Drug Development and Industrial Pharmacy, 22(9&10), 951-957 (1996)

Gelatin-Acacia Complex Coacervation as a Method for Ketoprofen Microencapsulation

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ABSTRACT

Ketoprofen microcapsules were prepared by complex coacervation between gelatin and acacia, and dried with different methods: isopropanol addition, spray-drying, and freeze-drying, Successively, microparticles were analyzed by infrared thermobalance, ultraviolet spectroscopy, optical and scanning electron microscopy, and sieves; and subjected to dissolution studies in order to examine parameters such as yield, moisture content, encapsulation percentage, morphology of solid particles, particle size, and dissolution behavior. Provided that encapsulation and drying methods did not affect ketoprofen dissolution profiles, the most appropriate drying method for industrial purposes was spray-drying.

INTRODUCTION

Ketoprofen is a drug having analgesic, anti-inflammatory, and antipyretic activities, and like other phenylpropionic acid derivatives, it is used in the treatment of rheumatoid arthritis and osteoarthritis (1). The molecule is practically water insoluble, but it is readily absorbed from the gastrointestinal tract. Unfortunately, this drug causes a certain irritation of gastric and nasal mucous membranes. Besides, it is also quite unstable to light (2). Thus, microencapsulation of drug in a polymeric shell could offer a solution to these drawbacks.

Many kinds of polymers have been used with a large number of procedures (able to encapsulate solid particles or fluid droplets) which can be summarized and simplified as coacervation–phase separation, solvent evaporation, atomization, interfacial polymerization, and fluid bed technique (3–17). The choice of one method rather

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than another depends on drug chemical–physical characteristics and on the final result desired.

In this case, as ketoprofen is water insoluble and no modification of absorption kinetics is wanted, the most appropriate microencapsulation method is coacervation of colloidal polymers from an aqueous drug suspension. This method has been widely studied (18–21) but it is not easy to reproduce on the industrial scale because of difficulty in recovering single microcapsules, avoiding sticking phenomena at the end of the procedure.

The aim of this work is to microencapsulate ketoprofen as model drug by a coacervation process with colloidal polymers using a method able to avoid the sticking phenomena which normally occur during the drying phase; for this reason, it is reproducible also on an industrial scale. The drug is microencapsulated by complex coacervation between gelatin and arabic gum, and microcapsules are dried and recovered using three different methods: coacervate dehydration with isopropanol and subsequent filtration, spray-drying, or freeze-drying of the same coacervate.

MATERIALS AND METHODS

Gelatine Characterization

In order to point out the isoelectric point of the gelatin utilized (Nuova Astrochimica, Milan, Italy), isoelectrofocalization has been performed with a Rotofor Cell under the following experimental conditions: 50 ml of 1% of gelatin and 2.5 ml of 40% Ampholines (I.P. 3–10) as a sample; 0.1 N NaOH as an anodic solution; 0.1 N H_3PO_4 as a cathodic solution; focalization room temperature 4°C; generator power 12 W; time course 5 hr.

Encapsulation Process

The whole procedure is represented in Fig. 1. A certain amount of powder of ketoprofen (Unibios, Trecate, Italy) was put in a 10% (w/v) gelatin solution at 40°C, and the suspension was homogenized with Ultraturrax (Janke & Kunkel Labortechnik, Staufen, Germany) at 10,000 rpm for 2 min. Then, an equal volume of a 10% (w/v) acacia (Nuova Astrochimica, Milan, Italy) solution at 40°C was added to the suspension, and the system was rehomogenized under the same conditions. The final weight ratio between ketoprofen and the two polymers altogether was 1:1. This new suspension was next

