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Kinetics of in vitro release of a model nucleoside deoxyuridine from crosslinked insoluble collagen and collagen–gelatin microspheres

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Abstract

The objective of this research is to investigate the effect of highly crosslinked insoluble collagen and collagen–gelatin hybrid matrices as platforms for controlled release of a highly water soluble model nucleoside, deoxyuridine as well as a high molecular weight model compound fluoroscienisothoicyanate (FITC)-dextran. Collagen and gelatin can be considered as biodegradable proteinous materials. Microspheres of deoxyuridine and FITC-dextran were prepared by emulsification solvent evaporation technique using collagen alone and collagen–gelatin combination. The microencapsulation efficiency, particle size and in vitro release profiles were compared. Microencapsulation efficiency of $\approx 10\%$ was obtained with collagen while 20% encapsulation efficiency was obtained when collagen was used in combination with gelatin. Particle size range became wider when only collagen was used as compared to collagen–gelatin combination. A slower release profile was observed for crosslinked as compared to noncrosslinked microspheres. This study demonstrated diffusion controlled release of both compounds from the two polymers used. A good correlation was obtained between theoretically predicted and experimentally obtained in vitro release rates for both deoxyuridine and FITC-dextran using Higuchi's square root model.

Keywords: Collagen and gelatin microspheres; Microencapsulation; Deoxyuridine; FITC-dextran; Controlled release

1. Introduction

Collagen is an insoluble fibrous protein found in all multicellular organisms. It is the most important structural protein in human and animal tissues with a mass of 285 kD. The molecular structure consists of three polypeptide chains of similar lengths, which are in helical conformation winding around each other. Several types of collagen can be formed by variation of the two major peptide chains α one and α two and their subtypes. The most important structural collagens are type one and two collagen consisting of two α one

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and one α two chains, respectively. Both chain types have similar amino acid contents rich in glycine (every third amino acid in a chain is glycine) and the presence of rare amino acids, 4-hydroxy proline and 5-hydroxylysine. Beside these, other amino acids like proline, lysine, and serine are also present but they represent only a small segment of the collagen molecule. It is a well tolerated and a completely biodegradable material. The physical properties of this protein have created new interest in collagen as one of the most useful biomaterials in medical use. It has been used as the basic material for drug delivery systems such as implantable matrix system, sponges, and ocular lenses (Robertson et al., 1984; Phinney et al., 1988; Jayanthi and Panduranga, 1990; Fujioka et al., 1995; Rossler et al., 1995; Li, 1996). Collagen contains a large number of reactive terminal and side chain residues such as amino and hydroxyl groups (Marvin, 1985; Gorham, 1991). Therefore, it is possible to prepare sustained-release dosage forms by crosslinking a defined chain of collagen (Marvin, 1985; Gorham, 1991). Soluble monomolecular collagen or atelocollagen has been used as drug carrier system. Their subsequent crosslinking has been reported to minimize dissolution and swelling at 37°C (Wolfgang et al., 1996). A novel sustained release drug delivery system was used in vivo in which liposomes and a collagen gel was injected together. Release of the drug into the systemic circulation was slower from liposomes sequestered in collagen gel than from vesicles alone (Weiner et al., 1985). Collagen molecules decrease liposome permeability by an antioxidant effect and also by a specific interaction with phospholipids (Pazo et al., 1989; Pajen et al., 1991). Gelatin microspheres were prepared by crosslinking with 20% glutaraldehyde but no difference in release rate was observed between noncrosslinked and crosslinked microspheres (Ugwoke et al., 1997). Microcapsules composed of soluble collagen and chondroitin sulphate were prepared by complex co-acervation (Shao and Leon, 1995). Studies were conducted to couple transforming growth factor beta (TGF-β) to collagen via bifunctional polyethylene glycol (Bentz et al., 1995). The diffusion of macromolecules can be controlled by the fabrication technique, the crosslinking agent and the chemical nature of crosslinks (Gilbert et al., 1998). Studies have shown that soluble collagen can be used as a new biodegradable carrier for preparing films of long acting pilocarpine delivery system (Vasantha et al., 1988).

