Journal of Macromolecular Science, Part A
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597274

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To cite this Article Bajpai, A. K. and Choubey, Jyoti(2005) 'Release Study of Sulphamethoxazole Controlled by Swelling of Gelatin Nanoparticles and Drug-Biopolymer Interaction', Journal of Macromolecular Science, Part A, 42: 3, 253 — 275
To link to this Article: DOI: 10.1081/MA-200050357
URL: http://dx.doi.org/10.1081/MA-200050357

Journal of Macromolecular Science®, Part A: Pure and Applied Chemistry, 42:253–275, 2005

ISSN 1060-1325 print/1520-5738 online
DOI: 10.1081/MA-200050357

Release Study of Sulphamethoxazole Controlled by Swelling of Gelatin Nanoparticles and Drug-Biopolymer Interaction

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Gelatin (Type A) particles were prepared by a single W/O emulsion technique and characterized by infrared (IR) spectra, scanning electron microscopy (SEM) and particle size analysis. Whereas the IR spectra clearly confirmed the presence of gelatin and sulphamethoxazole (SM) in the loaded nanoparticles, the scanning electron micrograph (SEM) image depicts smooth surface, spherical shape and uneven size of nanoparticles (100 to 300 nm). The nanoparticles were evaluated for their potential to act as a carrier of sulphamethoxazole drug. It was found that the amount of released SM increases with increasing percent loading of the drug in the range 18 to 39%. The chemical architecture of nanoparticles was also found to influence its drug-releasing capacity. It was observed that in the case of an increase in gelatin and crosslinker (gluteraldehyde) concentrations in the range 4.0–9.0 g and 5.3–31.8 mM, respectively, the amount of released SM initially increases up to 8.0 g of gelatin and 10.6 mM of crosslinker concentrations and thereafter decreases. It was also noticed that a greater release of SM occurs when type B gelatin is used as drug carrier. The influence of experimental conditions such as pH and temperature of the release medium were also investigated on the release profiles of SM. It was noticed that an optimum release is obtained at pH 7.4, while in the case of a variation of temperature in the range 10 to 35°C, a maximum release is found at 25°C. Beyond 25°C, a fall in the released SM was observed. The drug was also found to be chemically stable at pH 1.8 (gastric juice) as confirmed by UV spectral study.

Keywords gelatin, nanoparticles, drug, release, kinetics

Introduction

Controlled drug delivery technology represents one of the frontier areas of science, which involves multidisciplinary scientific approach, contributing to human health care (1). These delivery systems offer numerous advantages compared to conventional dosage forms, which include improved efficacy, reduced toxicity, and improved patient compliance and convenience. Among different dosage forms reported, nanoparticles have been identified as a noble class of matrices possessing a great potential as drug carriers.

Received June 2004; Accepted August 2004
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Nanoparticles are defined as submicron (size $<1\,\mu m$) colloidal carriers (2) which were initially devised for carrying vaccines and anticancer drugs. Compared to other drug carriers, such as liposomes (3), polymeric nanoparticles present a higher stability when in contact with biological fluids, and their polymeric nature makes it possible to obtain the desired controlled and sustained release. Nanoparticles have the advantage of being more stable. Many types of drug targeting depends on good stability. A better penetration of the particles inside the body following administration, as well as longer shelf storage life, are several benefits of the good stability of nanoparticles. Another significant advantage of nanoparticles is that due to their smaller size, they could easily reach the sensitive and deeper part of the body such as brain, bone marrow, etc. and deliver encapsulated or adsorbed drug into the targeted sight. Nanoparticles have attractive physicochemical properties such as size, surface potential, hydrophilic-hydrophobic balance, etc (4). For this reason, they have been recognized as potential drug carriers for bioactive ingredients such as anticancer drugs (5), vaccines (6), oligonucleotides (7), peptides (8), etc. Their widespread use for oral delivery also aims at improving the bioavailability of drugs with poor absorption characteristics (9), reducing GI mucosa irritation caused by drugs (10) and assuring stability of drugs in the GI tract (11). Thus, all these and many more such characteristics of nanoparticles qualify them as a promising candidate in drug-delivery technology.

Although various biodegradable nanoparticles of natural polymers such as starch (12), chitosan (13), liposomes (14), etc. are largely in use as drug carriers in controlled drug-delivery technology, those derived from gelatin are of prime importance because of their biocompatible, non-toxic and non-carcinogenic nature (15). These nanoparticles have also been richly documented in the literature. For example, Kaul and Amiji (16) prepared poly(ethylene glycol) modified gelatin nanoparticles for intracellular delivery and found them quite beneficial as long-circulating delivery system *in vivo*. Leo et al. (17) prepared gluteraldehyde crosslinked nanoparticles of gelatin and evaluated their drug-release potential taking doxorubicin as the experimental drug. Employing genipin as a crosslinking agent, gelatin nanospheres were prepared by Liang et al. (18) and their efficiency as a drug carrier was examined for intramuscular administration, both *in vitro* and *in vivo*. Biodegradable hydrophilic nanoparticles of gelatin were prepared by Cascone et al. (19) adopting a single solvent evaporation method based on a single water-in-oil emulsion and investigated the release of methotrexote, an anti-cancer drug, from the drug loaded nanoparticles. The effects of parameters such as particle size and drug-encapsulation efficiency was studied on the dry-release profiles. A crosslinked biodegradable matrix of gelatin was prepared by Kuijper et al. (20) and controlled release of antibacterial protein was investigated. A new two-step desolvation method for preparation of gelatin nanoparticles was given by Coester and co-workers (21) and two fluorescent dyes were attached to them for studying cell-uptake.

The above discussion reveals that although gelatin has been widely employed as a micro or nano drug carrier in various controlled release applications, its nanoparticles in colon-specific drug delivery have not been investigated. Thus, being motivated by the application potential of gelatin in biomedical and pharmaceutical fields, we in the present are reporting results on controlled release of sulphamethoxazole from the drug-loaded gelatin nanoparticles. Since gelatin dissolves rather rapidly in aqueous environments, it presents difficulty in the production of long term drug-delivery systems (22). This adverse aspect is avoided by using a crosslinking agent, such as gluteraldehyde that results in an insoluble network (23). The drug chosen for the study is sulphamethoxazole that belongs to "sulpha-drug" family, which are well known for their antibacterial property (24).

Experimental

Materials

Acid processed gelatin (Type A, isoelectric point 7.6) in yellowish granular form, was supplied by Loba Chemie, Mumbai, India and used without any pretreatment. Type B gelatin (Bloom no. 240, isoelectric point 4.8) extracted from human bone was a kind gift from Shaw Wallace Gelatins Ltd., Jabalpur, India. Glutaraldehye was employed as a crosslinker of gelatin and obtained from Research Lab, Pune, India. Polymethylmethacrylate (Sigma Aldrich Co., average $M_w \sim 120{,}000$ Da, inherent viscosity 0.20) was used for preparing oil phase. Other chemicals and solvents were of analytical reagent grade. Sulphamethoxazole was used as a model drug and obtained from Septron Tablet (400 mg, IP, Burroughs Wellcome Ltd., Mumbai, India).

Preparation of Nanoparticles

The preparation methods of nanoparticles for pharmaceutical use are divided broadly into two categories, those based on physicochemical properties such as phase separation (25) and solvent evaporation (26), and these based on chemical reactions such as polymerization and polycondensation. In the present study, solvent evaporation technique has been followed as published by Cascone et al. (19) Briefly, the method may be described as below.

