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Design and evaluation of a mucoadhesive therapeutic agent delivery system for postoperative chemotherapy in superficial bladder cancer

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Abstract

The most common treatment method is known as the transurethral resection (TUR) for the therapy of bladder cancer. Unfortunately, because of the recurrency of the tumoral tissues after TUR the chemotherapy or immunotherapy should be performed. In these kind of therapies the pharmacotherapeutics are infused intravesically into the bladder after TUR periodically (i.e. upto 6–36 weeks, each week). But these therapies are having very big problems (i.e. disturbancy to patients, adjustment of the suitable dosage, loss of active agents without using etc.). An alternative approach can be proposed as to design a pharmacotherapeutic agent delivery system, which will supply the suitable dosage of the agent for a certain time period to solve those problems. In this study; the pharmacotherapeutic agent (i.e. Mytomycin-C) delivery system was prepared by using a mucoadhesive polymer (i.e. chitosan) in the form of cylindirical geometry to facilitate the insertion of the carrier for in vivo studies. The chitosan carriers were prepared by cross-linking during the solvent evaporation technique. In the preparation of the chitosan carriers the chitosan polymers with different molecular weights, different amounts of cross-linker (i.e. glutaraldehyde) and different amounts of pharmacotherapeutic agent were used to obtain desired attachment onto the bladder wall and optimum release rate of the agent. On the other hand because of the gelous structure of the chitosan, the swelling behaviour of the polymer was evaluated by gravimetric determinations in aqueous media periodically.

Keywords: Bladder cancer; Transurethral resection (TUR); Chitosan; Mitomycin-C; Controlled release

1. Introduction

Carcinoma of the bladder occurs within the domain of human neoplasms by many different types of etiological factors such as some aromatics (i.e. xylamine, β-naphtylamine, benzydine, 4-nitro

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diphenyl) and smoking cigarette (Case et al., 1954; Wynder and Goldsmith, 1977). In the comparison with other types of cancer, the carcinoma of bladder is the seventh causing the cancer death in man and the ninth in women (Thompson et al., 1987). Transurethal resection (TUR) is the most common treatment method that consists the removal of the tumoural tissues as the modular lesions. However, the recurrency of the formation of tumoral tissues after the operation of TUR is in the range of 50–70% (Prout, 1976). The most widely used the treatment type is consisted of the formacotherapeutics (Mitomycin-C, doxorubicin, thiotepo, BCG, interferon α-2b etc.) directly (or intravesically) introduced into the bladder after the TUR periodically (i.e. up to 6 or 36 weeks, for each week) (Lamm et al., 1982; Catalona et al., 1987; Highley et al., 1999). Due to the difficulties in the establishment of the suitable dosage (i.e. the bladder is filled up and discharges periodically and the farmacotherapeutic agent leaves from bladder), the disturbance that's given to the patient in each agent application and the loss of pharmacotherapeutic agent without being used the therapeutic procedure needs much more improvement for practical applicability.

In the present study, the main aim is to prepare a reservoir type pharmacotherapeutic agent carrier which can release the agent in suitable dosage for a certain time period as an alternative way for pharmacotherapy after TUR. The inner surface of bladder has a mucosal structure (in other words, this surface is covered by a mucus layer) therefore in the preparation of pharmacotherapeutic agent reservoir, a mucoadhesive polymeric structure (i.e. chitosan) was used. Chitosan carriers were prepared by cross-linking during the solvent evaporation technique. In the cross-linking a bifunctional chemical (i.e. glutaraldehyde) was used. The pharmacotherapeutic agent was loaded into the polymers during the preparation process. The bioadhesion tests were performed by using the bladder of an animal (i.e. sheep) as ex vivo studies. The pharmacotherapeutic agent release experiments were followed in physiological buffers as in vitro studies.

2. Materials and methods

2.1. Materials

Chitosan polymers were obtained from Fluka (Switzerland) with different molecular weights (i.e. 150,000: LMW; 450,000: MMW; and 650,000: HMW). Model pharmacotherapeutic agent (i.e. Mitomycin-C) was purchased from Kyowa Hakko KCGYO Co., Japan. Aqueous acetic acid (Carlo Erba, Italy) solutions were used as the solvent for chitosan polymers and glutaraldehyde (Fluka, Switzerland) was used as the cross-linker. All the other chemicals used in this study were in analytical grade and no further purification was required.

