Enteric microparticles coated with smart polymers

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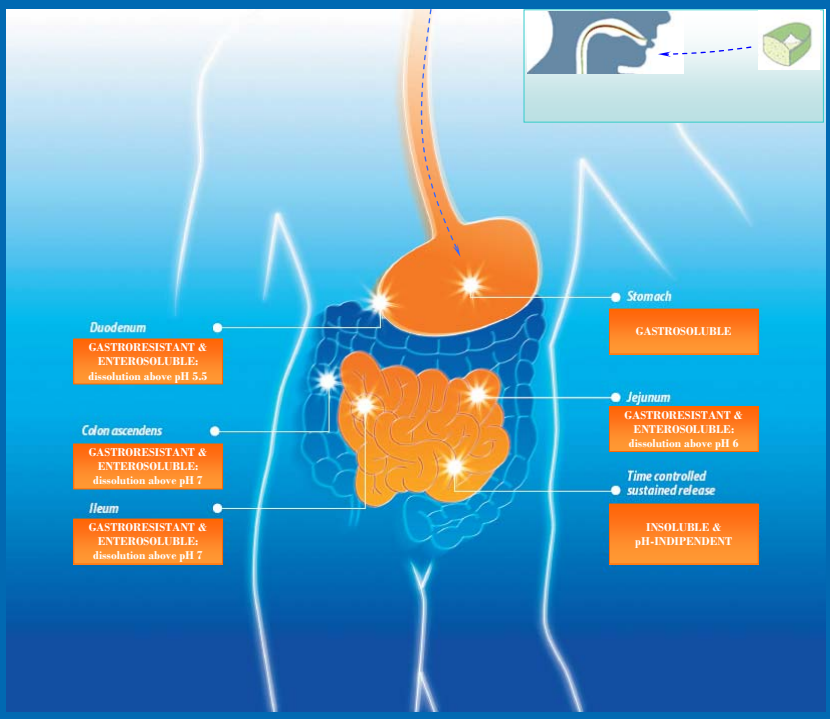

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Enteric microparticles coated with smart polymers for controlled drug delivery applications

Microparticelle enteriche rivestite con "*smart polymers*"
per applicazioni di rilascio controllato di farmaci

Tesi in

Principi di Ingegneria Chimica

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Ai miei genitori

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Contents

Figures index

Tables index

Abstract

The aim of this work was to produce enteric microparticles (Enteric: pertaining to the gastro-intestinal tract, referred to the oral way of administration) for controlled and tailored drug delivery applications. In order to achieve this goal, the literature dealing with microencapsulation processes was analyzed. The attention was focused on the different methods of microencapsulation (especially single or double emulsions: water in oil-W/O; oil in water-O/W; water-in oil-in water-W/O/W) and their influence on final properties of the product. Furthermore, the analysis of studies about enteric coated microparticles (Cellulose Acetate Phthalate-CAP and Eudragit were selected as coating polymers) was reported. A description of the polymers used to coat microparticles followed, especially with the reference to the synthesis and characterization of the novel poly(methylmethacrylate-acrylic acid)-poly(MMA-AA) copolymers, which were one of the results of the present thesis. The two used monomers, the hydrophobic methylmethacrylate and the hydrophilic acrylic acid, are the same functional groups of pH dependent Eudragit grades available on market. This new class of copolymers gave the desired dissolution pH simply changing the volume ratio of the hydrophobic to hydrophilic monomer. Thus, it was able to dissolve at tailored pH values, then they would give the drug release in correspondence of the desired target.
Then microencapsulation of theophylline (TP) was achieved by using a traditional enteric material, CAP, and two kinds of the novel poly(MMA-AA). The chosen method of encapsulation was the double emulsion solvent evaporation process because of water solubility of theophylline. The encapsulation in CAP microparticles was successful and gave the expected behaviour of theophylline release: a low percentage of TP in the acidic stage, then a complete and

instantaneous TP release immediately after reaching pH 6.8 owing to sudden dissolution of CAP. However studies related to the behaviour of such pharmaceutical system, require a fast and accurate method to assay the drug concentration in the dissolution medium. To measure the TP concentration in dissolution medium by UV-visible spectrometer, the blank (reference solution) and the sample solutions should contain the same substances, of course with the exception of TP in the blank solution. However, during the dissolution of CAP microparticles, the actual quantity of dissolved polymer was unknown, thus the blank could not be properly prepared. This problem in evaluation of drug concentration because of polymer presence was a great limitation for release studies. Hence, a simple method to assay, by UV analysis, theophylline and CAP concentrations in the dissolution medium, phosphate buffer pH 7.0, was proposed and validated.

Furthermore, the replacement of CAP with poly(MMA-AA), having an high volumetric percentage of MMA and thus dissolving at pH above 6, as enteric coating was tried. The great problem in poly(MMA-AA) microparticles preparation was that the polymer was soluble in a combination of hydrophobic and hydrophilic solvents, owing to the different features of the constituting monomers. Therefore, the organic solution preparation needed to use an hydrophilic co-solvent, such as ethanol, whose full miscibility in water caused a rapid diffusion of the solvent itself into the external water phase. This caused the immediate polymer precipitation before the organic solution could be dispersed as droplets, thus achieving unstable emulsion and the loss of theophylline before polymer hardening. To limit the early diffusion of solvent into the external aqueous phase, the viscosity of this latter was increased by adding hydroxypropylmethyl cellulose (HPMC). A more promising result and a similar theophylline release to CAP microparticles was thus obtained with a poly(MMA-AA) containing 60% of MMA (dissolution pH ~ 6). However, a more careful investigation about this phenomenon will have to be undertaken.

Summarizing, the main outcomes of this work are:

- the synthesis and the characterization of a novel class of copolymers which overcomes the problem of using polymers with different chemical structure, thus different polymer formulations, to give the dissolution at different pH values; in fact, the copolymer poly(MMA-AA) which dissolves at the pH

of interest, for any physiological compartment, can be easily synthesized by varying the MMA percentage;

- the pointing out of a novel method for simultaneous measurements of theophylline and cellulose acetate phthalate concentrations in phosphate buffer by means of UV spectrometry, in order to overcome the problem in evaluation of drug concentration because of polymer presence;
- the investigation of the operating conditions for production, in the field of controlled drugs delivery, of enteric microparticles coated with two different kind of polymers: the traditional CAP and the novel poly(MMA-AA).

