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Design of gelatin nanoparticles as swelling controlled delivery system for chloroquine phosphate

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Abstract Gelatin 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 prepared nanoparticles were loaded with chloroquine phosphate (CP), a well known antimalarial 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 processes was analyzed kinetically using Ficks power law and a correlation was established between the quantity of released drug and swelling of the nanoparticles.

Introduction

The treatment of acute disease chronic illness has been achieved by delivery of drug to the patients for many years. These drug delivery system include tablets, injectables, suspension, cream, ointments, liquids and aerosols. The term drug delivery can be defined as techniques that are used to get the therapeutic agents inside the human body [1]. Conventional drug therapy requires periodic doses of therapeutic agents. These agents are formulated to produce maximum stability activity and bioavailability. For most drugs conventional methods of drug administration are effective, but some drugs are unstable or toxic and have narrow therapeutic ranges. Some drugs also possess solubility problem. In such cases a method of continuous administration of therapeutic agents is desirable.

To overcome these problems, controlled drug delivery systems were introduced three decades ago [2]. Controlled drug delivery is delivery of drug at a rate or at a location determined by needs of body or Disease State over a specified period of time [3]. The principal advantage of this technology is that the carrier polymer matrix systems allow much less active agents to be used for the desire of activity. The field has grown and diversified rapidly in recent years and currently it is the subject of intensive research [4]. As a consequence, new and newer delivery systems are being explored frequently and finding exhaustive applications in pharmaceuticals [5] biomedical fields' [6] and agriculture [7]. Among different dosage forms reported nanoparticles have been identified as a noble class of matrices possessing a great potential as a drug carriers [8].

Polymeric nanoparticles can be identified as submicronic (size $< 1\mu$m) colloidal carriers. Compared to other colloidal carriers polymeric Nanoparticles hold significant promise for the advancement of treating diseases and disorders. They have attractive physicochemical properties such as size, surface potential, hydrophilic-hydrophobic balance etc. [9] and for this reason they have been recognized as potential drug carriers for bioactive ingredients such as anticancer drugs [10], vaccines [11], oligonucleotides [12], peptides [13], etc. Their widespread use for oral delivery also aims at improving the bioavailability of drugs with poor absorption characteristic [14], reducing GI mucosa irritation caused by drugs [15] and assuring stability of drugs in the GI tract [16]. Thus, all these and many more such characteristics of nanoparticles qualify them as a promising candidate in drug-delivery technology. Although various biodegradable nanoparticles

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of natural polymers such as starch [17], chitosan [18], liposomes [19] 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 nanoparticale material, it is a natural macromolecule, nontoxic and non carcinogenic nature (20) it possesses a relatively low antigenicity and it has a great deal of experience for its use parental formulations (21). Gelatin nanoparticles have been richly documented in literature also. For example, Kaul and Amiji [22] prepared poly(ethylene glycol) modified gelatin nanoparticles for intracellular delivery and found them quite beneficial as long-circulating delivery system *in vivo*. Leo and co-workers [23] 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 and co-workers [24] and their efficiency as a drug carrier was examined for intramuscular administration, both *in vitro* and *in vivo*. Yan and Li [25] prepared gluteraldehyde crosslinked gelatin microspheres with an average diameter of 70 μm and loaded them with mitomycin C, an anticancer drug, together with a radioisotype ^{131}I. The authors noticed that the loaded microspheres accumulated in the specific site and embolize the hepatic arteries after the hepatic intra-arterial infusion. Biodegradable hydrophilic nanoparticles of gelatin were prepared by Cascone *et al.* [26] adopting a single solvent evaporation method based on a single water-in-oil emulsion and investigated the release of methotrexate, an anticancer drug, from the drug loaded nanoparticles. The effect of parameters such as particle size and drug-encapsulation efficiency was studied on the dry-release profiles. Akin and Hasirel [27] prepared gluteraldehyde crosslinked microspheres of gelatin and characterized them by FTIR and SEM techniques. A crosslinked biodegradable matrix of gelatin was prepared by Kuijpers *et al.* [28] and controlled release of antibacterial protein was investigated. The contribution of collagen based materials in medical and drug-delivery applications were also reviewed by Rao [29]. Morita [30] prepared gelatin microparticles by co-lyophilization with poly(ethylene glycol) as a protein microionization adjuvant.

Thus being motivated by the application potential of gelatin in biomedical and pharmaceutical fields, we, in the present paper, are reporting results on controlled release of antimalarial drug, chloroquine phosphate from the drug loaded gelatin nanoparticles. The drug chosen for the study is chloroquine phosphate that belongs to antimalarial drug. Chloroquine is very rapidly and completely absorbed after ingestion. Toxic doses of chloroquine can be fatal. As little as 1gm may be fatal in children. Toxic symptoms can occur within minutes. These consist of headache drowsiness, visual disturbances, nausea and vomiting, cardiovascular collapse, shock and convulsions followed by sudden and early respiratory and cardiac arrest. To overcome from this overdose effect of chloroquine phosphate (31) controlled drug delivery of this drug is desirable.

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., USA, Average $M_W \sim$ 1,20,000 Da, inherent viscosity 0.20) was used for preparing oil phase. Other chemicals and solvents were of analytical reagent grade. IPCA Laboratories Ltd. used chloroquine phosphate as a model drug obtained from chloroquine phosphate injection (Lariago) made in 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 [32] and solvent evaporation [33], 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.* [26]. Briefly, the method may be described as below:

'Aqueous phase' was prepared by dissolving 8 g of gelatin in 25 ml of distilled water while for preparing 'oil phase', 7g of Polymethylmethacrylate was dissolved into a mixture of 25 ml of chloroform and 25 ml of 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 air tight polyethylene bags.

