In Vitro Release Dynamics of an Anticancer Drug from Swellable Gelatin Nanoparticles

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ABSTRACT: Gelatin (Type A) nanoparticles were prepared by a single W/O emulsion technique and characterized by infrared (IR) spectra, scanning electron microscopy (SEM), and particle size analysis. The IR spectra clearly confirmed the presence of gelatin and cytarabine in the loaded nanoparticles while the scanning electron micrograph (SEM) image depicts smooth surface, spherical shape and uneven size of nanoparticles (100–300 nm). The prepared nanoparticles were loaded with cytarabine, a well-known anticancer drug, and the release dynamics of entrapped drug was investigated as a function of various experimental factors, such as percent loading of the drug, chemical architecture of the nanocarriers, and pH, temperature, ionic strength, and nature of the release medium. The nanoparticles were also studied for their water sorption capacity by optical microscopic method taking advantage of the aggregation of nanoparticles. The drug release process was analyzed kinetically using Ficks power law, and a correlation was established between the quantity of released drug and swelling of the nanoparticles. © 2006 Wiley Periodicals, Inc. J Appl Polym Sci 101: 2320–2332, 2006

Key words: gelatin; nanoparticles; swelling; cytarabine; release

INTRODUCTION

Most anticancer drugs have limitations in clinical administration due to their poor solubility and other physicochemical and pharmaceutical properties.[1] Many times the drug delivery requires the use of adjuvants or excipients,[2] which often cause serious side effects. Moreover, intravenous injection and infusion are unavoidably associated with considerable fluctuations of drug concentration in the blood. Therefore, the drugs can only be administered over a limited dosage and time period. Thus, an alternate drug administration method becomes inevitable, which could not only reduce the harmful side effects of chemotherapeutic agents, but also maintain adequate drug levels in the body. One possible and effective way to achieve nearly an ideal drug delivery system for anticancer drugs may be the controlled drug delivery technology, which has been the subject of research by a great number of researchers.[3]

Controlled drug delivery systems have a number of advantages over traditional systems, such as improved efficacy, reduced toxicity, and improved patient convenience. The main goal of controlled drug delivery systems is to improve the effectiveness of drug therapies. Controlled drug delivery is the delivery of drug at a rate or at a location determined by needs of body or disease spread over a specified period of time. Ideally, two main objectives exist for these systems. These are spatial delivery, which is related to the control over the location of drug release, and temporal delivery in which the drug is delivered over an extended time period during treatment. The design of the controlled release system depends on various factors, such as the route of delivery, the type of drug delivery systems, the disease being treated, the patient, the length of the therapy, and the properties of the drug. These factors are related to each other, but the formulation mostly depends upon the physiological or biological properties of the drug.

Nanoparticles of biodegradable polymers[4] and other bioadhesive materials[5] might be an ideal alternative carrier for controlled delivery of anticancer drugs. Because of their small size and attractive physicochemical properties, nanoparticles may be injected intravenously and used to target drugs to particular organs. Most of the early investigations into the science of solid particulate drug delivery used nonbiodegradable polymers, such as polystyrene.[6] However, recent studies have quite rightly focused on the development of biodegradable or at least bioerodible particles. Polylactic acid, polyglycolic acid,[7] poly β-hydroxy butyrate,[8] and fibrin[9] are biodegradable polymers used to make drug delivery particles while the alkyl cyanoacrylates are bioerodible polymers. These solid nanoparticles and microparticles may be used to

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prepare sustained release parenteral formulations or to achieve drug targeting. Numerous investigations have shown that both tissue and cell distribution profiles of anticancer drugs can be controlled by their entrapment in submicronic colloidal systems (nanoparticles). The rationale behind this approach is to increase antitumor efficacy, while reducing systemic side-effects. Polymeric particulate nanocarriers, able to deliver drugs or other compounds to specific sites of action for a prolonged time, represent a potential therapeutic approach for several diseases. Nanoparticles are usually prepared by the controlled precipitation of polymers solubilized in one of the phases of an emulsion.[10–12] Precipitation of the polymer out of the solvent takes place on solvent evaporation, leaving particles of the polymer suspended in the residual solvent.

Although various biodegradable nanoparticles of natural polymers, such as starch,[13] chitosan,[14] liposomes,[15] etc., are largely in use as drug carriers in controlled drug-delivery technology, however, gelatin nanoparticles represent a promising carrier system for controlled drug delivery. Gelatin is a basic material that can be used for the production of nanoparticles. Gelatin has a number of advantages as a nanoparticle material; it is a natural macromolecule, nontoxic, and noncarcinogenic nature;[16] it possesses a relatively low antigenicity and has a great deal of experience for its use parenteral formulations.[17] Gelatin nanoparticles have been richly documented in literature also. For example, Kaul and Amiji[18] 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.[19] 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.,[20] and their efficiency as a drug carrier was examined for intramuscular administration, both *in vitro* and *in vivo*. Yan and Li[21] prepared gluteraldehyde crosslinked gelatin microspheres with an average diameter of 70 μm and loaded them with mitomycin C, an anticancer drug, together with a radioisotope.

Cytarabine (ara-C) is the most effective drug in the treatment of acute leukemia that interferes with DNA replication.[22] However, such drugs increase the bone marrow insufficiency and cause drastic secondary effects. When ara-C doses are very high, neurotoxicity and convulsions are observed. The administration of high dose of ara-C results partly from the short half-life of the compound.[23] Accordingly, it appears useful to develop drug delivery system that can reduce the toxicity of ara-C by maintaining adequate drug levels in the body.

Thus, being motivated by the possible application of degradable nanoparticles as carriers for clinical administration of anticancer drugs, the proposed investigation aims at studying the dynamics of the controlled delivery of cytarabine (ara-C) from the nanoparticles of crosslinked gelatin.

EXPERIMENTAL

Materials

Cytarabine (ara-C) was gifted from the Dabur Research Foundation (New Delhi, India) and used as received. 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., USA; 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.

