
Mitomycin-C-Loaded Alginate Carriers for Bladder Cancer Chemotherapy

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Mitomycin-C-loaded Alginate Carriers for Bladder Cancer Chemotherapy

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ABSTRACT: In this study Mitomycin-C-loaded alginate carriers were prepared as an alternative system in the postoperative chemotherapy in bladder cancer. Alginate was preferred because of its constructive properties and it was prepared in a cylindrical shape to facilitate the insertion of the carrier for *in vivo* studies. The alginate carriers were prepared as calcium alginate gel as well as cross-linking agents. In the preparation of the alginate carriers, precipitation medium concentration, cross-linker concentration, and Mitomycin-C/alginate ratio were changed to obtain drug attachment to the inner wall of bladder and/or optimum release rate of the agent. Due to the hydrogel structure of the alginate, the swelling behavior of the polymer was evaluated by gravimetric determinations in aqueous media periodically. Swelling ratios of the alginate carriers were changed from 30 to 65% based on precipitation medium, cross-linker concentration, and swelling medium pH. For prediction of the bioadhesion of the alginate carriers to the inner surface of bladder, *in vitro* bioadhesion tests were performed by using fresh bladders which were taken from New Zealand rabbits. It was observed that the *in vitro* Mitomycin-C release and bioadhesion values were significantly changed with changing swelling ratios.

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KEY WORDS: bladder cancer, transurethral resection (TUR), alginate, mitomycin-C, controlled release.

INTRODUCTION

Bladder cancer is the seventh greatest cause of death in men and the ninth in women [1]. Treatment consists of removing the tumorous tissue and the modular lesions via transurethral resection (TUR). Although it is the most common treatment, most of the time TUR is not an absolute solution for bladder cancer because the recurrence of the tumorous tissue is 50–70% [2]. Intravesical pharmacotherapeutics infusion is the most common protective therapy for recurrency after TUR; treatment is once a week for 6–36 weeks [3–5]. Unfortunately, there are some difficulties in this protective therapy such as the establishment of a suitable dosage and loss of bioactive agent; in the latter case, the bladder is filled and discharged periodically, consequently, the pharmacotherapeutic agent is eliminated from the bladder. The disturbance to the patient with each application and the loss of pharmacotherapeutic agent without being used effectively suggest that the current treatment needs improvement for practical applicability.

The main aim of the present study was to prepare a reservoir-type carrier that can release the agent in suitable amounts for a known time period after TUR. The inner surface of the bladder has a mucosal structure, therefore, in the preparation of a reservoir, alginate which is a mucoadhesive polymeric structure was used. Mitomycin-C is one of the most popular pharmacotherapeutic agents used in bladder cancer chemotherapy [20–22]. Therefore it was selected as a model pharmacotherapeutic agent for the design of anticancer-agent-loaded alginate carriers for postoperative chemotherapy in bladder cancer. Alginates have demonstrated bioadhesive potential [6–8], therefore, it was logical to examine the possibility of their use in bladder cancer pharmacotherapy; particularly with regard to mucosal adhesion in bladder. Additionally, alginate has been widely used in pharmaceutical and food industries because they are nontoxic and biodegradable [9–15] and have been approved by the Food and Drug Administration for human use. It is used as wound dressing material and as an injectable cell delivery vehicle for chondrocytes in the urinary tract in animals and humans [16–18].

In this study, alginate carriers were prepared by precipitation in $CaCl_2$ solution followed by cross-linking with ethylene glycol diglycidyl ether, EGDGE. In the preparation of the alginate carriers, precipitation medium, cross-linker type and concentration, and Mitomycin-C/alginate ratio were varied to obtain good attachment to the inner wall of bladder and optimum release rate of the agent.

EXPERIMENTAL

Materials and Reagents

Mitomycin-C was purchased from Kyowa Hakko KCGYO Co, Japan. Sodium alginate, calcium chloride, and ethylene glycol diglycidyl ether, EGDGE, were supplied by Fluka, Switzerland. All the other chemicals used in this study were analytical grade and no further purification was required.

