# A New Method for the Preparation of Gelatin Nanoparticles: Encapsulation and Drug Release Characteristics

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**ABSTRACT:** A new and simple method was developed to produce gelatin nanoparticles of ~ 30–40 nm for use as carriers for drug release applications. The nanoparticles were uniform in size and well dispersed. An anticancer drug, 5-fluorouracil, was encapsulated with an efficiency as high as 85%. The nanoparticles showed sustained release of 5-fluorouracil, and release rates varied with amount of crosslinking in the nanoparticles. © 2011 Wiley Periodicals, Inc. J Appl Polym Sci 121: 3495–3500, 2011

**Key words:** gelatin; nanoparticles; encapsulation; 5-fluorouracil

## INTRODUCTION

Over the past few decades, there has been considerable interest in developing biodegradable or biocompatible polymers as drug delivery systems.[1–5] Among the different types of delivery systems, nanoparticles have become promising carriers because of the improved uptake of the drug, site targeting capability and overall, the improved delivery efficiency and reduced side-effects of drug toxicity.[6–8] Furthermore, nanoparticles can passively accumulate in tumors through a mechanism known as the enhanced permeability and retention effect and their biodistribution is largely determined by their physical and biochemical properties. Various biopolymers such as chitosan, alginate, guar gum, hydroxyethyl cellulose, gellan, carboxymethyl cellulose, dextrans, and gelatin have been used for developing drug delivery systems.[3,7,9–11]

Of the biopolymers, gelatin forms a versatile class as it is seen in numerous food products and is indispensable in modern pharmaceuticals and medical applications. Gelatin is a protein obtained by the denaturation of collagen. The properties of a given type of gelatin depend on the method of preparation, origin, the type, and number of amino acids present and the molecular weight. There are three types of gelatin; Type A, which is obtained from porcine sources by acid treatment, Type B obtained from bovine sources by caustic treatment and a third type is from fish and marine sources. Gelatin A has an isoelectric point (IP) around 9 whereas gelatin B has an IP around 4.8.

Gelatin has enjoyed much success recently as a carrier for drugs and bioactive molecules.[12,13] Different forms of carriers such as hydrogels, drug/or polymer conjugates, micro/nanoparticles, cocktails, and core-shells have been fabricated and used for medical applications. The structure and functionality of gelatin offers advantages for modification. Gelatin modified with poly(ethylene glycol), thiolated derivatives of gelatin,[14] chitosan conjugated gelatin,[15] PAA/gelatin complexes,[16] and poly(*N*-isopropylacrylamide) grafted gelatin[17] have been reported as gelatin-based carriers for potential medical applications. Gelatin nanospheres as gene delivery vehicles have also been reported.[18] In addition, gelatin is also used in the field of tissue engineering. Gelatin and gelatin-based matrices incorporated with bone morphogenetic protein 2 (BMP-2) have shown enhanced bone formation and may therefore find use as carriers for prolonged release of bioactive proteins for bone tissue engineering.[19]

Several methods have been used to prepare gelatin and its derivatives as nanoparticles including desolvation techniques,[14,20] coacervation,[21] and water-in-oil emulsion techniques,[22] γ-ray irradiation,[23] and template polymerization.[16] All of these methods have several advantages, however their

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**TABLE I**
**Drug Loading and Encapsulation Efficiency of 5-FU-Loaded Gelatin Nanoparticles ($n = 3$)**

| Formulation code | 5-FU used (% (w/w) against polymer weight) | GA (% (w/w) against polymer weight) | % of 5-FU in Nanoparticles ± S.D. | % Encapsulation efficiency ± S.D. |
|---|---|---|---|---|
| Gel-1 | 20 | 0.01 | 17.04 ± 0.19 | 85.22 ± 0.95 |
| Gel-2 | 20 | 0.02 | 16.07 ± 0.20 | 80.39 ± 1.02 |
| Gel-3 | 20 | 0.03 | 15.31 ± 0.16 | 76.51 ± 0.80 |
| Gel-4 | 10 | 0.02 | 7.78 ± 0.16 | 77.79 ± 0.81 |
| Gel-5 | 30 | 0.02 | 25.07 ± 0.17 | 83.56 ± 0.83 |