Sintesi

Lo scopo di questo lavoro è la produzione di microparticelle enteriche (Enterico: riferito al tratto gastro-intestinale, in relazione alla via di somministrazione orale) per applicazioni di rilascio mirato e controllato di farmaci. Al tal fine, sono stati analizzati i lavori di letteratura che affrontano l'argomento della microincapsulazione. Una particolare attenzione è stata rivolta ai metodi di microincapsulazione (specialmente singola e doppia emulsione: acqua in olio-W/O; olio in acqua-O/W; acqua-in olio- in acqua-W/O/W) e all'effetto degli stessi sulle proprietà finali del prodotto. Inoltre, è stata messa in evidenza l'analisi, condotta in questa tesi, di studi riguardanti microparticelle a rivestimento enterico (i polimeri scelti sono Cellulose Acetate Phthalate-CAP e Eudragit). Segue una descrizione dei polimeri utilizzati per ricoprire le microparticelle, in particolare della sintesi e caratterizzazione dei copolimeri innovativi poly(metilmetacrilato-acido acrilico)-poly(MMA-AA), che sono stati uno dei risultati di questo lavoro di tesi. I due monomeri usati, il metilmetacrilato idrofobo e l' acido acrilico idrofilo, sono gli stessi gruppi funzionali presenti nei vari Eudragit disponibili sul mercato. Questa nuova categoria di copolimeri si scioglie al pH di dissoluzione desiderato cambiando semplicemente il rapporto volumetrico tra il monomero idrofobo e quello idrofilo. Di conseguenza, il copolimero riesce a sciogliersi a valori di pH specifici, per cui potrebbe fornire il rilascio del farmaco in corrispondenza dell' obiettivo desiderato.
In seguito la teofillina (TP) è stata microincapsulata utilizzando un materiale enterico tradizionale, CAP, e il poly(MMA-AA) sintetizzato. Il processo di evaporazione del solvente da doppia emulsione è stato scelto come metodo di incapsulamento a causa della solubilità in acqua della teofillina. L'incapsulamento in microparticelle di CAP ha avuto buon esito e ha fornito il comportamento atteso riguardo al rilascio di teofillina: basse

percentuali di TP in ambiente acido e un rilascio completo e istantaneo, per l'immediata dissoluzione del CAP, subito dopo aver raggiunto il pH 6.8. Inoltre, studi legati al comportamento di un tale sistema farmaceutico, richiedono un metodo veloce e accurato di analisi della concentrazione di farmaco nel mezzo di dissoluzione. Per valutare la concentrazione di TP nel mezzo di dissoluzione attraverso uno spettrofotometro UV-visibile, il bianco (soluzione di riferimento) e le soluzioni da analizzare devono contenere le stesse sostanze, naturalmente con l'assenza di TP nel bianco. Tuttavia, durante la dissoluzione delle microparticelle di CAP, non è nota l'effettiva quantità di polimero disciolto, cosicché il bianco non può essere preparato correttamente. Questo problema di valutazione della concentrazione di farmaco a causa della presenza del polimero rappresenta un grande limite per gli studi di rilascio. Per tale motivo, è stato proposto e validato un metodo semplice di misura, tramite analisi UV, delle concentrazioni di CAP e teofillina nel mezzo di dissoluzione, tampone fosfato a pH 7.

Inoltre, per il rivestimento enterico, si è cercato di sostituire il CAP con poly(MMA-AA), avente un'elevata percentuale di MMA e quindi in grado di sciogliersi a pH superiore a 6. Il problema maggiore incontrato nella preparazione delle microparticelle di poly(MMA-AA) deriva dal fatto che il polimero è solubile in una combinazione di solventi idrofobi e idrofili, a causa delle caratteristiche diverse dei monomeri costituenti. Di conseguenza, per la preparazione della soluzione organica, si è verificata la necessità di utilizzare un co-solvente idrofilo, ad esempio etanolo, la cui completa miscibilità in acqua ha causato una rapida diffusione del solvente stesso verso la fase acquosa esterna. Ciò ha provocato una precipitazione immediata del polimero prima che la soluzione organica potesse disperdersi in gocce, producendo una emulsione instabile e la conseguente perdita di teofillina prima dell'indurimento del polimero. Per limitare la precoce diffusione del solvente nella fase acquosa esterna, una quantità nota di idrossipropil metil cellulosa (HPMC) è stata aggiunta a quest'ultima per aumentarne la viscosità. Sono stati così ottenuti un risultato più promettente e un rilascio di teofillina simile alle microparticelle di CAP utilizzando un poly(MMA-AA) contenente il 60% di MMA (pH di dissoluzione ~ 6. Tuttavia, è necessaria un'indagine futura riguardo al fenomeno in questione.

Riassumendo, i principali risultati di questo lavoro sono:

- la sintesi e la caratterizzazione di una nuova classe di copolimeri che supera il problema di utilizzare polimeri con diversa struttura chimica, quindi formulazioni polimeriche diverse, per fornire la dissoluzione a differenti valori di pH; infatti il copolimero poly(MMA-AA) che si scioglie al pH di interesse, per ciascun compartimento fisiologico, può essere facilmente sintetizzato variando la percentuale di MMA;
- la messa a punto di un nuovo metodo per la misura simultanea delle concentrazioni di teofillina e CAP in una soluzione tampone fosfato tramite spettrometria UV, con lo scopo di superare il problema della difficoltà di misurare la concentrazione di farmaco a causa della presenza del polimero;
- la ricerca delle condizioni operative per la produzione, nel campo del rilascio controllato di farmaci, di microparticelle enteriche rivestite con due polimeri differenti: il tradizionale CAP e l'innovativo poly(MMA-AA).

Appendices

A.1 List of publications

Chirico S., **Dalmoro A.**, Lamberti G., Russo G., Titomanlio G., "Analysis and modeling of swelling and erosion behavior for pure HPMC tablet", *Journal of Controlled Release*, 122 (2) 181-188 (2007)

Chirico S., **Dalmoro A.**, Lamberti G., Russo G., Titomanlio G., "Radial water up-take in pure HPMC tablet analysis and model prediction", proceedings of Pharmaceutical Sciences World Congress, Amsterdam (NL), (2007)

Barba A., Chirico S., **Dalmoro A.**, Lamberti G., "Simultaneous measurement of Theopylline and Cellulose Acetate Phthalate in Phosphate Buffer by UV analysis", in press on *The Canadian Journal of Analytical Sciences & Spectroscopy* 53 (6) 2009

Barba A., **Dalmoro A.**, De Santis F., Lamberti G., "Synthesis and characterization of P(MMA-AA) copolymers for targeted oral drug delivery", in press on *Polymer Bulletin* (2009), doi: 10.1007/s00289-009-0040-4

Barba A., D'Amore M., **Dalmoro A.**, Lamberti G., Titomanlio G., "Enteric coated micro-particles for targeted and controlled release", to be presented to 36th Meeting of Controlled Release Society, Copenhagen (DK), (2009)

Barba A., Chirico S., **Dalmoro A.**, Galzerano B., Lamberti G., "Water and drug mass fraction profiles in HPMC/TP matrices", to be presented to 36th Meeting of Controlled Release Society, Copenhagen (DK), (2009)

A.2 Enclosed publications

1. *Synthesis and characterization of P(MMA-AA) copolymers for targeted oral drug delivery*
2. *Simultaneous measurement of Theopylline and Cellulose Acetate Phthalate in Phosphate Buffer by UV analysis*
3. *Enteric coated micro-particles for targeted and controlled release*

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ORIGINAL PAPER

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Synthesis and characterization of P(MMA-AA) copolymers for targeted oral drug delivery

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Abstract This paper describes the development of pH-sensitive poly(methyl methacrylate-acrylic acid) copolymers for the enteric coating of pharmaceutical products for oral administration. To obtain the dissolution at the desired pH level, different pH-sensitive polymers are available on the market. Usually, for each desired dissolution pH, an ad hoc polymer is designed. Thus, different dissolution pH values could ask for completely different polymers. Instead, the materials proposed in this work are copolymers of the same two monomers, and the different dissolution pH was obtained by changing the volume fraction of the hydrophobic methyl methacrylate monomer to the hydrophilic acrylic acid monomer. Increasing the volumetric percentage of methyl methacrylate causes the polymer to dissolve at increasing pH, until the dissolution does not take place at all, and it was replaced by a slow swelling phenomenon. The copolymers obtained were characterized by differential scanning calorimetry, in order to evaluate their glass transition temperature, and these latter were related to %MMA. The molecular weights of the pure polymers (PAA, PMMA) were measured by intrinsic viscosity, to further validate the glass transition temperatures observed. The dissolution of the copolymers was carefully tested in buffer solutions for a dense set of pH values. A linear relationship between dissolution pH and volumetric percentage of methyl methacrylate was obtained from these measurements. As a result, for any physiological compartment, the copolymer which dissolves at the pH of interest can be easily synthesized.