2.2. Preparation of chitosan carriers

In the preparation of pharmacotherapeutic agent delivery system chitosan was selected as the polymeric matrix, is especially with regard to the mucoadhesive properties of the chitosan polymers. In addition chitosan has many significant biological properties (i.e. biodegradable, biocompatible, bioactive) and chemical properties (polycationic, hydrogel, contains reactive groups such as OH vs. NH_2) (Sandford, 1988). Chitosan can be produced by alkaline deacetylation of chitin (Yao et al., 1995) and chitin is the secondly most abundant natural polymer after cellulose, therefore it is quite well known that it is a very cheap biopolymer (Hirano, 1986). In this study, different molecular weight of chitosan (i.e. LMW: 150,000; MMW: 450,000; and HMW: 650,000) were used to prepare the pharmacotherapeutic agent delivery system (or carrier). Chitosan carriers were prepared in the form of cylindrical rod to facilitate it's insertion intravesically with a catheter. Chitosan rods were prepared by the technique of cross-linking during solvent evaporation. In a typical procedure; certain amount of chitosan (i.e. 250 mg) was dissolved in aqueous acetic acid solution (i.e. 5% v/v) in an ultrasound bath for 6 h. All the obtained gelous chitosan was filled into an injector. Chitosan solution was injected vertically into the plastic pipette (with the internal diameter of 5 mm) filled with cross-linker (i.e.

glutaraldehyde) solution. The excess glutaraldehyde was taken out from the upper end of the plastic pipette during injection. After filling the chitosan solution, plastic pipettes were dried in vertical position at room temperature for 48 h. The concentration of glutaraldehyde solution was changed (i.e. 5 or 25% v/v) to achieve different cross-linking densities of the chitosan rods. The obtained chitosan rods displayed acidic structure and therefore the rods were neutralized with sodium hydroxide solution of different concentrations (i.e. 0.5–4.0 g NaOH/100 ml distilled water) for 1 h. After neutralization the chitosan rods were left to dry at room temperature until further analysis.

In the case of pharmacotherapeutic agent (i.e. Mytomycin-C) loaded chitosan rod fabrication, the same procedure mentioned above was applied just by the addition of certain amount of Mytomycin-C (i.e. 0.25 and 0.50 μg/g chitosan) into the aqueous acetic acid–chitosan solution at the beginning of the procedure. In all studies the most effective parameters were selected as the molecular weight of chitosan, glutaraldehyde concentration and the relative amount of Mytomycin-C. Different preparation procedures were summarized in Table 1 based on effective parameters.

2.3. Characterization of chitosan carriers

The prepared chitosan carriers were characterized by using different techniques based on morphology, swellability and bioadhesion properties. These studies were summarized in following subsections.

2.4. Morphological characterization

Chitosan carriers were evaluated with scanning electron microscope (SEM) for morphological characterization. For this purpose, both dry and swollen chitosan rods (swollen and lyophylized) were evaluated and in addition surface and cross-sectional evaluation was made. In a typical evaluation, a small piece of chitosan rod (i.e. 1–2 mm height) was cut and fixed onto the sample holder (a stap) and placed in a vacuum oven at room temperature for 24 h to dry. The samples were coated with gold, and then SEM micrographs were obtained.

Table 1
The formulations used for the preparation of different chitosan rods

Sample no.	Molecular weight	Glutaraldehyde concentration (%)	NaOH concentration (g/100 ml)	Mytomycin-C (mg/g chitosan)
Effects of molecular weight[a]				
1	LMW	5	0.5	0.00
2	MMW	5	0.5	0.00
3	HMW	5	0.5	0.00
Effects of glutaraldehyde concentration				
4	LMW	5	0.5	0.00
5	LMW	10	0.5	0.00
6	LMW	25	0.5	0.00
Effects of NaOH concentration in neutralization medium				
7	LMW	5	0.5	0.00
8	LMW	5	4.0	0.00
Effects of Mytomycin-C and chitosan molecular weight				
9	LMW	5	0.5	1.00
10	LMW	5	0.5	2.00
11	MMW	5	0.5	2.00
12	HMW	5	0.5	2.00

[a] *Note*: LMW, low molecular weight; MMW, medium molecular weight; and HMW, high molecular weight.