Preparation of Alginate Carriers

Alginate carriers were prepared by precipitation of the polymer in $CaCl_2$ solutions and formed into cylindrical rods to facilitate insertion intravesically with a catheter for *in vivo* applications. In a typical procedure, 250 mg of sodium alginate was dissolved in aqueous solution (5% v/v) in an ultrasound bath for 6 h. The gelatinous sodium alginate was introduced into a needle-less syringe and slowly injected into a beaker containing the $CaCl_2$ solution. The alginate rods were allowed to cure in the same medium for 30 min. The rods were then taken out, washed, and dried at room temperature to constant weight. They were then stored in a dessicator at 4°C for further analysis and applications. The size of the dried alginate rods were ~ 0.2 mm in diameter. In addition, the alginate was cross-linked with different amounts of EGDGE (1–20% v/v) to achieve different cross-linking densities of the alginate rods cross-linked previously with $CaCl_2$.

The same procedure as above was applied with certain amounts of Mitomycin-C (0.2, 1, and 2 mg/g alginate) in the initial sodium alginate solution.

Characterization of Alginate Carriers

The alginate carriers were characterized by different techniques based on geometry and size, swelling ability, and bioadhesion properties. The carriers were evaluated using optical and scanning electron microscopes (SEM, JEOL, Japan) for shape, size, and morphological characterizations. Both dry and swollen alginate rods were evaluated. In addition the effect of calcium chloride concentration on the swelling behavior of the alginate rods was evaluated with an optical microscope.

Swelling Measurements

Dynamic swelling properties (or behavior) of the gels were determined as follows: samples of gel (50 mg) were dried and placed in phosphate buffers with different pH values (6.0, 7.0, and 8.0) for selected periods of time. The swollen carrier was collected and the wet weight of the swollen carrier was determined by first blotting the carrier with filter paper to remove adsorbed water and then weighing immediately using electronic balance with ±0.1 mg accuracy. Weights were recorded at a predetermined time period. The percentage of swelling of the carrier in the media was calculated from the following equation,

$$S_{\mathrm{ac}} = \frac{w_l - w_0}{w_0} \times 100$$

where S_{ac} is the percentage of the swelling ratio of the alginate carrier, w_t denotes the weight of the carrier at time t, and w_0 is the initial weight of the carrier.

Bioadhesion Tests

Bioadhesion tests were carried out using an apparatus constructed according to a method improved by Smart [19]. The apparatus included a platform (which can be lowered automatically at a 2 mm/min rate), a 5 × 5 × 10 cm glass cuvette filled with urine obtained from a fresh rabbit bladder and a microbalance. A schematic representation of the apparatus is given in Figure 1. Here, the size of the alginate carrier was 2 mm in diameter and 1 cm in height and the size of the bladder was 2 cm in width and 5 cm in length.

Typically, a gel sample was attached (using a cyanoacrylate adhesive, Loctite Superglumatic) to a rope suspended from a top pan microbalance (Mettler AB 204, Switzerland) and the suspended alginate carrier was immersed into the bladder sample. The platform was then lowered at a rate of 2 mm/min until the carrier detached from the membrane and the weight changes (observed in microbalance) were recorded every 10 s. The data was normalized to 1 cm^2 surface area of the carrier in contact with the bladder sample and finally converted to mN/g of carrier.