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[24] and solvent evaporation,[25] and those 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.[26] Briefly, the method may be described as follows:

"Aqueous phase" was prepared by dissolving 8.0 g of gelatin in 25 mL of distilled water, and 7.0 g of polymethylmethacrylate was dissolved into a mixture of chloroform and toluene (1:1 v/v) for preparing "oil phase." The abovementioned 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 air-tight polyethylene bags.

FTIR spectra

The FTIR spectra of gelatin nanoparticles were recorded on a FTIR spectrophotometer (Shimatzu 8201 PC) by preparing a pellet of the particles with KBr.

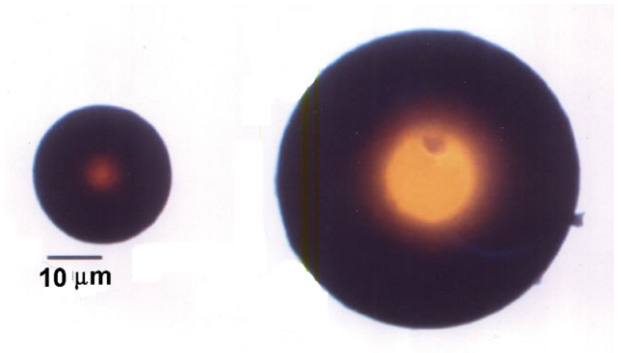


Figure 1 An optical microscope photograph of swelling gelatin-nanoparticle (a) dry, and (b) fully swollen nanoparticle. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Environmental scanning electron microscope

Morphological features of unloaded and cytarabine loaded nanoparticles were studied using environmental scanning electron microscope (ESEM) (Philips 515) in which no sample preparation is needed for the analysis.

Particle size analysis

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

Surface potential measurements

To understand the nature of the drug (cytarabine), 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 g 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 solution state form.[27,28] In the present case, because of 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 average dimension of 20 μm as shown in Figure 1.

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

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

Loading of cytarabine

Loading of cytarabine was performed by allowing 0.1 g of nanoparticles to swell in the freshly prepared drug solution till equilibrium, and then drying to obtain the release device. 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 PBS (pH 7.4, 1.2 m*M* KH_2PO_4, 1.15 m*M* Na_2HPO_4, 2.7 m*M* KCl, 1.38 m*M* NaCl) and distilled water (pH maintained to 7.4), and it was found that the nanoparticles get dissolved in the PBS probably due to inter action of salt ions present in the buffer and the polymer chains and low crosslinking of gelatin by gluteraldehyde. Therefore, all the release experiments (except pH effect) were carried out in aqueous medium (distilled water) only. To determine the released amount of the cytar-

abine, to 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 predetermined time period. After shaking was over, 3 mL of supernatant was withdrawn, and 3 mL distilled water was added again to the suspension, and previously withdrawn 3 mL supernatant was assayed for cytarabine spectrophotometrically.[29]

Kinetics of release process

For monitoring the progress of the release process, 3 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 cytarabine was determined as described earlier.

For achieving mechanistic insights into the release process of cytarabine, the following equation was used,[30]

$$\frac{W_t}{W_\infty} = kt^n \tag{3}$$

where W_t/W_∞ is the fractional release at time t, and k is the 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 drug releasing systems.

Assuming the diffusion of cytarabine 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] where L, is the diameter of dry nanoparticle.

$$\frac{W_t}{W_\infty} = 4\left[\frac{Dt}{\pi L^2}\right]^{0.5} \tag{4}$$

Chemical stability of drug

Chemical stability of drug in acidic media (pH 1.8) was judged by UV spectrophotometric method, as explained elsewhere[32] (Double Beam UV-VIS Spectrophotometer—-2201, Ahmedabad, India). In brief, the UV spectra of the pure drug solution (pH 1.8) and released drug solution (pH 1.8) were separately recorded in the range to nanometer, respectively.

Statistical analysis

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

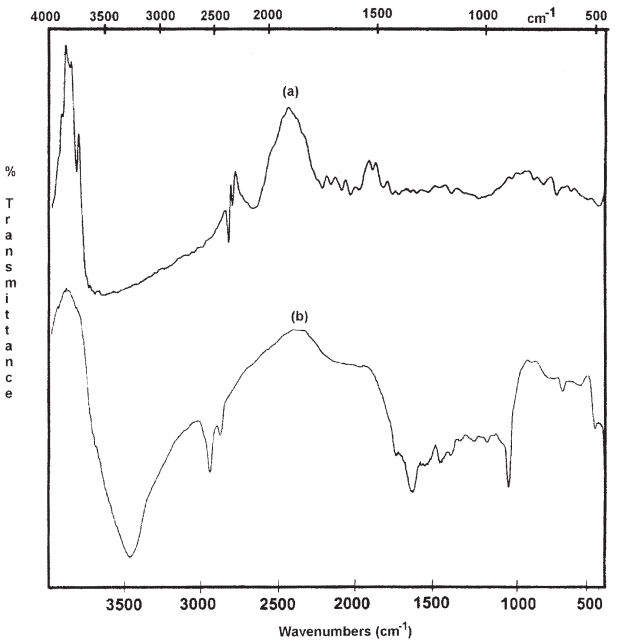


Figure 2 IR spectra of (a) pure gelatin, and (b) cytarabine-loaded crosslinked gelatin nanoparticles.

RESULTS AND DISCUSSION

Characterization of nanoparticles

FTIR spectral analysis

The FTIR spectra of uncrosslinked gelatin and cytarabine loaded nanoparticles are presented in Figure 2. The spectra of cytarabine loaded nanoparticles clearly confirm the presence of gelatin, glutaraldehyde, and cytarabine in the drug loaded nanoparticles. The presence of —CH_2 group at 2920 cm^{-1} due to C—H stretching vibration of furane ring and ketonic group at 1637 cm^{-1} due to C═O stretching vibration confirm the presence of drug molecule in the nanoparticles. Similarly, the peaks observed at 1464 cm^{-1} due to C═N stretching vibration of pyrimidine and at 1031 cm^{-1} due to O—H streching of primary and secondary alcholic group also present evidence for cytarabine.