IR spectra

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

Scanning electron microscope (SEM)

Morphological features of unloaded and chloroquine phosphate 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 chloroquine phosphate (CP) — 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 nanoparticle 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 [34–35].

In the present case because of 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 Fig. 1.

Thus, being inspired by the above 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 dimension of swelling 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 was sprayed on a petridish and the microscope was focused on a single nanoparticle reading its dimension on ocular micrometer scale. Now, a single drop of phosphate buffer saline (PBS, pH 7.4) was added to the particle so that it instantaneously starts swelling which is clearly seen on the microscope. Thus, the dimension of the swelling particle is noted and the degree of water sorption is quantified in terms of 'swelling ratio' as calculated below:

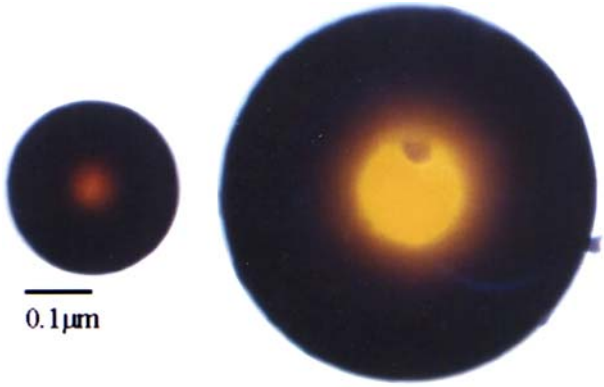


Fig. 1 an optical microscope photograph of swelling gelatin-nanoparticle (a) dry, and (b) fully swollen nanoparticle.

$$\text{Swelling Ratio} = \frac{\text{Diameter of swollen particle}}{\text{Diameter of Dry Particle}} \quad (1)$$

Optical microscopy

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

Loading of chloroquine phosphate (CP)

For loading of Nanoparticles, known volume of drug injection were taken and diluted with appropriate amount of doubly distilled water and shaked vigorously for mixing of drug injection and distilled water.

The loading of CP was performed by allowing the 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 eq.

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

where W_d and W_o 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 (CP) were almost same in both the 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 chloroquine phosphate, 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 predetermined time period. After shaking was over, 1 ml of supernatant was withdrawn and 1ml distilled water was added again to the suspension and 1 ml supernant which was withdrawn assayed for CP spectrophotometrically [36] as given below:

In a corning flask, 1.0 ml of the drug solution was taken and to that was added 9 ml of acetone and 3ml of freshly prepared ammonium molybdate solution and kept that solution for 5 minutes and the amount of the drug determined by a calibration curve.

Kinetics of release process

For monitoring the progress of the release process, 1ml of aliquots were withdrawn at desired time intervals and instantly replaced by fresh release medium (distilled water). In the aliquots withdrawn, the amount of CP was determined as described above.

For achieving mechanistic insights into the release process of CP the following equation was used [37],

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

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 CP 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 [38], where L is the diameter of dry nanoparticle.

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

Chemical stability of drug

Chemical stability of drug in acidic media (pH-1.8) was judged by UV spectrophotometric method as explained elsewhere [39] (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

(i) IR spectral analysis

The IR spectra of uncrosslinked gelatin and CP loaded nanoparticles are presented in Fig. 2. The spectra of CP loaded nanoparticles clearly confirms the presence of gelatin, glutaraldehyde and CP in the drug loaded nanoparticles. The strong band observed at 3561 cm^{-1} due to N-H stretching of amide group confirms the presence of gelatin while bands at 2930 cm^{-1} (C-H stretching of methyl and methylene group), 1729 (C=O stretching) confirms the presence of gluteraldehyde. The bands at 1545 cm^{-1} (C-N stretching vibration) 1459 cm^{-1} (C-C stretching and C=N ring stretching) and 1026 cm^{-1} (due to presence of phosphate ion) clearly indicate the presence of chloroquine phosphate in the drug loaded nanoparticles.

(I) Analysis of SEM

A scanning electron micrograph (SEM) of CP-loaded nanoparticles is shown in Fig. 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 surface. This obviously presents a morphological evidence for solid and smooth nanoparticles.

(ii) Particle size analysis

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

(iii) Surface potential measurements

The value of ζ potential for unloaded nanoparticles and drug loaded nanoparticles are summarized in

