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Original Article

Preparation and In Vitro Evaluation of a Microballoon Delivery System for Theophylline

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Abstract

A multiple-unit oral floating system was prepared using the emulsification-solvent diffusion method to prolong the gastric emptying time of theophylline. For this purpose, theophylline, ethyl cellulose and dibutyl phthalate were dissolved in an ethanol/dichloromethane mixture, added to 0.1 M HCl containing NaCl (20%) or saturated theophylline and/or different concentrations of polysorbate 80 and polyvinyl alcohol. The mixture was stirred at different speeds for 3 h. The resulting microspheres were separated from the solution by filtration. Physical characteristics, including the shape and size distribution, floating capability, drug loading and drug release of the resulting theophylline microspheres were investigated.

The prepared microspheres tended to float over the simulated gastric medium for over 12 h. Addition of NaCl (20%) to the aqueous phase increased the drug loading of microballoons. The mean geometric diameter of microspheres decreased, as the stirring speed rate or the polysorbate 80 concentration were increased. Microballoons prepared at higher stirring rates released their drug content faster. Also, it is concluded that particle size and floating capability of microballoons could be adjusted by altering the stirring rate during microencapsulation.

Keywords: Floating drug delivery systems; Microballoons; Emulsion-solvent diffusion; Hollow microspheres.

Introduction

Floating drug delivery systems offer a good protection against early and random gastric emptying of non-digestible forms (1). These systems remain buoyant on the gastric content for extended periods of time because of their low densities compared to that of the gastric fluid (2).Floating dosage forms can be classified as single-and multiple- unit system (3).

For conventional oral sustained or prolonged-release dosage forms, multiple units are more advantageous than single units because they disperse widely and uniformly along the gastrointestinal tract and could lessen intra- and inter-subject variability. For gastric-retentive systems, multiple units may have the advantage of avoiding all- or –nothing emptying, and increase the probability that some of the dosage form will remain in the stomach (3). Approaches devising multiple unit floating systems include multiple unit HBS (3), polycarbonate microspheres (4), alginate beads (5-7), charged ion exchange resins with bicarbonate (8-11), air compartment multiple unit systems (7), coated granules with a dual effervescent layer (3, 12) and emulsion-solvent diffusion (3, 13-16). At present, hollow microspheres are considered to be one of the most promising buoyant systems, because

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they combine the advantages of multiple unit systems and good floating properties (17). These systems are also called "microballoons" due to their low-density core (14, 16, 18). Generally, techniques used to prepare hollow microspheres involve simple solvent evaporation or solvent diffusion/evaporation methods. Polycarbonate, Eudragit S®, cellulose acetate, calcium alginate, agar and low methoxylated pectin are commonly used as polymers. Buoyancy and drug release are dependent on the quantity of polymer, the plasticizer-polymer ratio and the solvent used (19, 20).

In this study, an emulsion –solvent diffusion/ evaporation technique was used to prepare a floating controlled-release system for theophylline and the influence of several factors on various physical characteristics, including the particle size, drug loading , dissolution and floating properties of the resulting microspheres, were investigated.

Theophylline is a bronchodilator used in the treatment of asthma (21). So far different kinds of floating dosage forms have been prepared for theophylline. Atyabi et al. developed the ion-exchange resin beads loaded with theophylline and bicarbonate, coated with a semipermeable membrane (8). Carbon dioxide was released on contact with the acidic gastric juice. Studies in human volunteers, using gamma-scintigraphy, showed a prolonged residence time for beads (9). Stithit et al. used a novel emulsion–solvent evaporation process to obtain microspheres containing theophylline. The drug–polymer (cellulose acetate butyrate and Eudragit RL®100 [1:1]) dispersions are pressurized under CO_2, which dissolves within them and forms bubbles upon the release of the pressure, giving microspheres with round cavities enclosed in the dispersed drug polymer droplets. They float for more than 24 h in pH 1.2 and 7.5 buffers (22). Streubel et al. developed foam-based floating microparticles consisting of polypropylene foam powder, drug (chlorpheniramine maleate, diltiazem HCl, theophylline or verapamil HCl) and polymer (Eudragit RL® or polymethyl methacrylate). They were prepared by soaking the microporous foam carrier with an organic solution of drug and polymer, followed by subsequent drying. The mixture was poured into an organic liquid (ethanol or methylene chloride), forming a suspension. The polypropylene foam particles acted like microsponges, absorbing the organic liquid, and becoming free-flowing, low-density microparticles following solvent evaporation. Good in vitro buoyancy was observed in most cases and a broad variety of drug release patterns could be achieved by varying drug loading and the type of polymer used, with more than 77% or 98% of particles remain floated for at least 8 h depending on the polymer type (Eudragit RS® or polymethyl methacrylate, respectively) and initial drug loading of the system (10% or 23%) (23). Chitosan microcapsules containing theophylline and sodium carboxymethyl cellulose prepared by using an emulsion-phase separation method. The acetic acid and sodium carboxymethyl cellulose concentrations played an important role in controlling the floating property of the microcapsules (24).

