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Chitosan gel beads as drug carriers

2. Release of 8-hydroxy-7-iodoquinoline-5-sulfonic acid and 2,5-dihydroxybenzenesulfonic acid

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Summary
It is shown that chitosan gel beads are suitable carriers of 8-hydroxy-7-iodoquinoline-5-sulfonic acid (SQ) and 2,5-dihydroxybenzenesulfonic acid (DHBSA) - substances applied in medicine. Drug release has been investigated with respect to the nature of the crosslinking agent (epichlorohydrin or glutaraldehyde) and pH of the medium. Considerable differences in the release rate of DHBSA and SQ have been found. The total amount of the desorbed SQ, as well as the release rate of the drug depends on pH of the medium - they decrease from alkaline to acidic medium. The antimicrobial and antimycotic activity of the loaded with SQ beads is demonstrated.

Introduction

The application of chitosan as carrier of bioactive substances (1, 2) is determined by the combination of valuable properties (3)- biocompatibility and biodegradability, low toxicity, possibility to undergo chemical modification. Chitosan behaves as weak polybase (pK_a=6.5 (4)) due to the presence of free amino groups. SQ has antimycotic, antiamoebic and antiprotozoic effects and is applied in the medical treatment of dysentery, ulcers, urological and gynaecological troubles (5). DHBSA salts are applied as effective haemostatics (5). DHBSA is stable in acidic medium (pH<4), while under neutral and especially under alkaline conditions it is easily oxidized (6).

In the first stage of our investigation (7) it has been established that the interaction between SQ and DHBSA with chitosan gel beads (CGB) depends on the type of the crosslinking agent, the ionic strength and pH of the medium. It has been found that DHBSA and SQ are sorbed almost stoichiometrically with respect to the free amino groups for the CGB non-crosslinked or crosslinked with epichlorohydrin (ECC), while the CGB crosslinked with glutaraldehyde (GA) sorb much higher amount of SQ.

In the present work the release of SQ and DHBSA from CGB, non-crosslinked or crosslinked with GA and ECC, has been studied with respect to the way of beads preparation and pH of the medium. These two substances have been selected as model compounds because of their bioactivity and chemical structure (both substances possess

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sulfonic acid groups; they differ in solubility - HBSA dissolves easily in water while the condensed aromatic structure of SQ limits its water solubility).

Experimental

Materials

High molecular weight chitosan (MW 6.10^5 with 0.75 deacetylation degree), SQ and the cross-linking agent epichlorohydrin (ECC) were purchased from Fluka. Glutaraldehyde (GA) (25% aqueous solution, Merck) was used. Acetic acid, sodium hydroxide, the salts used for the preparation of buffer solutions (sodium hydrogen carbonate, sodium hydrogen phosphate and potassium dihydrogen phosphate) were of analytical grade. DHBSA of pharmaceutical grade of purity was obtained from Faculty of Pharmacy, Academy of Medicine, Sofia.

Preparation of CGB

The preparation and characterization of CGB, non-crosslinked and crosslinked with ECC or GA, and their loading with SQ or DHBSA have been already described (7). Briefly, the beads were obtained by simple coacervation. Chitosan solution (1.5% in 0.5% acetic acid) was dropped in 5% sodium hydroxide. The beads were crosslinked with ECC or with GA. The crosslinked beads were washed with water for 24 h to remove the residual free crosslinking agent. A part of the prepared beads (crosslinked or not) were dried under reduced pressure till constant weight. The rest of the beads were kept in water. The mean weight of a single dry and non-crosslinked bead is $2.8x10^{-4}$ g. Beads as prepared will be called "coacervate beads" in order to be distinguished from the swollen ones after having been dried. The diameter values depend slightly on pH of the medium (7) and are in the ranges of 2.7-3.2 mm for the coacervate beads and 1.0-1.5 mm for the swollen ones.