"Aqueous phase" was prepared by dissolving a definite amount of gelatin in distilled water while for preparing "oil phase"; polymethylmethacrylate was dissolved into a mixture of chloroform and toluene. The above two solutions were mixed with vigorous shaking (shaking speed 300 RPM, 0.5 HP motor capacity) (Toshniwal, India) for 30 min. and to this suspension was added with constant shaking, 2 mL of glutaraldehyde emulsion prepared in toluene (1 : 1 v/v). The crosslinking reaction was allowed to take place for 10 h at 4°C in an ice bath. Nanoparticles so prepared were cleaned by centrifuging and resuspending in toluene three times and then twice in acetone. The final product was dried at room temperature to obtain a fine yellow powder, which was stored in airtight polyethylene bags.

IR Spectra

The IR spectra of gelatin nanoparticles was recorded on a FTIR spectrophotometer (Shimadzu 8201 PC).

Scanning Electron Microscope (SEM)

Morphological features of unloaded and sulphamethoxazole loaded nanoparticles were studied using SEM (Philips 515).

Particle Size Analysis

Particle size analysis of unloaded gelatin nanoparticles was performed on a particle size analyzer (Malvern Mastersizer, 2000).

Surface Potential Measurements

In order to understand the nature of the drug sulfamethoxazole (SM)-nanoparticle interaction surface potential studies were performed with a digital pH meter (Systronics Model No. Digital pH Meter MK VI, Ahmedabad, India). In a typical experiment, 0.2 gm nanoparticles were dispersed into 20 mL of respective pH solution and emf was recorded using a compound electrode system. A similar experiment was also repeated for drug loaded nanoparticles.

Swelling of Nanoparticles

It is widely reported that nanoparticles undergo aggregation in solid and in solution state form (27, 28).

In the present case, due to the presence of multifunctional charged groups and hydrophobic regions in gelatin molecules, the possibility of aggregation cannot be ruled out. To confirm the state of aggregation in gelatin nanoparticles, they were viewed by an optical microscope fitted with an ocular micrometer (Olympus, India). The nanoparticles in aggregated form were clearly visible with an average dimension of 20 μm as shown in Fig. 1.

Thus, being inspired by the above observation we adopted a novel method of monitoring the progress of swelling microparticles of gelatin.

For determining the progress of the swelling process, the change in dimension of swelling microparticle was constantly monitored up to about 20 min using an optical microscope fitted with an occular micrometer. In a typical experiment, 1 mg of nanoparticles were sprayed on a petri dish and the microscope was focused on a single aggregated nanoparticle (microparticle) reading its dimension on a occular micrometer scale. Now, a single drop of phosphate buffer saline (PBS, pH 7.4) was added to the micro particle so that it instantaneously starts swelling, which was clearly seen on the microscope. Thus, the dimension of the swelling micro particle is noted and the degree of water

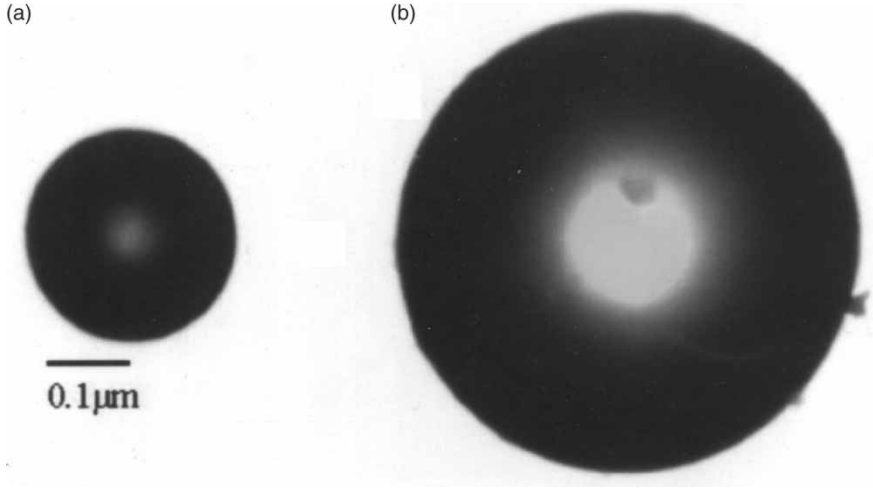


Figure 1. An optical microscope photograph of swelling gelatin-nanoparticle (a) dry, and (b) fully swollen nanoparticle.

sorption is quantified in terms of "swelling ratio" as calculated below:

$$\text{Swelling ratio} = \frac{\text{Diameter of swollen particle}}{\text{Diameter of dry particle}} \quad (1)$$

Optical Microscopy

The dry and swollen aggregated nanoparticles were photographed by a Trinacular Microscope (Lica, made in Germany) as shown in Fig. 1.

Loading of Sulphamethoxazole (SM)

A known number of drug tablets were ground to a fine powder, pasted with 2 drops of 10% hydrochloric acid and diluted to 20 mL with twice distilled water. The solution so prepared was filtered off with Whatman No. 41 filter paper to remove any insoluble matter.

A varying degree of SM loaded nanoparticles were prepared by allowing 0.2 gm of nanoparticles to swell till equilibrium in freshly prepared drug solutions (20 mL) containing 0.2 to 0.8 gm of SM. The loaded and swollen nanoparticles were frequently washed with triple distilled water to ensure the complete removal of adsorbed excipients. Such completely washed nanoparticles were dried at room temperature for a week and stored in airtight polythene bags.

The percent loading of drug was calculated by the following equation

$$\%\text{Loading} = \frac{W_d - W_0}{W_0} \times 100 \quad (2)$$

where W_d and W_0 are the weights of loaded and unloaded nanoparticles, respectively.

In Vitro Release Experiment

Release experiments were performed in both phosphate buffer saline (PBS) (pH 7.4, 1.2 mM KH_2PO_4, 1.15 mM Na_2HPO_4, 2.7 mM KCl, 1.38 mM NaCl) and distilled water (pH maintained to 7.4) and it was found that the amounts of released drug (SM) were almost the same in both cases. Therefore, all the release experiments (except pH effect) were carried out in aqueous medium (distilled water) only.

In order to determine the released amount of the sulphamethoxazole, into 100 mg of drug-loaded nanoparticles was added 5 mL of distilled water as a release medium (pH 7.4) and the resulting suspension was gently shaken for a predetermined time period. After shaking was completed, 2 mL of supernatant was withdrawn and assayed for SM spectrophotometrically (29) as given below.

In a corning flask, 2.0 mL of the drug solution was taken and to that was added 15.0 mL of the buffer solution (pH 3.0) followed by the addition of 2.0 mL of a freshly prepared 0.2% 4-N-methylamino-phenol sulphate solution and 3.0 mL of a potassium dichromate solution. The solution was further diluted to 25.0 mL by distilled water in a standard flask and the amount of the drug determined by a calibration curve.

Kinetics of Release Process

For monitoring the progress of the release process, 2 mL of aliquots were withdrawn at desired time intervals and instantly replaced by fresh release medium (distilled water). In the aliquots withdrawn, the amount of SM was determined as described above.

For achieving mechanistic insights into the release process of SM, the following equation was used (30):

$$W_t/W_\infty = kt^n \quad (3)$$

where W_t/W_∞ is the fractional release at time t and k is rate constant. The exponent n, called as diffusional exponent, is an important indicator of the mechanism of drug transport and, in general, has a value between 0.5 and 1. When $n = 0.5$, the release is taken to be Fickian. When $n = 1$, the release is zero order (Case II transport), and in between these values, i.e., $0.5 < n < 1$, the release is described as anomalous. When $W_t/W_\infty = 0.5$, t is the half life, another extremely useful parameter in comparing systems.

Assuming the diffusion of SM across the nanoparticle surface as one-dimensional, the following early time equation ($0 \leq W_t/W_\infty \leq 0.6$) can be used to calculate the diffusion coefficient (D) of the drug (31):

$$W_t/W_\infty = 4(Dt/\pi L^2)^{0.5} \quad (4)$$

where L is the diameter of dry nanoparticle.