Limited investigations have been conducted on the preparation of floating microparticulate systems based on the emulsion –solvent diffusion/ evaporation technique (3, 13-16). The major problem with the o/w emulsification technique is the low encapsulation efficiency of moderately water-soluble drugs such as theophylline, caffeine and salicylic acid. In cases like this, the drug can diffuse from the organic dispersed phase into the aqueous continuous phase, which results in poor entrapment (21). The choice of theophylline, as the model drug, is based on: (i) the biopharmaceutics classification system, since it is classified as a Class I drug that has a high solubility as well as a high permeability (25); and (ii) the fact that it is a targeted drug for sustained delivery (20, 22). The aim of this study was to devise this technique in order to prepare microballoons with a high theophylline encapsulation efficacy, as a water-soluble drug model.

Experimental

Materials

Materials were obtained from commercial sources: ethylcellulose 100 cps (Dow Chemicals), theophylline anhydrous, polyvinyl alcohol (PVA), dichloromethane, polysorbate 80 and dibutyl phthalate (Merck, Germany).

Methods

Preparation of microspheres

For preparation of the base formula, theophylline (200 mg), ethyl cellulose (200 mg) and dibutylphthalate (40 mg) were dissolved in a mixture of dichloromethane and ethanol 96% (3: 7). The solution was added to 100 ml 0.1 M hydrochloric acid (as an aqueous solution) and stirred for 3 h at 600 rpm. The resulting microspheres (formula F) were separated from the solution by filtration.

In order to find an appropriate formulation, various aqueous media using PVA, NaCl and polysorbate 80 at concentrations outlined in table 1 within a constant base of 0.1 M HCl, were prepared and investigated. Formulas D_1, D_2 and D_3, which had a similar composition to A_2, were prepared at different stirring rates of 500, 700 and 800 rpm, respectively (Table 1). Each formulation was prepared three times.

Solubility of theophylline in different concentrations of NaCl

One gram theophylline was added to 100 ml 0.1 M HCl containing 0, 5, 10, 15 and 20% (w/w) NaCl and stirred for 3 h at room temperature. The resulting mixtures were filtered through 0.45-µm membrane filters and the concentration of theophylline within the filtrate solutions was assayed spectrophotometrically at 270 nm.

Characterization of the microspheres

Floating ability

Floating behavior of the prepared microspheres was studied in a 0.1 M HCl solution containing 0.02% polysorbate 80. The solution was stirred at 100 rpm for 12 h and the buoyant beads counted every hour.

Size and shape

Size distribution of the prepared microspheres was measured by the sieve analysis method. The logarithm of the particle size was plotted against the percentage of cumulative frequency on a probability scale and the logarithm of the geometric mean diameter, which is the particle size equivalent to 50%, on the probability scale was obtained (18).

Table 1. The type and amount of ingredients present within the aqueous medium, as well as the stirring rate of various formulations.

Formulation	PVA (%)	NaCl (%)	Polsorbate80 (%)	Theophylline	Stirring rate (rpm)
F	-	-	-	-	600
V_1	0.5	-	-	-	600
V_2	2	-	-	-	600
V_3	5	-	-	-	600
M_1	-	-	-	Saturated	600
M_2	0.5	-	-	Saturated	600
M_3	2	-	-	Saturated	600
M_4	5	-	-	Saturated	600
A_1	-	20	-	-	600
A_2	-	20	0.2	-	600
A_3	-	20	0.5	-	600
A_4	-	20	1	-	600
D_1	-	20	0.2	-	500
D_2	-	20	0.2	-	700
D_3	-	20	0.2	-	800

For morphological examination, the microspheres were sputtered with platinum and viewed under a scanning electron microscope.

