Encapsulation of Water-Insoluble Drug by a Cross-linking Technique: Effect of Process and Formulation Variables on Encapsulation Efficiency, Particle Size, and In Vitro Dissolution Rate

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ABSTRACT

Ibuprofen-gelatin micropellets were prepared by the cross-linking technique using formaldehyde. Spherical micropellets having an entrapment efficiency of 65% to 85% were obtained. The effect of core to coat ratio, speed of agitation, temperature, and volume of oil phase was studied with respect to entrapment efficiency, micropellet size, and surface characteristics. Fourier transform infrared spectroscopy and differential scanning calorimetric analysis confirmed the absence of any drug-polymer interaction. X-ray diffraction patterns showed that there is a decrease in crystallinity of the drug. The micromeritic properties of micropellets were found to be slightly changed by changing various processing parameters to give micropellets of good flow property. The in vitro release profile could be altered significantly by changing various processing parameters to give a controlled release of drug from the micropellets. The stability studies of the drug-loaded micropellets showed that the drug was stable at storage conditions of room temperature, 37°C, 25°/60% relative humidity (RH) and 45°/60% RH, for 12 weeks.

KEYWORDS: ibuprofen, micropellets, gelatin micropellets

INTRODUCTION

The nonsteroidal anti-inflammatory drug ibuprofen is a good candidate for the development of oral controlled-release formulation. Adverse gastrointestinal reactions have been observed, and the short biological half-life requires administration 3 times a day.¹ Considerable research efforts have been spent on oral sustained-release

drug delivery systems, with the majority of the systems being solid dosage forms. However, such single unit solid dosage forms could be disastrous if they fail to release the drug at the desired rate and in the desired amount, or if they release the entire amount of drug so as to cause dose dumping.² Microencapsulation has been employed to sustain the drug release, and to reduce or eliminate gastrointestinal irritation.^{3,4} In addition, multiparticulate delivery systems can be distributed widely throughout the gastrointestinal tract providing a possibility of achieving a longer lasting and more reliable release of drugs. Unwanted intestinal retention of the polymeric material and local irritation, which may occur with nondisintegrating polymeric matrix tablets, can also be avoided.⁵

It is generally understood that gelatin is digested in the human gastrointestinal tract, and the rate of hydrolysis of the gelatin can be varied by hardening. This suggests that if medication is dissolved or dispersed in a gelatin solution and the gelatin is treated with formaldehyde-isopropanol, the rate of hydrolysis in the gastrointestinal tract would decrease.⁶ These considerations led to the objective of this study, which was to prepare and evaluate oral multiparticulate sustained-release delivery system of the ibuprofen using gelatin by cross-linking technique. The effect of concentration of drug and polymer on the physical properties of micropellets and the drug release was evaluated. Various process parameters such as drug polymer ratio, stirring speed, stirring time, and volume of oil were optimized. These spherical micropellets were evaluated for drug content, in vitro drug release, particle size distribution, and other micromeritic properties. Drug-polymer interactions in the solid state were studied by Fourier transform infrared spectrophotometry (FTIR), differential scanning calorimetry (DSC), x-ray diffraction studies (XRD), and scanning electron microscopy (SEM).

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MATERIALS AND METHODS

Materials

Ibuprofen was a gift sample from Shekhsaria Chemicals Ltd. (Mumbai, India). Gelatin was purchased from Himedia Chemicals (Mumbai, India). Sunflower oil (food grade) was a gift sample from Godrej oil Limited (Mumbai, India). All other reagents and solvents used were of analytical grade.

Methods

The micropellets containing ibuprofen were prepared by cross-linking technique using gelatin polymer. The gelatin (1.0 g) was soaked in 15 mL of distilled water for 15 minutes. It is then heated on a water bath to 60°C to 65°C until it completely dissolved. The drug (0.5-1.5g, previously passed through no. 200 sieve) was added to the gelatin solution with stirring. The drug-loaded micropellets were formed by dropping the drug-polymer dispersion by a jacketed glass syringe (provided with hot water [60°C to 65°C] supply) into previously cooled sunflower oil (10°C, maintained by ice-salt freezing mixture) with constant stirring at 75 to 150 rpm. The formaldehyde (10% vol/vol) solution (1.0 mL) was added to cross-link the gelatin, and the stirring was further continued for 2 hours. The resulting micropellets were decanted, freed of sunflower oil by repeated washings with isopropyl alcohol (200 mL) and finally air dried over a period of 24 hours. The dried micropellets were stored in a dessicator at ambient temperature until further evaluation.

