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Preparation of Prolonged Release Clarithromycin Microparticles for Oral Use and Their *In Vitro* Evaluation

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Prolonged release microparticles of clarithromycin (CL) were prepared using Eudragit *RL 100* and *RS 100* by spray-drying and casting-drying techniques. For the characterization of those microparticles, preparation yield, particle size distribution, X-ray diffraction, thermal behavior, active agent content and *in vitro* dissolution from the microparticles were performed. HPLC was used for the assay of clarithromycin and the assay method was validated. All the formulations obtained showed prolonged release when compared to pure clarithromycin. Microparticles prepared by spray-drying method had a slower release compared to those of casting-drying method. Spray-drying method seems to be a more suitable method to prepare microparticles for prolongation in release.

Key words: Prolonged release, Microparticle, Clarithromycin, Eudragit *RL 100* and *RS 100*, Spray-drying, Casting-drying

INTRODUCTION

Clarithromycin (CL) is a semi-synthetic macrolide antibiotic. It exerts its antibacterial action by binding to the 50S ribosomal subunit of susceptible organisms and by inhibiting protein synthesis. It is also used in the treatment of leprosy, upper tunistic mycobacterial infections, respiratory tract, skin and soft-tissue infections. It is administered orally, 250 mg twice daily, increased to 500 mg twice daily if required. It is rapidly absorbed from the gastrointestinal tract and its biovailability is about 55% following oral administration and undergoes first-pass metabolism (Barradell *et al.*, 1993; Conte *et al.*, 1995; Louis, 1997). High-performance liquid chromatography (HPLC) is routinely used for the selective and accurate determination of CL in pharmaceutical matrices (Morgan *et al.*, 1991; Chu *et al.*, 1991; Rotsch *et al.*, 1991).

There are several benefits for using controlled drug delivery systems over the conventional pharmacetical dosage forms: reduction in drug plasma level fluctuations, reduction in adverse side effects and improvement in tolerability, patient comfort and compliance and, finally, reduction in healthcare costs. For this purpose, Bele *et al.* have prepared silica-coated particles of CL. The decrease in the drug dissolution from the formulation of silica and cetylpyridinium chloride (CPC) in comparison to the other samples can be explained by the less permeability of the coating formed in the presence of CPC, either due to the inherent lower porosity or increased thickness of the coating owing to the better coverage of the side planes of CL particles (Bele *et al.*, 2005).

The efficacy and tolerability of CL modified release tablets (MR) (500 mg) administered once daily versus immediate-release tablets (IR) (250 mg) administered twice daily for 5 days were compared. MR was found to be as effective as standard IR in the treatment of lower respiratory tract infections (Adam *et al.*, 2001). IR and the granules for suspension formulations are dosed twice daily for respiratory tract infections. An extended release CL formulation (ER) has been developed to facilitate acceptability by reducing dosing frequency and perhaps improve gastrointestinal (GI) tolerability (Guay *et al.*, 2001).

In order to characterize the amorphous CL obtained by grinding and spray-drying, physicochemical properties were evaluated by Yonemochi *et al.*, 1999. From powder X-ray diffraction, it was estimated that the crystalline state

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of CL was changed to an amorphous state by grinding and spray-drying.

A common method for the controlled release of drugs is the use of microparticles of carrier-drug composites with different polymers using supercritical fluid (Martin *et al.*, 2002) and spray-drying technology (Kim *et al.*, 2005; Mu *et al.*, 2005).

In this study, 8 formulations of microparticles were prepared, using two different vehicles, two different drug-vehicle ratios and 2 different methods, to investigate the effects of vehicle and preparation method used on the release of CL. The polymers used were Eudragit *RL 100* and *RS 100* and the methods were spray-drying and casting-drying. For the characterization of the formulations, preparation yield, particle size distribution, X-ray diffraction, thermal behavior, active agent content and *in vitro* dissolution from the microparticles were performed. Flow-through-cell method defined in USP XXVI was used in *in vitro* dissolution tests. The quantification method, HPLC, was validated (Morgan *et al.*, 1991; USP 26-NF 21, 2003).

MATERIALS AND METHODS

Materials

Clarithromycin (Ambfar, The Netherlands), Eudragit *RL 100* and *RS 100* (Röhm pharma), sodium dihydrogen phosphate (Carlo Erba), phosphoric acid (Merck) and methanol (Merck) were used. All other chemicals were of analytical grade.