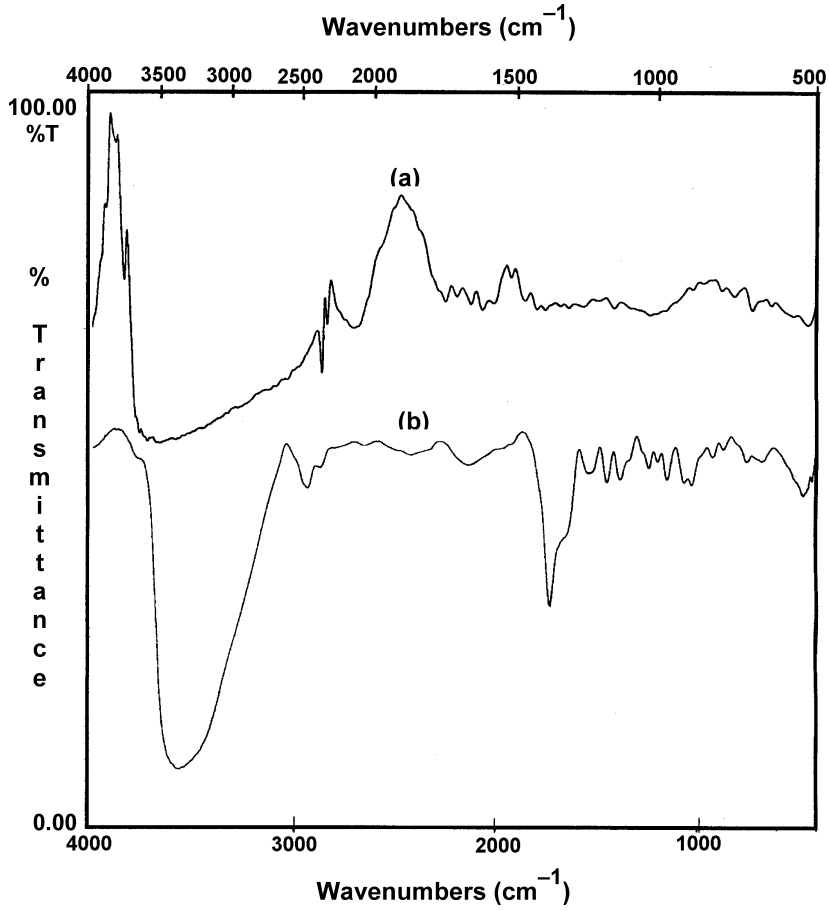


Fig. 2 IR spectra of (a) pure gelatin, and (b) chloroquine phosphate-loaded crosslinked gelatin nanoparticles.

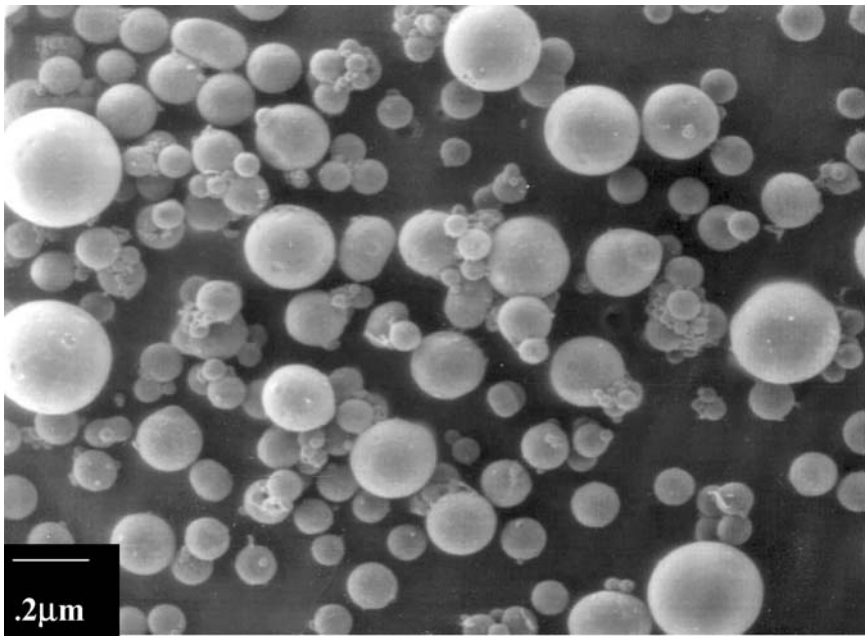


Fig. 3 Scanning electron micrograph (SEM) of CP-loaded nanoparticles.

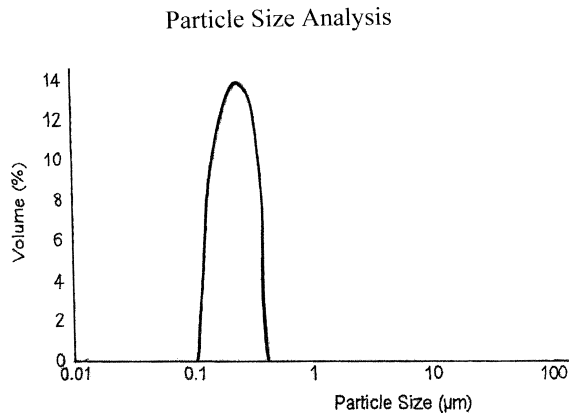


Fig. 4 A graph showing the particle size distribution of unloaded gelatin nanoparticles.

Table 1 which clearly indicate that upon loading of CP-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 an evidence of drug surface interaction.

Mechanism of drug release

A swollen nanoparticle may be imagined as a three dimensional polymer network structure between the strands of which are water-filled permeation channels [40]. 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.* [41] 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 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 carrying almost equal number of positive ($-NH_3^+$) and negative ($-COO^-$) charges (because pH 7.4 is isoelectric point also). At pH7.4 the drug (CP) will also be present in 100%-ionized state according to the following equilibrium:

Table 1 Surface potentials of unloaded and loaded gelatin nanoparticles

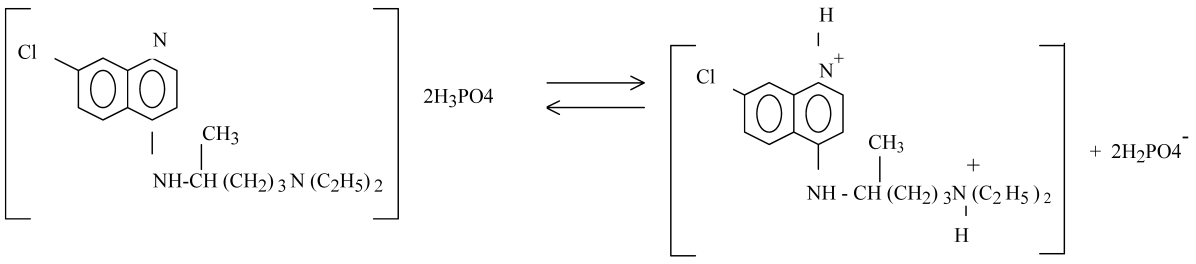
Particles	Medium	ζ Potential (mV)
Unloaded Nanoparticles	1.8 pH	201
	4.0 pH	116
	7.4 pH	193
Drug Loaded Nanoparticles	1.8 pH	204
	4.0 pH	136
	7.4 pH	205