Drug loading of coacervate beads, crosslinked or non-crosslinked, was carried out in drug aqueous solutions at 25°C. Sorbed quantities of SQ (mol SQ/g chitosan) were: $4.24x10^{-3}$ for the coacervate non-crosslinked beads, $3.94x10^{-3}$ for the crosslinked with ECC beads and $3.88x10^{-3}$ for the crosslinked with GA beads. The sorbed quantity of DHBSA in CGB crosslinked with ECC is $3.41x10^{-3}$ mol DHBSA/g chitosan. Non-crosslinked CGB were not used because they were soluble in DHBSA solutions. Because of DHBSA instability in solution, its sorption was performed in dark. A part of the loaded coacervate beads was treated by soaking for 5 min in PEG400 containing 0.05 $mol.l^{-1}$ DHBSA.

Release measurements

The release of SQ and DHBSA was estimated from: a) coacervate beads directly after their loading; b) loaded and dried beads and c) in the case of DHBSA, loaded and pre-treated with PEG400 beads. SQ desorption was performed at pH 7 and 9 from the non-crosslinked beads, at pH 3.9, 7 and 9 from the crosslinked ones; $\mu = 0.1$, 37°C. DHBSA release was carried out at pH 3.9 or 7, $\mu = 0.1$, 37°C. The drug content in the solutions was measured spectrophotometrically at regular time intervals. SQ and DHBSA electron spectra were registered on a spectrophotometer Specord UV-VIS 71. The solution

concentrations were determined using calibration. Beer's law was obeyed in the working concentration range (given in brackets): for SQ solution in: a) acetate buffer (pH 3.9) and phosphate buffer (pH 7.0) λ_{max} 435 nm (10^{-4} - 9.10^{-4} $mol.1^{-1}$); b) carbonate buffer (pH 9.0) λ_{max} 366 nm (10^{-5} - 2.10^{-4} $mol.1^{-1}$); for DHBSA solutions in an acetate buffer (pH 3.9) and phosphate buffer (pH 7.0) λ_{max} 300 nm (10^{-4} - 5.10^{-4} $mol.1^{-1}$).

Determination of the diffusion coefficients of SQ and DHBSA release from chitosan beads

The following relationship derived for device with spherical form (8) was used for the calculation of the diffusion coefficients of drug release from chitosan beads:

$$\frac{m_t}{m_0} = \frac{6}{r}\sqrt{\frac{D}{\pi}}\sqrt{t} \qquad (1)$$

where: m_t - the amount of the released drug in moment t, m_0 - the total drug amount sorbed in the beads, r - radius of the bead, D - the diffusion coefficient, t - time interval. The diffusion coefficient was determined from the slope of the straight part of the curve obtained from the experimental release data presented in coordinates m_t/m_0- $t^{1/2}$. The results were treated by the least square procedure.

Microbiological tests

Tests were carried out with *S. aureus, Y. enterocolitica* and *E. coli,* incubated for 24 h at 37°C and with *C. albicans,* incubated for 48 h at 37°C. Minimum inhibitory concentrations (MIC) were determined in meat-peptone broth by the serial dilution method. The microbiological effect of the loaded beads was estimated by the width of the sterile zone around a row of five beads arranged closely one to another on a Petri dish filled with meat-peptone agar. The given values of the sterile zone width were the mean values from 10 measurements.

Results and discussion

Drug release

DHBSA release from CGB (coacervate, swollen and treated with PEG400) has been studied at pH 3.9 (acetate buffer) and pH 7 (phosphate buffer), μ = 0.1, 37°C. Buffer solutions with higher pH have not been used because of DHBSA instability under alkaline conditions. It was found that soaking of coacervate beads in PEG400 enhances the stability of the system CGB-DHBSA. Beads remain colourless on storage (2 weeks), while non-treated with PEG beads become pink after 24 h. Desorption proceeds rapidly and completely for 30 min in all cases. During the release process the dry beads swell and almost reach the dimensions of the coacervate beads. Drying of the DHBSA loaded beads effects slightly the rate of drug release. For the beads pre-treated with PEG400 a small induction period is observed (Fig. 1).