Apparatus

HPLC (Shimadzu LC-10AT VP), flow-through-cell apparatus (Sotax CE 7), pH-meter (Orion, Shimadzu), mastersizer (Malvern, Hydro 2000S), differential scanning calorimeter (Shimadzu-DSC 60), FT-IR spectrometer (Perkin Elmer, Spectrum 2000), mini spray-dryer (Büchi, Model 190), X-ray diffractometer (Rigaku, Rint 2200) and vibration sieve (Elektro Mag, Türkiye) were the equipments used in this study.

Preparation of microparticles

Two different methods, namely casting-drying and spray-drying methods, were used for the preparation of CL microparticles. Active agent-vehicle ratios and the vehicles were kept the same for the two methods. The microparticle formulations are given in Table I.

Casting-drying method

The vehicle was solubilized in dichloromethane with 500 rpm magnetic stirring and at a temperature of 25°C. CL was added into the vehicle solution under the same conditions. The solution obtained was poured into petri dishes and evaporated in a 50°C oven. The dry residue was collected and sieved through 100 μm at 30 rpm for 5 min with the vibration sieve.

Table I. Microparticle formulations of clarithromycin

Vehicle	Preparing Method	Drug/vehicle Ratio (w/w)	Code Number
Eudragit RS 100	Casting-drying	1:1	F1
		1:2	F2
	Spray-drying	1:1	F3
		1:2	F4
Eudragit RL 100	Casting-drying	1:1	F5
		1:2	F6
	Spray-drying	1:1	F7
		1:2	F8

Spray-drying method

The same procedures were conducted to obtain the active agent-vehicle solution as described in Table I. The total concentration of the polymer and drug in the 4% organic solution was stirred at room temperature using a magnetic stirrer until all the components were completely dissolved. A mini spray-dryer with a standard nozzle (0.7 mm diameter) was used to produce the dry powders of various formulations. The compressed air for atomization was 1 atm. The liquid feed was pumped continuously with the rate of 7 $mL \cdot min^{-1}$. Both the inlet and the outlet temperatures were measured and controlled manually; inlet air temperature of 50±2°C, outlet temperature of 38±2°C (Mu *et al.*, 2005).

All the formulations obtained were kept in a dessicator with $CaCl_2$ at atmospheric pressure and room temperature until analysis and use in dissolution tests.

Tests on microparticles

Preparation yields, CL quantification, particle size, X-ray, IR, thermal analyses and *in vitro* dissolution studies were performed.

Assay method and its validation

HPLC method with a 210 nm DAD detector and 150 × 6.0 mm Shim-pack CLC-ODS column was used for CL assay (Morgan *et al.*, 1991; USP 26-NF 21, 2003). Flow rate was about 1.0 $mL \cdot min^{-1}$, column temperature was 50.0°C and injection volume was 20 μL. Methanol and 0.067 M monobasic potassium phosphate (650:350) mixture was used as the mobile phase (adjusted to pH 4 with phosphoric acid) (Morgan *et al.*, 1991; Chu *et al.*, 1991; Rotsch *et al.*, 1991; USP 26-NF 21, 2003). Validation i.e. the performance characteristics of the analytical method require accuracy, precision, specificity, detection limit, quantitation limit and linearity (USP 26-NF 21, 2003;

Beuving, 2001; Pav *et al.*, 2002; Reiley and Fell, 1996).

Linearity

10.0 mg CL was weighed accurately and dissolved in 10 mL 0.1 M sodium acetate buffer (pH 5) (5000 $\mu g \cdot mL^{-1}$ stock solution). Six samples of 10.0-100.0 μL were taken from this stock solution and diluted to 1.0 mL with the mobile phase (50-500 $\mu g \cdot mL^{-1}$ solutions). CL peak responses of these samples were determined. Regression equation and regression coefficients were calculated (n=3).

Accuracy

It was calculated as the percentage of recovery by the assay of the known amount of analyte which contained 50%, 100% and 150% active agent in the sample, using the regression equation (n=6).

Precision

50, 300 and 500 $\mu g \cdot mL^{-1}$ solutions were prepared using stock solution of CL. The peak responses of these samples were measured. The relative standard deviation (coefficient of variation) of a series of measurements was calculated. The same procedure was performed on consecutive days (k=3).

Specificity

Method selectivity was assessed by the analysis of eight placebo formulations at the same assay conditions.