In another experiment cross-linking of gelatin micropellets was achieved by exposing it to 10% formalin-isopropanol vapors in a closed vessel for varying periods of time.

Drug Loading Assay

About 50 mg of accurately weighed drug-loaded micropellets were added to 100 mL of phosphate buffer, pH 7.2. The resulting mixture was kept shaking on mechanical shaker for 24 hours. Then, after the solution was filtered (0.45 μ m pore size) and 1 mL of this solution was appropriately diluted using phosphate buffer, pH 7.2, and analyzed spectrophotometrically at 264 nm using Milton Roy UV-VIS spectrophotometer (Milton Roy USA, Ivyland, PA), the method was checked for precision and accuracy.

Size Distribution of Micropellets

Size distribution of the micropellets was determined using standard test sieves (Filterwel, Mumbai, India). Particles that passed through 1 sieve but were retained on the other were collected and weighed, and the distribution was analyzed based on the weight fraction on each sieve.

Differential Scanning Calorimetry

The DSC analysis of pure drug, blank micropellets, and drug-loaded micropellets was carried out using Perkin Elmer DSC 7 model (Perkin Elmer Inc, Boston, MA) to evaluate any possible drug-polymer interaction.⁷ Blank gelatin micropellets and drug-loaded micropellets were triturated to get a finely divided powder. This powder was passed through no. 100 sieve. In a similar way, pure drug was also passed through no. 100 sieve. Samples (2-8 mg) were accurately weighed using a Sartorius 4503 electronic microbalance (Sartorius, Edgewood, NY) and heated in sealed aluminium pans at a rate of 10°C/min from 50°C to 250°C temperature range under a nitrogen flow of 40 mL/min. Reproducibility was checked by running the sample in triplicate.

Fourier Transform Infrared Spectroscopy

Drug-polymer interactions were studied by FTIR spectroscopy. The spectra were recorded for ibuprofen, blank gelatin micropellets and drug-loaded micropellets using Buck Scientific IR spectrophotometer, (model 500, Buck Scientific Inc, Norwalk, CT). Samples were prepared in KBr disks (2 mg sample in 200 mg KBr) with a hydrostatic press at a force of 5.2 τ cm⁻² for 3 minutes. The scanning range was 450-4000 cm⁻¹ and the resolution was 4 cm⁻¹.

X-Ray Powder Diffractometry

X-ray powder diffractometry was carried out to investigate the effect of microencapsulation process on crystallinity of the drug. Powder XRD patterns were recorded on a Jeol PW 17291 powder XRD (Jeol Inc, Peabody, MA) using Ni-filtered, CuK α radiation, a voltage of 35 Kv, and a current of 25 mA. The scanning rate employed was 1° min⁻¹ over the 10° to 40° diffraction angle (2 θ) range. The XRD patterns of ibuprofen crystals, empty gelatin micropellets, and drug-loaded micropellets were recorded. Micropellets were triturated to get fine powder before taking the scan.

Table 1. Effect of Various Processing and Formulation Parameters on the Entrapment Efficiency of Drug-Loaded Micropellets*

Processing and Formulation Parameters		Theoretical Content (%)	Actual Content (%)	Entrapment Efficiency (%)
Polymer-Drug Ratio	1:0.50	33.34	23.47	70.42 ± 0.365
	1:0.75	42.86	29.51	68.87 ± 1.874
	1:1.00	50.00	42.40	84.80 ± 0.775
	1:1.25	55.56	45.56	82.00 ± 1.869
	1:1.50	60.00	45.00	75.00 ± 0.995
Volume of Sunflower Oil (mL)	300	50.00	42.40	84.80 ± 3.289
	400	50.00	39.02	78.05 ± 0.658
	500	50.00	43.00	86.00 ± 0.548
Temperature of Sunflower Oil (°C)	10	50.00	42.40	84.80 ± 2.669
	60	50.00	32.45	64.90 ± 0.339
Stirring Speed (rpm)	75	50.00	42.35	84.70 ± 0.448
	100	50.00	42.40	84.80 ± 0.885
	150	50.00	43.20	86.40 ± 0.104

*Each observation is the mean (± SD) of three determinations.