Drug loading

To assess the drug loading, specific amounts of microballoons were dissolved in small volume of ethanol and added to a large amount of water. The precipitated ethyl cellulose was filtered and the remaining solution analyzed specterophotometrically at 271 nm.

Drug release

Drug release from 100 mg of microballoons was studied using a USP dissolution apparatus I, containing 900 ml of 0.1 M HCl (pH 1), preheated and maintained at 37 ± 0.5°C. The basket was rotated at a speed of 50 ± 2 rpm. Samples were removed periodically for 24 h, followed by the replacement of an equal volume of the test medium and analyzed at 270 nm after dilution.

Assay for NaCl in microspheres

To calculate the NaCl content of formulations A_2 and D_3, 50 mg samples were dispersed in 10 ml distillated water and stirred at 100 rpm for 48 h. microballoons were filtered and the concentration of NaCl within the filtrate solution was determined using a solution containing 0.1 N $AgNO_3$ in 100 ml glacial acetic acid, 75 ml methanol and 0.5 ml eosin Y TS (19).

Results and Discussion

Particle size and shape

With application of 0.1 M HCl alone as the aqueous external phase of the emulsion (formula F), spherical microballoons were obtained. In the presence of PVA, with or without saturated theophylline (V-series and M-series), no microspheres were produced. The applicable range of PVA (as an emulsifier) for the preparation of microspheres, using the o/w emulsion-solvent evaporation technique, in terms of various utilized drugs and polymers is very different (28). The levels used in this work resulted in the preparation of an aqueous colloidal dispersion of ethyl cellulose after evaporation of dichloromethane and ethanol.

When using a solution containig 20% NaCl in 0.1 M HCl, formation of microballoons was practicable (formula A_1).

Presence of a low amount of polysorbate 80 (0.2%) accompanied with 20% NaCl in 0.1 M HCl improved the shape uniformity of microspheres. As can be seen in figure 1, the increase in the level of polysorbate 80 within the external phase led to a decrease in the particle size of microspheres, such that no microspheres were formed at the concentration of 1% (A_4). This was due to a fall in surface tension of the solution and enhancement of drug and polymer solubilization within the medium. The mean particle size of microspheres decreased, as the stirring speed increased (Figure 2). Statistical analysis of data , using ANOVA (single factor),

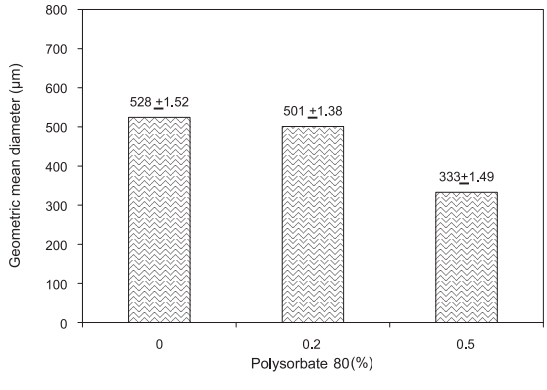

Figure 1. Effect of polysorbate 80 concentration present within the external phase on the particle size (n=3, mean ± SD).

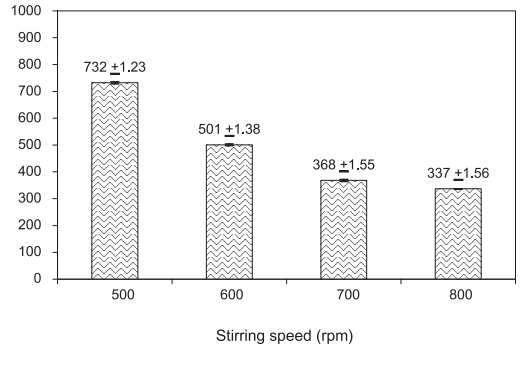

Figure 2. Effect of stirring rate on the particle size distribution of microspheres (n=3, mean ± SD).

Table 2. Theophylline content and drug loading efficiency of various formulations (n=3, mean±SD).