In vitro Mitomycin-C Release Studies

For the release experiments, Mitomycin-C-loaded alginates were placed in phosphate buffer solutions (20 mL) at different pH (6 and 8)

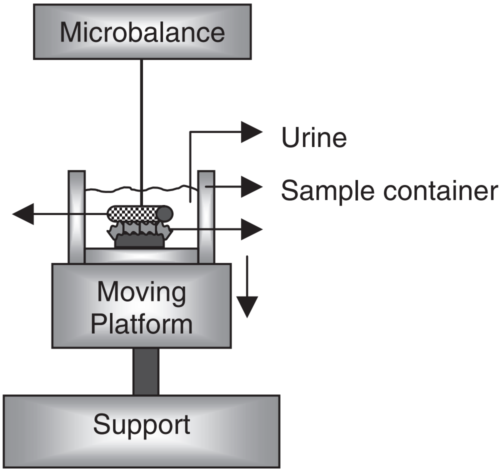

Figure 1. Test apparatus for bioadhesion tests of alginate carriers.

and the release amounts were determined with a UV–Visible spectrophotometer (Schimadzu, Japan). Absorbances were measured at 217 nm and concentrations were calculated by using the absorbance–concentration calibration curve performed previously [23]. Release behavior of the alginate carriers was obtained by following the amount of Mitomycin-C released periodically. The release medium was kept at 37°C and shaken in a constant temperature bath for sink conditions. In the release studies, $CaCl_2$ concentration in the precipitation medium and Mitomycin-C content of the carrier were selected as the effective parameters on release behavior of alginate carriers based on preliminary studies.

RESULTS AND DISCUSSIONS

Preparation and Characterization of Alginate Carriers

The morphology of the alginate carriers was evaluated using SEM. Surface and cross-section micrographs of the dried carrier are shown in Figure 2. The surface of the carrier seems to be smooth and there is no pores on either the surface or the cross-section. In the case of wet swollen carriers, the size of the pores increased three or more times according to the amount of cross-linking density (or the amount of $CaCl_2$ and/or EGDGE).

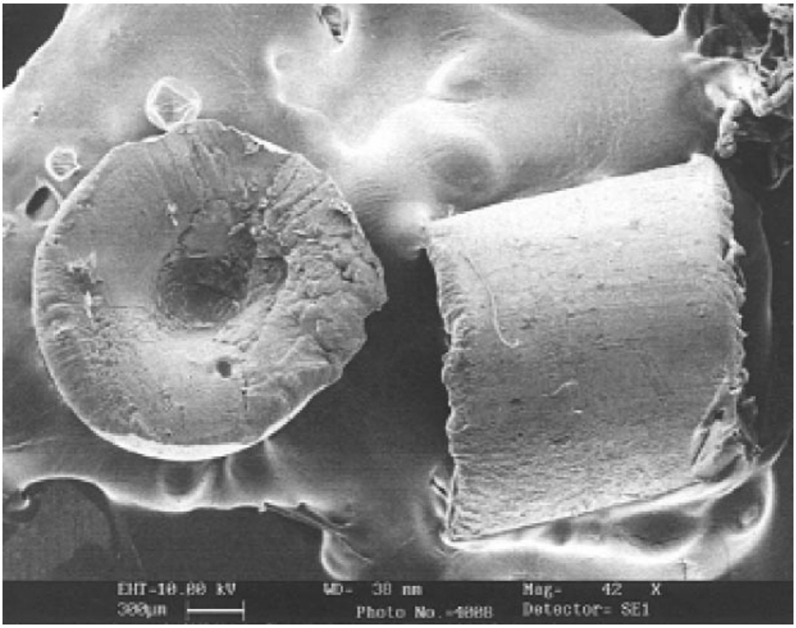

Figure 2. SEM micrograph of alginate carrier: surface (right hand) and cross-section (left hand).

Swelling Behavior of Alginate Carriers

The amount of cross-linkers ($CaCl_2$ and/or EGDGE) and the pH of the medium were the parameters used to effect the swelling behavior of the alginate carriers. According to the results obtained, almost all the alginate carriers reached saturation (or maximum swelling ratio) after 2 h and the swelling ratios changed 30–60% according to the selected parameters. The swelling ratios and weight parameters were changed as illustrated in Table 1. These formulations were used to investigate both the swelling behavior and the bioadhesion characteristics comparatively. During the preparation of alginate carriers, $CaCl_2$ concentration was varied from 10 to 40% changing the cross-linking densities. The swelling ratios were determined and are given in Figure 3. Swelling increased by decreasing the amount of $CaCl_2$ in precipitation medium as seen in Figure 3. This expected situation is due to the higher amount of cross-linking densities when the $CaCl_2$ concentration was increased.