Keywords P(MMA-AA) · Drug delivery · Enteric coating

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Article No. : 40 | LE | TYPESET
MS Code : PB-08-0420 | CP | DISK

Introduction

Controlled drug delivery systems, which are intended to deliver drugs at predetermined rates for predefined periods of time, have been used to overcome the shortcomings of conventional drug formulations. It would be highly beneficial if the active agents were delivered by a system that sensed the signal caused by disease, judged the magnitude of signal, and then acted to release the right amount of drug in response. Such a system would require coupling of the drug delivery rate with the physiological need by means of some feedback mechanism [1]. The environment-sensitive polymers, called "smart" polymers, are ideal candidates for developing self-regulated drug delivery systems.

Maybe the most important physiological stimulus is the change in the environment pH. The pH sensitive polymers contain pendant acidic (e.g., carboxylic and sulfonic acids) or basic (e.g., ammonium salts) groups that either accept or release protons in response to changes in environmental pH. The polymers with a large number of ionizable groups are known as polyelectrolytes: polyanions and polycations. The pendant acidic or basic groups on polyelectrolytes undergo ionization just like acidic or basic groups of monoacids or monobases. The pH-sensitive polymers have been most frequently used to develop enteric coated formulations for oral administration. The pH in the stomach (<3) is quite different from the neutral pH in the intestine, and such a difference is large enough to elicit pH-dependent behavior of polyelectrolyte polymer. Enteric coated products are designed to remain intact in the acidic juices of the stomach and then to release the drug at the higher pH of the small intestine (above pH 5.5) or at the even higher pH in the colon (above pH 6.5): the effectiveness of the drug will be reduced by stomach acids or enzymes if they were left unprotected [2].

Polymers made of poly(acrylic acid) (PAA) or poly(methacrylic acid) (PMA), which are polyanions, can be used to develop formulations that release drugs in a neutral pH environment [3].

Hydrophobic modifications affect both the segmental mobility of polyelectrolytes and the pH range over which the ionization takes place. Different comonomers provide different hydrophobicity to the polymer chain, leading to a different pH-sensitive behavior. Hydrogels made of PMA grafted with poly(ethylene glycol) (PEG) have unique pH-sensitive properties [4]. At low pH, the acidic protons of the carboxyl groups of PMA interact with the ether oxygen of PEG through hydrogen bonding, and such complexation results in shrinkage of the hydrogels. As the carboxyl groups of PMA become ionized at high pH, the resulting de-complexation leads to swelling of the hydrogels.

Copolymers of methacrylic acid (MAA) have shown conformational transition that shifted progressively towards higher pH values with increasing hydrophobicity and/or content of hydrophobic co-monomers (e.g., styrene or alkyl(meth)acrylate derivatives) [5].

The enteric coating polymers commonly available on the market are anionic polymethacrylates, that is copolymers of MAA and either methylmethacrylate or ethyl acrylate (Eudragit®, Kollicoat®); cellulose based polymers, e.g., cellulose

Journal : Small-ext 289 Dispatch : 16-1-2009 Pages : 10
Article No. : 40 LE TYPESET
MS Code : PB-08-0420 CP DISK

acetate phthalate or CAP (Aquateric®) and polyvinyl derivatives, e.g., polyvinyl acetate phthalate (Coateric®).

Polymers with different chemical structure were available to give the dissolution at different pH values. Therefore, to obtain the release in different body compartments, different polymer formulations have to be chosen. It would be better to have a single class of copolymers, of similar physical properties, which can dissolve at tailored pH values, giving the drug release in correspondence of the desired target.

Aim of this work is to propose and to characterize a class of copolymers obtained from two monomers, which gives the desired dissolution pH simply changing the volume ratio of the hydrophobic to hydrophilic monomer. Thus, the class of copolymers produced and characterized in this work should be able to fulfill the requirement mentioned above.

Experimental

Materials

For the co-polymer synthesis: methylmethacrylate (MMA, CAS number: 80-62-6) and acrylic acid (AA, CAS number: 79-10-7) were purchased from Sigma-Aldrich, Initiator 2,2′-azobis 2,4-dimethylvaleronitrile (AMVN, CAS number: 4419-11-8) was a Cayman Chemical Company product. Potassium biphthalate (CAS number: 877-24-7), hydrochloric acid (HCl, CAS number: 7647-01-0), sodium hydroxide (NaOH, CAS number: 1310-73-2) and monobasic potassium phosphate (CAS number: 7778-77-0), were used for buffer solution preparation; Acetone (CAS number: 67-64-1), for viscosity measurements; all purchased from Sigma-Aldrich.

Methods

Synthesis

The poly(MMA-AA) copolymers were obtained by a free radical polymerization method described by Abusafieh et al. [6], where they developed a cross-linked poly(MMA-AA) copolymer for potential applications in bone implants. The polymerization was carried out in bulk, using AMVN as initiator whose amount was fixed at 0.4 g/100 mL of total mixture. The initiator was added to various volumetric ratios of MMA/AA monomers and mixed thoroughly by sonication (Vibra-Cell™ Ultrasonic Processor, Sonics, Newtown, CT). The reaction mixture was poured into glass tubes, sealed and placed vertically in a water bath which provided a uniform and accurate temperature control. The assembly was maintained at 30 °C for 5 h, then the temperature was raised gradually (5 °C/h) to 70 °C and kept at this temperature for 10 h, this step was followed by overnight cooling. The tubes were then taken out from the bath and broken under slight clamp pressure. The samples were removed from the glass tubes and placed in an oven in which the

Journal : Small-ext 289 | Dispatch : 16-1-2009 | Pages : 10
Article No. : 40 | LE | TYPESET
MS Code : PB-08-0420 | CP | DISK

temperature was raised slowly (1 °C/min) to 150 °C and left for 3 h, followed by overnight cooling. The samples were crushed and stored at room temperature.

T_g measurements

Differential scanning calorimeter (DSC, Mettler Toledo 822) was used to measure the glass transition temperatures (T_g) of the synthesized samples. The thermal cycle was characterized by a first heating stage from 0 to 170 °C at a rate of 10 °C/min, then the temperature of 170 °C was kept for 5 min, a cooling stage from 170 to 0 °C at a rate of 10 °C/min came after, the temperature of 0 °C was maintained for 5 min after the cooling step and at the end a second heating stage from 0 to 170 °C at a rate of 10 °C/min was imposed.