Scanning Electron Microscopy Analysis

The surface topography of the micropellets was examined by SEM.⁸ SEM analysis was carried out using a Cambridge stereoscan-150 scanning microscope (Altran Corp, Boston, MA). Prior to examination, samples were gold sputter-coated to render them electrically conductive.

Measurement of Micromeritic Properties of Micropellets

The flow properties were investigated by measuring the angle of repose of drug-loaded micropellets using fixed-base cone method. The bulk and tapped densities were measured in a 10-mL graduated measuring cylinder as a measure of packability of the micropellets. The sample contained in the measuring cylinder was tapped mechanically by means of constant velocity rotating cam with the change in its initial bulk density to a final tapped density when it has attained its most stable form (ie, unchanging arrangement). Each experiment was carried out in triplicate.

Measurement of Drug Release Rate from Micropellets

The in vitro dissolution studies were carried out in 900 mL of phosphate buffer, pH 7.2, maintained at 37 ± 0.5°C and 100 rpm by using *United States Pharmacopeia* XXIII paddle-type dissolution apparatus (Electrolab, Mumbai, India). Micropellets containing 200 mg of the drug were employed in each case. Aliquots were withdrawn at regular intervals and analyzed spectrophotometrically at 264 nm using Milton Roy UV-VIS spectrophotometer. The dissolution studies were carried out

in triplicate and the mean values are plotted as percentage cumulative release versus time.

Stability Studies

The drug-loaded micropellets (1:1) were stored at various storage conditions (room temperature, 37°C, 25°/60% relative humidity [RH], and 45°/75% RH) in airtight sealed vials. The drug content of the drug-loaded micropellets was determined as described, at regular intervals (0, 14, 28, 60, and 90 days), and the drug release profiles were studied at weeks 0 and 12.

RESULTS AND DISCUSSION

The Effects of Various Process and Formulation Parameters on the Drug Entrapment Efficiency and Particle Size Distribution

The effects of various process and formulation parameters on the drug entrapment efficiency of micropellets is shown in Table 1. Good entrapment efficiency was achieved by increasing polymer-drug ratio from 1:0.5 to 1:1. This result indicates that 1:1 polymer-drug ratio has the optimum capacity for drug encapsulation. With further increase in polymer-drug ratio from 1:1 to 1:1.5, no significant change was observed on encapsulation efficiency of ibuprofen. The extent of loading appears to influence the particle size distribution of microspheres as shown in Figure 1. When the loading is high, the proportion of larger particles formed is also high. The viscosity of the polymer solution at such high drug loadings is very high and is responsible for the formation of large

Table 2. Effect of Various Processing and Formulation Parameters on the Micromeritic Properties of Drug-Loaded Micropellets

Processing and Formulation Parameters		Angle of Repose* (°)	Bulk Density* (g / ml)	Tapped Density* (g / ml)
Original Drug Crystals	-	43.71 ± 1.33	0.384 ± 0.032	0.575 ± 0.028
Polymer-Drug Ratio	1:0.50	28.07 ± 3.17	0.483 ± 0.034	0.527 ± 0.032
	1:0.75	24.19 ± 2.38	0.571 ± 0.012	0.592 ± 0.022
	1:1.00	21.06 ± 1.25	0.594 ± 0.024	0.634 ± 0.020
	1:1.25	27.28 ± 2.65	0.602 ± 0.066	0.637 ± 0.019
	1:1.50	21.64 ± 5.68	0.605 ± 0.011	0.613 ± 0.015
Volume of Sunflower Oil (mL)	300	21.06 ± 1.25	0.594 ± 0.003	0.634 ± 0.008
	400	33.07 ± 0.90	0.469 ± 0.003	0.492 ± 0.022
	500	28.14 ± 2.86	0.616 ± 0.170	0.638 ± 0.025
Temperature of Sunflower Oil (°C)	10	32.15 ± 3.17	0.594 ± 0.014	0.634 ± 0.021
	60	21.06 ± 1.26	0.538 ± 0.015	0.552 ± 0.023
Stirring Speed (rpm)	75	28.51 ± 0.92	0.340 ± 0.011	0.382 ± 0.024
	100	27.87 ± 0.84	0.430 ± 0.016	0.477 ± 0.032
	150	21.06 ± 1.25	0.594 ± 0.013	0.634 ± 0.033