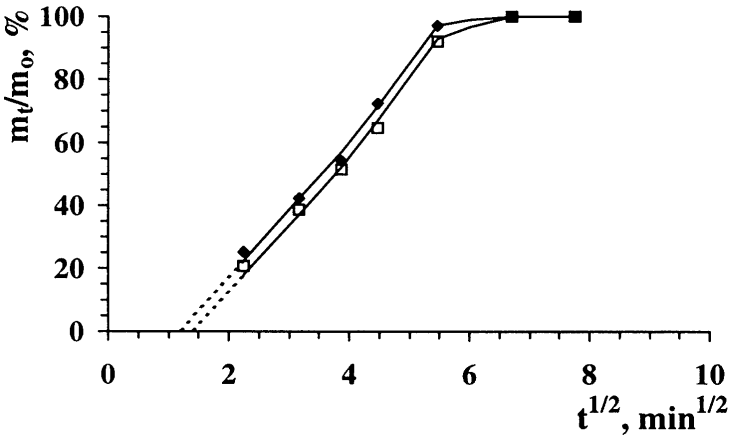


Fig.1. DHBSA release (m_t/m_0) as function of square root of time ($t^{1/2}$) from pre-treated with PEG400 beads crosslinked with ECC at $\mu = 0.1$, 37 ^{0}C: □ pH 7, ◆ pH 3.9.

A quantitative release of SQ has not been observed in non of the cases. The amount of the desorbed SQ depends on the CGB type and especially on the nature of the crosslinking agent. Non-crosslinked beads release more than 80%, beads crosslinked with ECC release approximately 80% while those crosslinked with GA up to 60% of the loaded drug. These data are in accordance with the sorption experiments (7). The introduction of hydrophobic groups into chitosan gel through its crosslinking with GA gives an increase of the sorptivity due to hydrophobic interactions. The crosslinking of the beads with ECC effects insignificantly their sorption (7) and release behavior (see also Table 1).

The effect of pH of the medium on the total amount of released SQ from a given CGB type is less noticeable. It increases with 6-12% on changing pH of the liquid phase from acidic to alkaline. For example, at pH 3.9 swollen beads crosslinked with ECC release 77% from the sorbed drug and at pH 9 - 83%.

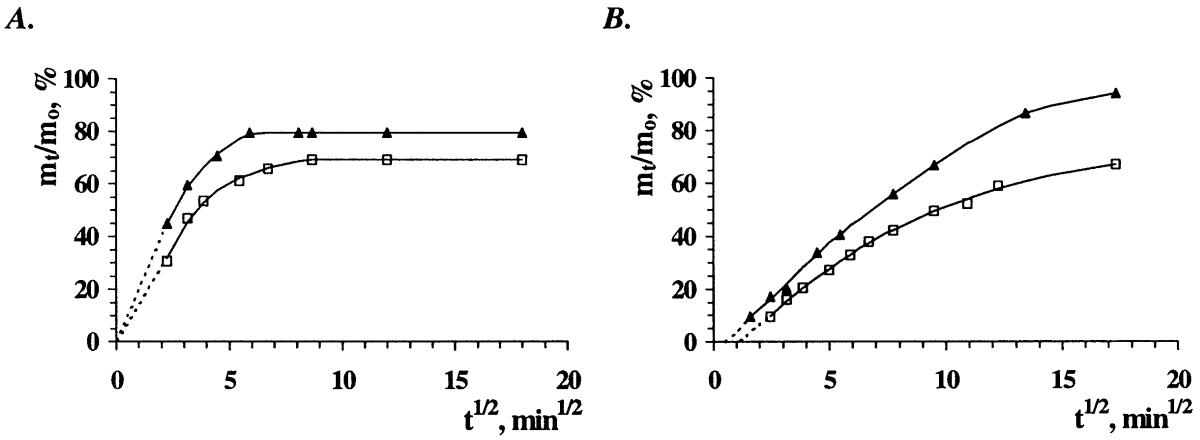


Fig.2. SQ release (m_t/m_0) as function of square root of time ($t^{1/2}$) from non-crosslinked beads, $\mu = 0.1$, 37^{0}C: A - coacervate and B - swollen beads; □ pH 7, ▲ pH 9.