Viscosity measurements

The viscosity measurements of polymers' dilute solution, carried out following the standard ASTM D2857-95, were made with a Cannon Ubbelohde B409 dilution viscometer (instrument constant $c_v = 0.002110$ cS/s, calibrated at 40 °C). Two polymeric solutions were prepared: PMMA in acetone and PAA in sodium hydroxide water solution, 2 M.

For each couple solute/solvent, the efflux time (t) was measured out starting with 15 mL of 0.002 g/mL solution, then adding 3 mL of solvent (the concentration was thus reduced to 0.00167 g/mL), then adding 3 mL of solvent once more (obtaining a concentration of 0.00143 g/mL). Therefore, the efflux times for three different concentrations were obtained. All the runs were carried out in triplicate.

Starting from the efflux time measurements, the solution viscosity η can be evaluated as the product between the efflux time (t) and instrument constant ($c_v = 0.002110$ cS/s); then the solution reduced viscosity, η_r (also called "viscosity number") can be calculated as:

$$\eta_r = \frac{\eta - \eta_0}{c \times \eta_0} \tag{1}$$

where η_0 is solvent viscosity and c is the solution polymer concentration. Then, the intrinsic viscosity, η_{int} (also called "limiting viscosity number"), can be calculated, since it is defined as:

$$\eta_{int} = \lim_{c \to 0} \frac{\eta - \eta_0}{c \times \eta_0} \tag{2}$$

The value of η_{int} can be easily evaluated from the intercept of a straight line fitting the η_r versus c values. At last, the polymer molecular weight, M_W can be calculated through Mark-Houwink-Sakurada equation (MHS):

$$\eta_{int} = K_W \times M_W^a \tag{3}$$

whose costants (K_W, a), reported in Table 1, were taken from section VII of "Polymer handbook" [7]. Both the ASTM D2857 and the Polymer Handbook define the concentration range (no more than 0.002 g/mL) to be used to avoid the non-newtonian effect of polymer solutions, and the tests were performed accordingly.

Journal : Small-ext 289 Dispatch : 16-1-2009 Pages : 10
Article No. : 40 LE TYPESET
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Table 1 Values of Mark-Houwink-Sakurada equation constants [7]

	T_{rif} (°C)	$K_W \times 10^3$ (mL/g)	a
PMMA in acetone	25	9.6	0.69
PAA in NaOH 2 M	25	42.2	0.64

Dissolution measurements

Measurements of pH for dissolution and swelling, in order to study the influence of the hydrophilic/hydrophobic ratios on the behavior of the synthesized copolymers, were performed in standard buffer solutions prepared according to USP 28. A dense range of pH values from 4 to 8 was investigated. Table 2 describes buffer solutions preparation. All the pH values were confirmed by Crison GLP 22 pH-meter.

The measure of dissolution pH was performed by putting a small amount (of the order of 1 g) of copolymer powder in a beaker containing 50 mL of any buffer solutions, at room temperature, in presence of a magnetic stirrer. After 24 h of stirring, for each sample: (1) the complete dissolution was assumed if there were not any trace of copolymer in solution; (2) the swelling was assumed if the powder were given up to swelled grains (increase in size and formation of a transparent surface layer of gel); (3) the copolymer was taken as pH-resistant if the powder was found un-dissolved.

Results and discussions

Synthesis

Several copolymers with different volumetric ratios of AA and MMA were synthesized and they were reported in Table 3. All synthesized samples exhibited high levels of transparency; while pure PMMA samples were colorless, pure PAA

Table 2 Composition of standard buffer solutions according to USP 28

Acid phthalate buffer												
Place 50 mL of the potassium biphthalate solution in a 200 mL volumetric flask, add the specified volume of HCl solution, then add water to volume												
pH	4.0											
0.2 M HCl (mL)	0.1											
Neutralized phthalate buffer												
Place 50 mL of the potassium biphthalate solution in a 200 mL volumetric flask, add the specified volume of NaOH solution, then add water to volume												
pH	4.2	4.4	4.6	4.8	5.0	5.2	5.4	5.6	5.8			
0.2 M NaOH (mL)	3.0	6.6	11.1	16.5	22.6	28.8	34.1	38.8	42.3			
Phosphate buffer												
Place 50 mL of the monobasic potassium phosphate solution in a 200 mL volumetric flask, add the specified volume of NaOH solution, then add water to volume												
pH	5.8	6	6.2	6.4	6.6	6.8	7	7.2	7.4	7.6	7.8	8
0.2 M NaOH (mL)	3.6	5.6	8.1	11.6	16.4	22.4	29.1	34.7	39.1	42.4	44.5	46.1

Journal : Small-ext 289 Dispatch : 16-1-2009 Pages : 10
Article No. : 40 LE TYPESET
MS Code : PB-08-0420 CP DISK

Table 3 Compositions of samples prepared along this work

Sample	Volumetric %								
	S1	S2	S3	S4	S5	S6	S7	S8	S9
MMA	0	25	30	40	50	60	70	75	100
AA	100	75	70	60	50	40	30	25	0

samples had a dark yellow color. The copolymer samples appeared yellow with the intensity of the color increasing with the amount of AA in the copolymer.

T_g measurements

Samples glass transition temperature was measured by DSC. Figure 1 shows the DSC signals for all samples recorded during the second heating step (the thermal history was summarized in the "Methods"). This choice is due to the fact that, in the first heating step, for polymers containing hydrophilic groups, a confusing peak due to adsorbed water can be found, of course this one disappears in the second heating [8].

In order to estimate the glass transition temperature, a Boltzmann equation (Eq. 4, with A_1, A_2, T_0 and dT as fitting parameters) was used to fit the DSC signal. The temperature corresponding to the center of the Boltzmann curve (the parameter T_0) was taken as T_g, as shown in Fig. 2 for a copolymer containing the 40% in volume of MMA (sample S4). Usually, the T_g is identified as the onset or as the midpoint of the transition. Here the center was selected as the most easily reliable data. However, in the following discussion the data obtained in this work will be

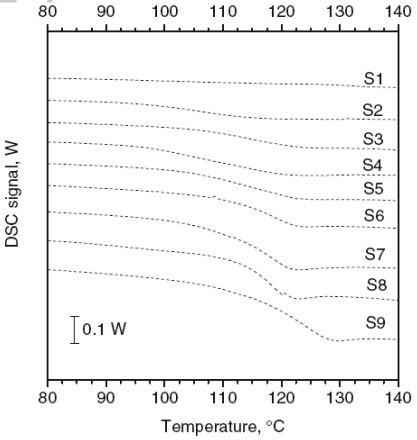

Fig. 1 DSC signal versus temperature, the graph is parametric in % of MMA

Journal : Small-ext 289 Dispatch : 16-1-2009 Pages : 10
Article No. : 40 LE TYPESET
MS Code : PB-08-0420 CP DISK

