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Egg Albumin Microaggregates Containing Colistin for Oral Administration: Organoleptic Characteristics

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ABSTRACT

In order to mask the bitter taste of drug, a microencapsulation method for the production of egg albumin microaggregates was employed. As model bitter-tasting drug, colistin sulfonate was used. The heat denaturation technique for egg albumin microaggregates (coagulation temperature: 60°C) is different from that for human serum albumin (coagulation temperature: 100°C), which is quite important for high temperature unstable drugs. This method was subsequently used to produce microaggregates with a size range of 108.76 ± 32 μm. The egg albumin microaggregates were able to improve the organoleptic characteristics of colistin sulfonate. Drug release from these microaggregates was confirmed by fitting the dissolution data to the equation of Peppas; this resulted in an n *value of 0.9791, signifying zero-order kinetics.*

INTRODUCTION

Egg albumin is widely used for the microencapsulation process (1,2). In a previous work (3), egg albumin was used to manufacture microaggregate egg albumin particles containing paracetamol. With the new microencapsulation method reported in this paper, it is not necessary to use either organic solvents (4,5) or oils (6) to prepare the albumin microspheres.

In recent years the number of drugs, including antibiotics or antibacterial agents, which have undesirable taste has increased. Taste-masking techniques, such as coating tablets or granules with a polymer, are relative easy and have been widely employed. However, it is more difficult to coat small particles efficiently without reducing the drug bioavailability while still masking the drug taste sufficiently. In spite of this, few studies on taste-masking methods have been reported.

Drug release from egg albumin microspheres depends on the polymerization technique. When glutaraldehyde is used as the cross-linking agent the drug release is slower (1); however, when oils and heat are used the drug release is faster (3).

The present work describes egg albumin microaggregates containing colistin sulfonate as a bitter-taste model drug. The study includes organoleptic characteristics, "in vitro" drug release, and the release kinetics of these egg albumin microaggregates containing colistin.

MATERIALS AND METHODS

Materials

The following products were used: colistin sulfonate (Impex, Spain), and ovoalbumin (Ovosec, Spain). All the other chemical were of analytical grade and were purchased from Panreac (Spain).

Methods

Formulations

1. *Preparation of microaggregated egg albumin particles containing colistin:* A previously reported method (7) was used. Colistin sulfate was added to an aqueous egg albumin solution. The system was vigorously stirred and then heated to coagulate the albumin and to promote the formation of microaggregated egg albumin particles. These particles were isolated by decantation, dried at 50°C for 12 hr, and then sieved to obtain a particle size smaller than 0.15 mm.
2. *Coagulated egg albumin particles*: An aqueous egg albumin solution was heated at 60°C for 1 hr. The coagulated egg albumin particles were dried in an over at 50°C for 12 hr and then sieved to obtain a particle size smaller than 0.15 mm.
3. *Colistin granulation*: Colistin and microcrystalline cellulose were granulated with water in a planetary mixer, dried, and sieved to obtain a particle size smaller than 0.15 mm.
4. *Reference sample*: Microcrystalline cellulose was granulated with water, dried, and then sieved to obtain a particle size smaller than 0.15 mm.

Scanning Electron Microscopy

A scanning electron microscope (JEOL-JSM-T-200, USA) was used to study the surface characteristics of the different materials.

Drug Release

The USP 23, dissolution testing method I was used. The dissolution medium was 250 ml of distilled water at 37°C. The stirring speed was 900 rpm. The samples were taken at different times, filtered, and then assayed by spectrophotometry at 210 nm.

Sensory Analysis of Colistin Formulations

The evaluation method was based upon standard methods for sensory analysis described previously (8,9). All the formulations have the same particle size, 108.76 $\pm$ 32 μm, and the formulations containing colistin have the same drug percentage.

Twenty adult volunteers participated in a single blind taste test. In each test, a total of three different formulations and a reference sample were compared. The reference sample was mixed with the formulations in order to obtain different drug concentrations. The formulations were kept for 1.5 min in the mouth, then disgorged and rinsed out with water. The bitter taste of the formulations was evaluated and ranked on a scale from 0 to 5.

Colistin Chemical Analysis

The samples containing colistin were suspended in water. The suspensions were maintained at 37°C and stirred at 100 rpm. The samples were taken at 24 hr, filtered, and analyzed spectrophotometrically at 210 nm. A part of the samples were used for the microbiological analysis.