Formulation	Weight of the product (mg)	Drug content (%)	Loading efficiency (%)
F	220 ± 1.23	9 ± 0.08	9.9 ± 0.05
A_1	396.8 ± 1.63	47.2 ± 0.34	93.6 ± 0.61
A_2	393.2 ± 1.89	46.8 ± 0.47	91.9 ± 0.71
A_3	361.6 ± 1.92	42.9 ± 0.51	77.6 ± 0.74
D_1	379.6 ± 2.03	42.9 ± 0.55	81.4 ± 0.82
D_2	390.4 ± 1.86	45.6 ± 0.46	89 ± 0.69
D_3	387.2 ± 1.95	44.8 ± 0.51	86.7 ± 0.76

showed a significant difference between size of formulations ($p<0.0001$). Droplet break-up by impaction on the baffles reduced the average size of the microspheres. Analogous results have also been observed by Garcia-Contreras et al. (29) for cisplatin microspheres prepared using the o/w emulsion-solvent evaporation technique. The results revealed that the mean particle size of microspheres was inversely related to the stirring rate and emulsifier concentration. Similar data were obtained by Jalil and Nixon (30).

Floating properties

To assess the floating properties, microspheres were placed in 0.1 M HCl containing 0.02% polysorbate 80, in order to simulate gastric conditions. The use of 0.02% polysorbate 80 was to account for the wetting effect of the natural surface-active agents, such as phospholipids, in the gastrointestinal tract (15). The resulting microspheres (A-and D-series) tended to float over the simulated gastric medium for more than 12 h, except for formulation D_1 which was prepared at the lowest stirring rate (500 rpm). Indeed, 90% of D_1 microballoons remained floated for at least 8 h. Rapid diffusion of alcohol into the aqueous medium and evaporation of dichloromethane was responsible for inducing an interfacial polymer deposition, leading to the formation of pores within the center and shell of microspheres as well as decreasing their densities (13, 14). Scanning electronic micrograph of D_3 microballoon (Figure 3) shows its porous and hollow structure.

Table 3. Solubility of theophylline in 0.1 M HCl containing different concentrations of NaCl.

NaCl (%)	0	5	10	15	20
Theophylline solubility (mg/ml)	5.52	3.67	2.23	0.82	0.16

Drug loading

Table 2 shows the drug loading efficiency of various formulations. The microspheres (Formulation), prepared using 0.1 M HCl alone as the aqueous external phase, almost had no theophylline.

Addition of NaCl (20%) to 0.1 M HCl (A_1) contributed to the preparation of microspheres with a desirable drug content. As can be seen at table 3, in high concentrations of NaCl, the saturated solubility of theophylline was significantly reduced. Hence, the presence of salt within the microencapsulation medium increased the theophylline encapsulation efficiency. Increasing the concentration of polysorbate within aqueous phase from 0-0.5% caused a reduction ($p<0.0001$) in the theophylline content of microspheres (table 2). Apart from enhanced drug solubilization, another reason attributed to a reduced drug content was stabilization of the polymer-droplet interface by the surfactant.

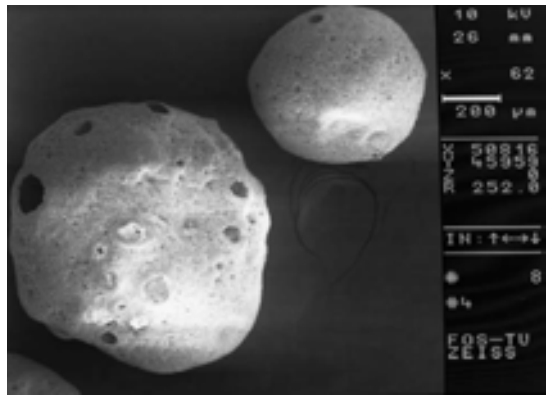

Figure 3. Scanning electron micrograph of D_3 microballoon.

Table 4. The NaCl content of microspheres (n=3, mean ± SD).

Formulation	NaCl (%)
A_2	14.4 ± 0.4
D_3	14.8 ± 0.8

This reduces solvent loss, thereby reducing the polymer precipitation rate, and thus allowing an increased loss of drug from the microspheres being formed, before an adequate polymeric diffusion barrier could be formed (21).

NaCl content

Table 4 shows the NaCl content of A_2 and D_3 formulations. Concerning the therapeutic dose of theophylline, only a little amount of NaCl exists in each dose of microballoons and this will not be harmful for the user.