The amount of additional cross-linker, EGDGE, was selected as another parameter to control the swelling ratio. EGDGE concentration in the precipitation medium was changed in the range of 1–20% (v/v). The results obtained with EGDGE concentration changes (Figure 4)

Table 1. The formulations used for the preparation of different alginate rods.

Sample No.	$CaCl_2$ Concentration (%)	Cross-linker, EGDGE (g/100 mL)	Mitomycin-C (mg/g Sodium Alginate)	Medium pH
Effects of calcium chloride concentration (%)				
1	10	0	1	7.0
2	20	0	1	7.0
3	40	0	1	7.0
Effects of EGDGE (%)				
1	10	0	1	7.0
4	10	1	1	7.0
5	10	10	1	7.0
6	10	20	1	7.0
Effects of mitomycin-C concentration (%)				
7	10	10	0.1	7.0
5	10	10	1	7.0
Effects of medium pH				
9	10	10	1	6.0
10	10	10	1	7.0
11	10	10	1	8.0

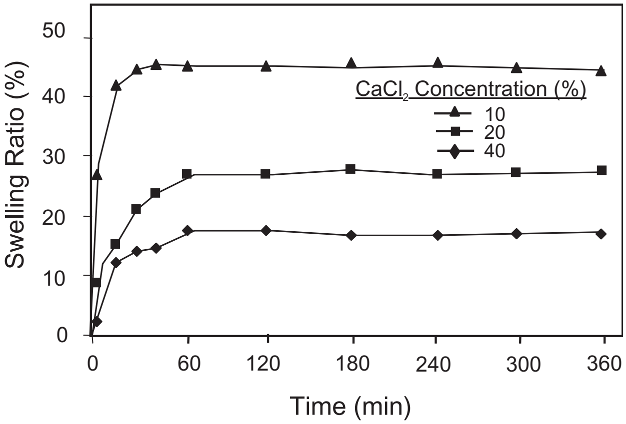

Figure 3. Effect of $CaCl_2$ concentration on the swelling ratio of the alginate carriers.

were similar to the effect with $CaCl_2$ concentration changes. It can be seen in this figure that the swelling ratio decreased with increasing EGDGE concentration due to the higher cross-linking densities in the case of higher amount of cross-linker.

Another effective parameter selected was the pH of the medium containing the alginate carrier. The urine pH changes are in the range of

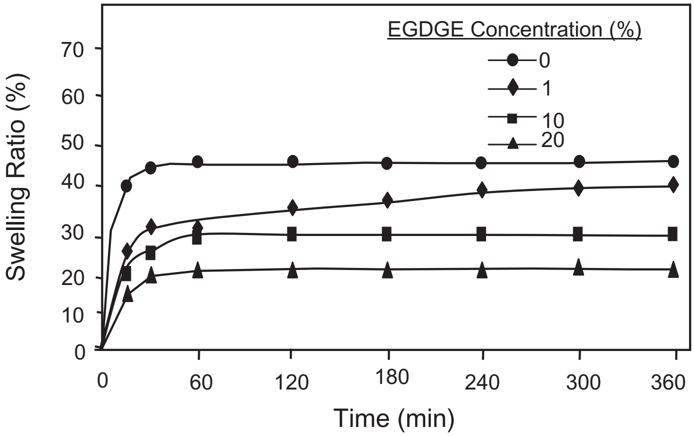

Figure 4. Effect of EGDGE concentration on the swelling ratio of the alginate carriers.