In the present investigation collagen and collagen–gelatin microspheres were prepared. Deoxyuridine and FITC-(fluorosceinisothiocyanate) dextran were selected as model low and high molecular weight hydrophillic compounds, respectively. The microspheres were prepared by emulsion solvent evaporation process using different percentages of glutaraldehyde as the crosslinking agent (Deasy, 1984b). In vitro dissolution study was conducted to investigate the mechanism of drug release from the crosslinked polymer matrix.

2. Materials and methods

2.1. Materials

Collagen (type II), deoxyuridine, polyvinyl alcohol (PVA)and FITC-dextran were obtained from Sigma Chemical, St Louis, MO. Gelatin, glutaraldehyde (GTA) and methylene chloride were purchased from Fisher Scientific (St Louis, MO).

2.2. Preparation of microspheres

Microspheres were prepared by solvent evaporation method (Krause and Rhodewald, 1985). In brief, the fibrilar collagen (1% w/v) was pre-swollen for 1 h in water with pH adjusted to 3.5 using acetic acid. Then deoxyuridine was dissolved in the collagen dispersion with 2 ml of 0.25% PVA and sonicated for 30 min. The generated slurry was poured into methylene chloride slowly with continuous homogenization to form water-in-oil emulsion. The resulting emulsion was then poured into 20 ml of 0.25% w/v PVA solution with constant stirring. Methylene chloride was evaporated and the suspension was mixed with varying concentrations of (8–18% v/v) glutaraldehyde. After stirring for 4 h, 2 ml of sodium metabisulphite solution was added to arrest the crosslinking

reaction. The mixture was stored in the freezer overnight at −5°C. The microspheres were separated by centrifugation at 5000 rpm for 15 min, washed with water and finally freeze dried.

For the preparation of gelatin-collagen microspheres, gelatin was first dissolved in warm water (50°C) and then cooled to room temperature. The solution was processed in a manner described previously.

2.3. Drug content

An accurately weighed (10 mg) amount of microspheres was dispersed in 15 ml of phosphate buffer, sonicated for 30 min and then filtered. The absorbence of the filtrate was measured at 262 and 493 nm for deoxyuridine and FITC-dextran, respectively.

2.4. Particle size analysis

Particle size distribution of microspheres was examined using standard sieve Nos 60 and 80. The particles that passed through sieve No. 80 were then measured microscopically with the aid of a stage, an eye piece micrometer, and camera (Carl Zeiss, Germany).

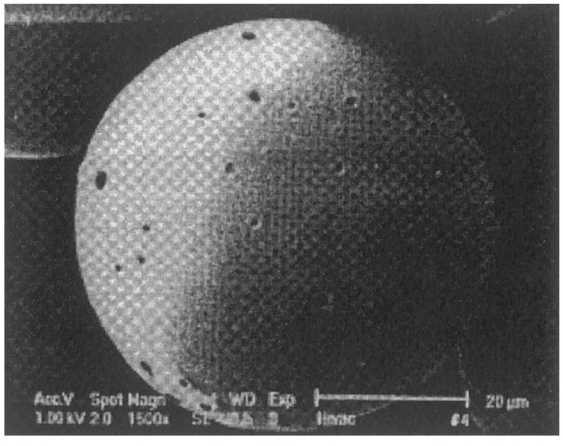

Fig. 1. Photomicrograph of microspheres prepared with collagen–gelatin combination.

2.5. In vitro release

Twenty five milligrams of microspheres was dispersed in 25 ml of pH 7.4 phosphate buffer in a 50 ml round bottom flask. The flasks were sealed to avoid solvent evaporation. They were then placed in a thermostated shaker water bath with a vibratory motion of 80 rpm and the temperature was maintained at 37°C. Samples (2 ml) were periodically withdrawn and immediately replaced with equal volumes of fresh dissolution medium to maintain sink conditions. The concentrations of the model compounds in the sample were determined by measuring the absorbence at 262 and 493 nm for deoxyuridine and FITC-dextran, respectively.

3. Results and discussion

3.1. Particle size

Microspheres were formed as clusters of particles. After freeze drying the solid agglomerates were passed through standard sieves (sieve Nos 60 and 80) and the resulting spheroid particles formed were composed of deoxyuridine dispersed in collagen or collagen/gelatin matrices. Selection of a suitable microsphere formulation requires optimization of the amount of emulsifier (PVA). The concentration of PVA was optimized at 0.25% based on the yield and particle size. Particle size appears to be inversely related to the concentration of emulsifier (Deasy, 1984a). Microspheres prepared with collagen generated larger particle size (mean diameter 300 μm) whereas microspheres prepared with gelatin and collagen combination resulted in smaller particle size (mean diameter 200 μm) and were more spherical in shape (Fig. 1).