Colistin Microbiological Analysis

Colistin released from microaggregated egg albumin particles containing colistin and the colistin granulations in a total period of 24 hr was tested according to the inhibitory minimum concentration (CMI) versus *E. coli* (bacteria sensitive to colistin) and *S. aureus* (bacteria resistant to colistin) in order to demonstrate that the drug maintained its antibacterial activity. The microbiological analysis was done according to the dilution technique.

RESULTS AND DISCUSSION

The particle shapes for colistin and albumin microaggregates containing colistin are summarized by scanning electron microscopy (SEM) in Figs. 1 and 2. The particle sizes as determined by laser diffraction are given

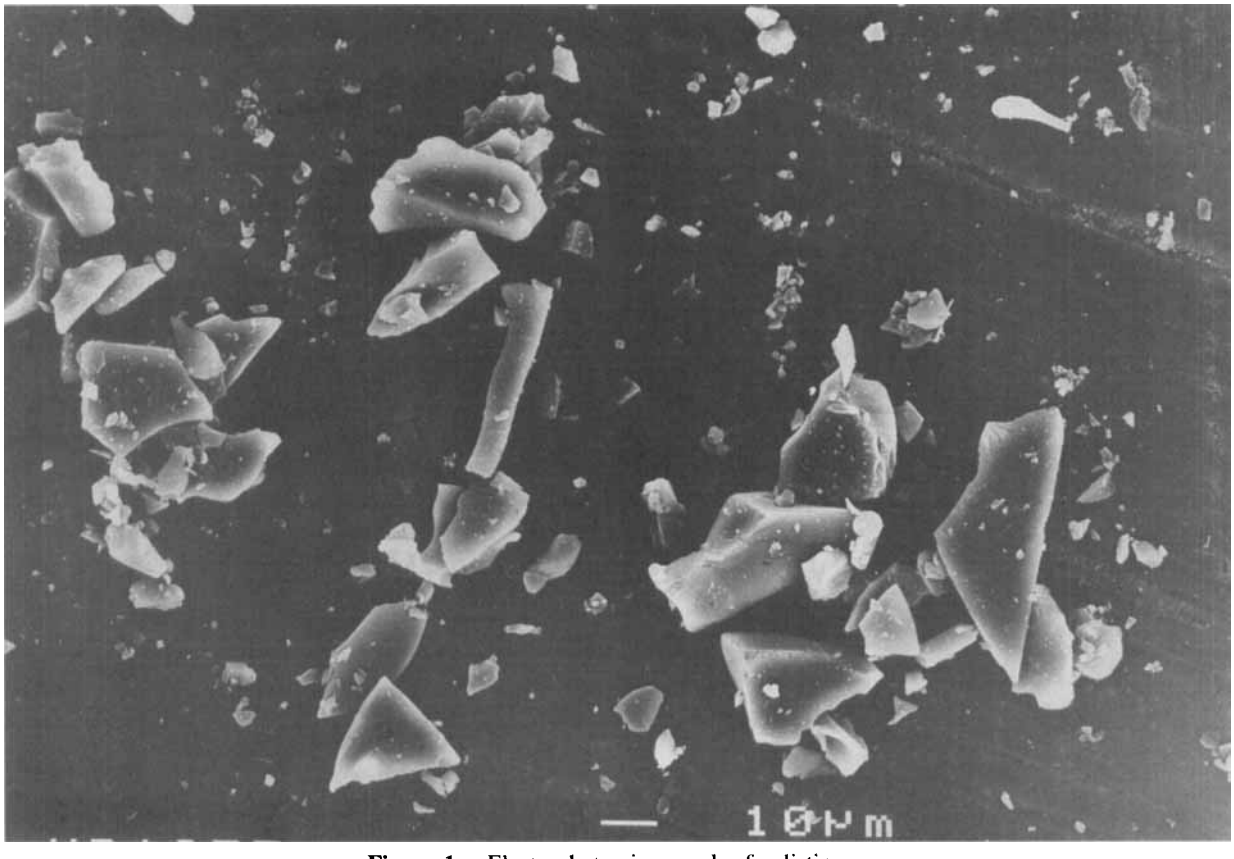


Figure 1. Electrophotomicrograph of colistin.

in Fig. 3. Colistin particles are generally crystalline and angular in shape. Microaggregates are relatively soft and rounded in shape.