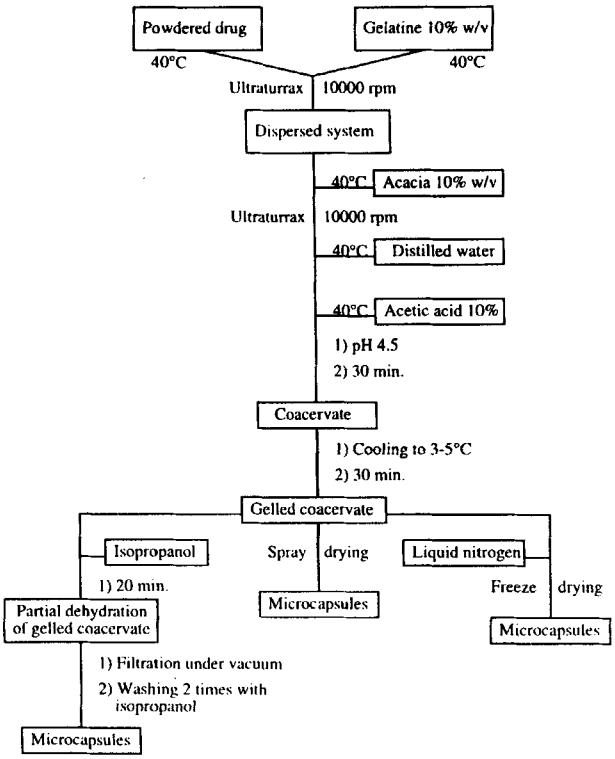

Figure 1. Coacervation procedure.

diluted with distilled water (prewarmed at 40°C) to reach a concentration of 1.5% (w/v) for each colloidal polymer. Finally, the addition of a volume of a 10% acetic acid solution (always prewarmed at 40°C) necessary to reduce the pH value to 4.5 gave rise to the coacervation process. After 30 min the system was cooled to 5°C and left for 1 hr at this temperature before drying and recovering the free-flowing microcapsules. During all this process, the system was continuously stirred at 150 rpm except during homogenization with the Ultraturrax.

Microcapsules were recovered using the following methods:

Dehydration with Isopropanol

An equal volume of previously cold isopropanol (5°C) was added to the system (maintained at 5°C) which was filtered under vacuum 20 min later. Then the partially dehydrated microcapsules were resuspended twice in fresh isopropanol, newly filtered, and finally air dried overnight before analysis.

Spray-Drying

The gelled system was directly spray-dried using a Niro Atomizer (Denmark) under the following conditions: inlet temperature 150°C, outlet temperature 85°C, feed rate 20 ml/min, pressure 4 kg/cm^2. The obtained powder was then collected and analyzed.

Freeze-Drying

An FTS Dura-Dry FD-14-84 freeze-drying apparatus (New York, USA) has been used for this process. The gelled system was shared into borosilicate glass containers which were plunged in liquid N_2 for a while and then put inside the freeze-dryer chamber previously cooled to -30°C. When the glass temperature was stabilized, the condenser temperature was set to -90°C and the vacuum pump was switched on. After the process had set out, the freeze-drying room was heated in the right proportion in order to avoid further cooling of the congealed mass, which could slow down or stop the sublimation process. When primary drying finished, the temperature of the freeze-dryer chamber was increased to 20°C and maintained for 30 min before stopping the process. The obtained powder was next collected and analyzed.

Moisture Content

Humidity of the obtained powders was verified using a Mettler PJ 300 infrared thermobalance.

UV Analysis

Ultraviolet (UV) analyses of microcapsules were performed to determine the percentage of encapsulated drug. An amount of each powder was extracted with 50 ml of ethanol for 12 hr under continuous stirring at room temperature. Then, the suspension was filtered and the drug content of the ethanol solution was assayed spectrophotometrically at 255 nm with a UV-2101 PC UV-VIS scanning spectrophotometer Shimadzu (Japan) connected to an AT 386 computer.

Optical and Scanning Electron Microscopy

For Optical microscopy, an Olympus 234682 microscope (Japan) was used to show particle transformation during the coacervation process. A Stereoscan 360 electron scanning microscope (Cambridge Instruments Limited) was used to point out morphology and surface structure of the microcapsules obtained with the three different drying methods.