flexibility in tailoring the properties of the nanoparticles is limited. Recently, a mini-emulsion technique has been reported to produce gelatin nanoparticles.[24] Size of the nanoparticles is important and smaller particles can easily penetrate into the cells and arterial walls. However, it is equally important to maintain a balance between size and properties such as stability, distribution, polydispersity, and uptake capacity of the nanoparticles for drugs/bioactive materials. In this article, we report a new method of making gelatin nanoparticles with good size distribution to serve as carriers for drug release applications. To this end, an anticancer drug, 5-fluorouracil (5-FU), was chosen as a model drug for encapsulation in the developed nanoparticles and their drug release kinetics were studied. The 5-FU has been successfully used for the treatment of cancer, in particular colorectal cancer. It acts principally as a thymidylate synthase inhibitor, interferes with nucleic acid synthesis, inhibits DNA synthesis, and eventually halts cell growth.[25]

## MATERIALS AND METHODS

### Materials

Gelatin Type A extracted from porcine skin (Bloom 300), 5-fluorouracil (5-FU), methanol and 25% aqueous glutaraldehyde (GA) were purchased from Sigma-Aldrich (Oakville, ON, Canada) and used as received. Distilled water was used for all the experiments.

### Preparation of nanoparticles

For drug release studies, a 0.5% (w/w) gelatin solution was prepared in distilled water. The solution was stirred overnight, and then heated gently on a hot plate under stirring to obtain a clear solution, after which it was cooled and then filtered. Twenty-five milliliter of the filtered solution was placed in a beaker with the desired amount of 5-FU (10–30% versus polymer weight, Table I). To this a predetermined amount of GA (0.01–0.03% versus polymer weight, Table I) was added and stirred well for 3–5 min. This mixture was added slowly under vigorous stirring along the walls of a beaker containing 50 mL of a 9 : 1 methanol-water mixture. After 1 h of stirring, about 500 μL of conc. HCl was added and the reaction was continued under stirring for another 5 h. The gelatin nanoparticles were obtained by adding the reaction mixture to double the amount of acetone in centrifuge tubes. The tubes were covered and left undisturbed for 1–2 h to allow settling of the particles. The acetone was then decanted, the particles were washed with water to remove acetone, unreacted gelatin, and GA, and recovered using a table top centrifuge at 5000 × *g* then air dried. The placebo nanoparticles were made in the same manner but without adding 5-FU. To determine the effect of GA crosslinker and FU loading on release rate from the nanoparticles, a total of five different drug-loaded particle formulations were made by (1); varying the amount of GA at a fixed 5-FU concentration, and (2) varying the amount of 5-FU at a fixed GA concentration (Table I).

### Transmission electron microscopy (TEM)

Approximately 10 mg $mL^{-1}$ nanoparticle dispersion was prepared in distilled water under stirring. One drop of dispersion was placed on a 400-mesh copper grid and allowed to air dry for 5 min. Excess solution was removed by delicately touching the edges of the grid with filter paper, followed by another 5–10 min of drying. TEM images of the particles were taken on a Tecnai T-12 microscope operated at 80 kV (FEI, Cambridge, England).

### Zetasizer measurements

The *z*-average diameter of gelatin nanoparticles dispersed in water (10 mg $mL^{-1}$) was measured using a Zetasizer, Model 3000HS (Malvern, UK).

### X-ray diffraction studies (XRD)

XRD patterns of pure 5-FU, drug-loaded and placebo nanoparticles were obtained with a Bruker D8 ADVANCE X-ray diffractometer (Bruker AXS,

Madison, WI) to determine the crystallinity of the samples. The XRD scans were recorded between 10° and 60° of 2θ.

### Differential scanning calorimetry (DSC) studies

DSC scans were recorded for pure 5-FU, drug-loaded, and placebo nanoparticles using a Q100 DSC (TA Instruments, New Castle, DE) at a heating rate of 10°C $min^{-1}$ under a nitrogen atmosphere.