Chemical Stability of Drug

Chemical stability of drug in acidic media (pH −1.8) was judged by a UV spectrophotometric method as explained elsewhere (32) (Double Beam UV-VIS Spectrophotometer-2201, Ahmedabad, India).

Statistical Analysis

All swelling and release experiments were performed in triplicate and swelling ratio vs. time and fractional release vs. time curves were plotted taking mean of the swelling ratio and released amount of three independent determinations.

Results and Discussion

Characterization of Nanoparticles

IR Spectral Analysis. The FTIR spectra of SM loaded nanoparticles clearly confirms the presence of gelatin, glutaraldehyde and SM in the drug loaded nanoparticles. The strong band observed at 3455 cm^{-1} and 2371 cm^{-1} due to N–H stretching and NH_3^+ stretching confirms the presence of gelatin while bands at 2965 cm^{-1} (C–H stretching of methyl group), 1633 cm^{-1} (N–H bending of NH_2) and 1394 cm^{-1} (S═O stretching) clearly indicate the presence of sulphamethoxazole in the drug-loaded nanoparticles. The broadness of band near 3500 cm^{-1} also suggest the presence of hydrogen bonded water molecules.

Analysis of SEM. A scanning electron micrograph (SEM) of SM-loaded nanoparticles is shown in Fig. 2, which clearly shows that smooth and spherical nanoparticles with an average diameter of 100–300 nm were produced. The photograph clearly indicates that no hair cracks or heterogeneity appear on the nanoparticles surface. This obviously presents a morphological evidence for solid and smooth nanoparticles.

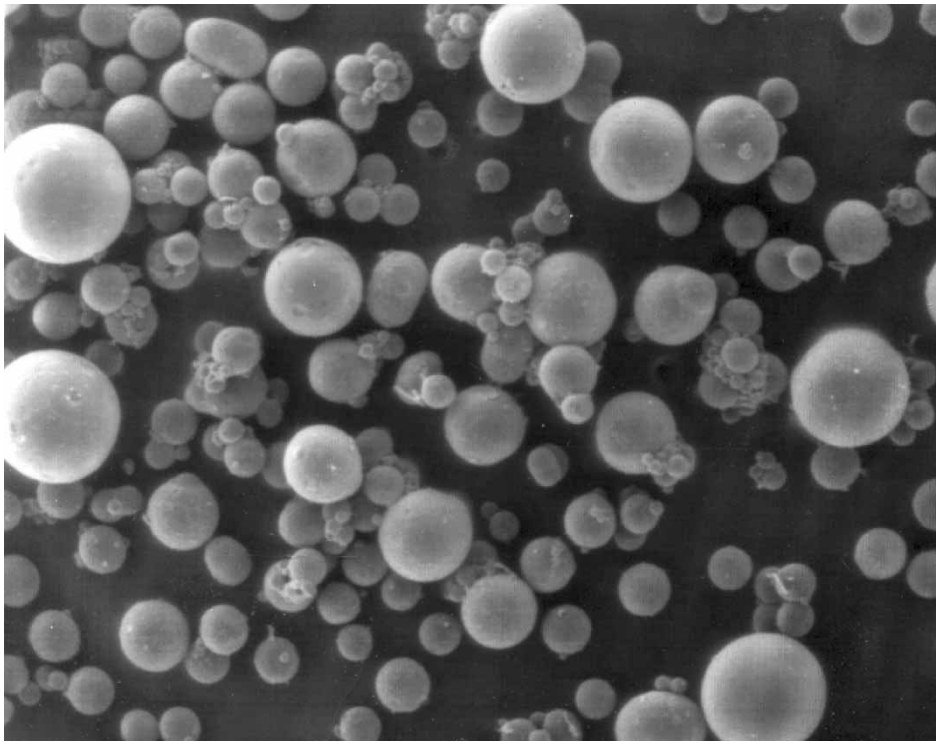


Figure 2. Scanning electron micrograph (SEM) of SM-loaded nanoparticles.

Particle Size Analysis. A typical particle size distribution curve is shown in Fig. 3, which implies that the dimensions of nanoparticles vary in the range 100 nm to 400 nm as also confirmed by SEM.

Surface Potential Measurements

The value of ζ potential for unloaded nanoparticles and drug-loaded nanoparticles are summarized in Table 1, which clearly indicates that upon loading of SM molecules on to the nanoparticle surface, a net increase occurs in positive potential of the particles surface. The observed increase is quite obvious and may be explained by the fact that drug molecule bear a positive charge and due to their loading on to the particle surface the positive charge increases on the surface which clearly provides evidence of drug surface interaction.

Release Study of Sulphamethoxazole (SM)

Mechanism of Drug Release. A swollen hydrogel may be imagined as a three-dimensional polymer network structure between the strands of which are water-filled permeation channels (33). The water occupies the permeation channels when the water soluble solutes diffuse out to the external receptor medium from within the gel. A free volume theory, developed by Yasuda et al. (34) assumes that the free volume of the water present in the hydrogel is available for the diffusion of water soluble solutes. The

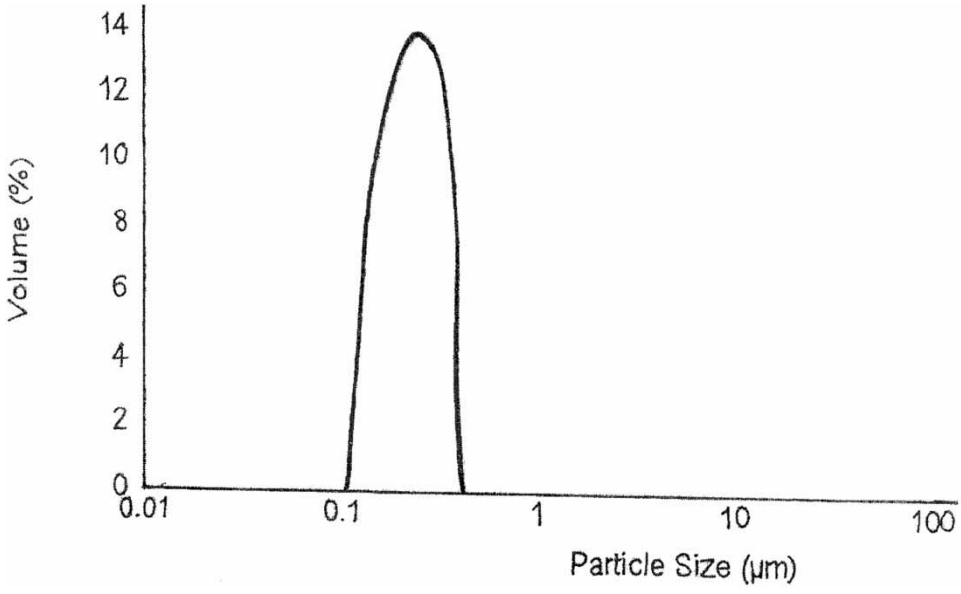
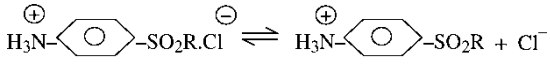


Figure 3. A graph showing the particle size distribution of unloaded gelatin nanoparticles.

theory implies that the free volume in a polymer may be thought of as a volume fraction of molecular sieve holes available for diffusion.

In the present case, the drug carriers are the crosslinked gelatin nanoparticles which in aqueous release medium (pH 7.4) will exist as crosslinked gelatin molecules with an almost equal number of positive ($-NH_3^+$) and negative ($-COO^-$) charges (because pH 7.4 is also isoelectric point). At pH 7.4, the drug (SM) will also be present in a 100% ionized state according to the following equilibria:



Thus, the positively charged SM molecules will be held up to the negatively charged $-COO^-$ groups via electrostatic attraction.