3.2. Microencapsulation efficiency

Microencapsulation efficiency was determined as the ratio of actual quantity of drug encapsulated within the microspheres to the theoretical quantity of drug added during emulsification phase and was expressed as percentage. Microen-

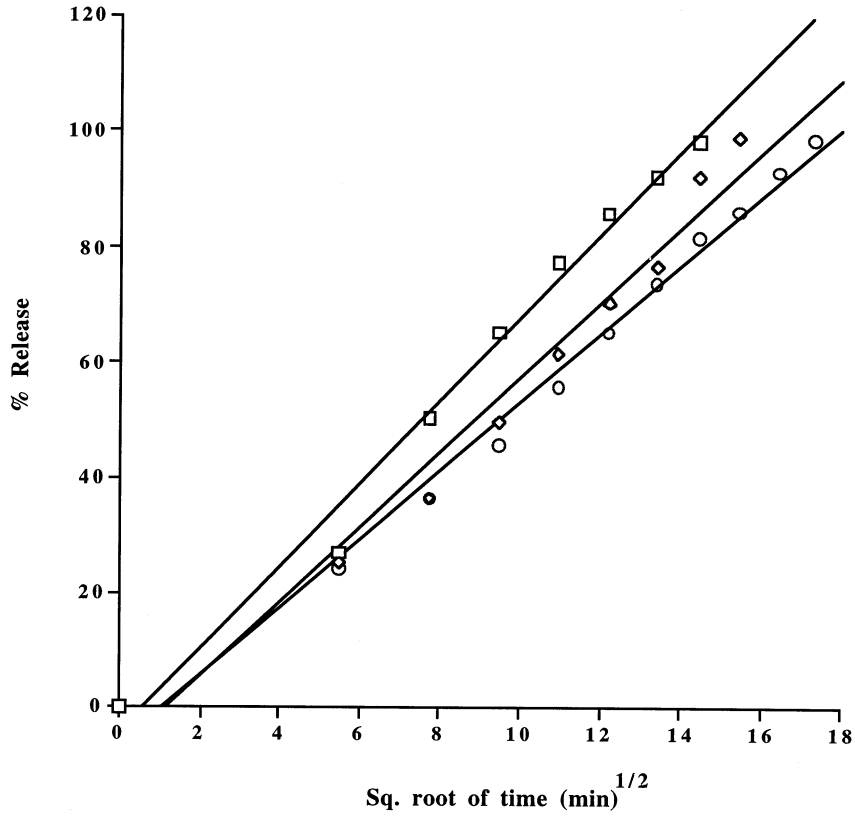

Fig. 2. Release of deoxyuridine from noncrosslinked microspheres prepared with (□) collagen; (◇) gelatin and (○) collagen–gelatin combination. Each point represents a mean of three determinations. Line drawn through the points does not represent any mathematical fit.

capsulation efficiency of three batches of collagen microspheres varied from 10 to 12% while efficiency of the batches of collagen–gelatin microspheres varied from 15 to 18%. A possible reason for this difference may be due to higher fraction of drug entrapped in gelatin as well as adsorbed onto the collagen surface. High solubility of the drug may be a reason for the low encapsulation efficiency. To remove any traces of glutaraldehyde, microspheres were washed with water. During washing a large percentage (2.5%) of the drug was washed away from the surface of the microspheres.

3.3. In vitro release

In vitro release studies of collagen, gelatin and collagen–gelatin microspheres indicated that deoxyuridine was released in a continuous manner over 3, 4 and 5 h, respectively (Fig. 2). Non crosslinked collagen adsorbs significant amount of water when soaked in phosphate buffer. In order to decrease water uptake GTA was added in various amounts (8–18% v/v) to collagen and collagen–gelatin dispersion. An increase in the degree of crosslinking may cause a reduction in the number of free amino groups. As the concentration of GTA increases water penetration into the highly crosslinked matrix is significantly retarded but not totally suppressed. In vitro study of crosslinked collagen microspheres indicated an increase in release over 6 h under the conditions employed (Fig. 3). Crosslinking reduces polymer chain mobility, increases glass transition temperature and decreases diffusivity (Deasy, 1984a). Since gelatin has chemical and physical properties