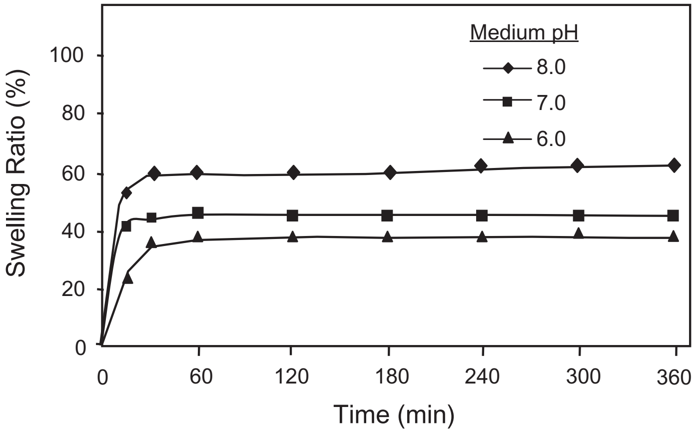

Figure 5. Effect of medium pH on the swelling behavior on the sodium alginate carriers.

4.5–8 [24]. Therefore, to represent the urine pH, swelling experiments were made at different pH values that varied from pH 6–8. The swelling ratio of the sodium alginate carrier is decreased by increasing the medium pH values as seen in Figure 5.

Bioadhesion Tests

Sodium alginate is a mucoadhesive polymer and has received significant attention for mucoadhesive drug delivery systems including different types of administration [25–27]. In this study, sodium alginate was specifically selected because of its mucoadhesive property as well as

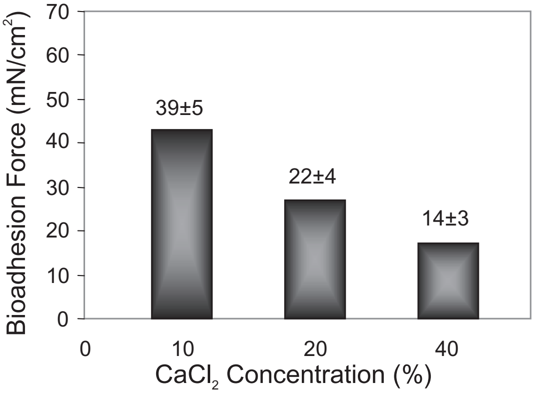

Figure 6. Effect of calcium chloride concentration on bioadhesion of the alginate carriers.

many other useful physicochemical properties, such as, biocompatibility and biodegradability. The bioadhesion tests were made using the apparatus shown in Figure 1. The sample used was a rabbit bladder. The bladder should be very fresh for significant results, otherwise the adhesion forces become weak and sodium alginate carriers are not retained in the bladder. The preliminary results showed that the cross-linking density was the most important parameter for bioadhesion. Therefore, the effect of calcium chloride concentration on bioadhesion was evaluated for this purpose and the results obtained are shown in Figure 6.

The adhesive forces decreased with increasing the calcium chloride concentration as seen in Figure 6. This can be explained by the fact that increasing cross-linking densities lead to less swelling and less surface area. Hence the total number of reactive groups that can play a role in bioadhesion were decreased.

In vitro Mitomycin-C Release Studies

Mitomycin-C was loaded into the alginate carriers before the cross-linking reaction of the carrier. However, during this procedure some of the Mitomycin-C escaped from the polymeric matrix (alginate carrier) because of the aqueous nature of the precipitation medium. The lost was calculated by spectrophotometer measurements. In the release studies, $CaCl_2$ concentration and the initial amount of Mitomycin-C were found to be the most effective parameters on adjusting the release behavior by the alginate carriers.