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

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 [42]. The whole mechanism of CP release is modelled in Fig. 5

Effect of percent loading on CP release

In the present study the physical loading was followed which involved swelling of preweighed nanoparticles into the chloroquine phosphate solution of concentration ranging from 15% to 19%. The loaded nanoparticles were allowed to release the entrapped CP into definite volume of the medium. The results are depicted in figure (6) which clearly indicate that the amount of released CP 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 molecule within the nanoparticles and this restrain the diffusion of water molecules into the loaded nanoparticles. This obviously results in a lower release of CP.



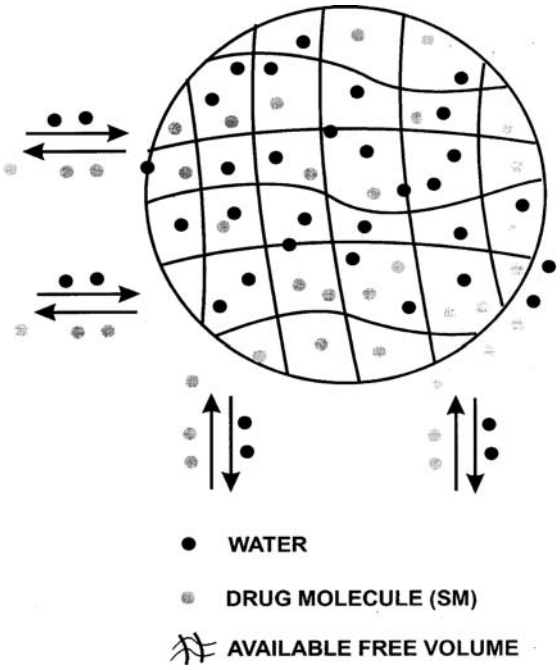


Fig. 5 A model depicting the release of CP from a swelling gelatin nanoparticle.

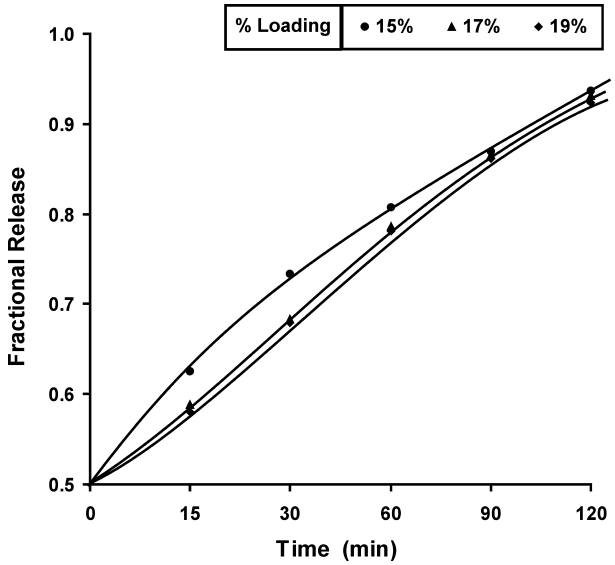


Fig. 6 Effect of %loading of CP on its release profile for a definite composition of nanoparticle [gelatin] = 8.0 g, [glutaraldehyde] = 10.6 mM, pH = 7.4, Temp. = 25 + 0.2°C.

Effect of gelatin on CP 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 concentrations of gelatin and glutaraldehyde in the feed mixture, molecular weight of polymethyl methacrylate and temperature and shaking time of emulsions.

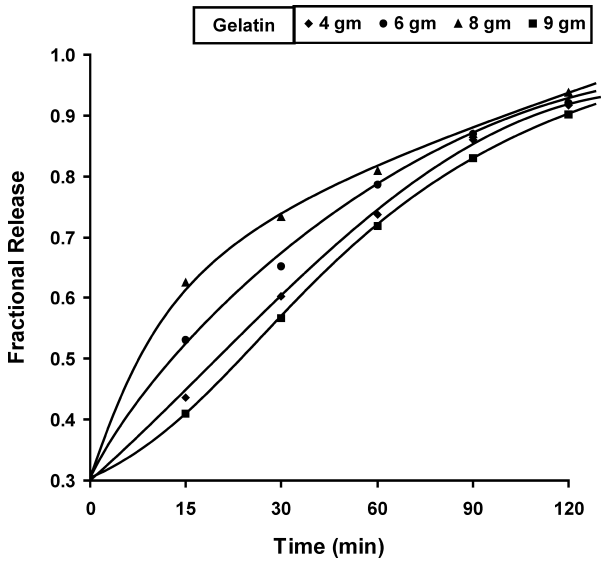


Fig. 7 Effect of varying amounts of gelatin in nanoparticles on release profiles of CP for a definite composition of nanoparticles [glutaraldehyde] = 10.6 mM, pH = 7.4, Temp. = 25 ± 0.2°C, %Loading = 15