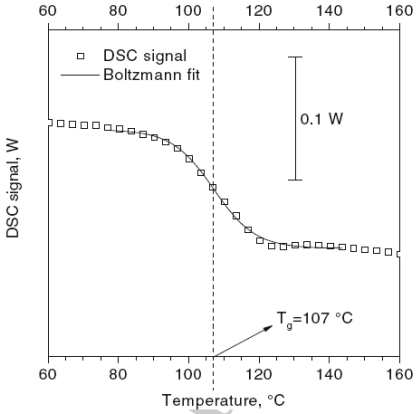

Fig. 2 DSC signal and Boltzmann fit for a copolymer with 40% (v/v) of MMA (S4)

critically compared with data from different source, accounting for the different method to estimate T_g

$$\mathrm{DSC} = \frac{A_1 - A_2}{1 + \exp\left(\frac{T - T_0}{\mathrm{d}T}\right)} + A_2. \tag{4}$$

The values of T_g as function of volumetric percentage of MMA were plotted in Fig. 3. These values for the copolymers were in good agreement with the values computed from the glass transition temperatures of the constituent homopolymers using the inverse weighted average rule:

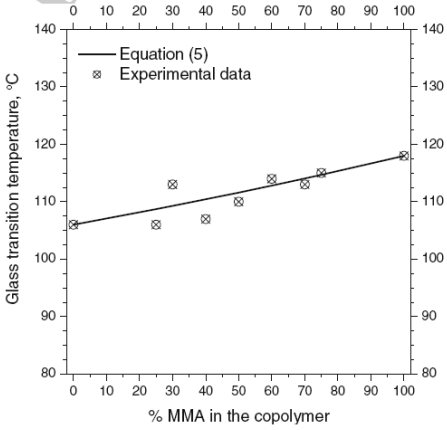

Fig. 3 Inverse weighted average rule (Eq. 4) and measured T_g values versus volumetric percentage of MMA in the copolymer

Journal : Small-ext 289 Dispatch : 16-1-2009 Pages : 10
Article No. : 40 LE TYPESET
MS Code : PB-08-0420 CP DISK

$$\frac{1}{T_g} = \frac{w_1}{T_{g1}} + \frac{w_2}{T_{g2}} \quad (5)$$

where w_1 and w_2 are the weight fractions of the monomer, and T_{g1} and T_{g2} are the glass transition temperatures of pure PMMA ($T_{g1} = 118$ °C) and of pure PAA ($T_{g2} = 106$ °C).

Viscosity measurements

The characterization of the polymers synthesized requires, at least, an estimation of their molecular weight. It can be done by MHS equation (Eq. 3), once the intrinsic viscosity, η_{int}, was measured. The constants in MHS equation were known for the two homopolymers (Table 1), thus the measurement is possible only for them (samples S1, S9). The intrinsic viscosity values found were 63.2 mL/g for PAA (S1) and 44.5 mL/g for PMMA (S9). By MHS equation the average molecular weight values found were 92,000 for PAA and 21,7000 for PMMA.

For commercial PAAs it is reported a glass transition temperature which is independent upon molecular weight on the value of 106 °C for polymers in the M_w range from 1,800 to 4,000,000 (materials with code numbers 323667, 181293, 181285, 306215, 306223, 306231 in the Sigma-Aldrich web-catalog), the same value of T_g we found for a M_w 92,000 (which falls in the M_w range mentioned above). Thus the molecular weight is coherent with the glass transition temperature.

The dependence of glass transition temperature upon the molecular weight for PMMA is roughly described by a straight line in the plane T_g versus $\log(M_w)$. In Fig. 4 a graph of such kind is reported, with several data for commercial PMMAs (taken from Sigma-Aldrich web-catalog, the code numbers are reported on the data point themselves). For three materials the onset of glass transition is reported, along with the molecular weight as determined by GPC (closed squares). From these three data points, the linear relationship was found (the continuous line), and then a line (dashed) with the same slope was assumed to describe the dependence of (midpoint of) T_g upon $\log(M_w)$. Of course this line has to pass through the single data available for the midpoint of T_g, the sample no. SA 445746, with $M_w = 350{,}000$ and $T_g = 122$ °C. The sample S9 produced in this work has to fall on the same line, and it nicely fits in. Thus, the molecular weight and the glass transition temperature were mutually strengthened.

Dissolution measurements

The results of dissolution measurements were shown in Fig. 5, in which the closed triangles identify the polymer dissolution, the open squares the polymer full swelling and the (lower) y-error bar identify the incipient swelling.

As expected, the increase of hydrophobic monomer (MMA) causes an increase of pH at which the copolymer swells or dissolves. For example, the copolymer containing the 60% (v/v) of MMA (sample S6) dissolves at pH = 6.2 (triangle), strongly swells at pH = 5.8 (square), but the swelling was observed up to pH = 5.6, as indicated by the error bar: under this pH value the polymer kept un-dissolved. For

Journal : Small-ext 289 Dispatch : 16-1-2009 Pages : 10
Article No. : 40 LE TYPESET
MS Code : PB-08-0420 CP DISK

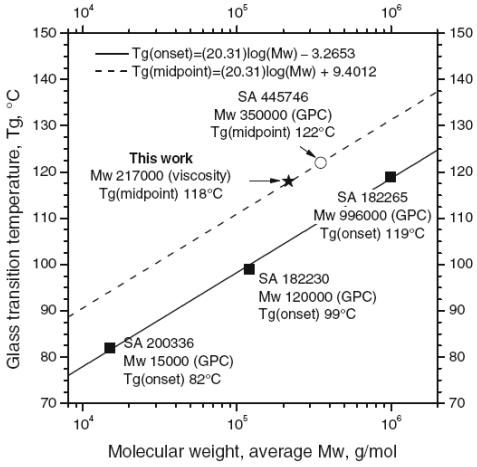

Fig. 4 Relationship between the glass transition temperature (onset or midpoint) and log (molecular weight), for some commercial PMMAs (SA, i.e., from Sigma Aldrich) and for the one obtained in this work

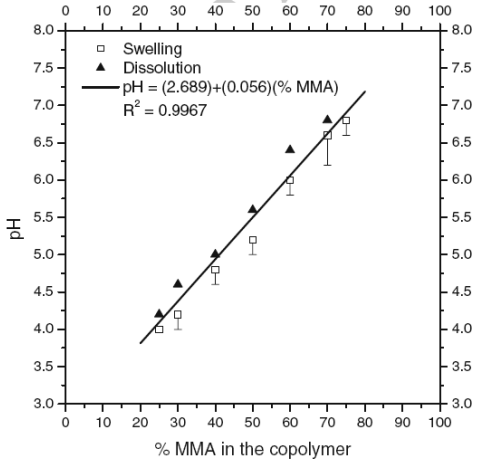

Fig. 5 Swelling and dissolution pH versus volumetric percentage of MMA in the copolymer

Journal : Small-ext 289 Dispatch : 16-1-2009 Pages : 10
Article No. : 40 LE TYPESET
MS Code : PB-08-0420 CP DISK

the sample S8 [the 75% (v/v) of MMA copolymer], only swelling was observed (from pH = 6.5 to 6.8): dissolution was not observed in the investigated range, that is up to pH = 8.

The pH interval between the swelling and dissolution is small and it is hard to investigate, since the two phenomena take place simultaneously. Thus, an arithmetic mean between the swelling and the dissolution pH values was taken as the measure of "incipient" dissolution, and these average data points (not reported in the graph) were taken as the basis for a linear fit, reported as a continuous line in Fig. 5. The equation is:

$$\mathrm{pH} = a + b\%\mathrm{MMA} \tag{6}$$

where $a = 2.689$, $b = 0.056$ and the values of R^2 was 0.9967. Therefore the desired pH of dissolution of a poly(methyl methacrylate-acrylic acid) copolymer (wished pH for targeted drug release) can be chosen in function of its MMA volumetric percentage by this linear equation.