2.5. Swelling behaviour of chitosan carriers

Dynamic swelling properties (or behaviour) of chitosan carriers were determined by gravimetric method. In this method, a chitosan carrier of a known amount (50 mg) was dried and placed in phosphate buffer with a pH of 6.0 (as an artificial medium of urine) for a required period 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 excess water on the surface and then weighing immediately on an electronic balance with ± 0.1 mg accuracy. The weight of the swollen chitosan carrier was recorded at a predetermined time period. The percent of swelling of the sponges in the media was then calculated from the following equation,

$$S_{cc} = \frac{w_t - w_0}{w_0} \times 100,$$

where, S_{cc} is the percentage of the swelling ratio of the chitosan carrier, where w_t denotes the weight of the carrier at time t, and w_0 is the initial weight of the carrier. The cross-linker concentration in dispersion medium, chitosan molecular weight and the neutralization medium concentration (i.e. based on NaOH concentration) were changed in order to obtain different swelling ratios as shown in Table 1.

2.6. Bioadhesion tests

The bioadhesion tests for chitosan carriers were made with an apparatus constructed according to the method improved by Smart reported in the literature (Smart, 1991). The apparatus included a platform (which can be lowered automatically with the rate of 2 mm/min), a glass container (with the size of $5 \times 5 \times 10$ cm) filled with urine (obtained from fresh sheep bladder) and a microbalance. Schematical representation of the apparatus for accessing bioadhesion tests of chitosan carriers is given in Fig. 1.

In a typical procedure, a chitosan carrier 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 chitosan carrier was immersed onto the bladder sample (a small piece of sheep bladder supplied freshly). Afterwards, the platform was lowered at a rate of 2 mm/min until the carrier pulled clear of the membrane and the weight changings (observed in microbalance) were recorded at each 10 s. The results were calculated in terms of a standard 1 cm^2 surface area of the carrier contacted with the bladder sample and finally converted to the form of mN/g carrier. In this section, chitosan molecular weight and the amount of cross-linker were evaluated as the effective parameter on the bioadhesion of chitosan carrier onto the bladder surface.

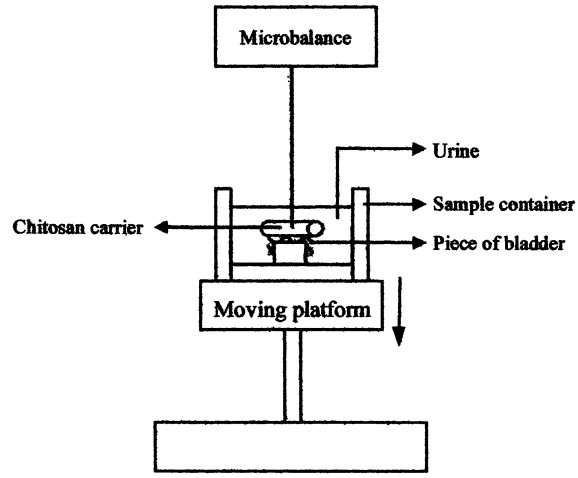

Fig. 1. Schematical representation of bioadhesion test apparatus for chitosan carriers.

2.7. Mitomycin-C loading and release studies

Mitomycin-C is one of the most popular pharmacotherapeutic agent used in bladder cancer chemotherapy (Huland et al., 1984; Herr et al., 1987; Dalton et al., 1991; Wientjes et al., 1993), therefore it was selected as a model pharmacotherapeutic agent for the design of anticancer agent loaded chitosan carriers for postoperative chemotherapy in bladder cancer. Mitomycin-C was loaded into the carriers during the preparation procedure of the carriers, meaning that, a certain amount of Mitomycin-C was added into the aqueous acetic acid solution of chitosan poly-

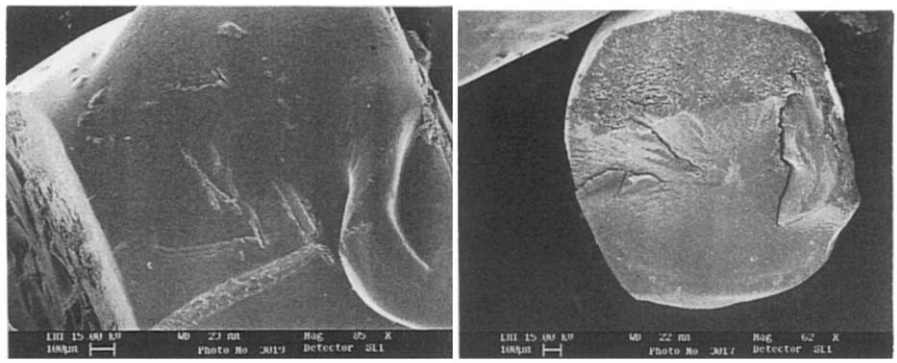

Fig. 2. SEM micrographs of chitosan carrier: (A) surface; (B) cross-section.