Table 1
Surface potentials of unloaded and loaded gelatin nanoparticles

Particles	Medium	ζ Potential (mV)
Unloaded nanoparticles	1.8 pH	201
	4.0 pH	116
	7.4 pH	193
Drug loaded nanoparticles	1.8 pH	242
	4.0 pH	209
	7.4 pH	207

When the drug loaded nanoparticles come into contact with a solvent, relaxation of gelatin chains takes place. This happens when the characteristic glassy-rubbery transition temperature (T_g) of the biopolymer is decreased below the experimental temperature. The dissolved drug passes into the external receiving medium, crossing the swollen polymeric layer formed around the matrix. Depending on the rate of the swelling process, the associated drug release may be Fickian or non-Fickian (35). The whole mechanism of SM release is modeled in Fig. 4.

Effect of Percent Loading on SM Release

An important parameter influencing the release profile of a loaded drug is the extent of loading which, in the present case, has been investigated by allowing the nanoparticles to swell till equilibrium in the SM solutions of varying concentrations. The release profiles of nanoparticles loaded to different extent are shown in Fig. 5, which clearly indicate that the fractional release of SM increases with increasing % loading. The observed increase in the release rate may be attributed to the fact that a larger loading of the nanoparticles facilitates a faster movement of the invading solvent front which as a consequence enhances the release of entrapped SM (36).

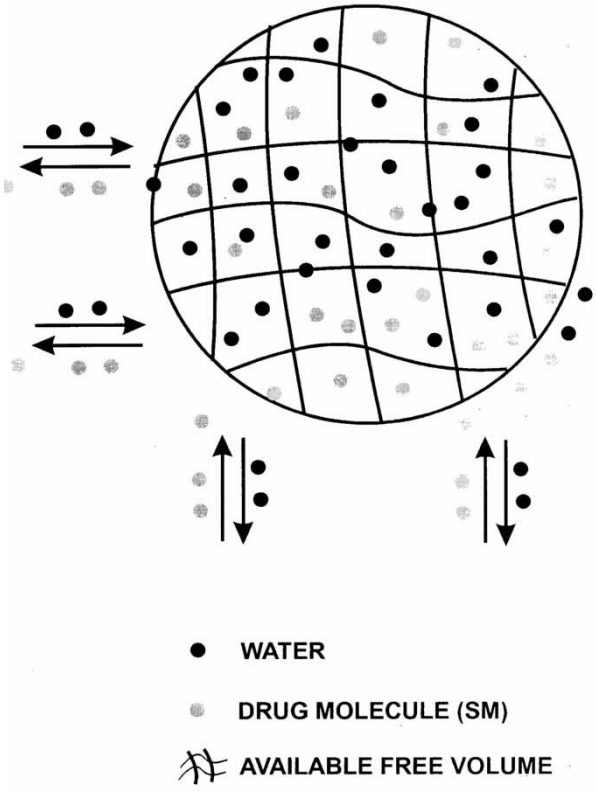


Figure 4. A model depicting the release of SM from a swelling gelatin nanoparticle.

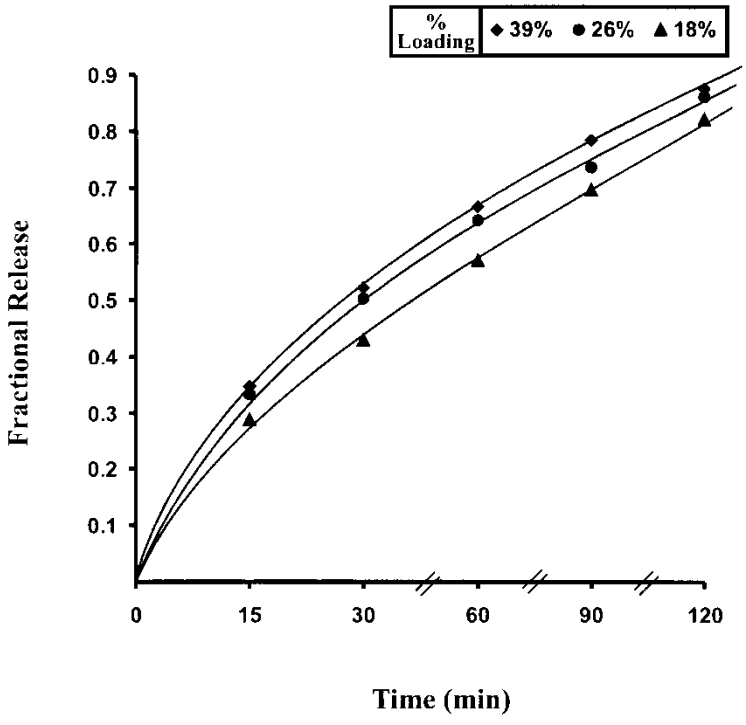


Figure 5. Effect of %loading of SM on its release profile for a definite composition of nanoparticle [gelatin] = 8.0 g, [glutaraldehyde] = 10.6 mM, pH = 7.4, temp. = 25 ± 0.2°C.

Effect of Gelatin on SM Release

Drug release profiles are often sensitive to chemical architecture of the carrier, as well as the experimental conditions of preparation of drug carrier. Furthermore, in the present study, the size and morphology of nanoparticles are greatly determined by the factors such as concentrations of gelatin and glutaraldehyde in the feed mixture, molecular weight of polymethyl methacrylate, and temperature and shaking time of emulsions. The effect of gelatin on the SM release has been investigated by varying its concentration in the range 4.0–9.0 g in the feed composition. The release and swelling results are shown in Figs. 6 and 7, respectively which clearly indicate that the fractional release of SM increases with increasing concentration of gelatin up to 8.0 g, whereas a decrease is noticed beyond 8.0 g. The observed findings may be explained on the basis of the swelling results of nanoparticles, which are also displayed in Fig. 8. The swelling results clearly reveal that the swelling ratio constantly increases up to 8.0 g of gelatin content and thereafter a decrease is noted. The reason for the observed enhanced swelling is that on increasing the concentration of gelatin, the nanoparticles of large size and wide pores are produced which obviously allow a greater number of water molecules to enter into the nanoparticles. This consequently results in larger release of SM into the release medium.

However, beyond 8.0 g of gelatin content, the volume fraction of gelatin increases significantly in the nanoparticles, and as a consequence, both the water and SM molecule will

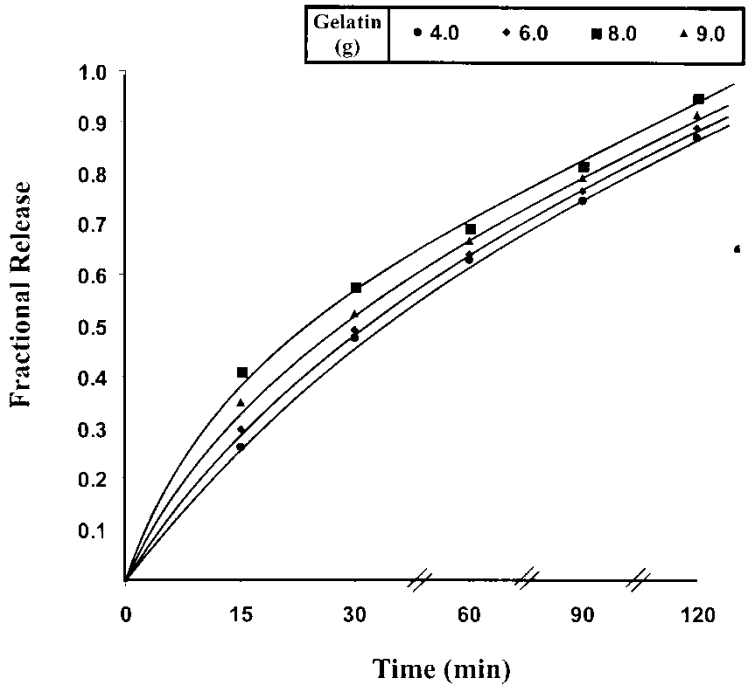


Figure 6. Effect of varying amounts of gelatin in nanoparticles on release profiles of SM for a definite composition of nanoparticles [glutaraldehyde] = 10.6 mM, pH = 7.4, temp. = 25 ± 0.2°C, %loading = 39.

have to travel a larger path in order to penetrate into and release from the nanoparticles. This obviously brings about a fall in both the swelling ratio as well as the released amount of drug.