Conclusions

In this work, copolymers of poly(methyl methacrylate-acrylic acid) with various ratios of the hydrophobic to hydrophilic monomers were synthesized and characterized. The glass transition temperature of each copolymer follows the inverse weighted average rule, confirming the production of copolymers with the right composition. The viscosity of the pure-polymer solution well agrees with the molecular weight expected on the basis of glass transition temperature measurements. The dissolution and swelling pH values were carefully determined, and a linear relationship was found between the %MMA in the copolymer and the dissolution pH. Thus, the copolymer which dissolves at the desired pH, for targeted oral drug delivery, can be easily prepared by using Fig. 5.

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Journal : Small-ext 289 Dispatch : 16-1-2009 Pages : 10
Article No. : 40 LE TYPESET
MS Code : PB-08-0420 CP DISK

Simultaneous measurement of theophylline and cellulose acetate phthalate in phosphate buffer by UV analysis

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Abstract

The oral administration of pH-sensitive drugs requires protecting the drug molecules from the acidic pH in the stomach: the simplest way is to use polymers as coating, especially polymers which are insoluble at low pH (in the stomach) and soluble under neutral conditions (in the intestine). The Cellulose Acetate Phosphate, CAP, is one of these polymers, and it is one of the most used coating polymers.

Studies related to the behavior of such pharmaceutical systems require fast and accurate methods to assay the released drug concentration in dissolution medium. However, both the drug and the coating polymer are present in the dissolution bulk with unknown concentration, and they can interfere each other in assaying.

In this communication, a simple method to assay, by UV analysis, Theophylline (TP) and Cellulose Acetate Phosphate concentrations in a dissolution medium, phosphate buffer pH 7.0 (BP), is proposed and validated.

Keywords: UV-visible spectroscopy, Theophylline, CAP

Résumé

L'administration orale de médicaments sensibles au pH requiert la protection de ces molécules des effets de l'acidité stomacale. La façon la plus simple est de se servir d'une protection par des polymères insolubles au pH acide de l'estomac mais soluble aux conditions neutres de l'intestin. Le phosphate acétate de cellulose, CAP, est l'un de ces polymères et il est l'un de ceux les plus utilisés comme agent protecteur. Les études qui rapportent le comportement de tels systèmes pharmaceutiques requièrent des méthodes rapides et exactes pour mesurer la concentration du médicament relâché dans le milieu de dissolution. Cependant, le médicament et le polymère de protection sont tous deux présents dans la solution brute en concentrations inconnues et ils peuvent interférer l'un sur l'autre dans la mesure finale. Nous proposons et validons ici une méthode simple par analyse UV, qui permet de mesurer les concentrations de théophylline (TP) et du phosphate acétate de cellulose dans le milieu de dissolution, un tampon phosphate à pH 7.0 (BP).

Introduction

In pharmacology, enteric coated products are designed to remain intact in the acidic juices of the stomach and then to release the drug at higher pH (above pH 5.5) of the small intestine. If the drug is unprotected, its effectiveness will be reduced by stomach acids or enzymes activity [1].

The polymers commonly used to achieve enteric properties are anionic polymethacrylates (copolymer of methacrylic acid and either methylmethacrylate or ethyl acrylate (Eudragit®)), cellulose based polymers, e.g. cellulose acetate phthalate or CAP (Aquateric®) and polyvinyl derivatives, e.g. polyvinyl acetate phthalate

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(Coateric®) [1].

Common methods to assay the quantity of drug released from a pharmaceutical form in a dissolution medium are UV-visible spectrometry and HPLC (High-performance liquid chromatography) [2]. The HPLC-based method has specific advantages over the spectrometric one: it achieves a higher degree of resolution, that is, the separation of constituents is more complete. In addition, the results of analysis are more highly reproducible. However HPLC is very expensive, so UV-visible spectrometry is the most used method.

If CAP is adopted as the enteric coating polymer and theophylline (TP) is the model drug, it would be useful to be able to measure the TP concentration in dissolution media by UV-visible spectrometer. By a two rays spectrometer, the blank and the sample solutions should contain the same substances, of course with the exception of TP in the blank solution. However, during the dissolution of a polymer-matrix pharmaceutical form, the actual quantity of dissolved polymer is unknown, thus the blank cannot be properly prepared [3]. Then, this problem in evaluation of drug concentration because of polymer presence is a great limitation for release studies from polymer-matrix pharmaceutical form. The aim of this work is to develop a method able to assay simultaneously TP and CAP in their mixtures by UV-visible spectrometry.

Experimental

Materials

Theophylline (TP, CAS no. 58-55-9, Sigma Aldrich), cellulose acetate phthalate (CAP, CAS no. 9004-38-0, Sigma Aldrich). Distilled water, potassium dihydrogen phosphate, and sodium hydroxide were used to prepare the phosphate buffer (BP, pH 7.0).

Methods

The measurements of UV-visible spectra were achieved by a Spectrophotometer Lambda 25 Perkin-Elmer, using quartz cuvettes with an optical length of 10 mm. The spectra were collected for wavelengths between 200 and 400 nm, with a step of 1 nm.

Data Analysis

Optimization strategy (1)

Spectra of pure compounds can be fitted by a number of Gaussian curves, each one characterized by three parameters: peak height (H_{0i}), peak center (λ_{0i}) and peak width (ω_i) [4,5]. The full spectrum was thus obtained by equation (1).

$$H(\lambda) = \sum_{i=1}^{N} H_{0i} \exp\left[-4\ln(2)\left(\frac{\lambda - \lambda_{0i}}{w_i} \right)^2 \right] \quad (1)$$

Three Gaussian curves (i.e. i = 1, …, N with N = 3) were used in order to fit CAP's spectra which were obtained for different CAP concentration in BP {10, 20, 30 and 40 mg/L}. For each spectrum, all the parameters were optimized by minimizing the sum of square differences between the measured absorbances, as obtained by UV-visible spectrometer, and the calculated values from equation (1). The same work was done to fit the TP's spectra, collected for the same range of concentration {10, 20, 30 and 40 mg/L}, using, in this case, four Gaussian curves (N = 4).

In this way, nine different parameter values are obtained to describe the spectrum collected for each CAP concentration. Furthermore, twelve different parameter values were obtained to describe the spectrum collected for each TP concentration.

Optimization strategy (2)

As second step, average values of λ_{0i} and ω_i (obtained by the arithmetic mean between λ_{0i} and ω_i got for each spectrum at different concentrations) were fixed in order to limit the optimization procedure only to H_{0i}. This method is applied to TP and CAP and the resulting fitting was very good for both pure substances in BP solutions, as reported in Figure 1. However, it is worth to note that the TP spectra cannot be correctly described for low wavelength values (200-225 nm).

At last, the absorbance has to be related to solute concentration (the Beer-Lambert Law), thus the obtained values of H_{0i} can be described by the equation (2).

$$H_{0i}(C) = k_i C \quad (2)$$

The fitted parameters were summarized in Table 1.

