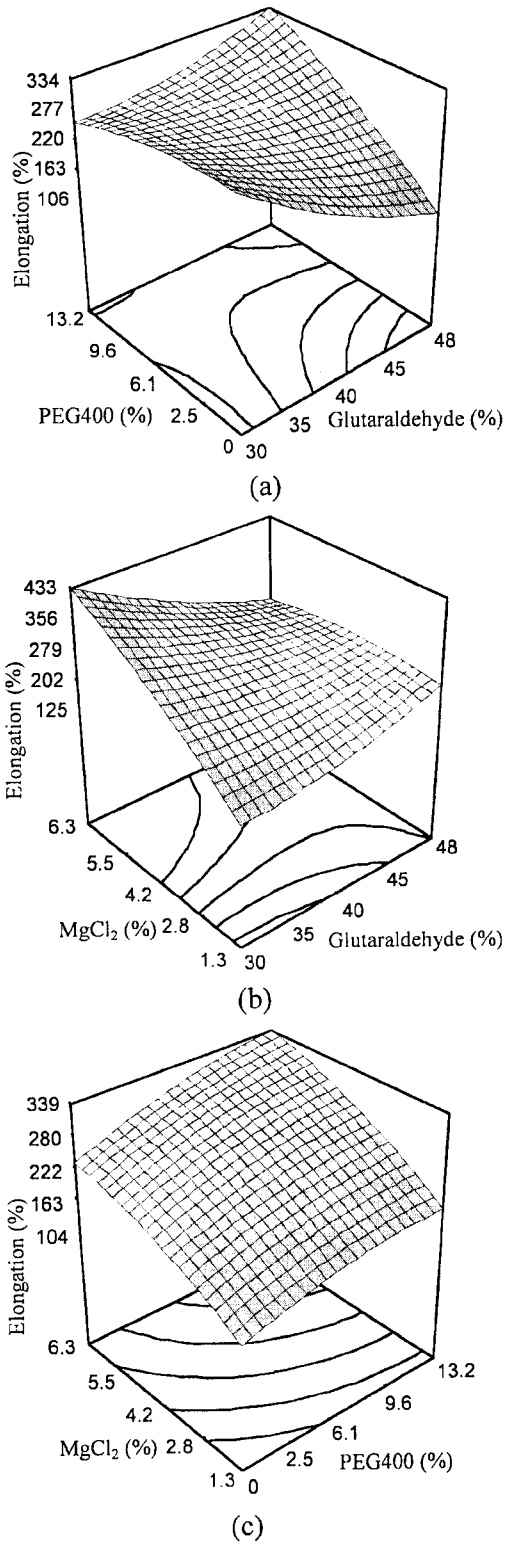

Figure 5. 3D response surface plot of elongations of dextran hydrogel as functions of (a) GA and PEG 400 concentrations ($MgCl_2$: 5.5%), (b) GA and $MgCl_2$ (PEG 400: 6.1%), and (c) PEG 400 and $MgCl_2$ (GA: 35%).

Swelling behavior was influenced significantly by PEG 400 and $MgCl_2$ concentrations. Mechanical strength was influenced significantly by the interaction term between GA and $MgCl_2$ concentrations and $MgCl_2$ concentration.

Acknowledgements. A Korea Research Foundation Grant (KRF-Y00-290) supported this research.

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