Effect of Crosslinker on SM Release

Glutaraldehyde (GA) is the most commonly used crosslinking agent in the preparation of bioprostheses (such as heart valves, vascular grafts, elastic cartilage, and artificial skin) in cell and enzyme immobilization, and in protein and polysaccharide stabilization. GA is presumed to crosslink by inter- and intramolecular covalent bonds. GA crosslinking of collagenous tissues significantly reduces biodegradation, making them biocompatible and non-thrombogenic while preserving anatomic integrity, strength and flexibility. Among aldehydes, which are used to crosslink a protein matrix, GA is advantageous because its reaction is rapid; less expensive; and is readily available and highly soluble in aqueous solution.

Although gelatin itself is nontoxic, however, the crosslinker used for the preparation of stable structures may create toxicity. It is reported in the literature that GA crosslinked matrix could release GA-related molecules and cause toxicity (37). The released molecules may be either unreacted GA present in the sample or products of gelatin-matrix degradation. Since in the present study, GA was employed in mM concentration range, its possible toxicity could be quite lower in effectiveness.

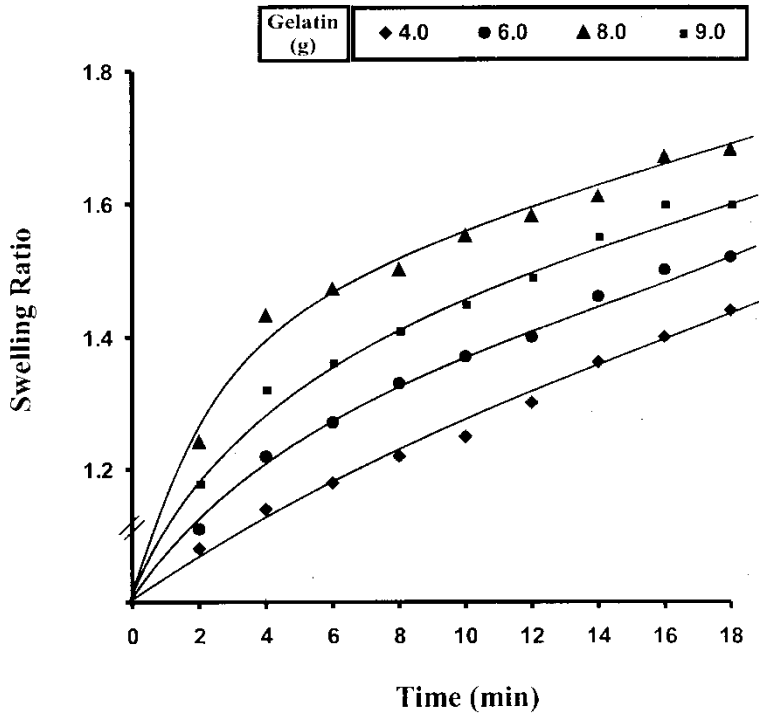


Figure 7. Effect of varying amounts of gelatin on swelling ratio of nanoparticles for a definite nanoparticle composition [glutaraldehyde] = 10.6 mM, pH = 7.4, temp. = 25 ± 0.2°C.

The effect of the crosslinker on the release and swelling profiles of SM has been investigated by varying the concentration of glutaraldehyde (GA) in the range 5.3 to 31.8 mM. The results are shown in Figs. 8 and 9, respectively which clearly reveal that both the fractional release of SM and swelling ratio increase respectively with increasing GA up to a 10.6 mM concentration while beyond it a fall in release and swelling is noticed. The results can be explained by the fact that since glutaraldehyde is a hydrophilic crosslinker, its increasing number of linkages in the nanoparticle enhances their hydrophilicity, which in turn, will allow an increasing number of water molecules into the nanoparticle and obviously the swelling ratio will increase. Thus, increased swelling will permit a greater number of SM molecules to diffuse out and the release of SM will also increase.

However, beyond 10.6 mM of GA, the size of nanoparticle will decrease due to enhanced crosslinking density of the nanoparticle and as a result, therefore, both swelling and release will fall.

Another explanation for the observed decrease in the swelling ratio and SM release may be that increasing the crosslinker concentration lowers the molecular weight between crosslinks and this, consequently, reduces the free volume accessible to the penetrant water molecules. Similar types of results have also been reported by other workers (38). Some authors (39) have, however, reported that introduction of crosslinker into the polymer matrix enhances its glass transition temperature (T_g) which may be because the glassy behavior of polymers restrains the mobility of network chains and, therefore, both swelling and SM release decreases.

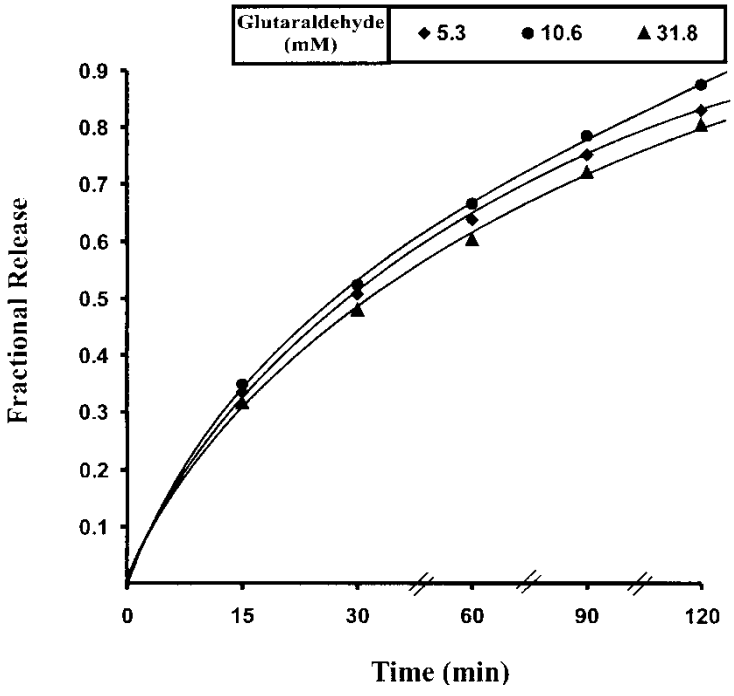


Figure 8. Effect of varying amounts of glutaraldehyde (crosslinker) on release profiles of SM for a definite nanoparticle composition [gelatin] = 8.0 g, pH = 7.4, temp. = 25 ± 0.2°C, %loading = 39.