### Encapsulation efficiency

The 5-FU content in the loaded particles was estimated by dispersing a known amount of particles in 10 mL of a pH 7.4 phosphate buffer solution and stirring vigorously for 24 h. The dispersion was centrifuged and the 5-FU content in the supernatant liquid was determined by measuring the absorbance at 270 nm with a UV–Vis spectrophotometer, and comparing it to a standard curve. The % drug loading and % encapsulation efficiency were calculated using eqs. (1) and (2), respectively.[5] The experiments were performed in triplicate.

$$\%\text{Drug loading} = \left(\frac{\text{weight of drug in NPs}}{\text{total weight of NPs}}\right) \times 100 \quad (1)$$

$$\%\text{Encapsulation efficiency} = \left(\frac{\text{actual loading}}{\text{theoretical loading}}\right) \times 100 \quad (2)$$

### *In vitro* drug release studies

*In vitro* drug release studies were carried out in 250 mL flasks, where 10 mg of drug-loaded nanoparticles were placed in 90 mL of pH 7.4 phosphate buffer, maintained at 37°C ± 0.5°C and the contents were stirred at 100 rpm using a mechanical stirrer. A 2-mL aliquot was withdrawn at regular time intervals and replaced with the same amount of fresh buffer solution. The samples collected were analyzed for 5-FU spectrophotometrically at 270 nm.[26] The dissolution experiments were carried out in triplicate.

## RESULTS AND DISCUSSION

### Preparation and particle size of nanoparticles

Many research groups[14,20] have employed a two-step desolvation method to produce gelatin nanoparticles in which the polymer solution is desolvated by adding a nonsolvent. In the present method, a dilute polymer solution (0.5% (w/w)) is added to 90% aqueous methanol to precipitate gelatin nanoparticles from the solution. This method can be termed as "nanoprecipitation" because nanoparticles were formed during the precipitation process. Without water in the precipitation medium, the gelatin precipitated instantaneously from the solution as a lump, indicating that water plays an important role in the nanoprecipitation process.

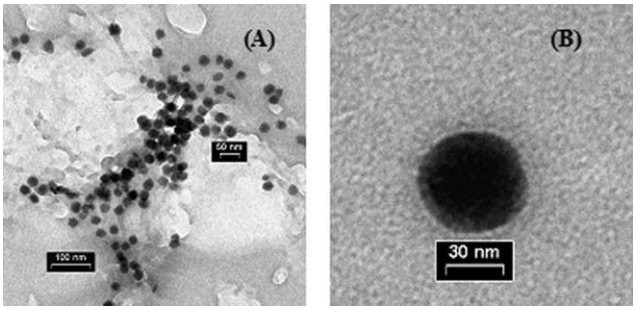


**Figure 1** TEM image of gelatin nanoparticles prepared with 0.02% GA in 90% aqueous methanol (Gel-2).

Figure 1(A,B) are TEM images of nanoparticles prepared in 90% aqueous methanol with 0.02% GA crosslinker. The nanoparticles were spherical and well dispersed, with an average size of ~ 30–40 nm. When dispersed in water, the nanoparticles (10 mg $mL^{-1}$) swelled to ~ 110–130 nm, as determined by Zetasizer. The previously reported desolvation method[14,20] produced wet particle sizes in the range of 230–290 nm whereas the mini-emulsion method developed by Ethirajan et al.[24] gives particle sizes in water in the range of 206–306 nm by changing the emulsification conditions. Compared to these methods, the present method is simpler because in the desolvation method the gelatin has to be precipitated and redissolved to make the nanoparticles, and in the case of mini-emulsions, further thorough cleaning is required to remove emulsifier to obtain pure gelatin nanoparticles. The present method produces smaller, well dispersed nanoparticles compared to previously reported methods. It was found that properties of nanoparticles can easily be controlled by adjusting the production parameters such as water-methanol ratio, polymer concentration and GA content. For example, adjusting the water–methanol ratio to 8 : 2 and increasing GA content to 0.04% reduced the particle size compared to particles prepared with a 9 : 1 water–methanol ratio and 0.02% GA (Fig. 2). Flexibility in altering the properties of the particles is desirable when targeting specific end use requirements. In addition, preliminary experiments in our lab indicate that this new method can be used to produce nanoparticles of polysaccharides such as chitosan, alginate, and blends of these polymers.