mers at initial stage, while the other parts of the procedure progressed similarly with the unloaded chitosan carriers. Chitosan molecular weight and Mitomycin-C content of the carrier were selected as the effective parameter on release behaviour of chitosan carriers based on preliminary studies.

In the release experiments, Mitomycin-C loaded chitosan carriers were placed in physological phosphate buffer solutions and the release amounts were determined spectrophotometrically. Absorbance measurements were made at a wavelength of 217 nm and the concentrations were calculated by using the absorbance–concentration calibration curve performed previously (Irmak, 2001). Release behaviour of chitosan carriers were obtained by following the released amounts of Mitomycin-C periodically (i.e. each hour for initial 6 h and each 6 h for the following 18 h).

3. Results

3.1. Preparation and characterization of chitosan carriers

3.1.1. Morphological evaluation

The morphological evaluation of the chitosan carriers were made with SEM micrographs. For this purpose, surface and cross-sectional micrographs of a dried carrier were obtained and these observations are given in Fig. 2. The surface of the carrier seems to be so smooth and there is no pore on both surface and cross-sectional micrographs as seen in figures. On the other hand in the case of wet (or swollen) chitosan carriers some holes and texturity occurred as seen in Fig. 3. This figure represents the cross-sectional view of the chitosan carrier when reaches the saturated swelling value. The hole size and the quantities of the holes especially depend on the amount of cross-linker (or the cross-linking density). These changes were also concluded in the section of swelling behaviour of chitosan carriers as given in following subparts.

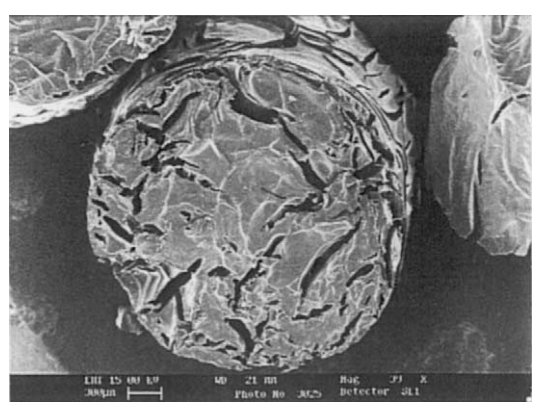

Fig. 3. SEM micrograph of swollen chitosan carrier (cross-sectional view).

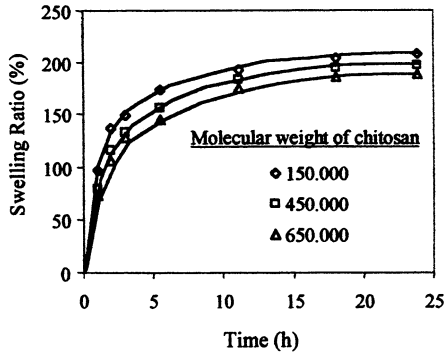

Fig. 4. Effects of chitosan molecular weight on swelling behaviour of chitosan carriers.

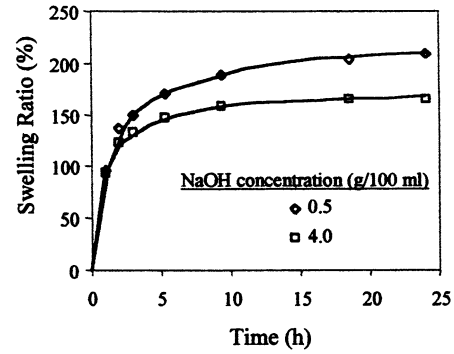

Fig. 6. Effects of NaOH in neutralization media on swelling behaviour of chitosan carriers.

3.2. Swelling behaviour of chitosan carriers

Chitosan molecular weight, amount of cross-linker (i.e. glutaraldehyde concentration) and NaOH concenrations in neutralization media were used as the effective parameters on the swelling behaviour of chitosan carriers. These parameters were changed as in Table 1. The obtained results are given in Figs. 4–6, respectively.