Effect of pH on SM Release

Drug delivery systems capable of a selected release of drugs in the colon have received much attention in recent past (40). Specific targeting of drugs to the colon is recognized to have several therapeutic advantages. Drugs, which are destroyed by the stomach acid/or metabolized by pancreatic enzymes, are slightly affected in the colon, and, thus, sustained colonic release of drugs can be an effective method to treat colonic diseases. In order to achieve successful colonic delivery, a drug needs to be protected from absorption and/or the environment of the upper gastrointestinal tract (GIT) and then to be abruptly released into the proximal colon, which is considered the optimum site for the colon-targeted delivery of drugs. Colon targeting is valuable in the treatment of diseases of colon such as ulcerative colitis, Chron's disease, carcinomas and infections whereby high local concentrations can be achieved while minimizing side effects that occur because of release of drugs in the upper GIT or unnecessary systemic absorption. The region of the colon is recognized as having a somewhat less hostile environment with less diversity and intensity of activity than the stomach and small intestine (41). The various strategies for targeting orally administered drugs to the colon include covalent linkage of a drug with a carrier, coating with pH-sensitive polymers, formulation of timed release systems, exploitation of carriers that are degraded specifically by colonic bacteria, bioadhesive systems and osmotic controlled drug delivery systems. Of all these approaches to execute colon targeted drug delivery, the method based on utilization of pH changes within the GIT has been majorily used and frequently cited in the literature (42). In the present investigation, the release

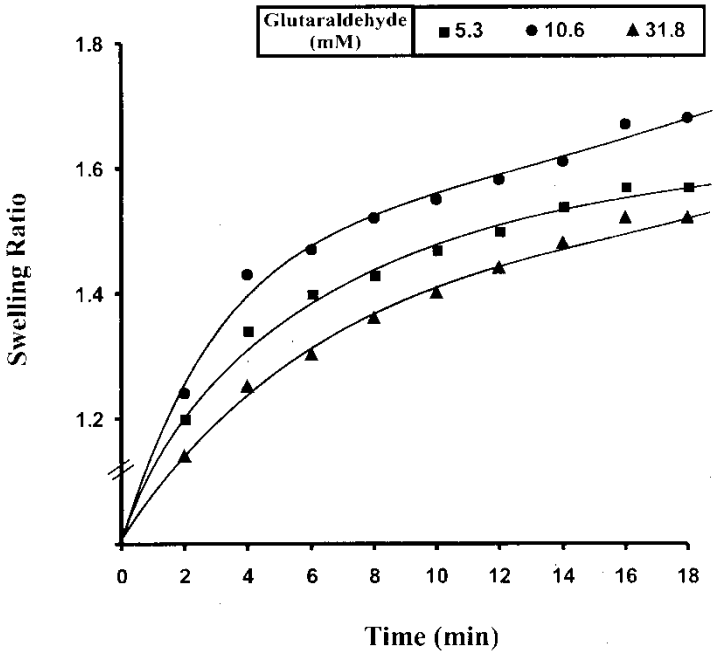


Figure 9. Effect of varying amounts of glutaraldehyde on swelling ratio of nanoparticles for a definite nanoparticle composition [gelatin] = 8.0 g, pH = 7.4, temp. = 25 ± 0.2°C.

dynamics of the SM has been observed under varying pH conditions as found in the GIT [e.g. stomach (gastric juice) 1.0, and small intestine 7.5 to 8.6]. The wide range of pH allows a specific drug to be delivered to a targeted site only. For example, the pH in the stomach (<3) is quite different from the neutral pH in the intestine and this pH difference could be used to prevent release of foul-tasting drugs into the neutral pH environment of the mouth while using polycationic hydrogels as drug carrier (43). Similarly, a polyanion hydrogel which shows a minimal swelling at acidic pH (such as in stomach) could be of potential use to increase in pH leading to ionization of the carboxylic groups (44).

The results obtained in the present study are depicted in Figs. 10 and 11 which clearly indicate that both the fractional release of SM and the swelling of nanoparticles increase with increasing pH up to 7.4. The results obtained may be explained as below.

Since the present drug delivery systems is swelling controlled, the extent of water sorption by the nanoparticles will determine the amount of the released SM. It has been well demonstrated by theoretical considerations (45) that a balance between osmotic pressure and the polymer elasticity sets the physical dimensions of the swelling polymer. The osmotic pressure (π) results from a net difference in concentration of mobile ions between the interior of the hydrogel and external swelling (or release) medium. Increasing the ionic concentration difference obviously results in an enhanced swelling of the polymer which, in turn, will bring about an increase in the amount of released drug. Thus, ignoring ion–ion, ion–polymer and ion–solvent interaction, we can write:

$$\pi_{\text{ion}} = \text{RT}\Sigma(\text{C}_\text{i}^\text{g} - \text{C}_\text{i}^\text{s})$$

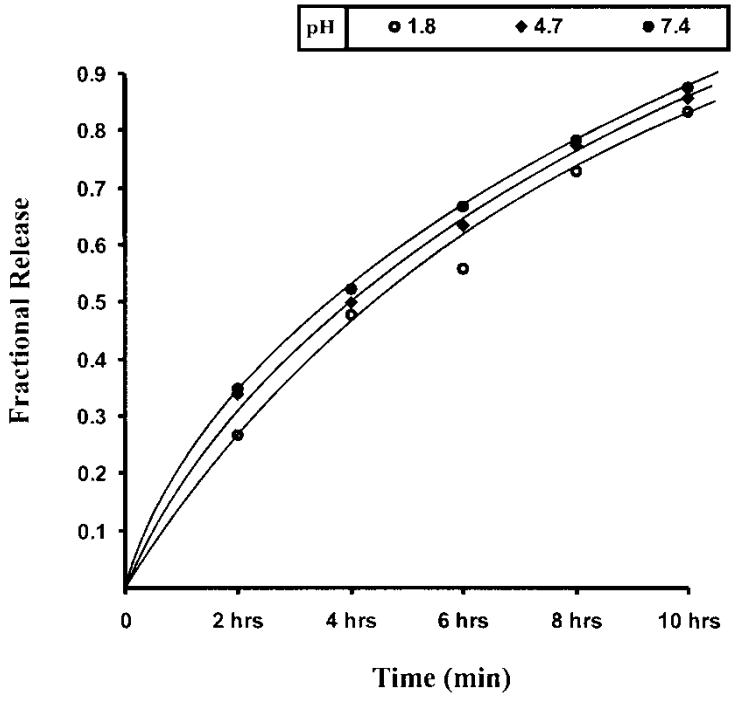
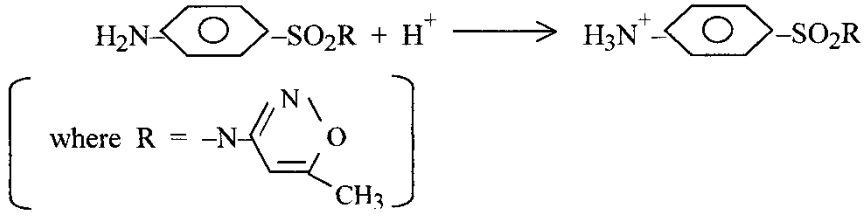


Figure 10. Variation in released amount of SM with varying pH of the release medium for a definite nanoparticle composition [gelatin] = 8.0 g, [glutaraldehyde] = 10.6 mM, temp. = 25 ± 0.2°C, %loading = 39.

where C_i^g and C_i^s represent the mobile ion concentration in the gel (nanoparticle) and release medium, respectively. The above equation clearly reveals that larger the difference between the nanoparticle and release medium, the greater would be the swelling and, therefore, the release.

When the SM loaded nanoparticles are placed at the lowest pH (1.8) of the studied range, the SM molecules entrapped within the nanoparticles will remain in protonated state as shown below:



while gelatin molecules will also possess a net positive charge due to predominance of the protonated amine groups ($-NH_3$) over carboxylateions ($-COO^-$) of their amino acids. Thus, ionic concentration will be greater in the external receptor medium than the interior of the nanoparticles, and as a result, the difference in ionic concentrations between the interior and external solutions will be less. This consequently results in a lower degree of swelling of nanoparticles, which produces a less release of the entrapped drug.