The organoleptic properties of all formulations are shown in Fig. 4. All volunteers identified the colistin granulation as the most unpleasant due to its bitter taste. For this formulation, colistin concentrations higher than 2.7% present a stronger bitter taste. The volunteers had difficulty in ranking the bitter taste of the colistin granulate formulation when concentrations were below 0.8% (umbral bitter taste).

The colistin microaggregates present an umbral bitter taste between 1.6% and 2.7%. It is important to note that in the case of albumin microaggregates alone, the volunteer could not rank the different concentrations. The advantage of this egg albumin microaggregate preparation method is that it avoids the oily taste of the albumin microaggregates obtained with oil (3) and the hot taste of the organic solvents used to wash the microspheres after their preparation (1).

Chemical and Microbiological Analysis

The egg albumin microaggregated particles containing colistin were assayed by spectrophotometry and the total colistin content was 16.66% (w/w).

The colistin released from the colistin granulates and microaggregates containing colistin is still capable of inhibiting the growth of *E. coli*. However, these samples were not able to inhibit the growth of *S. aureus* (bacteria resistant to colistin). The minimum inhibitory concentration (MIC) was 1.25 μg/ml for the colistin sulfonate and was the same for the egg albumin microaggregated particles containing colistin (for the same colistin amount).

So, the colistin has not lost its microbiological activity during the heat denaturation procedure (60°C during 60 min). Employing egg albumin avoids the use of the high temperatures (>100°C) needed for processing human serum albumin (5), which may affect the chemical stability or microbiological activity of the drug employed.