In the first part, Mitomycin-C-loaded alginate carriers were prepared in a medium containing different amounts of $CaCl_2$ and the release

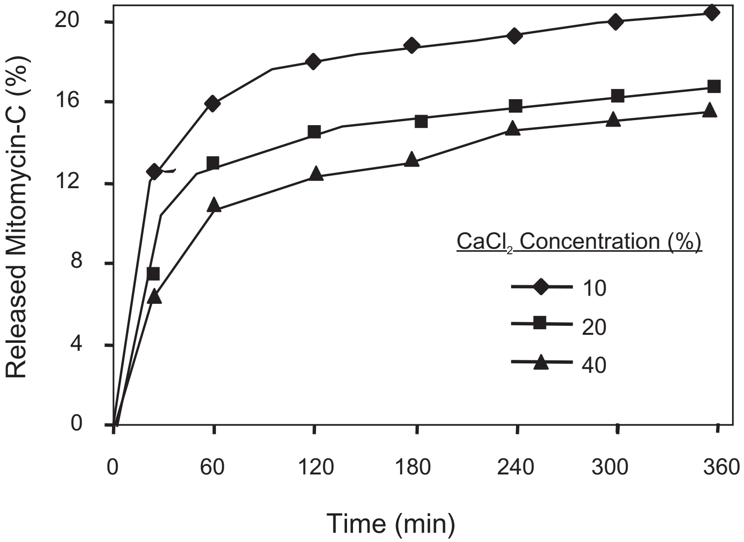

Figure 7. Effect of calcium chloride concentration on Mitomycin-C release behavior from alginate carriers.

data obtained is shown in Figure 7. Mitomycin-C was released rapidly in the case of lower $CaCl_2$ concentrations. This release behavior was very similar to the swelling behavior for the different $CaCl_2$ concentrations discussed previously. In the second part, the initial Mitomycin-C concentration was evaluated as another parameter that could affect the release behavior. Two types of alginate carriers were prepared by using two different initial Mitomycin-C concentrations. The release rate increased in the case of the higher initial Mitomycin-C content as seen in Figure 8. This can be explained with the holes (or spaces) generated by the released Mitomycin-C molecules, it implies that the released Mitomycin-C molecules left more spaces after they were released in the case of higher Mitomycin-C content. This hole generation is similar to the generation of the holes during the swelling process and, of course, the number of holes affects the release rate.

CONCLUSIONS

In bladder cancer therapy, TUR is the most common therapy. But due to the high recurrence risk and the formation of tumoral tissues after the TUR, postoperative pharmacotherapy seems unavoidable. A reservoir type of pharmacotherapeutic delivery system would be the most suitable alternative to postoperative therapy. Alginate was

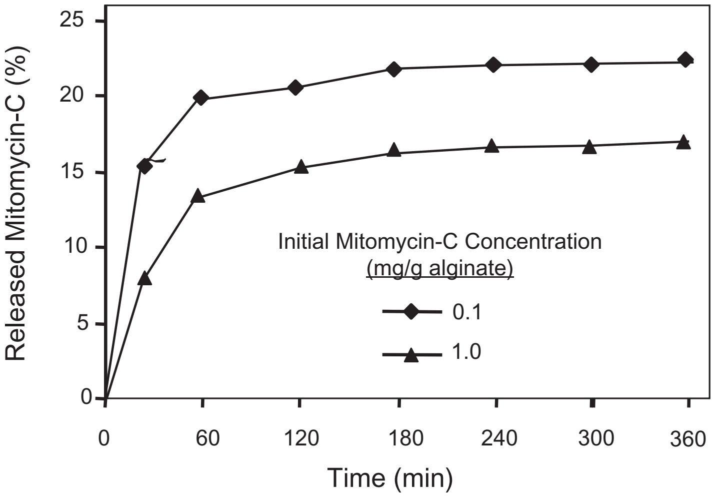

Figure 8. Effect of initial Mitomycin-C content on Mitomycin-C release behavior from alginate carriers.

considered as good matrix for the carriers because of its biocompatible, biodegradable, and mucoadhesive properties. Alginate and its degradation products are nontoxic, therefore it is widely used in the pharmaceutical and food industries for human applications. The biodegradability of alginate carriers facilitates their removal since, after treatment, the carrier would be degraded during the therapy [9–15]. Our bioadhesion tests and *in vitro* Mitomycin-C release characteristics showed that the proposed system could be a promising alternative for after TUR in bladder cancer therapy. Further *in vivo* studies such as, implantation of the carriers into the bladder and *in vivo* Mitomycin-C release, are still under investigation.