Analysis of esem

A scanning electron micrograph (ESEM) of cytarabine loaded nanoparticles is shown in Figure 3, 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

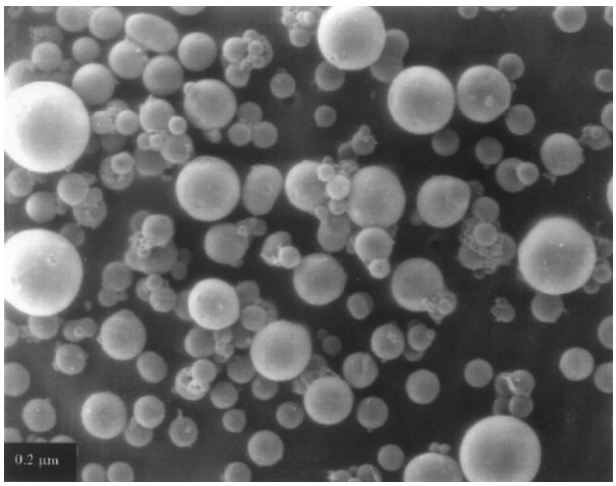


Figure 3 Scanning electron micrograph (SEM) of cytarabine-loaded nanoparticles.

surface. This obviously presents a morphological evidence for solid and smooth nanoparticles.

Particle size analysis

A typical particle size distribution curve is shown in Figure 4, which implies that the dimensions of nanoparticles vary in the range 100–400 nm as confirmed by SEM also.

Surface potential measurements

The values of ζ potential for unloaded nanoparticles and drug loaded nanoparticles are summarized in Table I, which clearly indicate that upon loading of cytarabine molecules on to the nanoparticle surface a net decrease occurs in the positive potential of the particle surface. The observed decrease is quite obvious and may be explained by the fact that this decrease in ζ potential depends on surface charge. This decrease in surface charge induces particle aggregation and instability of the suspension. It is worth mentioning here that in the case of unloaded gelatin nanoparticle a different trend is observed of their ζ potential, which could be due to the effect of isoelectric point of crosslinked gelatin on its surface potential.

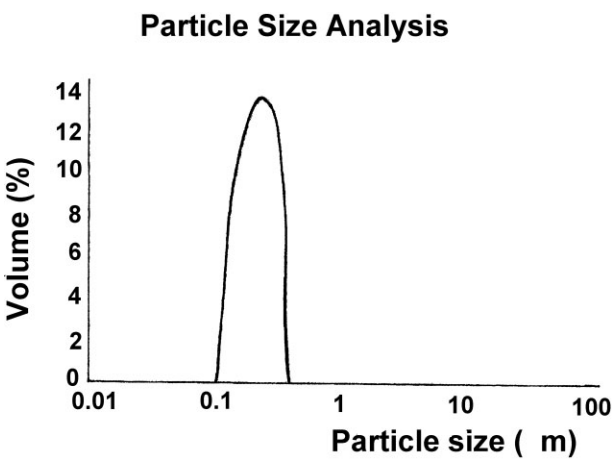


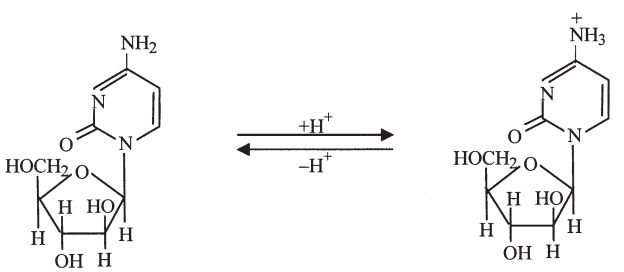
Figure 4 A graph showing the particle size distribution of unloaded gelatin nanoparticles.

TABLE I
Surface Potentials of Unloaded and Loaded Gelatin Nanoparticles

Particles	Medium (pH)	ζ-Potential (mV)
Unloaded nanoparticles	1.8	201
	4.0	116
	7.4	207
Drug loaded nanoparticles	1.8	190
	4.0	101
	7.4	63

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 theory implies that the free volume in a polymer may be thought of as a volume fraction of molecular size 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 carrying almost equal number of positive (—NH_3^+) and negative (—COO^-) charges (because pH 7.4 is isoelectric point also). At pH 7.4, the drug (cytarabine) will also be present in 100% ionized state, according to the following equilibrium



Thus, the positively charged cytarabine molecules may be held up to the negatively charged —COO^- groups via electrostatic attraction.

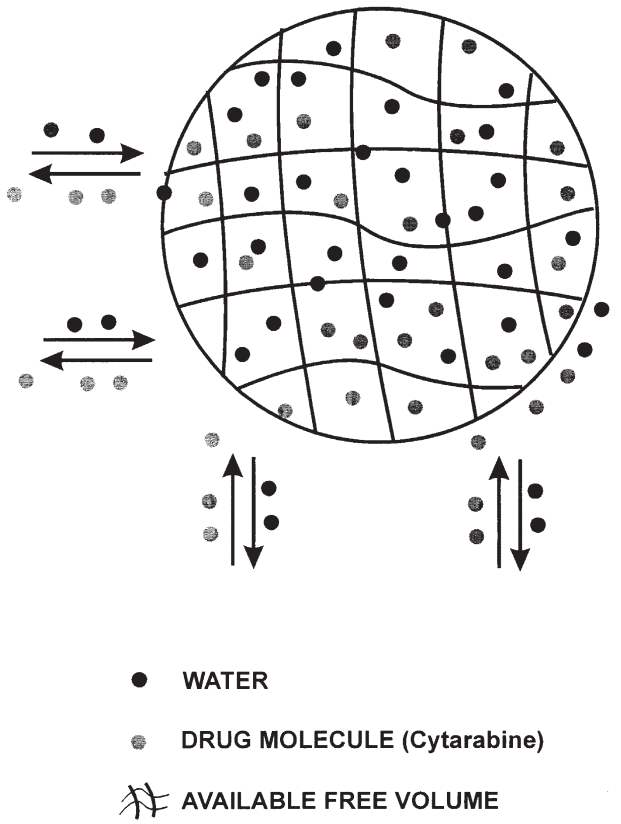


Figure 5 A model depicting the release of cytarabine from a swelling gelatin nanoparticle.