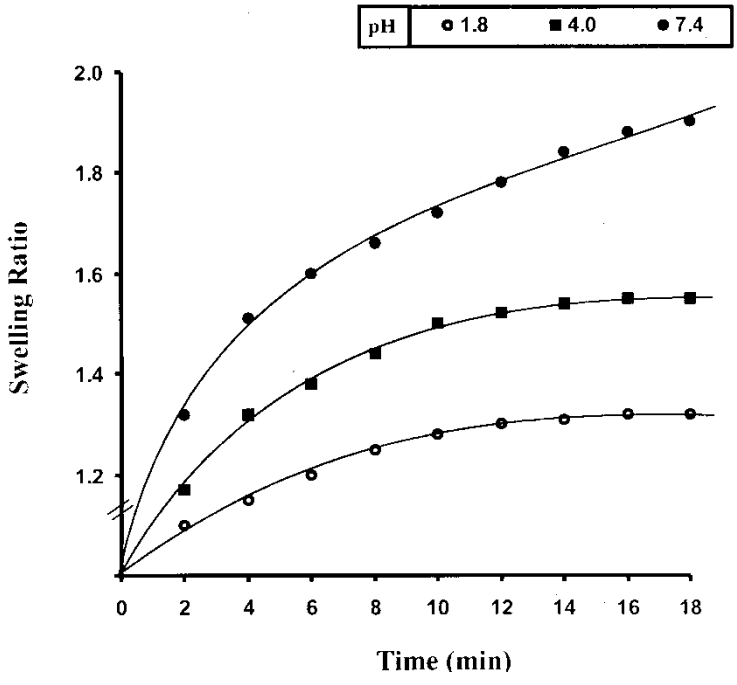


Figure 11. Effect of pH on swelling ratio of nanoparticles for a definite nanoparticle composition [gelatin] = 8.0 g, [glutaraldehyde] = 10.6 mM, temp. = 25 ± 0.2°C.

When the pH is raised to 4.6, about 75% of entrapped SM molecules become ionized ($pK_a = 4.51$)(46) and, therefore, ionic concentration within the nanoparticles becomes quite great. At this pH, the gelatin molecules still possess a net positive charge although negative charge due to $-COO^-$ ions of the amino acids has somewhat increased. Thus, an increase in the difference in ionic concentrations between the external solution and internal nanoparticle the degree of water sorption increases which obviously enhances the extent of SM release.

At pH 7.4, the SM molecules get 100% ionized and at the same time gelatin molecules also possess a net zero charge (isoelectric point is 7.6), because of an equal number of $-NH_3^+$ and $-COO^-$groups on the gelatin molecule. Thus, the nanoparticles become fully swollen at pH 7.4, and as a consequence, an optimum release of the entrapped SM is noticed. As no body fluids of the GI tract acquire a pH greater than 8.0, no release experiments were performed in a greater alkaline range.

The above discussion clearly reveals that while an optimum release of SM occurs at pH 7.4, a minimum release is observed at pH 1.8. This clearly provides an opportunity to explore the possibility of the present drug carrier in colon specific drug delivery methods.

Chemical Stability of Drug

In order to ascertain the chemical stability of sulfamethoxazole in a highly acidic pH medium such as gastric juice, the drug was left in a simulated gastric juice medium and its UV spectra was scanned and compared to that of SM in the aqueous medium. The spectra are shown in Fig. 12, which clearly indicates that they are nearly identical to

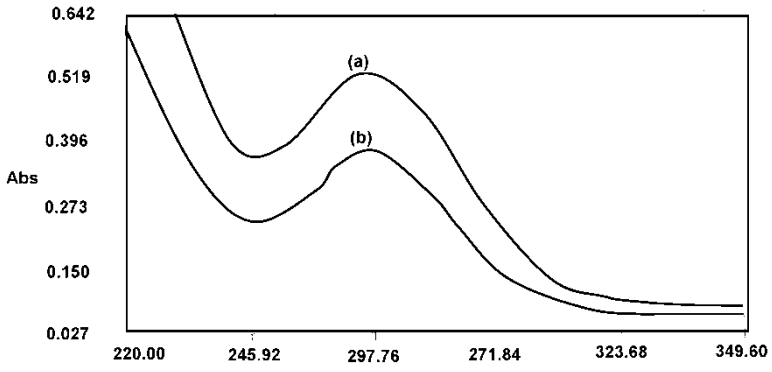


Figure 12. UV spectra showing the chemical stability of sulfamethoxazole in its (a) pure solution, (b) released medium.

each other. This obviously suggests that even in remaining in highly acidic media, the chemical nature of sulfamethoxazole does not change. Moreover, it was also found that even gelatin nanoparticles do not undergo any cleavage in gastric juice medium. This clearly explains the stability of drug carrier system in highly acidic media.

Effect of Temperature on SM Release

The effect of temperature on the release profiles of SM has been investigated in the range 10°C to 35°C. The results are shown in Figs. 13 and 14, which reveal that both the swelling

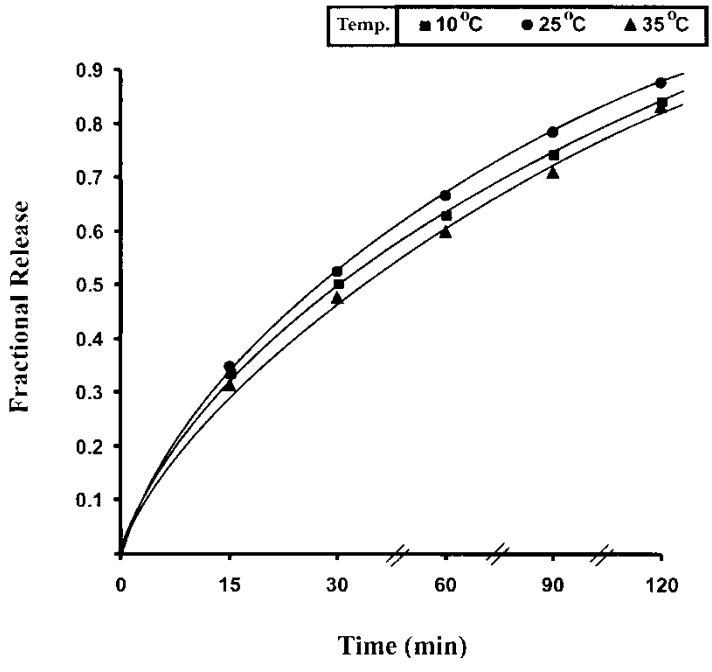


Figure 13. Effect of temperature on the released amounts of SM for a definite nanoparticle composition [gelatin] = 8.0 g, [glutaraldehyde] = 10.6 mM, pH = 7.4, %loading = 39.

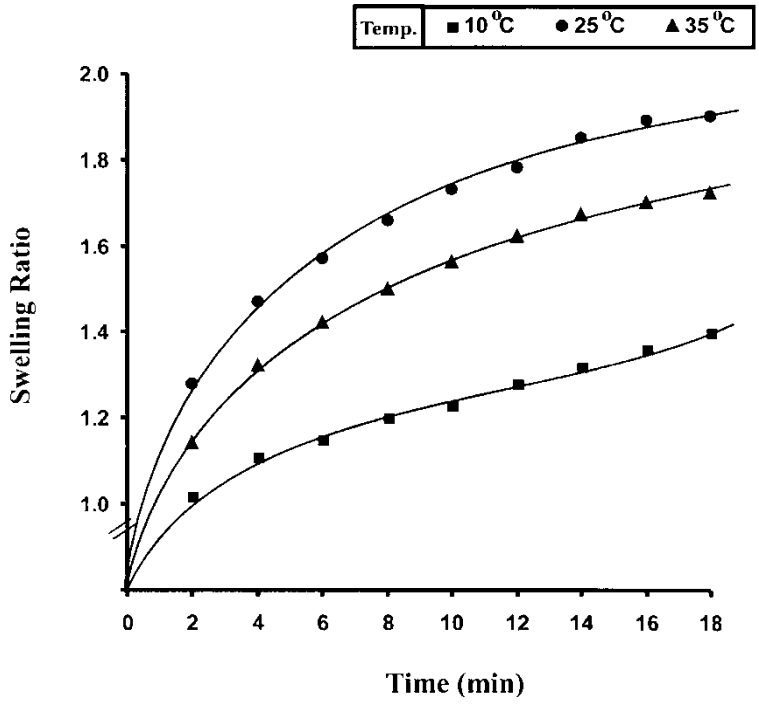


Figure 14. Variation in swelling ratio of nanoparticle with temperature of swelling both for a definite nanoparticle composition [gelatin] = 8.0 g, [glutaraldehyde] = 10.6 mM, pH = 7.4.