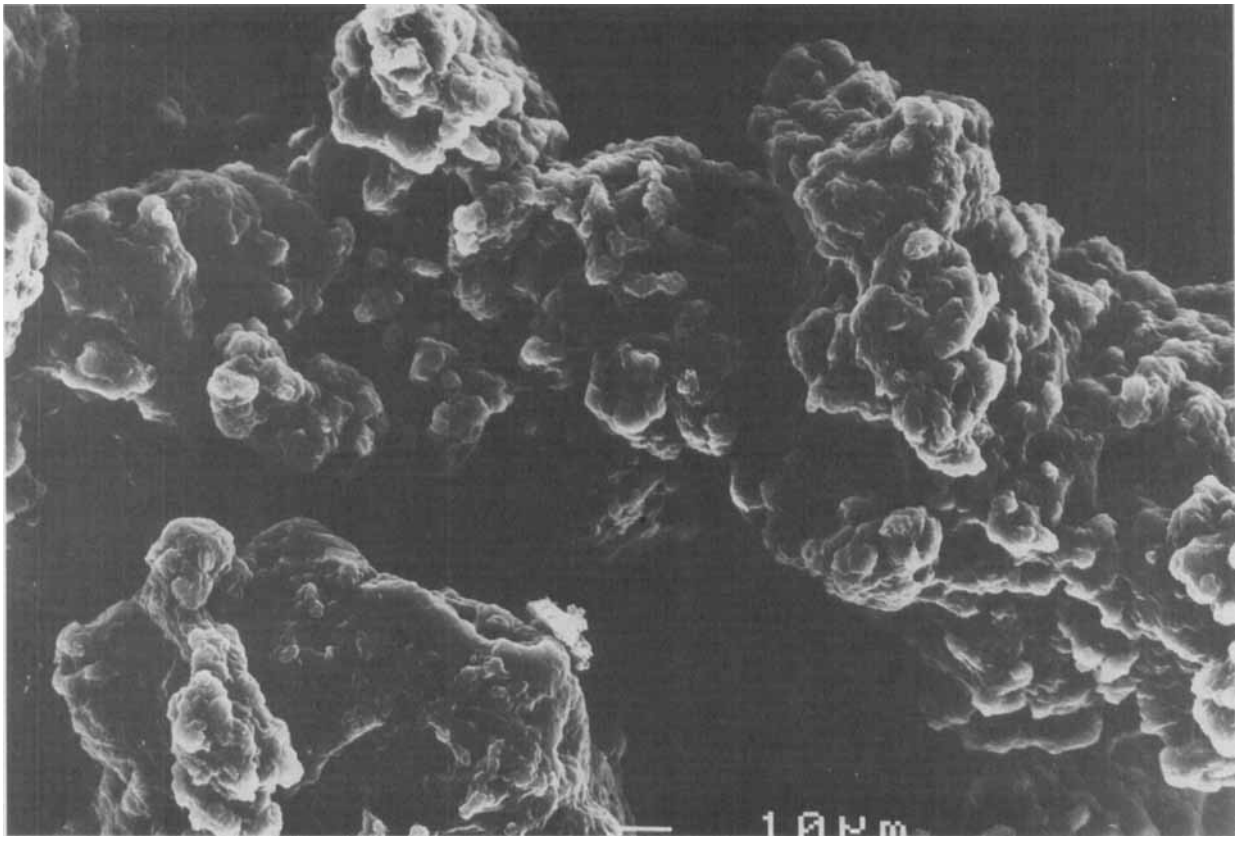


Figure 2. Electrophotomicrograph of albumin microaggregates containing colistin.

In Vitro Release Study

Figure 5 shows that the egg albumin microaggregates containing colistin have a significantly ($p < 0.01$) slower release than the colistin granulate for the first 10 min; after that, the drug release is not significantly different. The slower colistin release from the egg albumin microaggregates containing colistin during the first 10 min is sufficient to mask the unpleasant taste of colistin.

In order to analyze the drug release of these microaggregates, the dissolution data obtained were fitted to the Peppas equation (10):

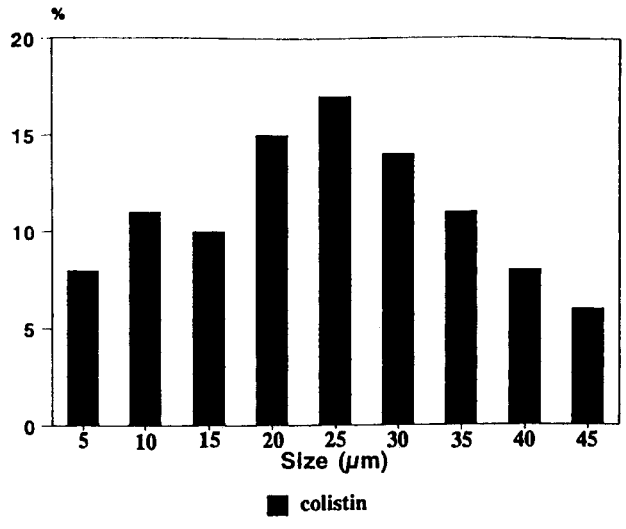
$$M_t/M_\infty = K\,t^n$$

where M_t/M_∞ is the drug release fraction, t is the release time, K is a constant that includes the drug and formulation component characteristics, and n characterizes the type of release mechanism operative during the dissolution process.

The linear regression values of K, n, and the coefficient of correlation (r^2) of the dissolution data are shown in Table 1. For colistin granulate, the values of n were close to 0.5, which indicates that the release mechanism was close to Fickian transport (case I). This release mechanism for the colistin granulate approaches the Higuchi equation type (Fig. 5).

The value of n for the egg albumin microaggregates containing colistin was close to 1.0, indicating that the release mechanism was close to Fickian transport (case II), so the release mechanism is approaching zero-order (Fig. 5). This same zero-order kinetic was the major release mechanism for the bovine albumin microspheres studied by Orienti et al. (11).

To elucidate the extent of the zero-order kinetic release, the data corresponding to $M_t/M_\infty > 0.6$ and < 1.0 were also fitted to Eq. (1). For various values of M_t/M_∞, the values of n were close to 1.0 (Table 1); therefore, these practically constant values of n for



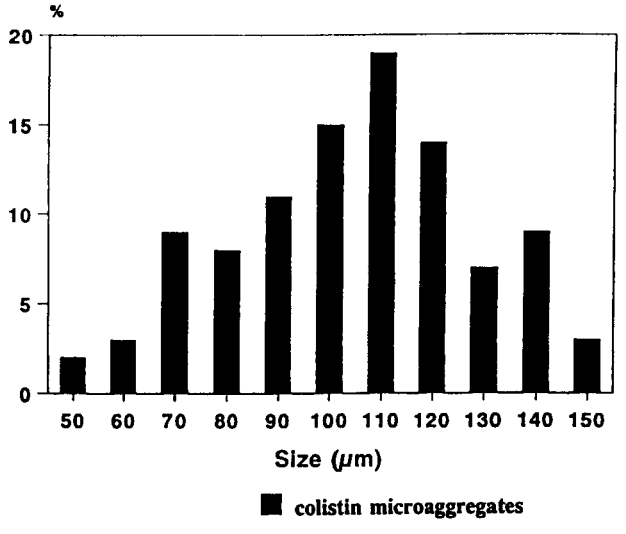


Figure 3. Drug size distribution of colistin and colistin microaggregates.