Results and Discussion

Mixtures of CAP/TP with known concentration (defined as theoretical concentrations) were analyzed by UV spectrometer, obtaining absorption spectra in the range 200-325 nm.

The curve used to fit mixtures spectra is expressed by equation (3), in which H(λ) is given by the sum of the two curves fitting TP and CAP spectra (i.e. $j = 1, \ldots, NC$

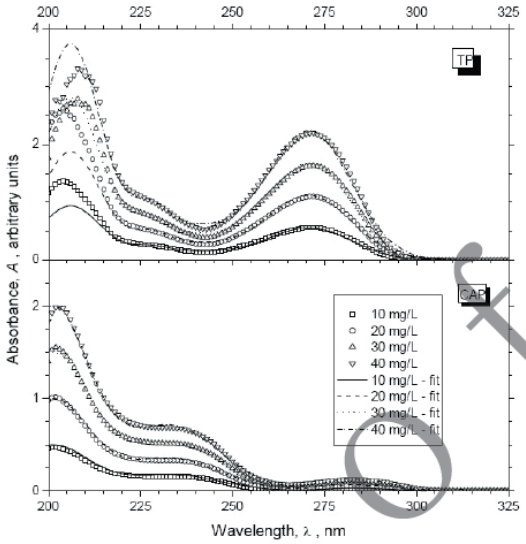

Figure 1. Experimental (symbols) and fitting (curves) spectra of TP and CAP for concentration of 10 mg/L, 20 mg/l, 30 mg/L, 40 mg/L.

Table 1. Curve fitting parameters (to be used in equation 3).

	TP	CAP
Gaussian 1		
λ_{01}	271.4	233.0
ω_1	26.0	31.6
k_1	0.05216	0.01764
Gaussian 2		
λ_{02}	205.7	283.6
ω_2	18.9	22.2
k_2	0.08705	0.00327
Gaussian 3		
λ_{03}	234.2	201.0
ω_3	53.1	23.3
k_3	0.01479	0.04903
Gaussian 4		
λ_{04}	227.8	
ω_4	11.3	
k_4	0.01004	

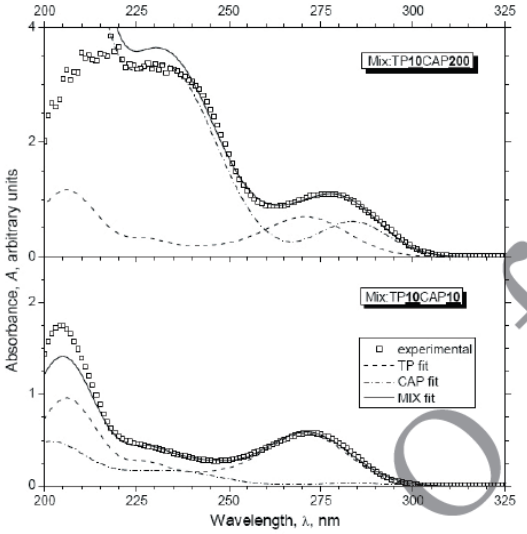

Figure 2. Fitting of experimental spectra for two different mixtures: a mix with 10 mg/L of TP and 10 mg/L of CAP (bottom) and a mix with 10 mg/L of TP and 200 mg/L of CAP (up).

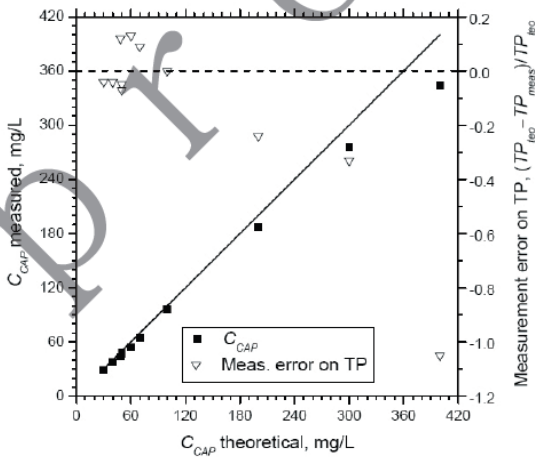

Figure 3. Trends of measured CAP concentration (on the left axis) and of measurement error on TP (on the right axis) against theoretical CAP concentration.

with $NC = 2$), respectively. The range of wavelength (λ) of good overlap between fitting curves and experimental spectra changes with CAP concentration: the range of 225-325 nm was used for low concentrations until 100 mg/L and the range of 250-325 nm was chosen for higher concentrations, as displayed in Figure 2. The fitting is shown in Figure 2, in which each curve was obtained by the sum of Gaussian distribution curves (N=3 for CAP and N=4 for TP), as before reported.

$$H(\lambda) = \sum_{j=1}^{NC} \left\{ \sum_{i=1}^{N_j} H_{0i,j}(C_j) \exp\left[-4\ln(2) \left(\frac{\lambda - \lambda_{0i,j}}{w_{i,j}} \right)^2 \right] \right\} \quad (3)$$

An Excel code was written to find the minimum of the sum of square differences between experimental absorbances of mixtures and the calculated values (by equation 3). The optimized parameters were the wanted concentration of both TP and CAP in mixture (C_j).

The method was highly reliable for CAP concentration less than 100 mg/L. This is clear from Figure 3, where the measured CAP concentration is reported versus the theoretical CAP concentration (on the left axis), as well as the percentage error in measuring the TP concentration (on the right axis). Thus, the method requires sample dilution if it gives CAP concentration higher than 100 mg/L.

Conclusion

A method for simultaneous measurements of theophylline and cellulose acetate phthalate concentrations in phosphate buffer by means of UV spectrometry was pointed out and presented. The spectra were fitted by the sum of two curves, one for each component, which in turn were made up by two sums of Gaussians, tuned by comparison with pure component spectra.

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Enteric coated micro-particles for targeted and controlled release

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ABSTRACT SUMMARY

This work is focused on production of enteric coated microparticles for oral administration, using a water-in oil-in water ($W_1/O/W_2$) solvent evaporation technique. The active agent theophylline (TP) is encapsulated in cellulose acetate phthalate (CAP), a pH-sensitive polymer available on the market, which is insoluble in acidic media, but dissolves at neutral pH (above pH 6). Some preliminary tests are carried out using a novel synthesized pH-sensitive poly(methyl methacrylate-acrylic acid) copolymer, P(MMA-AA), which is checked to replace CAP in theophylline enteric coated microparticles.

INTRODUCTION

Oral controlled-release multiple-unit dosage forms (e.g. pellets, granules or microparticles) offer several advantages over single-unit dosage forms (e.g. capsules or tablets). Local drug concentration and risk of toxicity can be avoided by the use of multiple-unit dosage forms which can spread uniformly throughout the gastrointestinal tract (GIT). Microencapsulation is used to modify or to retard drug release. It offers greater effectiveness, lower toxicity and more lasting stability than conventional formulations [1].

Many microencapsulation processes are modifications of the three basic techniques: solvent extraction/evaporation, phase separation (coacervation) and spray-drying. Spray-drying is relatively simple and of high throughput but must not be used for highly temperature-sensitive compounds. Moreover, control of the particle size is difficult, and yields for small batches are moderate. Coacervation is frequently impaired by residual solvents and coacervating agents found in the microspheres. Solvent extraction/evaporation neither requires elevated temperatures nor phase separation inducing agents [2]. The water-in oil-in water emulsion (W/O/W) solvent evaporation is the most used method to encapsulate water soluble drugs in order to increase the encapsulation efficiency [3].