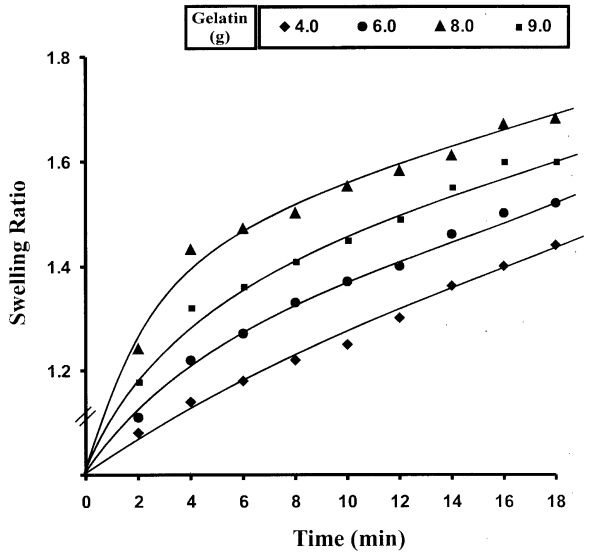


Fig. 8 Effect of varying amounts of gelatin on swelling ratio of nanoparticles for a definite nanoparticle composition [glutaraldehyde] = 10.6 mM, pH = 7.4, Temp. = 25 ± 0.2°C.

The effect of gelatin on the CP release has been investigated by varying its concentration in the range 4.0 to 9.0 g in the feed composition. The release and swelling results are shown in Fig. 7 and Fig. 8 respectively which clearly indicate that the fractional release of CP increases with increasing concentration 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 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 greater number of water molecules to enter into the nanoparticles. This consequently results in larger release of CP 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 CP molecule will 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 CP 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; it is less expensive; it is readily available and highly soluble in aqueous solution.

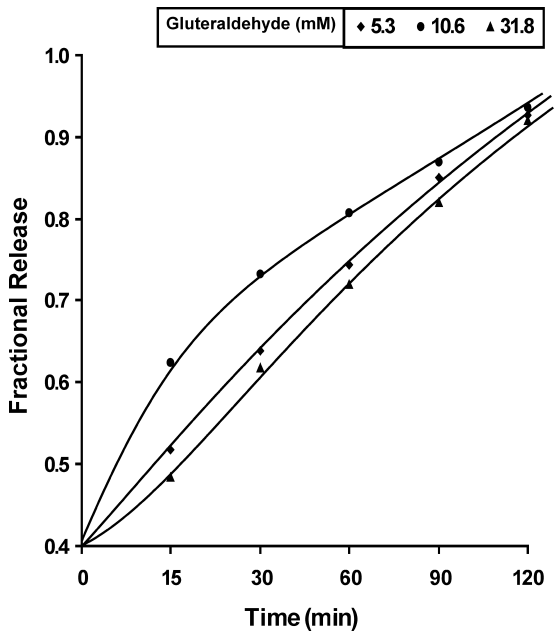


Fig. 9 Effect of varying amounts of glutaraldehyde (crosslinker) on release profiles of CP for a definite nanoparticle composition [gelatin] = 8.0 g, pH = 7.4, Temp. = 25 ± 0.2°C, %Loading = 15.

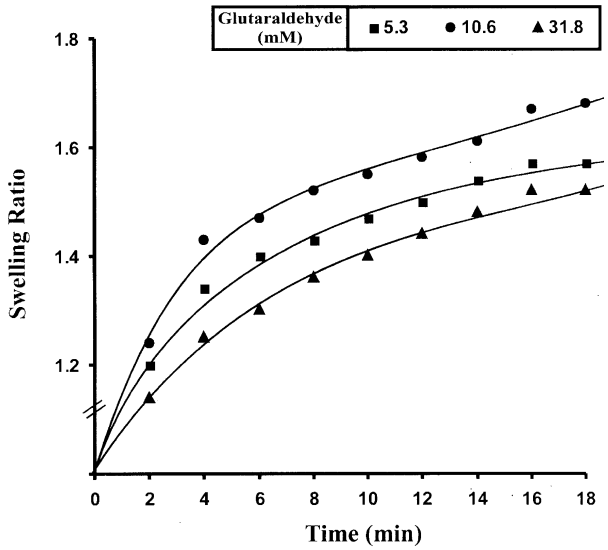


Fig. 10 Effect of varying amounts of glutaraldehyde on swelling ratio of nanoparticles for a definite nanoparticle composition [gelatin] = 8.0 g, pH = 7.4, Temp. = 25 ± 0.2°C.

Although gelatin itself is non toxic, 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 [43]. 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.

The effect of crosslinker on the release and swelling profiles of CP has been investigated by varying the concentration of glutaraldehyde (GA) in the range 5.3 to 31.8 mM. The results are shown in Fig. 9 and Fig. 10 respectively which clearly reveal that both the released amount of CP and swelling ratio increase respectively with increasing GA up to 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 increasing number of water molecules into the nanoparticle and obviously the swelling ratio will increase. Thus, increased swelling will permit greater number of CP molecules to diffuse out and the release of CP 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 CP 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 [44]. Some authors (45) have, however, reported that introduction of crosslinker into the polymer matrix enhances its glass transition temperature (T_g) which because of glassy behaviour of polymers restrains the mobility of network chains and, therefore, both swelling and CP release decreases.