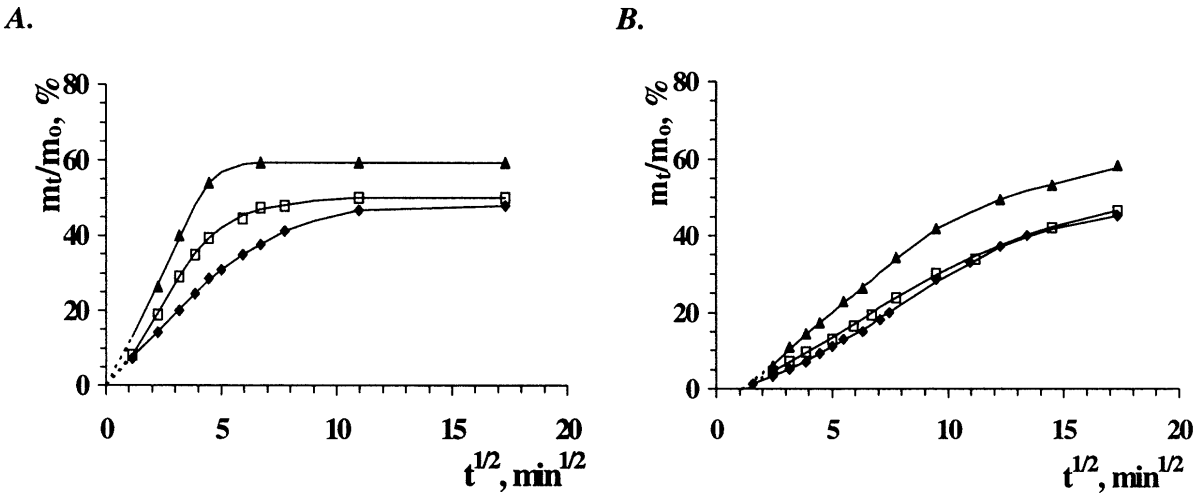


Fig.3. SQ release (m_t/m_0) as function of square root of time ($t^{1/2}$) from crosslinked with GA beads, μ = 0.1, 37^{0}C: A - coacervate and B - swollen beads; ◆ pH 3.9, □ pH 7, ▲ pH 9.

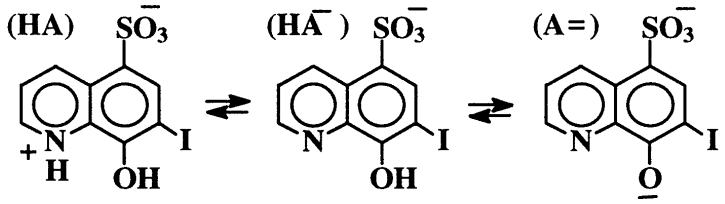
The linear dependence of the amount of drug released on square root of time is an evidence for diffusionally controlled release from the macromolecular carrier. Examples of release curves experimentally obtained for DHBSA and SQ desorption are presented in Figs. 1-3. The corresponding diffusion coefficients, given in Table 1, are calculated from the slope of the straight part of the curves. Their values express quantitatively the effect

Table 1. Diffusion coefficient (D) values of SQ and DHBSA release from chitosan beads depending on pH of the medium and chitosan matrix.

Chitosan beads-drug	D [m^2s^{-1}]×10^{11}		
	pH 3.9	pH 7	pH 9
non-crosslinked			
coacervate-SQ	a)	6.09 ± 0.06	8.58 ± 2.23
swollen-SQ		0.20 ± 0.02	0.46 ± 0.05
crosslinked with ECC			
coacervate-SQ	4.37 ± 0.63	5.05 ± 0.53	9.56 ± 3.53
swollen-SQ	0.63 ± 0.05	0.16 ± 0.02	0.31 ± 0.06
crosslinked with ECC			
coacervate-DHBSA	15.6 ± 1.7	17.7 ± 1.1	c)
coacervate -DHBSA (PEG)(b)	14.5 ± 2.8	12.4 ± 0.6	c)
swollen-DHBSA	10.3 ± 1.5	7.3 ± 1.6	c)
crosslinked with GA			
coacervate-SQ	1.03 ± 0.05	2.07 ± 0.15	4.17 ± 0.25
swollen-SQ	0.07 ± 0.01	0.04 ± 0.01	0.09 ± 0.01

a) the beads dissolve; b) beads treated with PEG400; c) not determined because of DHBSA instability at pH 9.