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 cytarabine release is modeled in Figure 5.

Effect of percent loading on cytarabine release

In the present study, the physical loading was followed, which involved swelling of preweighed nanoparticles into the cytarabine solution of concentration ranging from 0.5 to 2.5%. The loaded nanoparticles were allowed to release the entrapped cytarabine into definite volume of the release medium. The results are depicted in Figure 6, which clearly indicate that the amount of released cytarabine gradually decreases with increasing percent loading. The observed decrease in the release rate may be attributed to the fact that, with increasing percent loading, the pore size of nanoparticles become smaller due to accumulation of drug molecules within the nanoparticles and this restrains the diffusion of water molecules into the loaded nanoparticles. This obviously results in a lower release of cytarabine.

Effect of gelatin on cytarabine release

Drug release profiles are often sensitive to chemical architecture of the carrier as well as the experimental conditions of preparation of drug carrier. In the present study too, the size and morphology of nanoparticles are greatly determined by the factors such as amounts 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 cytarabine release has been investigated by varying its amounts in the range 4.0–9.0 g in the feed composition. The release and swelling results are shown in Figures 7 and 8, respectively, which clearly indicate that the fractional release of cytarabine increases with increasing amount of gelatin up to 8.0 g, whereas a fall 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 Figure 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 amount of gelatin, the nanoparticles of large size and wide pores are produced, which obviously allow greater number of water molecules to enter into the nanoparticles. This consequently results in larger release of cytarabine into the release medium.

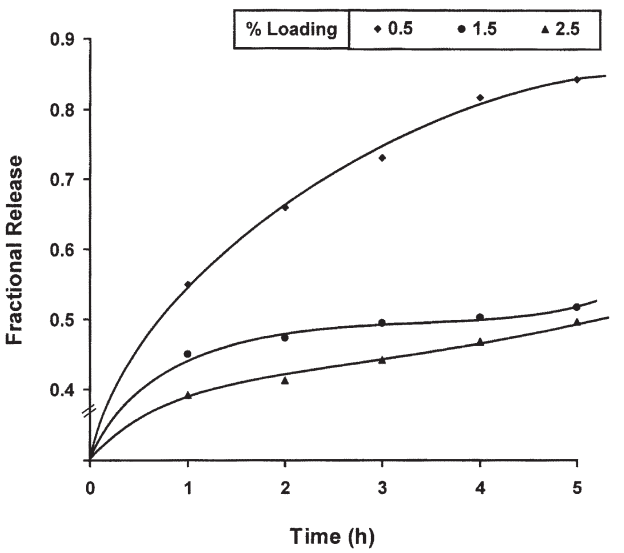


Figure 6 Effect of %loading of cytarabine on its release profile for a definite composition of nanoparticle [gelatin] = 8.0 g, [glutaraldehyde] = 10.6 m*M*, pH = 7.4, Temp = (25 ± 0.2)°C.

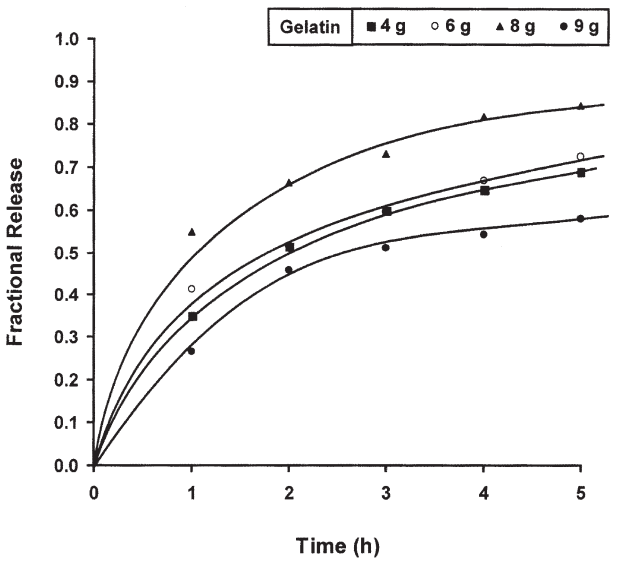


Figure 7 Effect of varying amounts of gelatin in nanoparticles on release profiles of cytarabine for a definite composition of nanoparticles [glutaraldehyde] = 10.6 m*M*, pH = 7.4, Temp = (25 ± 0.2)°C, %Loading = 0.5.

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 cytarabine molecules will have to travel a longer path through the nanoparticle to penetrate into the release medium. This obviously brings about a fall in both the swelling ratio as well as the released amount of drug.

Effect of crosslinker on cytarabine 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 nonthrombogenic 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; it is less expensive; it 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.[36] 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 millimolar concentration range, its possible toxicity could be quite lower in effectiveness.