### XRD analysis

X-ray diffraction studies are useful for investigating the crystallinity of the 5-FU in the crosslinked nanoparticle matrix. The XRD spectra for pure 5-FU (a),

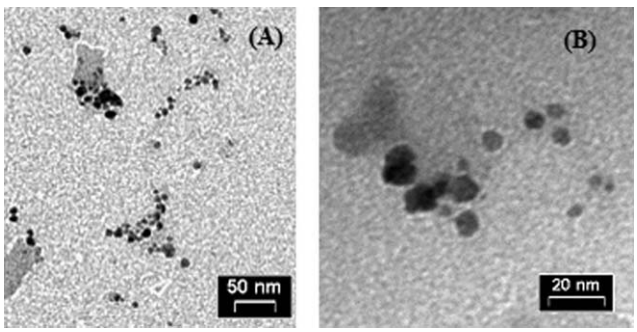


**Figure 2** TEM image of gelatin nanoparticles prepared with (A) 0.02% GA in 80% aqueous methanol, and (B) 0.04% GA in 80% aqueous methanol.

placebo nanoparticles (b) and 5-FU loaded nanoparticles (c) are presented in Figure 3. The pure 5-FU showed characteristic peaks at 2θ of 16, 19, 21, 28, 31, and 33° due to its crystalline nature.[26] However, these peaks were not observed in 5-FU-loaded nanoparticles, which showed a spectrum similar to the placebo nanoparticles. This suggests that 5-FU in the loaded nanoparticles was well dispersed and amorphous or of a crystal size too small to be resolved by the XRD instrument.

### DSC analysis

DSC thermograms of pure 5-FU (a), placebo nanoparticles (b), and 5-FU-loaded nanoparticles (c) are presented in Figure 4. Pure 5-FU exhibited a sharp melting peak at 285°C.[26] However, the thermogram for 5-FU-loaded nanoparticles did not show this peak and was instead quite similar to that of the placebo nanoparticles, which supports the interpretation of the XRD analysis.

### Drug loading and encapsulation efficiency

Drug loading and encapsulation efficiency values for each nanoparticle formulation are presented in Table I. As the amount of crosslinking agent, GA, increased from 0.01 to 0.03%, the percent loading decreased, which in turn decreased the encapsulation efficiency of the nanoparticles from 85.22 to 76.51%. It is apparent that as the crosslinking density increased, the available free volume for encapsulation or entrapment of the 5-FU molecules decreased, resulting in a reduction in the encapsulation efficiency of the 5-FU in the nanoparticle matrix. As expected, when the 5-FU content increased from 10 to 30% (w/w) at a fixed GA concentration of 0.02% the drug loading and encapsulation efficiency values were seen to increase.

### I*n vitro* release

The cumulative release over time of 5-FU from 20% (w/w) loaded nanoparticles as a function of GA concentration is presented in Figure 5. During the first 2 h ~ 35–45% of the 5-FU was released, depending on GA content, however in all the formulations the release extended beyond 15 h. The release profile was similar for all formulations, but as the amount of GA in the nanoparticle matrix increased, the release became slower. This was probably due to decreased swelling with increasing GA which in turn decreased the release of 5-FU from the nanoparticle matrix. For sustained release applications, the initial release may help to reach a therapeutic regime, while the sustained release could help maintain the plasma concentration level.

Generally, drug release from nanoparticles depends on many factors such as size of the particle, type and nature of the polymer matrix, surface characteristics of the particles, polymer molecular weight, swelling of the particles, degradation rate, nature of crosslinking agent, nature of the drug, rate of hydration, etc.[3]

Figure 6 shows the cumulative release of 5-FU from gelatin nanoparticles prepared with 0.02% GA and 10, 20, and 30% (w/w) of 5-FU. Again, an initial 40–45% release was observed within the first 2 h and release extended beyond 15 h. The rate of 5-FU release was greater as the encapsulation efficiency increased.