of the way of preparation of the chitosan matrix and pH of the medium on the rate of drug release. The non-crosslinked or crosslinked with ECC beads release SQ faster than those crosslinked with GA. Pre-drying slows down the SQ release (Figs. 2,3). This effect is noticeable comparing the behaviour of the coacervate and the swollen beads. The volume of the former is about 15 times larger than that of the swollen beads. As it is seen from the data listed in Table 1, the diffusion coefficient values of SQ release from coacervate spheres exceeds more than 10-fold those from swollen beads. The variation in the swelling degree for the dry beads is rather small (7). Its effect on the rate of SQ desorption is combined with that of pH of the medium. The latter affects both the swelling degree of the beads and the dissociation equilibria in the system: the ratio of the different SQ ionic forms (9) and the percentage of the charged NH_2-groups in chitosan macromolecules. Using the values of the SQ dissociation constants (pK_1 = 2.514 and pK_2 = 7.417 (9)) it is calculated that HA^- is the predominant form at pH 3.9 (96%), at neutral pH both forms HA^-(72%)



and $A^=$ (28%) present. At pH 9 the equilibrium is drawn towards $A^=$ (97%). Therefore the percentage of the charged SQ forms increases on increasing of pH which determines the better SQ solubility at higher pH. Moreover, under alkaline conditions the amino groups in chitosan macromolecules are mainly in neutral form which excludes the possibility for ionic interaction between chitosan-NH_3^+ and SQ^- and favours the release of SQ. All these factors are interrelated and their contributions to the changes of the release rate can not be separately evaluated. Generally, a tendency of increasing the diffusion coefficient values with the increase of pH of the medium is observed.

Comparing the desorption rate of the two bioactive substances from CGB crosslinked with ECC, it is seen that DHBSA desorbs faster (about 3.6 times) than SQ both in acid or neutral medium. SQ lower release rate may be assigned to its considerable hydrophobicity.

Microbilogical tests

Microbiological test data are listed in Table 2. Sterile zones have not been observed during the tests carried out with unloaded beads (non-crosslinked or crosslinked with ECC and GA). Clearly defined sterile zones have been observed at both sides of the rows of loaded with SQ beads. A dependence of the sterile zone width on the MIC values for the corresponding microorganism is noticed. The measured widths are smaller for microorganisms with higher MIC.

Table 2. Minimum inhibitory concentration (MIC) and width of the sterile zone resulting from SQ release from CGB, 37°C, incubation period 24 h, five beads, meat peptone agar.

	MIC mg/l	Width of the sterile zone (in mm)		
		Non-crosslinked	Crosslinked with ECC	Crosslinked with GA
E. coli	350	8	6	6
S. aureus	125	40	20	40
Y. enterocolitica	253	20	21	10
*C. albicans**	275	24	23	20

* Incubation period 48 h

Conclusion

With view of chitosan application in medicine the preparation of drug release systems with antimicrobial and haemostatic activity have been investigated. SQ and DHBSA have been used as model substances. The quantity of the desorbed drug depends mainly on the chemical nature of the drug and the crosslinking agent. DHBSA release proceeds completely while quantitative desorption of SQ has not been observed. Crosslinking with GA reduces both the total amount of the desorbed SQ and the rate of SQ release, while crosslinking with ECC effects slightly the release behavior of the beads in comparison with the non-crosslinked ones. The drying of the loaded beads slows down the rate of SQ release. Microbiological tests reveal that the loaded with SQ beads display antimicrobial and antimycotic activity.

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