Sieve Analysis

Granulometric distribution and mean diameter of microcapsules were determined by sieving 100 g of each powder with a Vibrotronic VE1 (Retsch, Germany) using sieves of the following aperture size (expressed in micrometers): 40, 80, 125, 160, 200, 250, 400, 500, 630, and 1000.

Dissolution Studies

In order to verify any variation in dissolution profile between unprocessed and encapsulated ketoprofen, dissolution studies were performed in triplicate with an Erweka DT6 dissolution test (Heusenstamm, Germany), in phosphate buffer at pH 7 and 37°C using the paddle method at the rotation speed of 75 rpm (USP XXIII, Apparatus 2). A certain amount of each powder, containing 50 mg of ketoprofen, was put into a vessel with 1000 ml of buffer. At 5-min intervals, 3 ml of liquid were withdrawn, passed through a 0.45-μm membrane filter (Millipore), and assayed spectrophotometrically with a UV-2101 PC UV-VIS scanning spectrophotometer at 255 nm to measure the concentration of ketoprofen present in solution. The initial volume of the vessel was maintained by adding 3 ml of buffer after each sampling.

RESULTS AND DISCUSSION

Gelatin Isoelectric Point

Gelatin used for microencapsulation process was composed by several fractions (Fig. 2) having isoelectric points ranging between pH 6.46 and pH 8.64. Particularly, the isoelectric point of the predominant fraction was 6.46.

Yield of Microencapsulation Procedures

Yield of the three drying methods has been calculated from the difference between the amount of material used and that recovered at the end of the process (Table 1). The yield was only 78.2% when microcapsules were dried with isopropanol, while the best results were obtained by freeze-drying (98.1%). Spray-drying gave

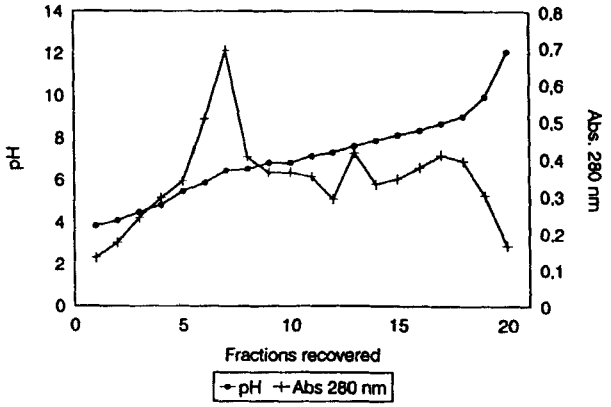

Figure 2. Gelatin isoelectrofocalization.

Table 1

Yield of Microencapsulation Procedures

Drying method	Yield (%)
Isopropanol	78.2
Spray-drying	90.2
Freeze-drying	98.1

intermediate results; in fact, a small quantity of microcapsules adhered to the spray-dryer chamber.

Moisture Content

Residual humidity of each series of dried microcapsules is reported in Table 2. Microcapsules dried with isopropanol possessed a certain residual humidity, while those dried by spray-drying and freeze-drying had considerably lower values. Anyway, this difference in moisture content decreased if powders were exposed to air. After 2 days all values were similar, with a humidity increase more sensible in microcapsules dried by spray- or freeze-drying.

UV Analysis

Table 3 shows the percentages of drug recovered in the powders and the consequent microencapsulation percentages (as was confirmed by microscopic analysis). All powders presented a very good percentage of microencapsulation although differences in values were noted. The lowest percentage was obtained when coacervate was dried with isopropanol, because this solvent partially extracted the drug from the core of microcapsules. On the other hand, after having freeze-dried the same coacervate, nearly 100% of the drug was found in the powder. Also in this case, spray-drying was in the intermediate position.

Optical Microscopy

Figures 3 and 4 show images of solid ketoprofen particles suspended in the colloidal dispersion and of the gelled microcapsules, respectively, as visible by optical microscopy (magnification 400). Irregular-shaped drug particles (Fig. 3) were transformed, after coacervation and coacervate gelling, into spheroidal agglomerates constituting the core of microcapsules (Fig. 4). Around this core was a very easily visible colloidal layer of coacervate which made the particles completely spherical.