The chitosan carriers reached the saturation (or maximum) swelling ratio after 4–6 h and the swelling ratios changed between 190 and 210% according to the molecular weight of chitosan. This is an expected result, because it is well known that the water diffusion is more difficult in the case of higher molecular weight because of the less molecular spaces than lower molecular weights for the same volume of polymer unit.

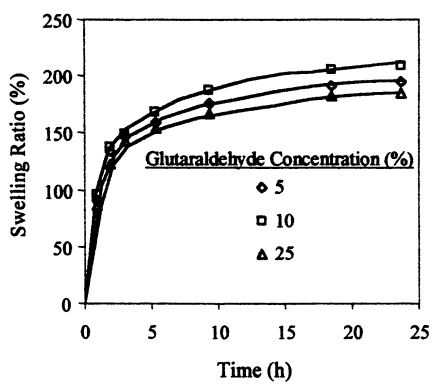

Fig. 5. Effects of cross-linker concentration on swelling behaviour of chitosan carriers.

Similar observations can be seen in Fig. 6, which shows the effects of the amount of cross-linker (i.e. glutaraldehyde concentration). It means that, more glutaraldehyde causes the higher cross-linking densities subsequently causing a smaller swelling ratio as expressed in related literature (Denkbaş and Odabaşı, 2000).

Generally, chitosan polymers do not dissolve in aqueous solutions while freely soluble in dilute (i.e. 1–10%) aqueous acetic (or formic) acid solutions (Rinaudo and Domard, 1988). When the dried chitosan carriers are placed the aqueous media, the carriers convert into gelous form because of the residual acidic groups in the polymer. Therefore, the chitosan carriers should be neutralized with alkaline solutions (i.e. by using NaOH solutions at different conditions). Here the concentration of NaOH solutions will change the amount residual acidic groups and hence it will change the swelling ratio as seen in Fig. 6. The swelling ratio is decrased by increasing NaOH concentration in neutralization media as expected.

3.3. Bioadhesion tests

Chitosan is a mucoadhesive polymer and it gains significant attention for mucoadhesive drug delivery systems including different types of administration in recent years (Lehr et al., 1992; Wong et al., 1999; Witschi and Mrsny, 1999). In this study, chitosan was especially selected because of its mucoadhesive property out of its useful physicochemical properties (i.e. biocompat-

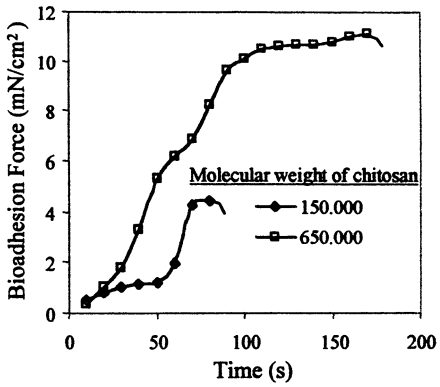

Fig. 7. Effects of chitosan molecular weight on bioadhesion of chitosan carriers.

ibility, biodegradability etc.) as expressed before. The bioadhesion tests were made by using an apparatus as expressed in Section 2. In this section, sheep bladder was used as the model bladder for bioadhesion tests because of the easy and fresh obtaining the sheep bladder. The bladder should be very fresh for healthy observations, otherwise the adhesion forces are very weak and chitosan carriers leave the bladder easily. The obtained results are summerized in Figs. 7 and 8 as the effects of chitosan molecular weight and the cross-linking densities (as different glutaraldehyde concentrations) on bioadhesion, respectively.

The adhesive forces are decreased by increasing the chitosan molecular weight as seen in Fig. 7. This can be explained with the length of polymeric chain carrying the functional (or reactive) groups, which is the predominant parameter for adhesive forces that increases by the increasing number of polymeric chains in the case of higher molecular weights. On the other hand, the cross-linking occurs via the functional groups of chitosan and glutaraldehyde. It means that if the amount of glutaraldehyde increases more functional groups are occupied with glutaraldehyde molecules and the decrease in the number of functional groups decreases the adhesive force as expected. This behaviour can be seen easily in Fig. 8.