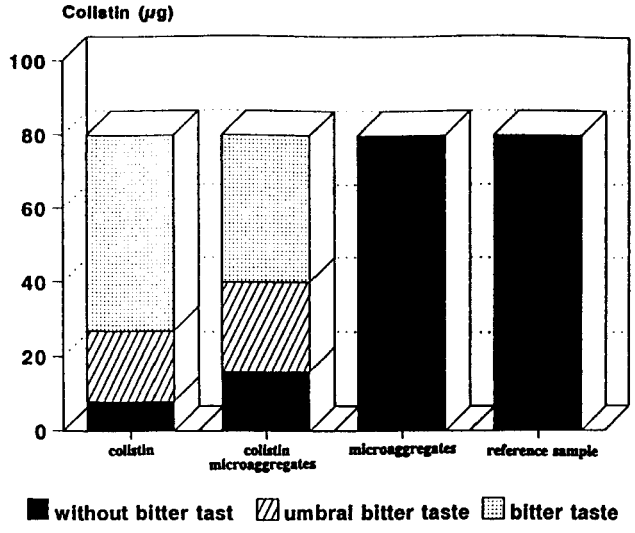


Figure 4. Bitter taste properties of the different formulations: colistin granulates, colistin microaggregates, coagulated egg albumin particles (microaggregates), and reference sample.

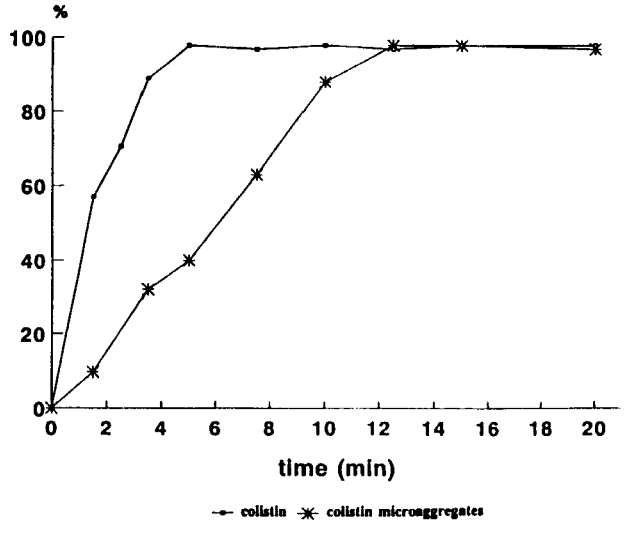


Figure 5. In vitro drug release of the colistin granulates and the albumin microaggregates containing colistin.

Table 1

Values of Kinetic Constant (k), Release Exponent (n) and Coefficient of Correlation (r^2) Following Linear Regression of Dissolution Data of Various Values of $M_t/M\infty$

Formulation	$M_t/M\infty$	Kinetic Constant (K, min^{-n})	Release Exponent (n)	Correlation Coefficient (r^2)
Granulated colistin	0.98	0.4282	0.5260	0.9900
Microaggregated colistin	0.63	0.1010	0.8941	0.9754
	0.88	0.0890	0.9791	0.9842
	0.98	0.0950	0.9381	0.9869

$M_t/M_\infty > 0.6$ and < 1.0 confirm that deviation from zero-order release is practically negligible until 90% of the drug is released from the egg albumin microaggregated particles containing colistin. This may be attributed to the unchanged characteristics of the egg albumin microaggregates during the dissolution test, so the diffusional path length for the colistin remains constant.

CONCLUSIONS

The heat denaturation method provides a convenient method for the production of egg albumin microaggregates containing temperature-sensitive drugs. These egg albumin microaggregates were able to improve the bitter taste of colistin sulfonate. For the egg albumin microaggregates, the release mechanism approached a zero-order kinetic during almost the entire release process.

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