Scanning Electron Microscopy

Figures 5, 6, and 7 show the final shape of microcapsules dried with isopropanol, spray-drying, and freeze-drying, respectively, as visible by electron scanning microscopy. Particles obtained by treatment with isopropanol were formed by agglomerates of single microcapsules (Fig. 5) and were greater than those obtained by atomization (Fig. 6). As expected, the spraying device of the atomizer broke every particle cluster so that single microcapsules were recovered. On the

Table 2

Moisture Content of Dried Microcapsules

	Humidity (%)	
Drying Method	10 Min After Drying	2 Days After Drying
Isopropanol	4.46	5.5
Spray-drying	1.2	4.18
Freeze-drying	1.05	4.15

Table 3

Microencapsulation Percentages

Drying Method	% of Ketoprofen	% of Encapsulation
Isopropanol	41.3	82.6
Spray-drying	46.2	92.4
Freeze-drying	49.4	98.8

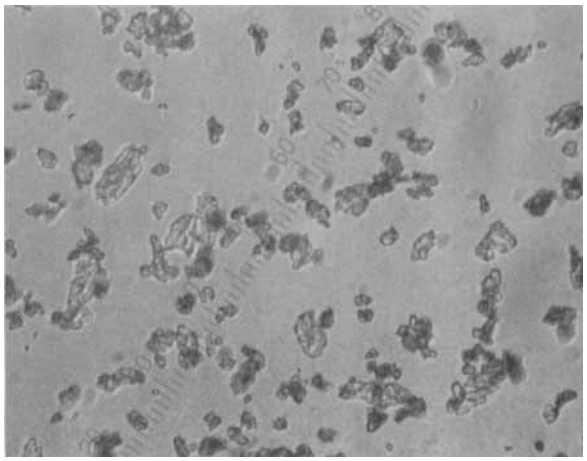

Figure 3. Solid ketoprofen particles suspended in the colloidal dispersion (optical microscope).

contrary, single microparticles could not be recovered using freeze-drying (Fig. 7) because the uncoacervated excess of colloidal polymers formed bridges between particles after water sublimation.

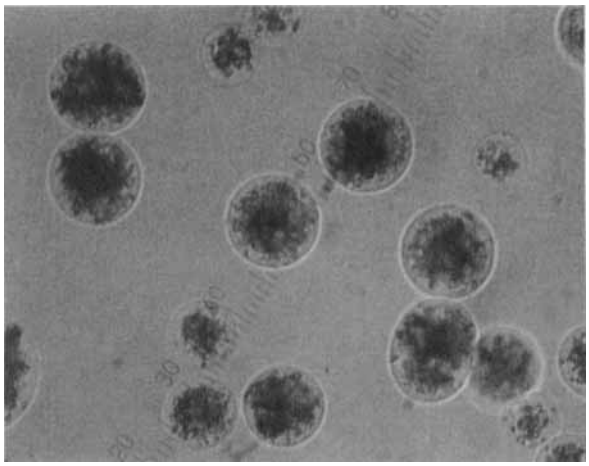

Figure 4. Gelled microcapsules (optical microscope).

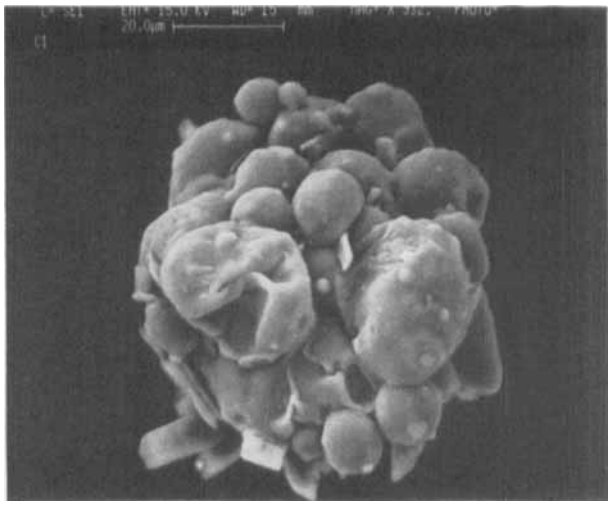

Figure 5. Final shape of microcapsules dried with isopropanol (SEM).