similar to collagen, two polymers were subsequently combined to prepare biodegradable microspheres. In vitro release profiles of gelatin-collagen microspheres show extended release up to 9.5 h (Fig. 4). Such extended release may be due to the fact that a higher fraction of drug is entrapped in the gelatin core as well as adsorbed onto the collagen surface. Subsequent to water penetration the collagen or collagen–gelatin sphere forms a gel that is separated from the unhydrated polymer by a partially hydrated and wetted layer. Depending on the amount of polymer added such gels tend to hinder the outward diffusion of deoxyuridine either by mechanically blocking it and/or by providing a more tortuous pathway or by direct interaction with the permeant. Similar adsorption and entrapment phenomena have been described with monomolecular collagen or gelatin (Krause and Rhodewald, 1985; Nastruzzi et al., 1994). Retention of drug appears to be related to a decrease in swellability of the polymer due to crosslinking by glutaraldehyde. Collagen microparticles were prepared with 25% glutaraldehyde but no release study was performed to examine the effect of crosslinking on permeant diffusion (Rossler et al., 1995). In another study no measurable differences were observed in release rates of small permeants from polymers crosslinked with various amounts of glutaraldehyde but the maximum concentration of glutaraldehyde used was only 0.0855% (Wolfgang et al., 1996). Release studies with FITC-dextran showed that all the compounds released fairly rapidly within 10 h (Figs. 5 and 6). Such rapid

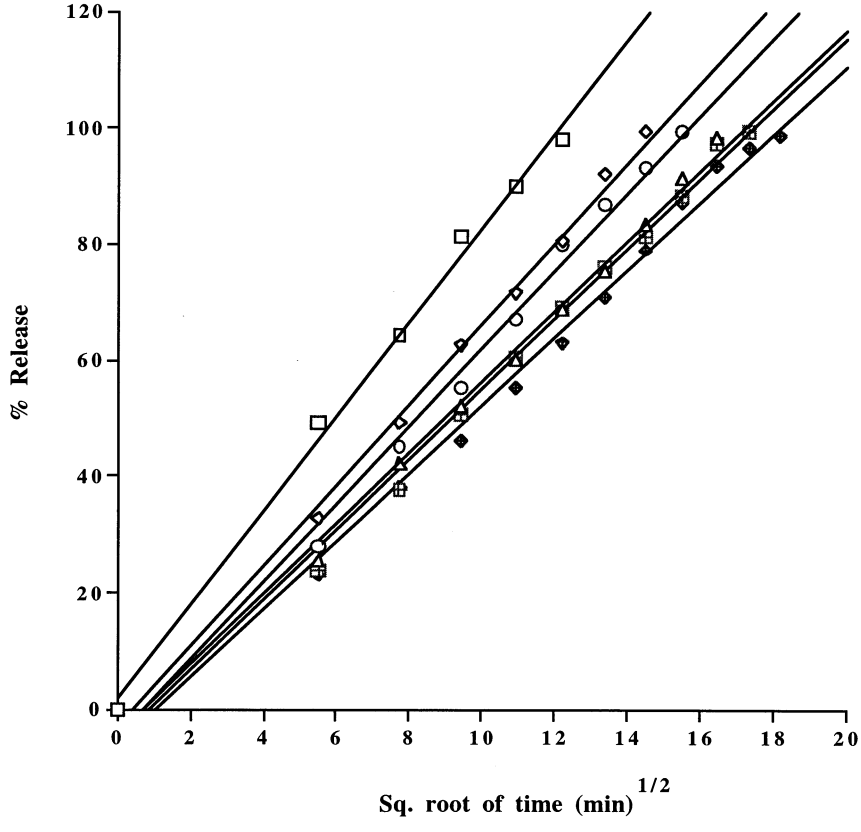

Fig. 3. Release of deoxyuridine from crosslinked collagen microspheres prepared with (□) 8% GTA; (◇) 10% GTA; (○) 12% GTA; (△) 14% GTA; (⊠) 16% GTA; (◆) 18% GTA. Each point represents a mean of three determinations. Line drawn through the points does not represent any mathematical fit.