The effect of crosslinker on the release and swelling profiles of cytarabine has been investigated by varying the concentration of glutaraldehyde (GA) in the range 5.3–31.8 m*M*. The results are shown in Figures 9 and 10, respectively, which clearly reveal that both the fractional release of cytarabine and swelling ratio increase respectively, with increasing GA up to 10.6 m*M* concentration while beyond it a fall in release and

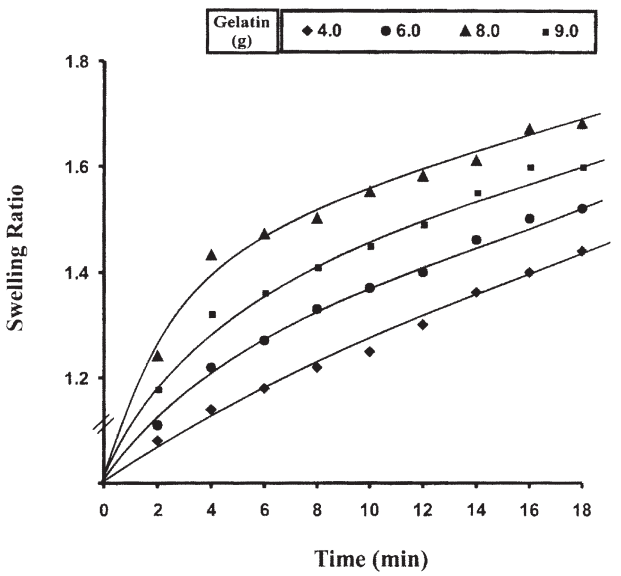


Figure 8 Effect of varying amounts of gelatin on swelling ratio of nanoparticles for a definite nanoparticle composition [glutaraldehyde] = 10.6 m*M*, pH = 7.4, Temp = (25 ± 0.2)°C.

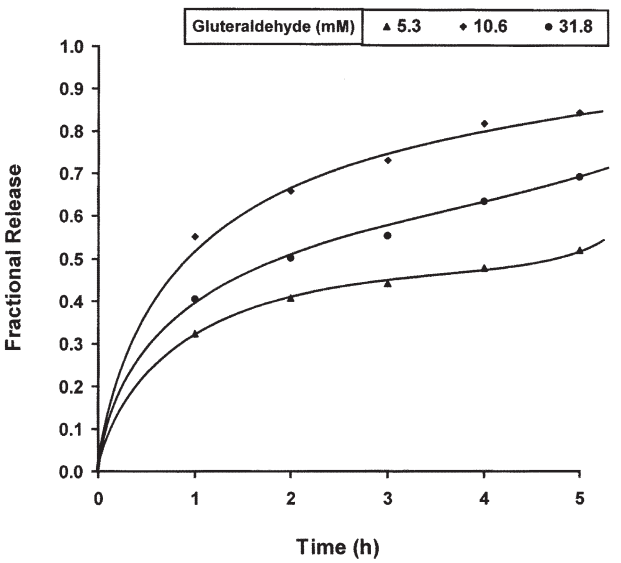


Figure 9 Effect of varying amounts of glutaraldehyde (crosslinker) on release profiles of cytarabine for a definite nanoparticle composition [gelatin] = 8.0 g, pH = 7.4, Temp = (25 ± 0.2)°C, %Loading = 0.5.

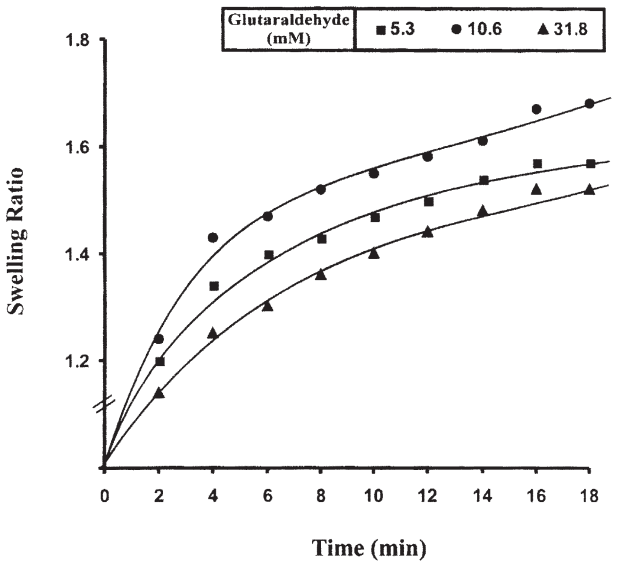


Figure 10 Effect of varying amounts of glutaraldehyde on swelling ratio of nanoparticles for a definite nanoparticle composition [gelatin] = 8.0 g, pH = 7.4, Temp = (25 ± 0.2)°C.

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 increasing number of water molecules into the nanoparticle and obviously the swelling ratio will increase. Thus, increased swelling will permit greater number of cytarabine molecules to diffuse out and the release of cytarabine will also increase.

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

Another explanation for the observed decrease in the swelling ratio and cytarabine 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 type of results have also been reported by other workers.[37] Some authors,[38] however, have reported that introduction of crosslinker into the polymer matrix enhances its glass transition temperature (T_g), which because of glassy behavior of polymers restrains the mobility of network chains and, therefore, both swelling and cytarabine release decreases.

Effect of pH on cytarabine release

Drug delivery systems capable of selected release of drugs in the colon have received much attention in recent past.[39] 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. 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.[40] 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 majority used and frequently cited in the literature.[41] In the present investigation, the release dynamics of the cytarabine has been observed under varying pH conditions as found in the GIT [e.g., stomach (gastric juice) 1.0, and small intestine 7.5–8.6]. 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.[42] 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.[43]

The results obtained in the present study are depicted in Figures 11 and 12, which clearly indicate that the fractional release of cytarabine decreases with increasing pH and the swelling of nanoparticles increases with increasing pH up to 7.4. The results obtained may be explained as follows.