Sieve Analysis

In Figs. 8, 9, and 10 are reported, in percent, the granulometric distributions (weight of powder retained by each sieve) of powders dried with the three different methods. The mean diameters of these powders calculated from data of the three figures were: 300 μm for isopropanol drying method (Fig. 8), 169 μm for spray-drying method (Fig. 9), and 310 μm for freeze-drying method (Fig. 10). In agreement with electron scanning

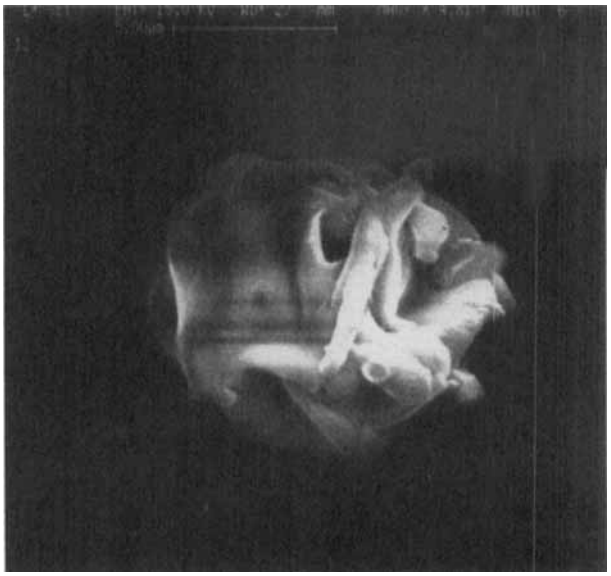

Figure 6. Final shape of microcapsules spray dried (SEM).

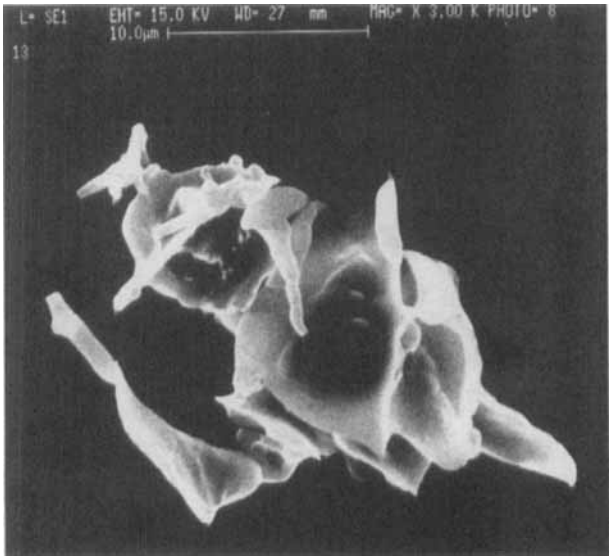

Figure 7. Final shape of microcapsules freeze dried (SEM).

microscopy, these results substantially confirmed the better effectiveness of spray-drying in recovering single microparticles.

Dissolution Studies

As expected, dissolution profiles of the three series of microencapsulated drug did not differ too much from that of pure unprocessed ketoprofen. Figure 11 shows dissolution curves of microcapsules dried with the three different methods compared with the curve of pure ketoprofen.