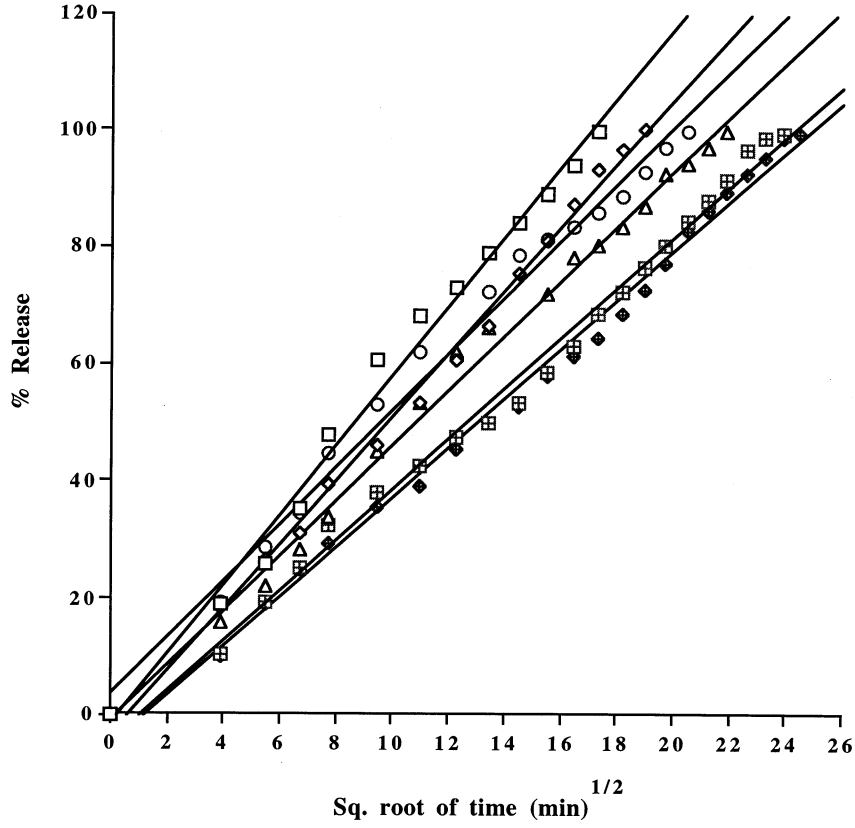

Fig. 4. Release of deoxyuridine from crosslinked gelatin –collagen microspheres prepared with (□) 8% GTA; (◇) 10% GTA; (○) 12% GTA; (△) 14% GTA; (⊠) 16% GTA; (◆) 18% GTA. Each point represents a mean of three determinations. Line drawn through the points does not represent any mathematical fit.

release rates may be due to the fact that the availability of free highly reactive amine groups in collagen were not enough to be appreciably crosslinked by glutaraldehyde and consequently there was little rise in viscosity. Increasing the degree of crosslinking has little effect on the release of compounds from the microspheres. When the concentration of collagen and gelatin was raised to 3% microspheres formed a spongy mass. High aqueous solubility of the dextrans may also be responsible for rapid release of compounds from the microspheres.

3.4. Release kinetics

Drug release from homogenous matrix assumes that dissolution is initiated at the solid–liquid boundary and gradually the front moves through the subsequent layers to the inner core. Release rates from such irregularly shaped microspheres apparently follow Higuchi equation (Martin, 1983). In the present study, three different mathematical equations were employed to model the dissolution profiles i.e. (a) first order equation; (b) Higuchi square root of time equation and (c) Hixson and Crowell cube root equation.

(a) First order model can be expressed as

$$\frac{M_t}{M_\infty} = 1 - e^{-kt} \quad (1)$$

(b)Higuchi square root of time model is given by

$$\frac{M_t}{M_\infty} = kt^{1/2} \quad (2)$$

(c) Hixson and Crowell cube root equation is denoted by

$$\left(1 - \frac{M_t}{M_\infty}\right)^{1/3} = 1 - kt \quad (3)$$

M_t is the amount of drug released at time t, M_∞ is the maximal amount of time released at infinite time, and k is the rate constant associated with the drug release.

The correlation coefficients obtained after fitting the in vitro release data to the above equations indicate that best fit is obtained with Higuchi square root of time equation rather than first order or Hixson and Crowell cube root equation. The data shown in Table 1 and Table 2 clearly suggest that the release of deoxyuridine and FITC-dextran from the microspheres follows a simple diffusion mechanism across the porous structures of gelatin or gelatin/collagen layers.