Since the present drug delivery systems is swelling controlled, the extent of water sorption by the nanoparticles will determine the amount of the released cytarabine. It has been well-demonstrated by theoretical considerations[44] 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 concen-

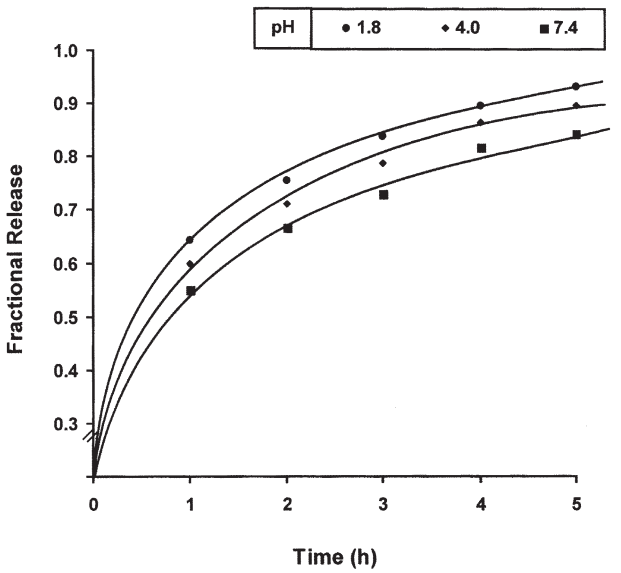


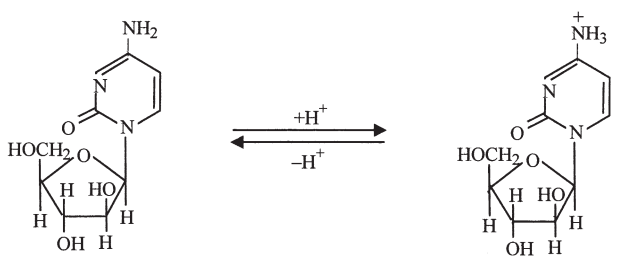
Figure 11 Variation in released amount of cytarabine with varying pH of the release medium for a definite nanoparticle composition [gelatin] = 8.0 g, [glutaraldehyde] = 10.6 m*M*, Temp = (25 ± 0.2)°C, %Loading = 0.5.

tration of mobile ions between the interior of the hydrogel and external swelling medium. Increasing the ionic concentration difference obviously results in an enhanced swelling of the polymer. Thus, ignoring ion–ion, ion–polymer, and ion–solvent interaction, we can write

$$\pi_{\text{ion}} = RT\Sigma[C_i^g - C_i^s]$$

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 ionic concentration of nanoparticle and release medium, greater would be the swelling.

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



while gelatin molecules will also possess a net positive charge due to predominance of the protonated amine groups ($-\overset{+}{\underset{}{N}}H_3$) over carboxylate ions ($-COO^-$) of their amino acids and cause repulsion among the entrapped drug molecule within the nanoparticles. This obviously results in a widening of the mesh sizes of the nanoparticle, which facilitate the release of the cytarabine molecules into the release medium.

The observed findings may also be explained on the basis of solubility of cytarabine at different pH media. Because of the basic nature of the drug, a greater solubility is observed at low pH while it slightly decreases with increasing pH of the medium. In this way at low pH, the entrapped cytarabine has a greater tendency to go into the release medium of low pH, while at higher pH lesser tendency of drug to pass into the release medium results in lower release.

It is also implied by the figure that with increasing pH the release mechanism shifts toward zero order, which can be interpreted as follows.

The swelling of a polymer matrix normally results in a rapid decrease in its glass transition temperature (T_g) to the temperature of the dissolution medium. Microscopically, there is a relaxation response of the polymer chains due to stresses introduced by the presence of the dissolution solvent. This results in an increase in the radius of gyration and end to end distances of the polymer chains, causing a significant increase in the molecular volume of the hydrated polymer.[45] This reduces the free volume due to the presence of the micropores, which may manifest itself as a shift in the drug release mechanism. Thus, the observed zero-order release may be attributed to a reduction in regions of low microviscosity and the closing of micropores in the swollen gel.

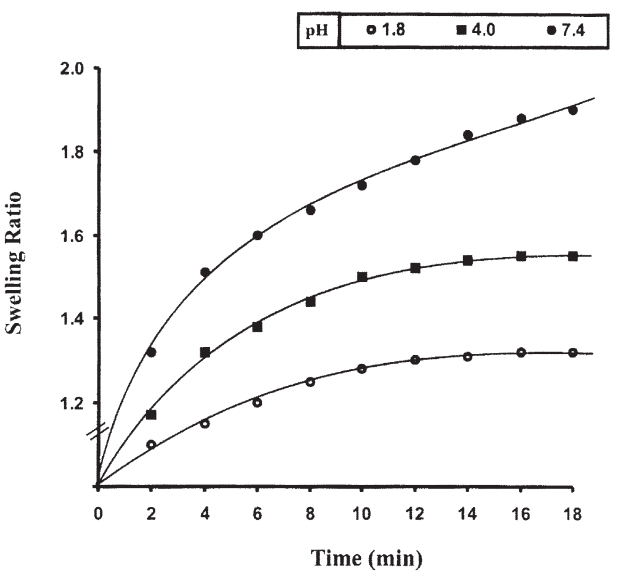


Figure 12 Effect of pH on swelling ratio of nanoparticles for a definite nanoparticle composition [gelatin] = 8.0 g, [glutaraldehyde] = 10.6 m*M*, Temp = (25 ± 0.2)°C.

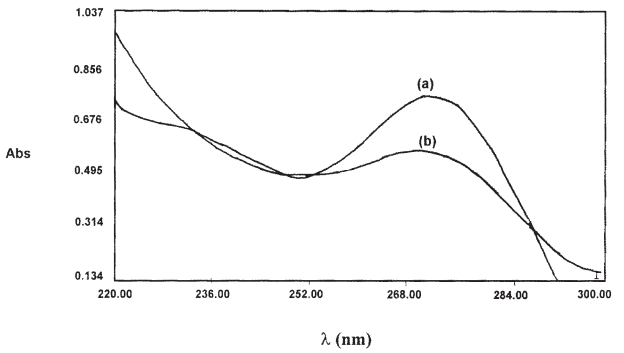


Figure 13 UV spectra showing the chemical stability of cytarabine in its (a) pure solution, (b) released medium.