Sensitivity (Detection and quantitation limits)

The limit of detection is the lowest concentration of the analyte which can be detected in a sample. Limit of quantitation is the parameter of quantitative assays for the low level compounds in sample matrices, such as impurities in bulk drug substances and degradation products in finished pharmaceuticals.

Particle size analyses

Particle size distributions of formulations (F1-F8) were analyzed using a laser diffraction technique. Samples prepared in distilled water were sonicated for 1 min before measurement (n=3).

X-ray analyses

X-ray diffraction patterns of CL, Eudragit *RS 100*, Eudragit *RL 100* and formulations (F1-F8) were measured by X-ray diffractometer at 5-40°. Crystallinity of powder, vehicles and the formulations were determined at 2 degree.min^{-1} scanning rate, 40 kV and 30 mA with Rigaku generator.

IR analyses

For infrared spectra, KBr disks were used for CL, Eudragit *RS 100*, Eudragit *RL 100* and the formulations (F1-F8). All samples were scanned in the range of 400-4000 cm^{-1}.

Thermal analyses (Differential scanning calorimetry, DSC)

DSC measurements were performed using an empty aluminum pan as a reference, at a nitrogen gas flow rate of 40 $mL \cdot min^{-1}$ and a temperature increase rate of $10°C \cdot min^{-1}$. The analysis was achieved at the range of 40-240°C.

In vitro dissolution studies

Parameters of the dissolution tests were as follows (USP XXVI): flow-through-cell method (Apparatus IV); 0.1 M sodium acetate buffer (pH 5); 37±0.5°C and flow rate of 8 $mL \cdot min^{-1}$. Amounts of formulations corresponding to 125 mg of CL were used for the dissolution tests. Samples were withdrawn automatically at appropriate time intervals and the amount of CL released into the medium was determined by HPLC (n=6). In the preparation of the flow-through-cells, equal volumes of glass beads and the microparticles were put in the cells.

RESULTS AND DISCUSSION

RSD values of the validation procedure have to be approximately 1% (USP 26-NF 21, 2003; Beuving 2001). As shown in Table II, the validation parameters obtained are suitable for the HPLC method employed in this study. Reproducibility refers to the use of HPLC assay method for CL in different laboratories, as in a collaborative study. An important step in the validation of any analytical method is the establishment of the relationship between released % (y) and the concentration of the analyte (x). When the correlation coefficient is above 0.9990, the assay method is acceptable. The satisfying recoveries confirm the suitability of the proposed method for the routine analysis of CL in pharmaceuticals (Table II) (USP 26-NF 21, 2003; Pav *et al.*, 2002; Reiley and Fell, 1996). In the

Table II. Validation results of HPLC

Concentration ($\mu g \cdot mL^{-1}$)	Precision RSD % (k=3, n=6)		Accuracy (%) ± SE (n=6)	Sensitivity ($\mu g \cdot mL$-1)
	Repeatability	Reproducibility		
50	4.83	5.29	99.47 ± 1.08	LOD 14.04
300	1.33	1.36	102.21 ± 0.72	LOQ 42.55
500	2.32	2.44	102.04 ± 1.07	
Linearity	Regression equation		y = 992.4 x + 417.5 (n=3)	
	Regression coefficient		r = 0.9998	

y: area (peak response), x: concentration, LOD: Limit of Detection, LOQ. Limit of Quantitation

Table III. Yield, CL content and mean particle size of microparticles

Formulations	Yield (%) ± SE (n=2)	CL content (%) ± SE (n=3)	Particle size (μm) ± SE (n=3)
F1	82.69 ± 6.66	46.50 ± 0.88	17.79 ± 0.17
F2	76.09 ± 16.66	28.33 ± 0.44	154.19 ± 0.81
F3	35.15 ± 1.14	46.78 ± 0.42	11.45 ± 0.16
F4	22.85 ± 12.32	32.17 ± 0.93	9.37 ± 0.04
F5	74.04 ± 15.47	60.78 ± 6.30	54.00 ± 0.35
F6	77.66 ± 8.82	32.28 ± 1.40	120.13 ± 0.72
F7	25.14 ± 18.37	47.44 ± 1.13	6.98 ± 0.02
F8	28.33 ± 14.75	41.17 ± 6.33	6.99 ± 0.01

specifity results, no interferences from the all placebo formulations were observed at the retention time and or the specific detection window of each analyte. According to the results, the proposed method is able to access to the analyte in the presence of common excipients and hence it can be considered specific. Results in Table II indicate good detection limit for CL.