ratio of nanoparticles and a fractional release of SM increase up to 25°C while beyond it a decrease is noticed. The results may be attributed to the fact that with an increasing temperature the diffusion of both the water and SM molecules and rate of relaxation of nanoparticles chain increases, which in turn results in greater water sorption and the SM release. However, beyond 25°C, a lower release of SM observed may be explained on the basis of the lower degree of swelling of nanoparticles at higher temperature. The lower value of swelling ratio at 35°C may be because of breaking of hydrogen bonds between water molecules and nanoparticle chains. A decreased swelling at higher temperature is widely reported in the literature (47).

Effect of Type of Gelatin

Gelatin is a natural polymer that is extracted from collagen by alkaline or acidic pretreatment and thermal denaturation (48). Depending on this pretreatment, two types of gelatin can be distinguished, A and B. Gelatin A is extracted from porcine skin, and processed by acidic pretreatment, while gelatin B is extracted from bovine skin, and processed by alkaline pretreatment. The alkaline pretreatment converts glutamine and aspargine residues into glutamic acid and aspartic acid, which results in a higher carboxylic acid content for gelatin B (118/1000 amino acids) than for gelatin A (77/1000 amino acids) (49).

The effect of type of gelatin on the release profile of SM has been investigated by loading the drug onto both gelatin A and B nanoparticles and following the released

amounts under identical experimental conditions. The results are shown in Fig. 15 which clearly indicate that the fractional release of drug is much higher in the case of type B than that by type A. The results may be explained by the fact that at the experimental pH (7.4) (which is above the isoelectric point 4.8) the gelatin B molecules will possess a net negative charge due to $-COO^-$ groups in the molecule. Thus, the SM molecules, which are almost fully ionized at pH 7.4, will attach to these negatively charged centers present along the gelatin molecules and, therefore, will result in a greater percent loading. When largely loaded type B nanoparticles are placed in the release medium the $-COO^-$ groups present along the gelatin chains repel each other, thus producing a greater relaxation in the nanoparticle. This obviously results in a larger swelling of the loaded nanoparticles which, in turn, produces greater release of SM in type B nanoparticles. A similar type of results have also been published elsewhere (50).

Analysis of Kinetic Release Data

When a drug-loaded polymeric carrier contacts a thermodynamically compatible solvent, such as water swelling of the polymer occurs as a result of diffusion of water molecules into the polymer network and relaxation of macromolecular chains. Both of these processes result in a release of the entrapped drug. It is known that drug release may be diffusion controlled or dissolution controlled, depending on parameters such as permeability of the polymer to water, the solubility of the drug in the polymer and in water, and size of the drug.

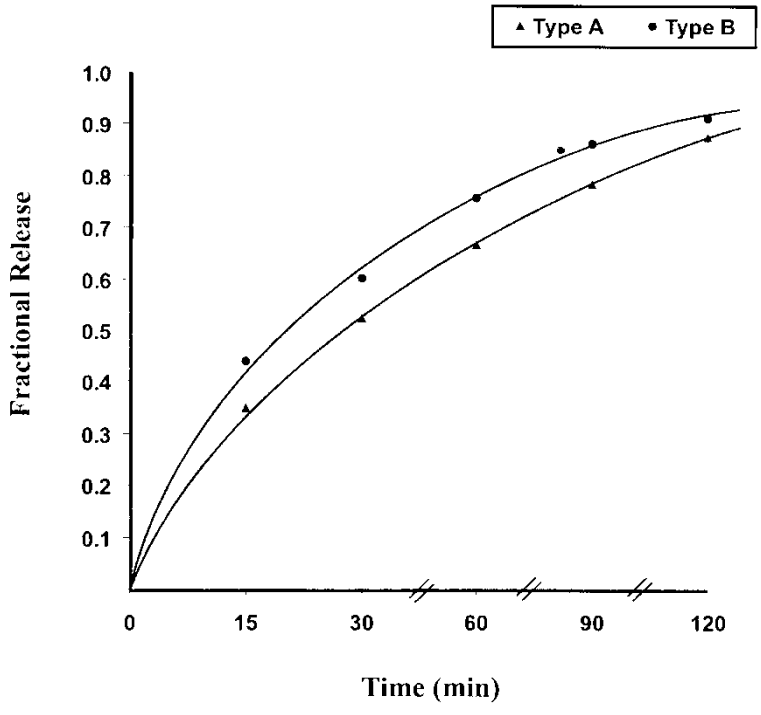


Figure 15. Effect of type of gelatin on the released amount of SM for definite nanoparticle compositions [gelatin] = 8.0 g, [glutaraldehyde] = 10.6 mM, pH = 7.4, temp. = 25 ± 0.2°C, %loading = 39.

Table 2
Data showing the release exponent and diffusion constant under varying experimental conditions

Gelatin (g)	Glutaraldehyde (mM)	pH	n	$D \times 10^4$ $cm^2\,min^{-1}$
4	10.6	7.4	0.51	3.1
6	10.6	7.4	0.66	3.6
8	10.6	7.4	0.5	1.5
9	10.6	7.4	0.5	4.3
8	5.3	7.4	0.51	6
8	10.6	7.4	0.5	1.5
8	31.82	7.4	0.5	3.7
8	10.6	7.4	0.5	1.5
8	10.6	4.4	0.52	3.6
8	10.6	9	0.52	1.8
8	10.6	11	0.5	2.4

In the present case, release by dissolution is not applicable as the SM is fully soluble in water. Moreover, the drug release due to the erosion of the matrix is also unlikely as crosslinked gelatin does not dissolve under existing experimental conditions and its biodegradation starts at a time longer than that taken into consideration. Thus, the mechanism of SM release could be either diffusion or relaxation controlled, which can be judged by the value of diffusion at exponent (n) predicted by Eq. (3).

The value of "n" has been calculated on the basis of Eq. (3) and summarized in Table 2. The data clearly reveal that the value of n is quite near to 0.5 and, therefore, the release of SM may be considered as Fickian or diffusion controlled. A similar type of release mechanism was also confirmed by other workers.

Conclusions

Crosslinked gelatin nanoparticles form a swelling-controlled drug release system which effectively delivers sulphamethoxazole via a diffusion controlled pathway. It is found that release profiles of SM are greatly influenced by %loading of SM, concentrations of gelatin and glutaraldehyde (crosslinker) in the nanoparticles. With an increase in percent loading of drug on nanoparticles, the released amount of SM constantly increases. In the case of gelatin, the release of SM increases when concentration of gelatin is increased from 4.0 g to 8.0 g, whereas the extent of release decreases beyond 8.0 g of gelatin content. The released amount of SM constantly decreases with increasing the glutaraldehyde content in the nanoparticles. It is noticed that the release behavior is directly regulated by the extent of swelling of gelatin nanoparticles. The type of gelatin has a profound effect on the release potential of nanoparticles and it is found that type B gelatin nanoparticles show a greater drug delivery than that by type A nanoparticles.

An optimum drug release is obtained near physiological pH (7.4), while a lower release is observed in the acidic pH range. It is also noticed that the extent of release of SM increases with an increasing temperature up to 25°C, while beyond 25°C a fall is observed.

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