Chemical stability of drug

To ascertain the chemical stability of cytarabine in highly acidic pH medium, such as gastric juice, the drug was left in simulated gastric juice medium and its UV spectra was scanned and compared with that of cytarabine in the aqueous medium. The spectra are shown in Figure 13, which clearly indicate that they are nearly identical to each other, which means that the two spectra have the same λ_{max} and appear almost similar in shape. This obviously suggests that even in remaining in highly acidic media, the chemical nature of cytarabine does not change. Moreover, it was also found that even gelatin nanoparticles do not undergo any cleavage in gastric juice medium. This clearly confirms the stability of drug carrier system in highly acidic media.

Effect of temperature on cytarabine release

In the present study, the temperature of the release medium was varied in the range 10–35°C and its effects on the release of cytarabine have been investigated. The results are shown in Figures 14 and 15, which indicate that with increasing temperature, release rate increases up to 35°C while swelling increases up to 25°C and then decreases.

The observed increase in the released amount of cytarabine up to 35°C can be explained by the fact that with increasing temperature the diffusion of both water and cytarabine molecules and rate of relaxation of nanoparticles chain increase, which, in turn, results in greater cytarabine release. 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.[46]

Effect of type of gelatin

Gelatin is a natural polymer that is extracted from collagen by alkaline or acidic pretreatment and thermal denaturation.[47] 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

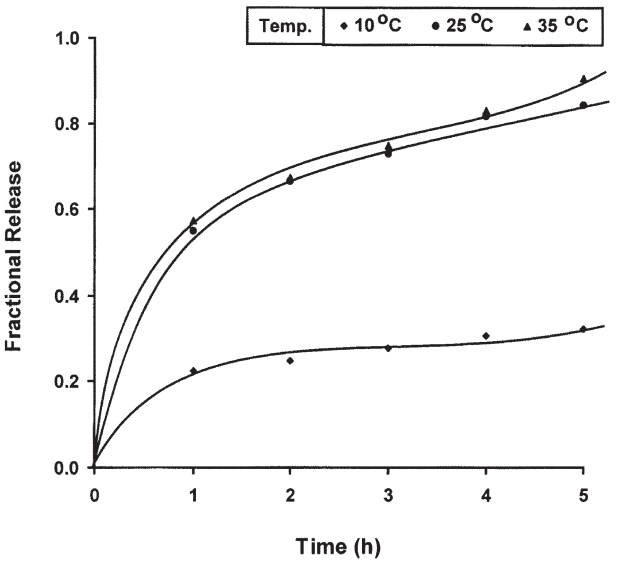


Figure 14 Effect of temperature on the released amounts of cytarabine for a definite nanoparticle composition [gelatin] = 8.0 g, [glutaraldehyde] = 10.6 m*M*, pH = 7.4, % Loading = 0.5.

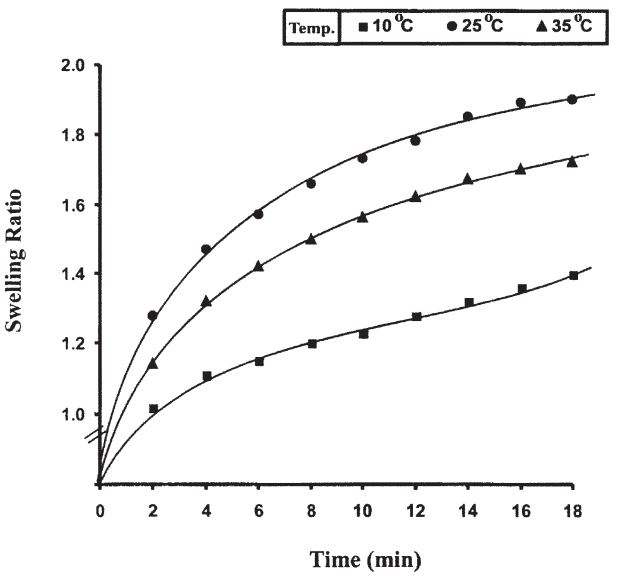


Figure 15 Variation in swelling ratio of nanoparticle with temperature of swelling both for a definite nanoparticle composition [gelatin] = 8.0 g, [glutaraldehyde] = 10.6 m*M*, pH = 7.4.

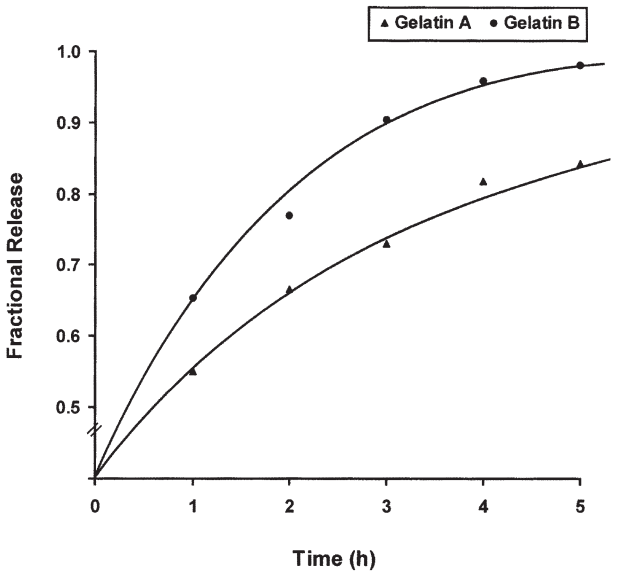


Figure 16 Effect of type of gelatin on the released amount of cytarabine for definite nanoparticle compositions [gelatin] = 8.0g, [glutaraldehyde] = 10.6 m*M*, pH = 7.4, Temp = (25 ± 0.2)°C, %Loading = 0.5.