*Each observation is the mean (± SD) of three determinations.

micropellets. There was no significant change on encapsulation efficiency of drug with increasing the speed of agitation. The volume of oil does not have any impact on entrapment efficiency of ibuprofen micropellets. As volume of oil increases, mean particle size of micropellets increases, which could be attributed to less collision between the micropellets while stirring because of the lower viscosity of the system. The temperature of the oil phase is one of the important process parameters, as good entrapment efficiency was found at low temperature (10°C), which also contributed to hardness of micropellets. As the temperature of oil increases to 60°C, a wide range of micropellet particle size distribution is found.

Figure 1. Particle size distribution of ibuprofen-gelatin micropellets. Polymer: drug ratio: (A) 1:0.5, (B) 1:0.75, (C) 1:1, (D) 1:1.25, and (E) 1:1.5.

The Micromeritic Properties of Micropellets

The micromeritic properties of micropellets are tabulated in Table 2. The flow properties, represented in terms of angle of repose of the micropellets, were much improved over those of pure drug crystals. The bulk and tapped densities in a 10 mL measuring cylinder were equivalent for the micropellets and drug crystals, but the micropellets were found to have higher packability. The improvements of micromeritic properties suggest that the micropellets can be easily handled.

Differential Scanning Calorimetry

The drug could be either dispersed in crystalline / amorphous form or dissolved in the polymeric matrix during the process of microencapsulation.^{9,10} Also, any abrupt or drastic change in the thermal behavior of either the drug or polymer may indicate a possible drug-polymer interaction. The thermal curves of pure components and of the drug-polymer micropellets are presented in Figure 2. A sharp endotherm ($T_{\text{peak}} = 77.4^{\circ}\text{C}$) was observed for ibuprofen at the temperature corresponding to its melting point. In the case of gelatin, exothermic peak was observed in the temperature range of 100°C to 250°C. The characteristic, well-recognizable thermal profile of the drug appeared at the temperature corresponding to its melting point in the physical mixtures of drug with gelatin. The same thermal behavior was observed in ibuprofen-gelatin micropellets showing thermal peak at 77°C but with the loss of its sharp appearance. It appears that there is a significant reduction of drug crystallinity in the polymer matrix.

Figure 2. DSC curves of (A) ibuprofen, (B) blank gelatin micropellets, (C) ibuprofen-gelatin physical mixture (1:1), and (D) ibuprofen-gelatin micropellets (1:1).

Fourier Transform Infrared Spectroscopy

As shown in Figure 3, there was no significant difference in the FTIR spectra of physical mixtures of drug and polymer as well as drug-loaded micropellets when compared to the spectra of individual components. The characteristic carbonyl-stretching band of isopropionic acid group of drug was unchanged in the case of micropellets. These studies revealed that there was no significant interaction between drug and polymer.

X-Ray Powder Diffractometry

The x-ray powder diffraction patterns of micropellets along with those of physical mixtures and raw crystals of drug and polymer are shown in Figure 4. The ibuprofen-gelatin system, in the form of micropellets, indicated the presence of the crystalline ibuprofen but with a dramatic decrease of the intensity of the signal because of both a dilution effect and a decrease in crystallinity of the drug.

Scanning Electron Microscopy Analysis

SEM of drug-loaded micropellets as shown in Figure 5 revealed that the micropellets were spherical in shape and had a rough surface due to higher concentration of drug in the micropellets as compared to the blank gelatin micropellets. This finding explains the fact that the crystallinity of the ibuprofen micropellets was higher as revealed by the DSC and XRD.

Figure 3. FTIR spectra of (A) ibuprofen, (B) blank gelatin micropellets, (C) ibuprofen-gelatin physical mixture (1:1), and (D) ibuprofen-gelatin micropellets (1:1).