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REFERENCES

1. Thompson, I.M., Peek, M. and Rodriguez, F.R. (1987). *J. Urol.*, **137**: 401.
2. Prout, G.R. Jr. (1976). *Urol. Clin. N. Am.*, **3**: 149.
3. Lamm, D.L., Thor, D.E., Stogdill, V.D. and Radwin, H.M. (1982). *J. Urol.*, **128**: 931.

4. Catalona, W.J., Hudson, M.A., Gıllen, D.P., Andriole, G.L. and Ratliff, T.L. (1987). *J. Urol.*, **137**: 220–224.
5. Highley, M.S., Oosterom, A.T., Maes, R.A. and Brujin, E.A. (1987). *Drug Deliv. Sys.*, **37**: 59–73.
6. Chen, J.L. and Cyr, G.N. (1970). In: Manley, R.S. (ed.), *Adhesion in Biological Systems*, pp. 163–181, Academic Press, New York.
7. Smart, J.D., Kellaway, I.W. and Worthington, E.C. (1984). *J. Pharm. Pharmacol.*, **36**: 295–299.
8. Batchelor, H.K., Banning, D., Dettmar, P.W., Hampson, I.G., Jolliffe, D.Q.M. and Craig, C. (2002). *Int. J. Pharm.*, **238**: 123.
9. Kim, C.K. and Lee, E.J. (1992). *Int. J. Pharm.*, **79**: 11.
10. Singh, O.N. and Burgess, D.J. (1989). *J. Pharm. Pharmacol.*, **41**: 670.
11. Downs, E.C., Robertson, N.E., Riss, T.L. and Plunkett, M.L. (1992). *J. Cell Phys.*, **152**: 422.
12. Edelman, E.R., Mathiowitz, E., Langer, R. and Klagsbrun, M. (1991). *Biomaterials*, **12**: 619.
13. Mumper, R.J., Hoffman, A.S., Puolakkainen, P.A., Bouchard, L.S. and Gombotz, W.R. (1994). *J. Control. Release*, **30**: 241.
14. Sandford, P.A. and Hutchings, G.P. (1987). In: Yalpani, M. (ed.), *Industrial Polysaccharides*, *Genetic Engineering, Structure/Property Relations and Applications*, 363 pp., Elsevier, Amsterdam.
15. Daly, M.M. and Knorr, D. (1988). *Biotech. Progr.*, **4**: 76.
16. Atala, A., Kim, W. and Paige, K.T. (1994). *J. Urol.*, **152**: 641.
17. Diamond, D.A. and Caldamone, A.A. (1999). *J. Urol., Suppl.,* **162**: 1185.
18. Atala, A. (2000). *J. Endourol.*, **14**: 49.
19. Smart, J.D. (1991). *Int. J. Pharm*. **73**: 69.
20. Huland, H., Otto, U. and Droese, M. (1991). *J. Urol.*, **132**: 27.
21. Herr, H.W., Laudone, V.P. and Whitmore, W.F. (1987). *J. Urol.*, **138**: 1363.
22. Wientjes, M.G., Badalement, R.A. and Wang, R.C. (1993). *Cancer Res.,* **53**: 3314.
23. Irmak, S. (2001). MSc Dissertation, Hacettepe University, Ankara, Turkey.
24. (1996). In: Seeley, R.R., Stephens, T.D. and Tate, P. (eds), *Essentials of Anatomy and Physiology*, **2nd edn**, WCB McGraw Hill Co., NY.
25. Lehr, C.M., Bouwstra, J.A., Schacht, E.H. and Junginger, H.E. (1992). *Int. J. Pharm.*, **78**: 43.
26. Wong, C.F. and Yuen, K.H. and Peh, K.K. (1999). *Int. J. Pharm.*, **180**: 47.
27. Witschi, C. and Mrsny, R.J. (1999). *Pharmaceut. Res.*, **16**: 382.