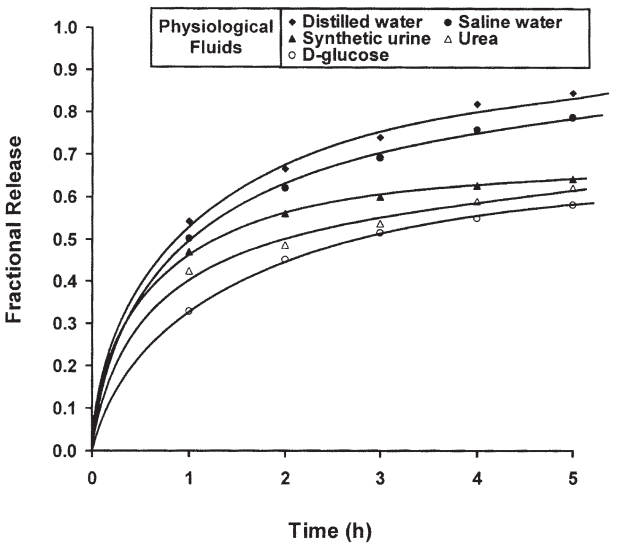


Figure 17 Effect of physiological fluids on the released amount of cytarabine for definite nanoparticle compositions [gelatin] = 8.0 g, [glutaraldehyde] = 10.6 m*M*, pH = 7.4, Temp = (25 ± 0.2)°C, %Loading = 0.5.

content for gelatin B (118/1000 amino acids) than for gelatin A (77/1000 amino acids).[48]

The effect of type of gelatin on the release profile of cytarabine 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 Figure 16, which clearly indicate that the fractional release of drug is quite higher in 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 cytarabine 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. Similar type of results has also been published elsewhere.[49]

Effect of physiological fluid on cytarabine release

The effect of nature of the medium on the swelling and release kinetics of cytarabine has been investigated by performing release experiments in various physiological fluids. The results are depicted in Figures 17 and 18, which reveal that the release and swelling of cytarabine is significantly suppressed in physiological fluids in comparison to that in the distilled water. The possible reason for the lower release and swelling of cytarabine in these fluids may be that the presence of salt ions in the release medium lowers the rate of penetration of water molecules into the loaded nanoparticles, thus resulting in a fall in the release amount of cytarabine. In the case of urea, its capacity to break

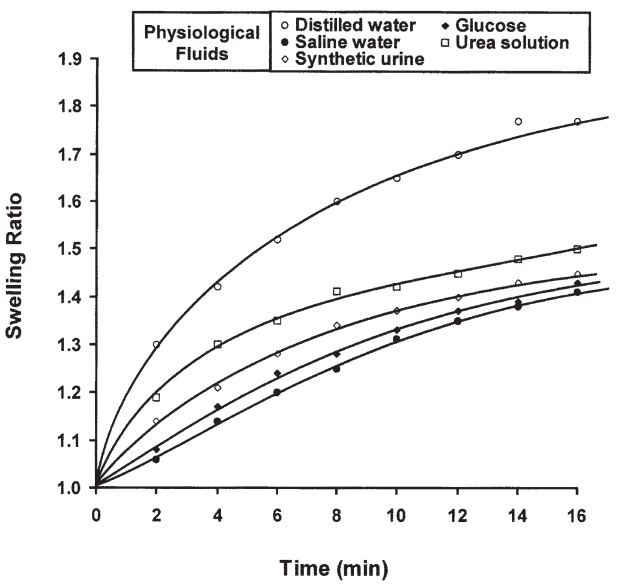


Figure 18 Effect of physiological fluids on the swelling ratio of nanoparticles for a definite nanoparticle composition [gelatin] = 8.0 g, [glutaraldehyde] = 10.6 m*M*, Temp = (25 ± 0.2)°C.

TABLE II
Data Showing the Release Exponent and Diffusion Constant under Varying Experimental Conditions

Gelatin (g)	Glutaraldehyde (m*M*)	pH	*n*	D × 10^4 (cm^2 min^{-1})
4	10.60	7.4	0.475	0.785
6	10.60	7.4	0.50	1.03
8	10.60	7.4	0.578	0.55
9	10.60	7.4	0.56	1.04
8	5.30	7.4	0.50	0.63
8	10.60	7.4	0.578	0.55
8	31.82	7.4	0.59	0.82
8	10.60	7.4	0.578	0.55
8	10.60	4.4	0.327	1.22
8	10.60	9.0	0.379	1.59

hydrogen bonds between water molecules and IPN chains may be responsible for the lower amount of water uptake[50] and consequently for the lower release of cytarabine also.

Analysis of kinetic release data

When a drug-loaded polymeric carrier contacts a thermodynamically compatible solvent, such as water, the swelling of 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 the 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.

In the present case, release by dissolution is not applicable as the cytarabine 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 cytarabine release could be either diffusion or relaxation controlled, which can be judged by the values of diffusion at exponent (n) predicted by eq. (3).

The values of n have been calculated on the basis of eq. (3) and summarized in Table II. The data clearly reveal that the value of n is quite near to 0.5 and, therefore, the release of cytarabine may be considered as Fickian or diffusion controlled.

CONCLUSIONS

Crosslinked gelatin nanoparticles form a swelling controlled drug release system, which effectively delivers cytarabine via diffusion controlled pathway. It is found that release profiles of cytarabine are greatly influenced by %loading of cytarabine, concentrations of gelatin and glutaraldehyde (crosslinker) in the nanoparticles. With increase in percent loading of drug on to the nanoparticles, the released amount of cytarabine constantly decreases. In the case of gelatin, the release of cytarabine increases when concentration of gelatin is increased from 4.0 to 8.0 g, whereas the extent of release decreases beyond 8.0 g of gelatin content. The released amount of cytarabine constantly decreases with increasing glutaraldehyde content in the nanoparticles. It is noticed that the release behavior is directly regulated by the extent of swelling of gelatin nanoparticles. 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 pH 1.8 while a lower release is obtained in pH 7.4. It is also noticed that the extent of release of cytarabine increases with increasing temperature. The extent of release of cytarabine is suppressed by the physiological fluids.

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