Drug Release Behavior of Micropellets

Factors such as microsphere size, drug loading, and polymer composition govern the drug release from microspheres.^{11,12}

The original drug crystals showed a faster release showing about 98% drug release within 2 hours in comparison to the microencapsulated form of the drug which showed sustained release as shown in Figure 6. At lower drug-polymer ratios, the mean particle size of the micropellets was less than that at higher drug-polymer ratios. Therefore, the drug release from micropellets prepared at lower drug-polymer ratios was faster than that of micropellets prepared at higher drug-polymer ratios because of the small size of the micropellets, which pro-

vided a large surface area for faster drug release. This finding is consistent with the general rule (ie, small size of microspheres provides large surface area for faster drug release). The effect of stirring during preparation of ibuprofen micropellets on the *in vitro* drug release was not significantly different. The volume of sunflower oil as the oily phase plays an important role in the cumulative release of ibuprofen from micropellets as shown in Figure 7. This finding can be attributed to the decrease in viscosity of the oily phase as the volume of oil increases, which in turn controls the micropellet size. See Figure 8 where micropellets prepared at 60°C (temperature of oil phase) showed faster drug release than the micropellets prepared at 10°C. This result may be attributed to the deterioration of the micropellet wall by longer exposure time to formaldehyde at higher temperature, which may give rise to burst effect; hence, rapid drug release would be expected.

Figure 4. XRD spectra of (A) ibuprofen, (B) blank gelatin micropellets, (C) ibuprofen-gelatin physical mixture (1:1), (D) ibuprofen-gelatin micropellets (1:1).

As evident from Figure 9, the micropellets treated with formalin vapors for a longer period of time showed slower drug release as compared to micropellets treated

for a shorter period of time. This result may be attributed to the higher degree of cross-linking of gelatin, when exposed to formalin vapors for a longer period of time, which retarded a drug release from the pellets.

Figure 5. SEM photographs of ibuprofen micropellets: (A) surface morphology before dissolution, (B) and (C) after dissolution, (D) blank gelatin micropellets, and (E) cross-section.

Figure 6. Effect of polymer-drug ratio on the cumulative release of ibuprofen from gelatin micropellets (speed of agitation, 150 rpm; volume of sunflower oil, 300 mL; and temperature of oil, 10°C were kept same for all batches).

Stability Studies

As described in Table 3, there was no significant change in the drug content of drug-loaded micropellets, stored at room temperature, room temperature, 37°C, 25%/60%

Table 3. The Results of Drug Content for Drug-Loaded Micropellets (Drug:Polymer; 1:1) at Various Storage Conditions for a Period of Twelve Weeks*

Days	RT (°C)	37°C	25°/60% RH	45°/75% RH
14	98.86 ± 3.14	98.36 ± 3.52	98.99 ± 3.41	94.32 ± 3.28
28	98.82 ± 2.65	97.36 ± 2.43	97.76 ± 3.52	95.28 ± 2.57
60	97.63 ± 2.57	97.23 ± 3.19	97.21 ± 2.56	94.07 ± 2.32
90	97.22 ± 4.62	96.56 ± 2.31	96.58 ± 3.32	93.04 ± 2.39

*RT indicates room temperature; RH, relative humidity. Results were calculated considering the initial actual percentage drug content of 42.4 as 100% for the micropellets of 1:1 drug:polymer proportion. Results are expressed as mean (± SD) of three independent determinations.

RH, and 45°/60% RH, after 12 weeks of study. The cumulative release of ibuprofen from micropellets stored at different storage conditions during weeks 0 and 12 showed that there was no significant effect of temperature of storage on the drug release.

Figure 7. Effect of volume of sunflower oil on the cumulative release of ibuprofen from gelatin micropellets (speed of agitation, 150 rpm; polymer-drug ratio 1:1; and temperature of oil, 10°C were kept same for all batches). **Figure 8.** Effect of temperature of oil phase on the cumulative release of ibuprofen from gelatin micropellets (speed of agitation, 150 rpm; polymer-drug ratio, 1:1; and volume of oil, 300 mL were kept same for all batches). **Figure 9.** Effect of exposure time of formaldehyde vapors on the cumulative release of ibuprofen from gelatin micropellets (speed of agitation, 150 rpm; polymer-drug ratio, 1:1; and volume of oil, 300 mL were used).

CONCLUSION

Micropellets containing the nonsteroidal, anti-inflammatory agent ibuprofen were successfully prepared by a cross-linking technique using gelatin. The physical properties of the pellets and their drug release rates varied according to the amount of drug entrapped in the pellets and the degree of cross-linking of the gelatin. The data suggest that gelatin is a potentially useful polymer for making controlled-release micropellets by the cross-linking technique.

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