Effect of pH on CP release

Drug delivery systems capable of selected release of drugs in the colon have received much attention in recent past [46]. 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

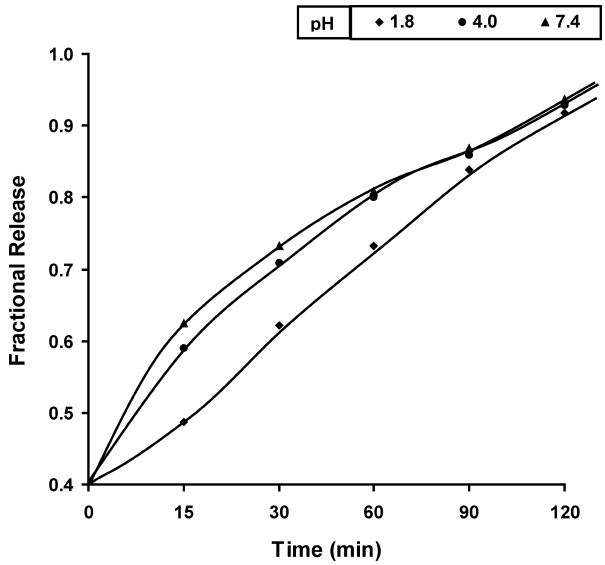


Fig. 11 Variation in released amount of CP with varying pH of the release medium for a definite nanoparticle composition [gelatin] = 8.0 g, [glutaraldehyde] = 10.6 mM, Temp. = 25 ± 0.2° C, %Loading = 15.

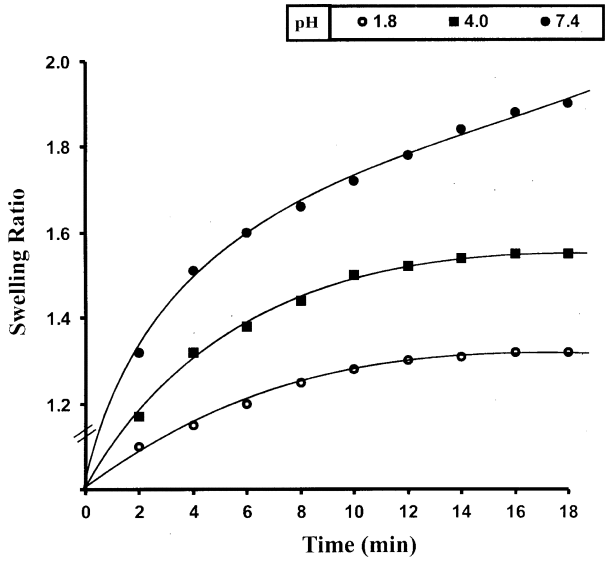


Fig. 12 Effect of pH on swelling ratio of nanoparticles for a definite nanoparticle composition [gelatin] = 8.0 g, [glutaraldehyde]= 10.6 mM, Temp. = 25 ± 0.2° C.

[47]. 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 [48]. In the present investigation, the release dynamics of the CP 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 [49]. 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 [50].

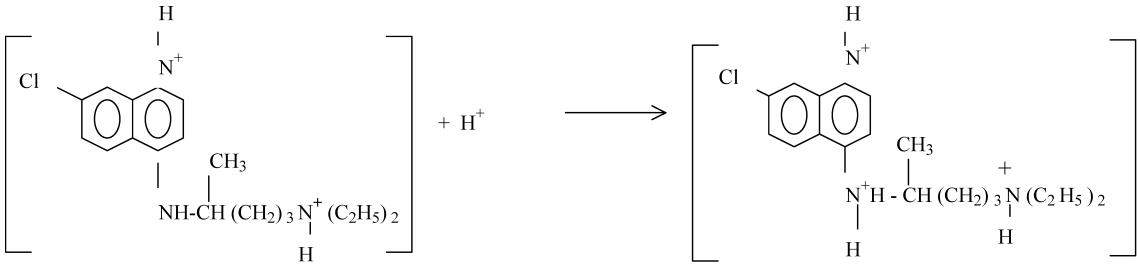
The results obtained in the present study are depicted in Fig. 11 and 12, which clearly indicate that both the release of CP and the swelling of nanoparticles increase with increasing pH up to 7.4. The results obtained may be explained as below:

Since the present drug delivery systems is swelling controlled, the extent of water sorption by the nanoparticles will determine the amount of the released CP It has been well demonstrated by theoretical considerations [51] 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

$$\pi_{\text{ion}} = \text{RT} \sum \left(C_i^g - C_i^s\right)$$

Where C_i^g and C_i^s represent the mobile ion concentration in the gel (nanoparticle) and release medium, respectively. The above eq. clearly reveals that larger the difference between the nanoparticle and release medium, greater would be the swelling and, therefore, the release. When the CP loaded nanoparticles are placed at the lowest pH (1.8) of the studied range, the CP 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 carboxylate ions ($-COO^-$) of their amino acids. Thus, ionic concentration will be greater in the external receptor medium then 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.

When the pH is raised to 4.6, about 75% of entrapped CP molecules become ionized ($pK_a = 4.51$) [52] 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 CP release.

At pH 7.4, the CP 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 get fully swollen at pH 7.4, and as a consequence, an optimum release of the entrapped CP is noticed. As no body fluids of GI tract acquire a pH greater than 8.0, no release experiments were performed in greater alkaline range.

The above discussion clearly reveals that while an optimum release of CP occurs at pH 7.6, 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 chloroquine phosphate 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 to that of CP in the aqueous medium. The spectra are shown in Fig. 13, which clearly indicate that they are nearly identical to each other. This obviously suggests that even in remaining in highly acidic media, the chemical nature of chloroquine phosphate 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 CP release

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

The observed increase in the released amount of CP up to 35°C can be explained by the fact that with increasing temperature the diffusion of both water and CP molecules and rate of relaxation of nanoparticles chain increases which in turn results in greater CP release. The lower value of swelling at 35°C may be because of breaking of hydrogen bonds between water molecules and nanoparticles chains. A decreased swelling at higher temperature is widely reported in the literature (53).