Drug release

Figure 4 compares theophylline release profiles of microballoons prepared at different stirring rates. Increasing the speed caused a faster drug release. One explanation for this phenomenon could be the reduction of particle size at higher speeds. The release rate will increase with a decrease in particle size, as a result of the increase in surface area (21). A linear relationship between the geometric mean diameter and the time for 50% of encapsulated drug to be released ($t_{50\%}$) was found in this study (figure 5). Analogous results have also been observed by Suzuki et al (31) and Pongpaibul et al. (31, 32). In all formulations, the initial release of theophylline was relatively fast, showing a burst effect in the first 1-2 h. This is attributed to the release of the drug from the surface of microballoons, as the drug might have migrated to the surface along with alcohol during the solvent diffusion and with water during the drying

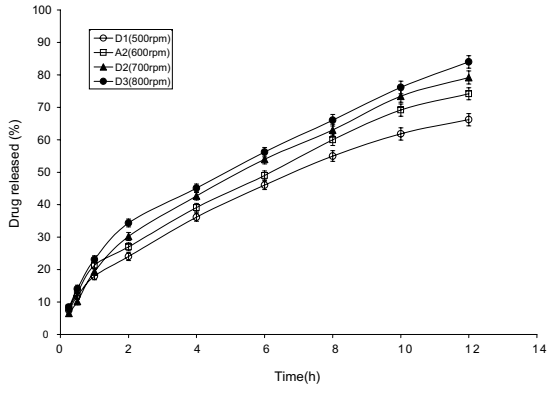

Figure 4. Effect of the stirring rate on the rate of drug release (n=3, mean ± SD).

process (21). For studying the kinetics of drug release, dissolution data were fitted to different kinetics models including the zero-order (M_t/M_∞ versus t), first-order ($-Ln(1-M_t/M_\infty)$ versus t), and square root of time (M_t/M_∞versus $\sqrt{t}$) using linear and nonlinear regression. The ratio of M_t/M_∞ represents the fraction of drug released at time t. Square regression coefficient (R^2) and F-statistic were calculated for each model (Table 5). As could be expected for granular matrix systems, all formulations followed a square root of time kinetics.

Conclusion

The present study reports the development of a novel multiple-unit floating dosage form for theophylline based on an o/w emulsion-solvent diffusion/evaporation method, using 100 cps ethylcellulose. This method has been applied for the preparation of microballoons system with other polymers and drugs (13-18).

Hollow theophylline microsheres were formed by preparing a solution of ethanol/ dichloromethane containing the drug and polymer. On pouring into 0.1M HCl solution

Table 5. Goodness of fitting indices of drug release data with different kinetics models.

Kinetics model		A2	D1	D2	D3
Zero-order	R^2	0.9920	0.9963	0.9959	0.9878
	F-statistic	869.1	1863	1697	565
First-order	R^2	0.9789	0.9629	0.9645	0.9703
	F-statistic	324.3	181.7	190.4	229.1
Square root of time	R^2	0.9955	0.9982	0.9987	0.9972
	F-statistic	1535	3796	5455	2465

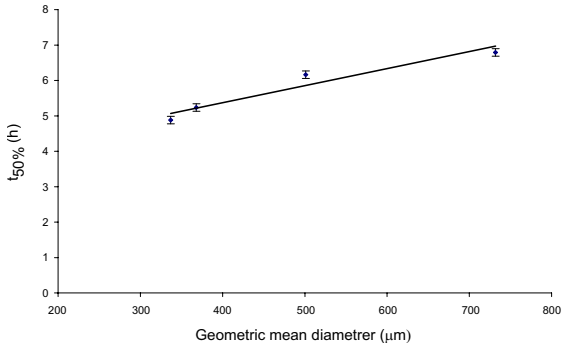

Figure 5. Relationship between particle size and 50% of drug to be released ($t_{50\%}$) (n = 3, mean ± SD).

containing 20% NaCl, an emulsion containing the dispersed polymer/drug/solvent particles, was formed, via a coacervation-type process. From this ethanol (a solvent for the polymer) was rapidly diffused, precipitating polymer at the surface of the droplet to give a hard-shelled particle enclosing the drug dissolved in the dichloromethane. At this point, a gas phase of dichloromethane was generated within the particle, which diffused through the shell.

In such a process, both drug and polymer should be insoluble in water. The problem existing with a low encapsulation efficiency of theophylline as a water-soluble drug was resolved by the use of NaCl. The presence of salt within the microencapsulation medium decreased the solubility of theophylline and the increased encapsulation efficiency. Polysorbate 80 (0.2%), as an emulsifier, accelerated the formation of droplets and stabilized them in the external phase. Particle size, floating capability and drug release of microballoons could be adjusted by altering the stirring rate during microencapsulation.

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