### Drug release kinetics

The release kinetics of 5-FU from gelatin nanoparticles were analyzed from cumulative release data ($M_t/M_\infty$) with respect to time by fitting the data to Eq. (3)[27]

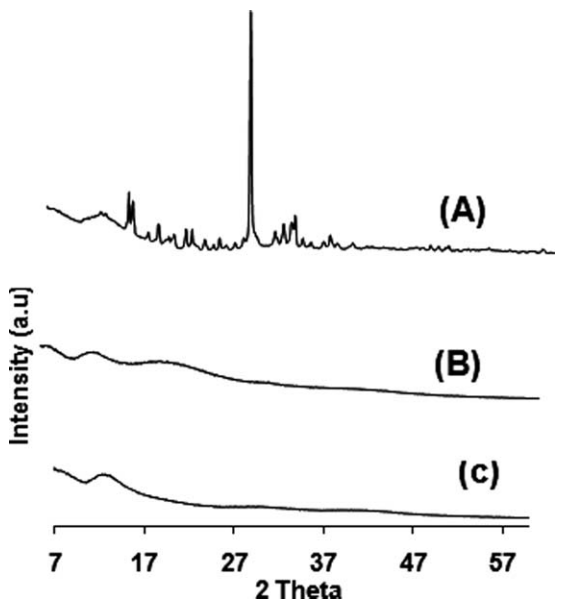


**Figure 3** X-ray diffraction curves of (A) pure 5-FU, (B) 5-FU loaded nanoparticles, and (C) placebo nanoparticles.

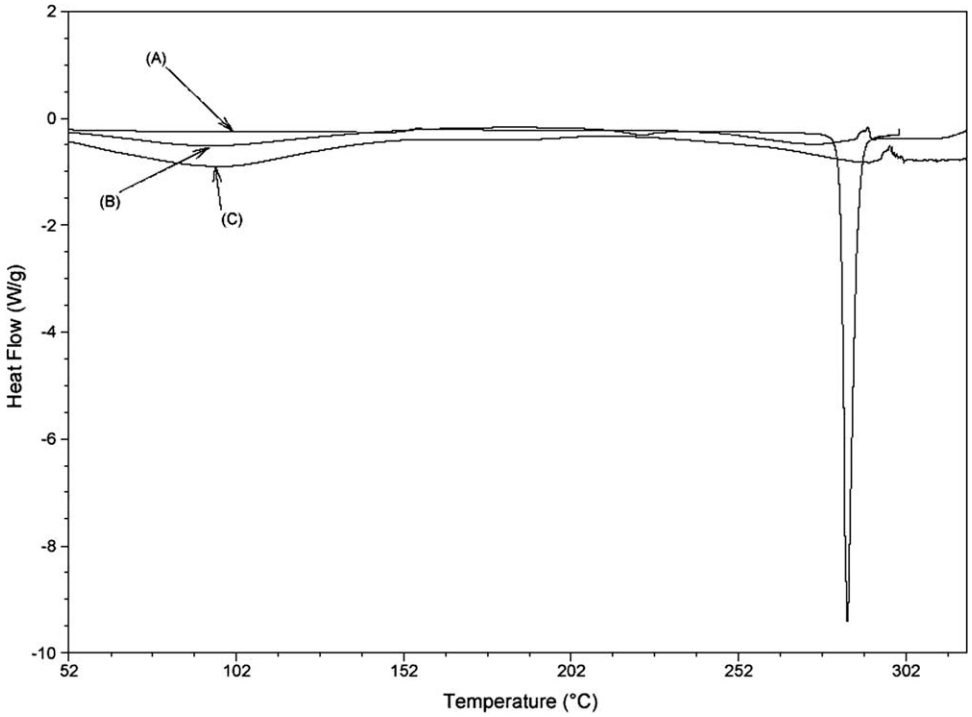


**Figure 4** DSC thermograms of (A) pure 5-FU, (B) 5-FU loaded nanoparticles, and (C) placebo nanoparticles.

$$\left(\frac{M_t}{M_\infty}\right) = \mathrm{kt}^{\mathrm{n}} \quad (3)$$

Here, $M_t/M_\infty$ represents the fractional drug release at time $t$, $n$ is a diffusion parameter characterizing the release mechanism, and $k$ is a constant characteristic of the drug-polymer system. Using the least squares procedure, the k and n values were estimated for the different formulations (Table II). If $n = 0.5$, the drug diffuses and releases from the polymer matrix following quasi-Fickian diffusion. For $n < 0.5$, an anomalous or non-Fickian type diffusion occurs. If $n = 1$, a completely non-Fickian case II or zero-order release kinetics is operative. Intermediary values between 0.5 and 1.0 are attributed to the anomalous type transport. The values of $n$ shown in Table II are in the range of 0.106–0.159 indicating that the release mechanism deviates from Fickian diffusion.[26]