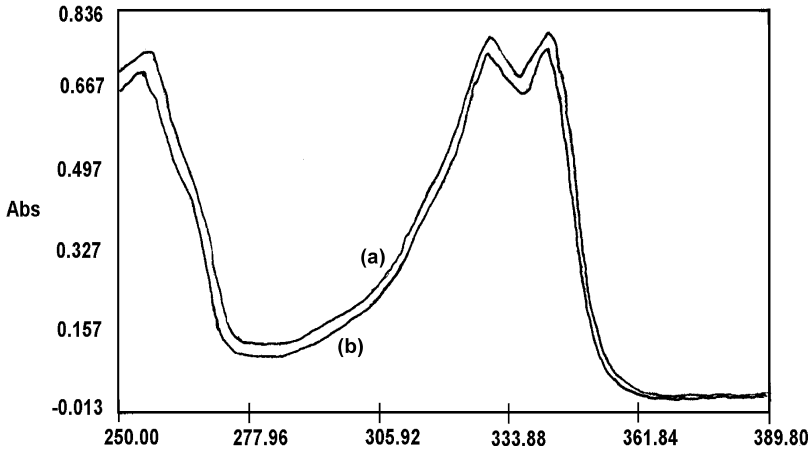


Fig. 13 UV spectra showing the chemical stability of chloroquine phosphate in its (a) pure solution, (b) released medium.

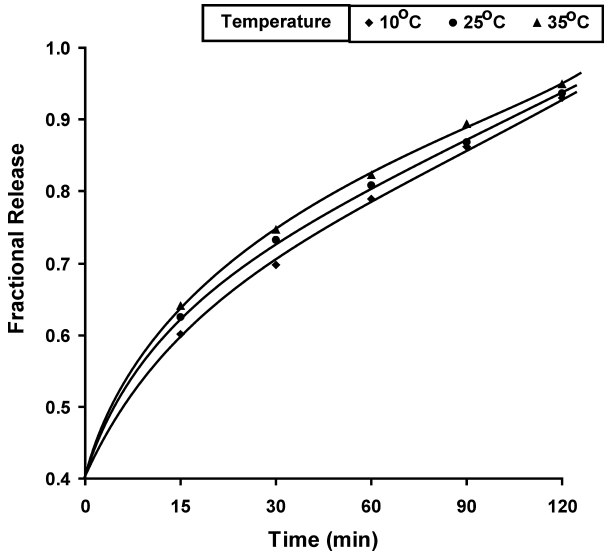


Fig. 14 Effect of temperature on the released amounts of CP for a definite nanoparticle composition [gelatin] = 8.0 g, [glutaraldehyde] = 10.6 mM, pH = 7.4, % Loading = 15.

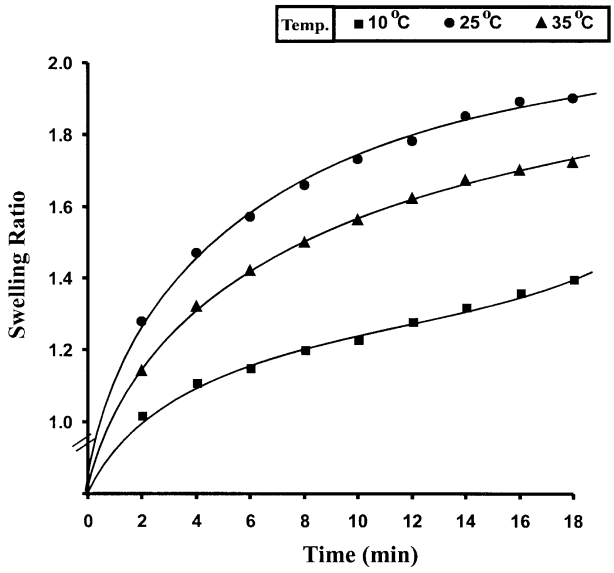


Fig. 15 Variation in swelling ratio of nanoparticle with temperature of swelling both for a definite nanoparticle composition [gelatin] = 8.0 g, [glutaraldehyde] = 10.6 mM, pH = 7.4.

Effect of type of gelatin

Gelatin is a natural polymer that is extracted from collagen by alkaline or acidic pretreatment and thermal denaturation [54]. 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) [55].

The effect of type of gelatin on the release profile of CP 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. 16 which clearly indicate that the 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 CP 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 CP in type B nanoparticles. Similar type of results have also been published elsewhere [56].

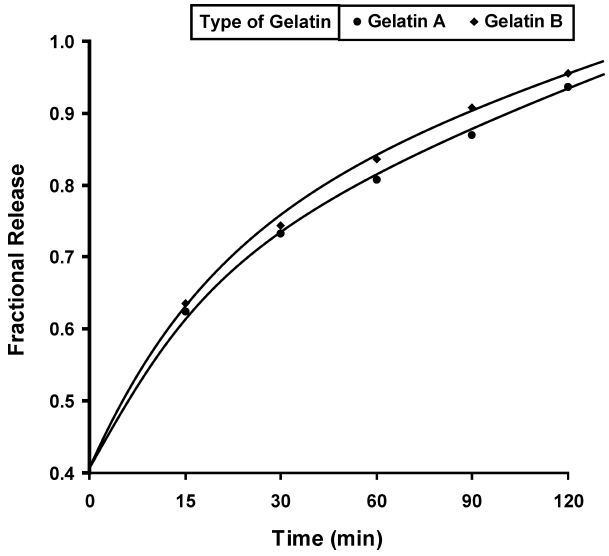


Fig. 16 Effect of type of gelatin on the released amount of CP for definite nanoparticle compositions [gelatin] = 8.0 g, [glutaraldehyde] = 10.6 mM, pH = 7.4, Temp. = 25 ± 0.2°C, %Loading = 15.