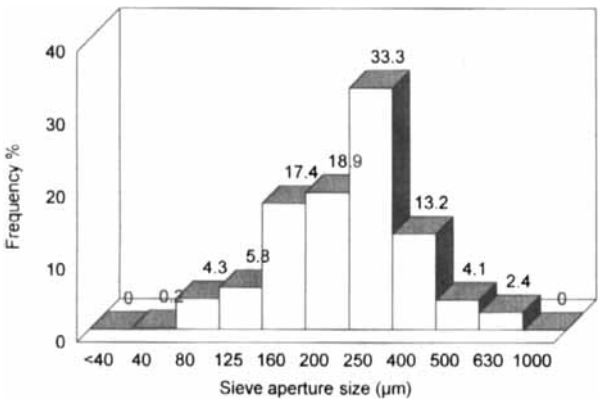

Figure 8. Granulometric distribution of powder dried with isopropanol.

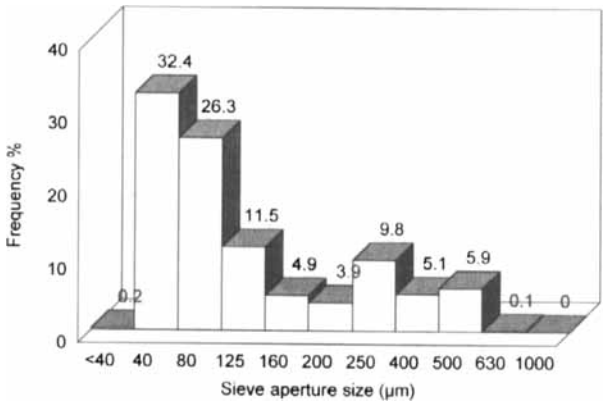

Figure 9. Granulometric distribution of powder spray-dried.

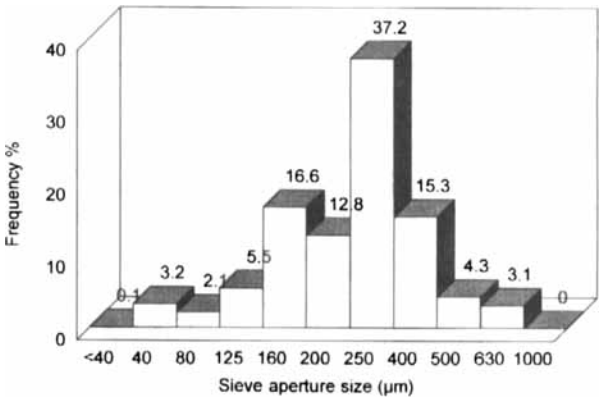

Figure 10. Granulometric distribution of powder freeze-dried.

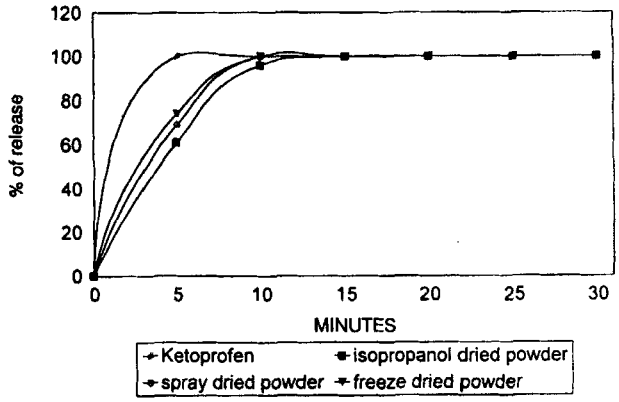

Figure 11. Dissolution studies.

CONCLUSION

Ketoprofen microcapsules can be easily prepared by gelatin–acacia complex coacervation, which forms a readily water-soluble shell. Among the three drying methods, the most indicated for applications on the industrial scale is surely spray-drying because it enables a high production speed, avoiding, at the same time, sticking phenomena between microcapsules. This allows recovery of a powder composed of single microcapsules. The only drawback is represented by the yield, which is not 100%. An optimization of the spray-drying operating conditions should be carried out in order to improve the yield of this drying process.

ACKNOWLEDGMENT

The authors gratefully acknowledge the financial support of Italian MURST (fondi 40%) for this work.

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