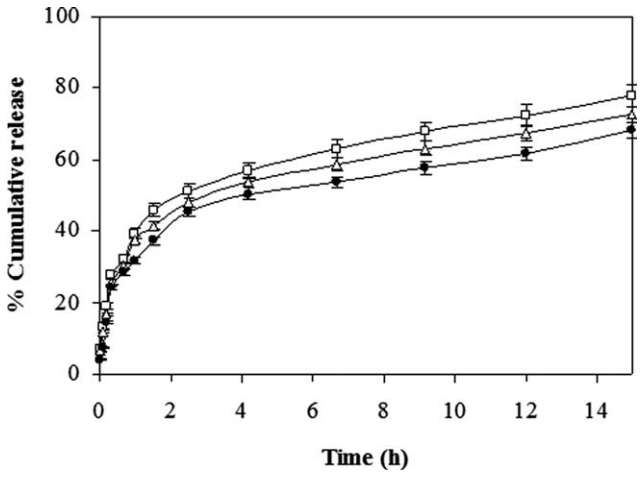


**Figure 5** Cumulative release profiles of 20% (w/w) 5-FU loaded nanoparticles with (□) 0.01% GA, (△) 0.02% GA, and (•) 0.03% GA. The data shown are the mean and standard deviation of triplicate determinations.

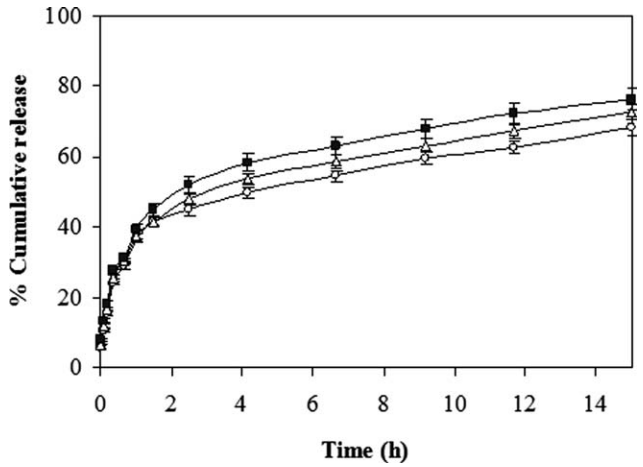


**Figure 6** Cumulative release profiles of nanoparticles prepared with 0.02% GA with different amounts of 5-FU: (○) 10% (w/w), (△) 20% (w/w) and (■) 30% (w/w). The data shown are the mean and standard deviation of triplicate determinations.

**TABLE II**
**Release Kinetic Parameters of Different Nanoparticle Formulations**

| Formulation code | $k \times 10^2$ | $n$ | Correlation coefficient, $r$ |
|---|---|---|---|
| Gel-1 | 0.34 | 0.106 | 0.9052 |
| Gel-2 | 0.32 | 0.134 | 0.8998 |
| Gel-3 | 0.31 | 0.148 | 0.8852 |
| Gel-4 | 0.33 | 0.159 | 0.8812 |
| Gel-5 | 0.31 | 0.115 | 0.8798 |

## CONCLUSIONS

The new nanoprecipitation method described produced well dispersed gelatin nanoparticles of uniform size and spherical shape. The method provides greater flexibility for altering the properties of nanoparticles than methods previously reported. The developed nanoparticles have excellent encapsulation efficiency of the anticancer drug 5-fluorouracil, and the *in vitro* release studies indicate that they may be useful for sustained drug release applications. Initial results suggest that this method can also be used to make nanoparticles of other biopolymers.

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