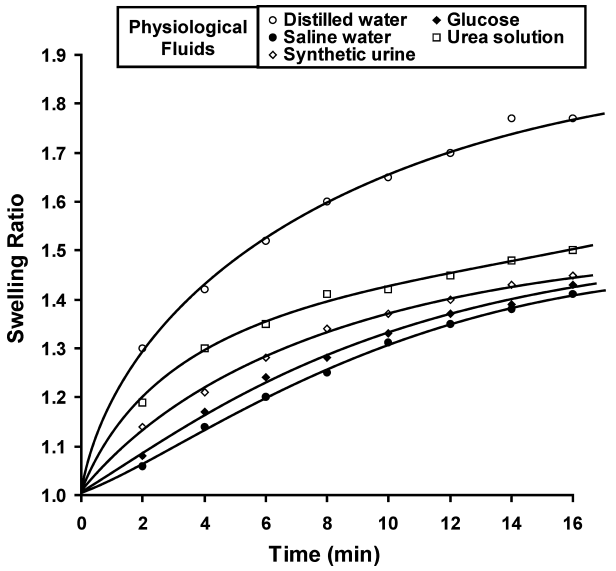


Fig. 18 Effect of physiological fluids on swelling ratio of nanoparticles for a definite nanoparticle composition [gelatin] = 8.0 g, [glutaraldehyde] = 10.6 mM, Temp. = 25 + 0.2°C.

Effect of physiological fluid on CP release

The effect of nature of the medium on the swelling and release kinetics of CP has been investigated by performing release experiments in various physiological fluids. The results are depicted in Fig. 17 and 18 which reveal that the release and swelling of CP is significantly suppressed in physiological fluid in comparison to that in the distilled water. The possible reason for the lower release and swelling of CP 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 released amount of CP. In the case of urea its capacity to break hydrogen bonds between water molecules and IPN chain may be responsible for the lower amount of water uptake (57) consequently for the lower release of CP.

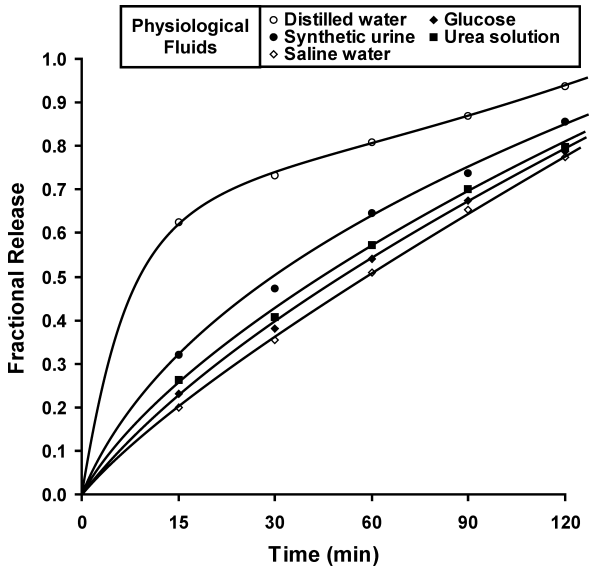


Fig. 17 Effect of physiological fluids on the released amount of CP for definite nanoparticle compositions [gelatin] = 8.0 g, [glutaraldehyde] = 10.6 mM, pH = 7.4, Temp. = 25 ± 0.2°C, %Loading = 15.

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 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 CP 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 CP 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' have been calculated on the basis of eq. (3) and summarized in Table 2. The data clearly reveal that

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

Gelatin (g)	Glutaraldehyde (mM)	pH	n	D × 10^4 cm^2 min^{-1}
4	10.60	7.4	0.50	3.8
6	10.60	7.4	0.54	3.6
8	10.60	7.4	0.55	1.85
9	10.60	7.4	0.60	4.4
8	5.30	7.4	0.52	5.1
8	10.60	7.4	0.55	1.85
8	31.82	7.4	0.50	3.8
8	10.60	7.4	0.55	1.85
8	10.60	4.0	0.50	3.6
8	10.60	1.8	0.51	1.99

the value of n is quite near to 0.5 in almost all cases and, therefore, the release of CP may be considered as Fickian or diffusion controlled. The reason for the observed fickian release may be attributed to the fact that the nanosized particles contain three-dimensional network of crosslinked gelatin chains creat voids with nanometric dimensions. Thus, due to narrow mesh sizes of the network the diffusion of CP molecules from within the loaded nanoparticles to the release medium becomes rate limiting and therefore, a diffusion controlled release process are achieved.

Conclusions

Crosslinked gelatin nanoparticles from a swelling controlled drug release system, which effectively delivers chloroquine phosphate via diffusion, controlled pathway. It is found that release profiles of CP are greatly influenced by % loading of CP concentrations of gelatin and gluteraldehyde (crosslinker) in the nanoparticles. With increase in percent loading of drug on nanoparticles, the released amount of CP constantly decreases. In the case of gelatin, the release of CP 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 CP constently decreases with increasing gluteraldehyde content in the nanoparticles. It is noticed that the release behaviour 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 physiological pH (7.4) while lower release is observed in acidic pH range. It is also noticed that the extent of release of CP increases with increasing temperature. The physiological fluids suppress the extent of release of CP.

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