Further analysis of data suggests that release rate can be reduced to two approximations which are valid for different portions of the dissolution curve. The early time approximation which holds over the initial portion of the curve is expressed by the Eq. (4), when percent released is $< 40\%$ (Benoit et al., 1984).

$$\frac{Q}{W_0} = 1 - \frac{6}{\pi^2} e^{\frac{\pi^2 Dt}{r^2}} \quad (4)$$

Q is the amount of drug released at time t, W_0 is the initial amount present in the microspheres, D

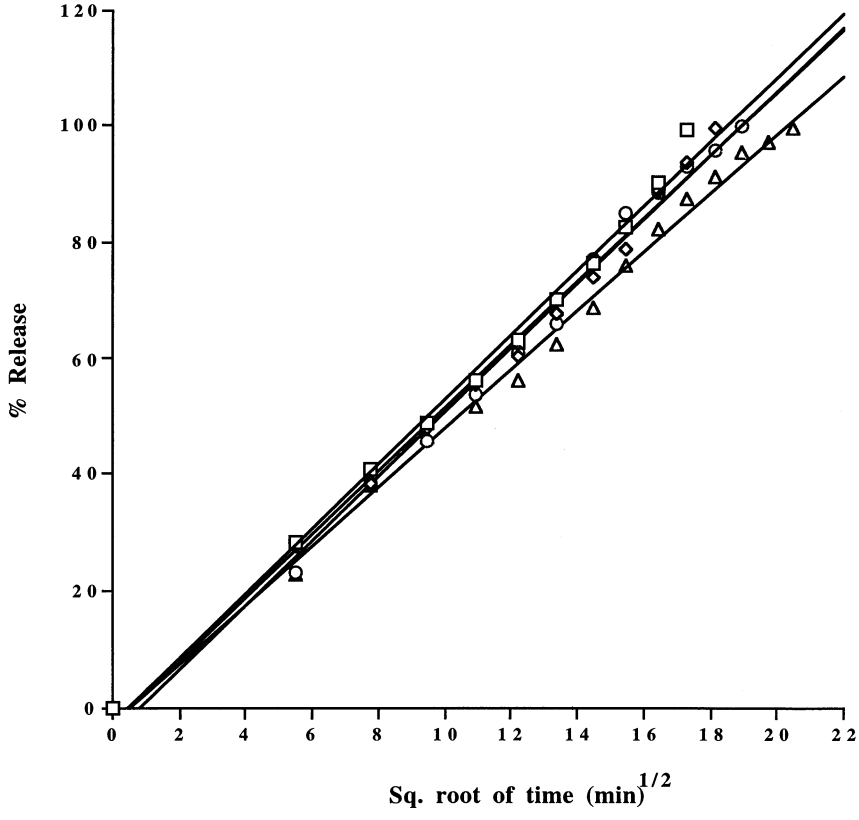

Fig. 5. Release of FITC –Dextran from crosslinked collagen microspheres prepared with (□) 8% GTA; (◇) 10% GTA; (○) 12% GTA; (△) 14% GTA. Each point represents a mean of three determinations. Line drawn through the points does not represent any mathematical fit.