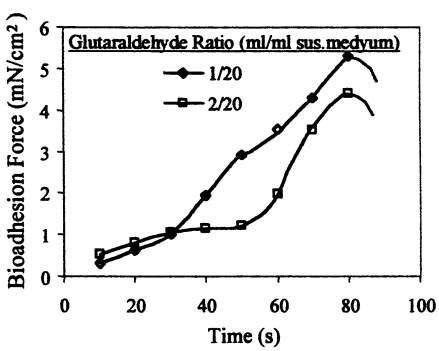

Fig. 8. Effects of glutaraldehyde concentration on bioadhesion of chitosan carriers.

3.3.1. Mitomycin-C loading and release studies

Mitomycin-C was loaded into the chitosan carriers during the preparation of the carrier. In fact because of the procedure all the Mitomycin-C should be kept in the carrier, but because of the neutralization procedure with NaOH solution some of the Mitomycin-C escaped from the polymeric matrix (i.e. chitosan carrier). This amount was calculated from the concentration value obtained from the spectrophotometric data. The release amount to the neutralization media was approximately 35–40% of the initial amount of Mitomycin-C and this value directly depends on the treatment time of chitosan carriers in NaOH media. Therefore, this time was kept absolutely as 1 h. The remaining Mitomycin-C in the carrier after neutralization was used as the initial amount of Mitomycin-C before release.

In the release studies, chitosan molecular weight and the initial amount of Mitomycin-C were selected as the most effective parameters on release mechanism according to the previous studies made with chitosan polymers (Denkbaş et al., 1999, 2000). In the first part, Mitomycin-C loaded chitosan carriers were prepared with different molecular weight chitosan polymers and the obtained release data were shown in Fig. 9. The Mitomycin-C release was decreased by increasing the chitosan molecular weight as seen in this figure. This behaviour depends on the higher tightness of the polymeric chains in the case of chitosan carriers prepared with higher molecular weight chitosan polymers. In this case, swelling ratio was also decreased as expressed before.

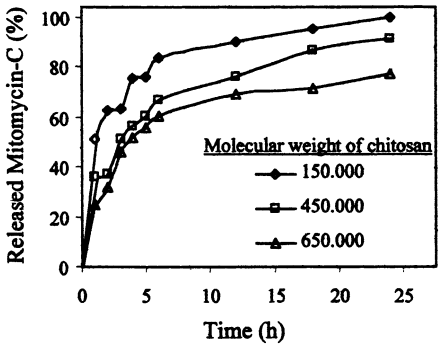

Fig. 9. Effects of chitosan molecular weight on Mitomycin-C release behaviour.

Another significant parameter was used as the initial amount of Mitomycin-C for the determination of release behaviour of Mitomycin-C from chitosan carriers. The obtained release data are given in Fig. 10. The release rate is increased in the case of higher initial Mitomycin-C content as seen in this figure. This can be explained with the generated holes (or spaces) by releasing the Mitomycin-C molecules, it means that the released Mitomycin-C molecules left more space after they released in the case of higher Mitomycin-C content. This hole generation is similar with the generation of the holes during the swelling process and of course the amount of the hole numbers will affect the release rate directly.

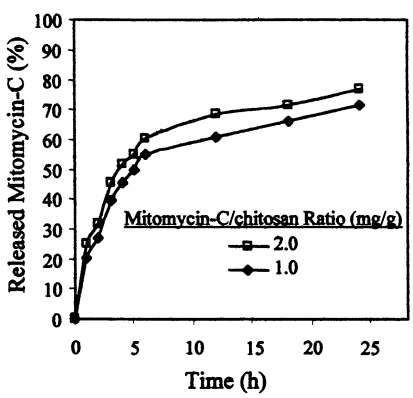

Fig. 10. Effects of initial Mitomycin-C content on Mitomycin-C release behaviour.

4. Discussion

In the literature, there is a lack of investigations about the drug delivery systems (or controlled release) for intravesical administration especially as implantation into the bladder. This because of the conventional approach to the drug delivery systems that almost all of them are consist of the transportation of the active agent to the systemic circulation via any drug delivery system (or any carrier), but the wall of bladder is not valid to the active agent pass through the wall into the systemic circulation. This behaviour is well characterized and published in related literature (Highley et al., 1999). But our approach is so different, that we do not want to transfer the active agent into the systemic circulation, oppositely we want to use active agent in the bladder according to the postoperative treatment of the bladder cancer after TUR. The in vivo experiments are under investigations (Irmak, 2001).

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