Microcapsules and microspheres can be engineered to gradually release active ingredients. A coating may also be designed to open in the specific areas of the body [4]. It would be highly beneficial if the active agents were delivered by a system that sensed the signal caused by disease, judged the magnitude of signal, and then acted to release the right amount of drug in response. Such a system would require coupling of the drug delivery rate with the physiological need by means of some feedback mechanism [5]. The environment-sensitive polymers, called "smart" polymers, are ideal candidates for developing self-regulated drug delivery systems.

Maybe the most important stimulus which can occur in physiology is the change in environment pH. The pH-sensitive polymers have been most frequently used to develop enteric coated formulations for oral administration. The pH in the stomach (< 3) is quite different from the neutral pH in the intestine, and such a difference is large enough to elicit pH-dependent behavior of polyelectrolyte polymer. Enteric coated products are designed to remain intact in the acidic juices of the stomach and then to release the drug at the higher pH of the small intestine (above pH 5.5) or at the even higher pH in the colon (above pH 6.5): the effectiveness of the drug will be reduced by stomach acids or enzymes if left unprotected [6].

Polymers with different chemical structure are available on the market to give the dissolution at different pH values. Recently, copolymers of MMA and AA were synthesized, and they can dissolve at tailored pH values only changing the volumetric percentage of methyl methacrylate (MMA), giving the drug release in correspondence of the desired pH target [7].

The ideal drug release should be insignificant at low pH, then controlled in neutral media. CAP dissolved immediately at pH above 6.0 [8], therefore the drug is immediately released in the intestine. The novel synthesized P(MMA-AA) [7], with 70% of MMA, first swells and then dissolves at neutral pH, therefore it could be useful in realizing controlled release devices to replace CAP as coating agent.

EXPERIMENTAL METHODS

Materials. For the co-polymer synthesis: Methylmethacrylate (MMA, CAS Number: 80-62-6) and Acrylic acid (AA, CAS Number: 79-10-7) are purchased from Sigma-Aldrich; initiator 2,2'-azobis 2,4-dimethylvaleronitrile (AMVN, CAS Number: 4419-11-8) is a Cayman Chemical Company product.

For micro-particles preparation: Cellulose acetate phthalate (CAP, CAS Number: 9004-38-0), Theophylline (TP, CAS Number: 58-55-9), Methyl ethyl ketone (MEK, CAS Number: 78-93-3), Tween 80 (CAS Number: 9005-65-6), Span 80 (CAS Number: 1338-43-8), all by Sigma Aldrich; and HydroxyPropyl-MethylCellulose (HPMC, Methocel K15M Premium Grade, kindly supplied by Colorcon) are used. Deionized water is used for all of the experiments.

Preparation. Microspheres are prepared by double emulsion ($W_1/O/W_2$) solvent evaporation method. The internal aqueous phase (W_1) was made of 40 mL of distilled water, 0.2 g of TP and 0.3 g of HPMC (the HPMC is added with the aims of increasing the viscosity

of W_1, of simplifying the micelle formation during the first mixing and of retarding the release after the pH raise in the intestine). 10 mL of W_1 are emulsified at room temperature with 40 mL of organic phase (O) for 1 minute using an ultrasonic disruptor (Vibra Cell 130 Watt Ultrasonic processor, Sonics & Materials, USA). The organic phase consisted of 400 mg of CAP dissolved in 40 mL of MEK. A drop of Span 80 was mixed with the oil phase. 5 mL of W_1/O are then emulsified with 45 mL of external aqueous phase W_2 (W_2: 100 mL distilled water with a drop of Tween 80 and some drops of hydrochloric acid). The resulting $W_1/O/W_2$ emulsion is mixed with a magnetic stirrer at room temperature for 24 hours and then placed in a rotavapor (Laborota 4002 control, Heidolph), under vacuum, with a temperature increase from 20°C to 50°C within 25 minutes and a speed of 18 rpm, to allow the solvent to evaporate. The suspended micro-particles are washed thrice with distilled water by centrifugation at 6000 rpm for 5 minutes. The final product is obtained by evaporation of residual water in an oven at 60°C.

Dissolution. To test the enteric nature of micro-particles and the drug release, a weighted amount is put in 75 mL of 0.1 N hydrochloric acid (pH 1), magnetically stirred at room temperature; after about 75 min, 25 mL of 0.2 M tribasic sodium phosphate are added in order to reach pH 6.8. The TP contents are assayed by fitting the spectra collected for wavelength between 200 and 400 nm, by an UV-visible spectrometer (Lambda 25 by Perkin Elmer), following a method pointed out in a previous work [9]. A similar protocol will be followed in testing the novel P(MMA-AA) micro-particles produced.

RESULTS AND DISCUSSION

Theophylline release from CAP coated micro-particles follows the expected behavior. Figure 1 showed a low percentage of TP released in the acidic media, then a complete and instantaneous TP release after reaching pH 6.8 owing to the sudden dissolution of CAP.

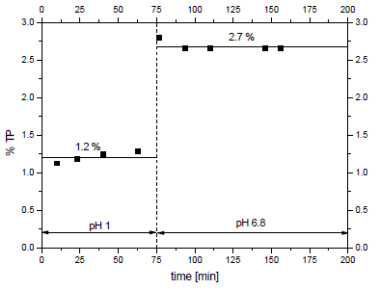

Figure 1. Dissolution profiles of CAP micro-particles: first at pH 1, after 75 min at pH 6.8. The percentage of TP released was referred to the ratio between mass of TP released and total mass of micro-particles putted in dissolution media.

The expected amount of micro-particles was 525 mg: 50 mg of TP (200/4), 75 mg of HPMC (300/4) and 400 mg of CAP. The obtained results are:

- Powder theoretical TP load ratio: 9.5% (50 mg/(525 mg = 50 mg TP + 75 mg HPMC + 400 mg CAP)
- Powder actual TP load ratio: 2.7 % (which is the TP fully released at neutral pH, from Figure 1);
- Powder encapsulated TP load ratio: 1.5% (which is the difference between the percentage of TP released at pH 6.8 and the percentage released at pH 1.0);
- Yield of encapsulation: 55% (ratio between encapsulated TP and loaded TP).

Once the preparation method has been proven able to produce enteric coated micro-particles, some preliminary tests are performed replacing the CAP with the P(MMA-AA) copolymer, produced in the frame of the present research [7]. Some difficulties arise because of the solvents which needs to dissolve the copolymer in the O solution (the solvent should dissolve the ionic AA fraction, but it should be not water miscible, to allow the production of the double emulsion). The first results seems encouraging, even if the desired release pattern is not obtained yet.

CONCLUSION

In this work, a method to prepare enteric coated micro-particles is pointed out using a conventional agent for coating, the Cellulose Acetate Phthalate. The method is pointed out with the aim of replacing this polymer with a novel synthesized copolymer of MMA and AA, which dissolution pH could be tailored by varying the MMA/AA ratio. The preliminary results are interesting.

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