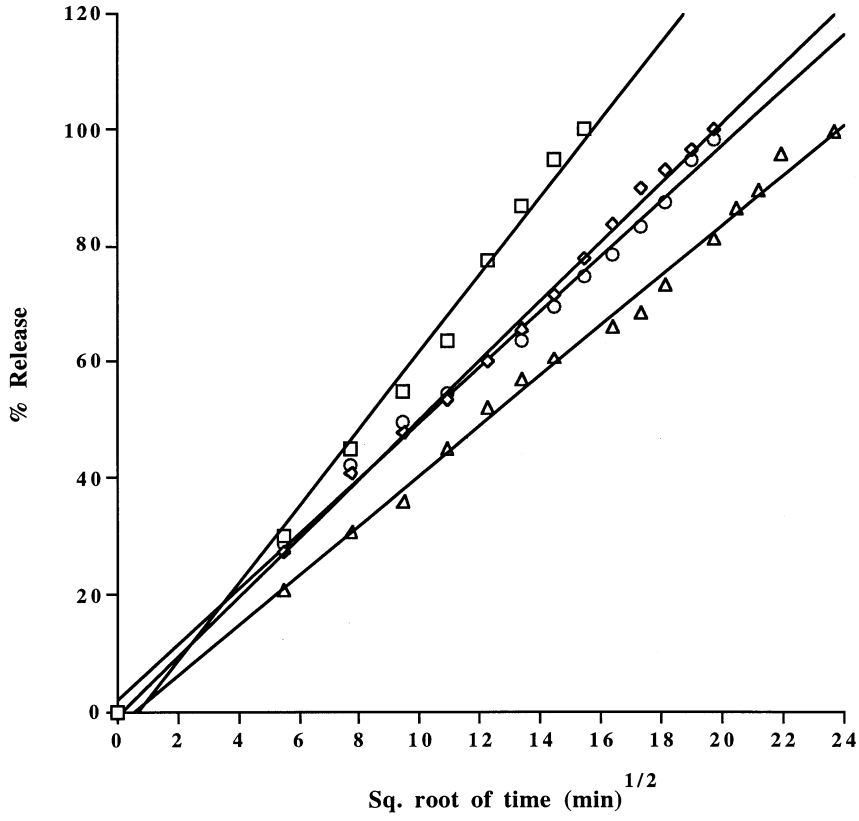

Fig. 6. Release of FITC-Dextran from crosslinked collagen–gelatin microspheres prepared with (□) 8% GTA; (◇) 10% GTA; (○) 12% GTA; (△) 14% GTA. Each point represents a mean of three determinations. Line drawn through the points does not represent any mathematical fit.

Table 1
Correlation coefficients of different mathematical models for deoxyuridine

% (v/v) Crosslinking agent (with collagen)	First order model	Higuchi square root of time model	Hixson–Crowell cube root equation
8	0.970	0.995	0.966
10	0.969	0.997	0.960
12	0.987	0.992	0.970
14	0.970	0.994	0.960
16	0.980	0.993	0.967
18	0.960	0.991	0.960
(With collagen and gelatin)			
8	0.991	0.990	0.987
10	0.973	0.996	0.962
12	0.980	0.989	0.970
14	0.978	0.994	0.970
16	0.974	0.993	0.980
18	0.970	0.992	0.964

Table 2
Correlation coefficients of different mathematical models for FITC-dextran

% (v/v) Crosslinking agent (with collagen)	First order model	Higuchi square root of time model	Hixson–Crowell cube root equation
8	0.982	0.993	0.972
10	0.984	0.995	0.967
12	0.978	0.993	0.973
14	0.983	0.995	0.968
(With collagen and gelatin)			
8	0.981	0.990	0.972
10	0.975	0.997	0.988
12	0.973	0.995	0.986
14	0.972	0.995	0.971

is the diffusion coefficient and r is the radius of the particle.

Later time approximation is expressed by Eq. (5), where percent released is $>60\%$ (Benoit et al., 1984)

$$\frac{Q}{W_0}=\left(\frac{Dt}{r^2\pi}\right)^{1/2}-\left(\frac{3Dt}{r^2}\right) \quad (5)$$

When release data was fitted to Eqs. (4) and (5), the dissolution profiles followed conditions of Eq. (4) up to 1 h and then followed Eq. (5). Eq. (4) follows first order model (Eq. (1)) whereas Eq. (5) is derived from Higuchi equation (Eq. (2)). Since permeant release up to 1 h can be explained by Eq. (4), the release mechanism probably suggests non-Fickian diffusion and the release after 1 h as can be best explained by Eq. (5) which suggests transition to Fickian diffusion. This observation is contrary to the findings of a similar study where the early time approximation was given by Eq. (5) and late time approximation was given by Eq. (4) (Benoit et al., 1984).

4. Conclusion

Highly crosslinked collagen–gelatin microspheres may be useful for sustaining the release of highly hydrophillic drugs. Molecular weight of the model compounds seems to have no effect on the release rate. Solubility of the compound should be considered before designing crosslinked microspheres. Upon mathematical modeling the release rate of the model compounds followed Higuchi's square root equation more closely than first order or Hixson and Crowell cube root equation. The mechanism of release from microspheres is likely to be non-Fickian diffusion up to 1 h and Fickian diffusion after 1 h of dissolution.

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