Spray-drying offers an one-step process with the advantages of the less time and cost consumption, less preparation steps and good process control. With those facts in mind, this method was selected among the many methods to prepare the microparticles of CL. Total CL loaded into the products were 28-60% and 32-47% for the casting-drying and spray-drying, respectively. Preparation yields were higher for the casting-drying method (74-82%) compared to spray-drying method (22-35%). The low yield with the spray-drying technique may be due to the small quantity of the formulation leading to adherence of microparticles to the glass collector of the spray-dryer. When the size distribution profiles of powder CL and microparticle formulations were investigated, mean particle sizes of microparticles prepared with casting-drying and spray-drying method were found to be 17-154 μm and 6-11 μm, respectively. While preparation yields and particle sizes were higher for the casting-drying method compared to spray-drying method, CL content did not show any significant difference between the two methods (Table III).

Smaller particle size of spray-dried products results in higher porosity and poor flowability. Spray-drying has been succesfully used in the preparation of drug delivery systems made from biodegradable polymers, such as polylactic acids, methacrylic polymers like commercial Eudragits®, cellulose derivatives and some natural polymers (Oneda and Ré 2003).

According to the IR analysis results, preparing method, excipient and the active substance did not interact. The IR spectra of CL showed characteristic "C=O" (carbonyl) stretching bands at 1733-1692 cm^{-1}, -O- ether function bands at 1170-1053 cm^{-1}, alkyl-CH_3 substitution bands at 2974-2940 cm^{-1}, OH bands at 3473 cm^{-1} region. OH bands changed according to the preparation method and drug-polymer ratio, since OH can make H bands which has different numbers in different formulations. For this reason, microparticles formulated showed different solubility properties because of varying 0H bands, which is in accordance with a previous study (Perng *et al.*,1998).

Crystallization behavior of the drug and polymers after microparticle formation was examined by X-ray diffractometry. X-ray patterns of pure powder drug, polymers and microparticle formulations were compared. The results obtained are shown in Fig. 1 and Fig. 2. X-ray diffraction data of CL and the polymers have led to the fact that CL has the crystalline state, while Eudragit *RL 100* and *RS 100* have amorphous states. When X-ray patterns were examined, cristallinity of the drug was found to decrease in formulations F1, F2, F3, F5 and F6, F4, F7 and F8 which showed similar patterns with polymers. This showed that CL did not maintain its physical state. It seems that preparation parameters affect the crystallinity of CL (Yonemochi *et al.*, 1999; Li, *et al.*, 2005).

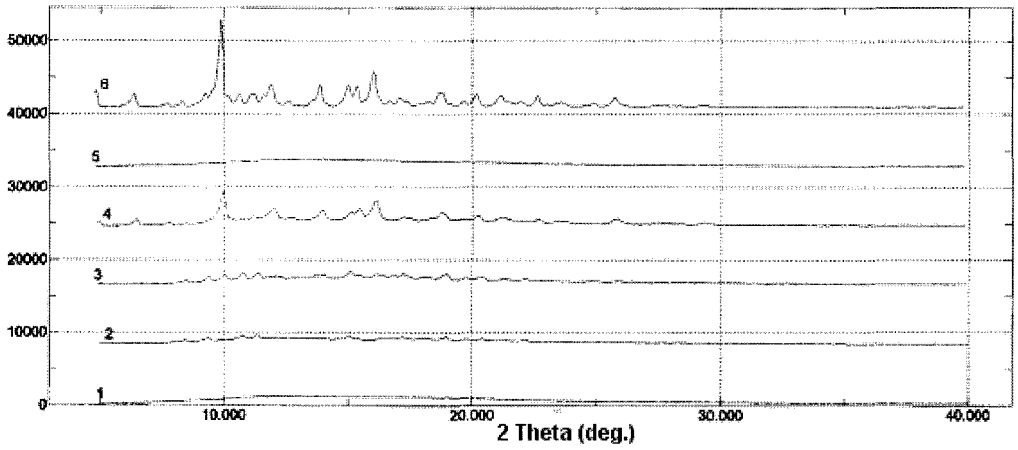

Fig. 1. X-ray diffraction patterns of CL (6), Eudragit RS 100 (5), F1 (4), F2 (3), F3 (2), and F4 (1) formulations

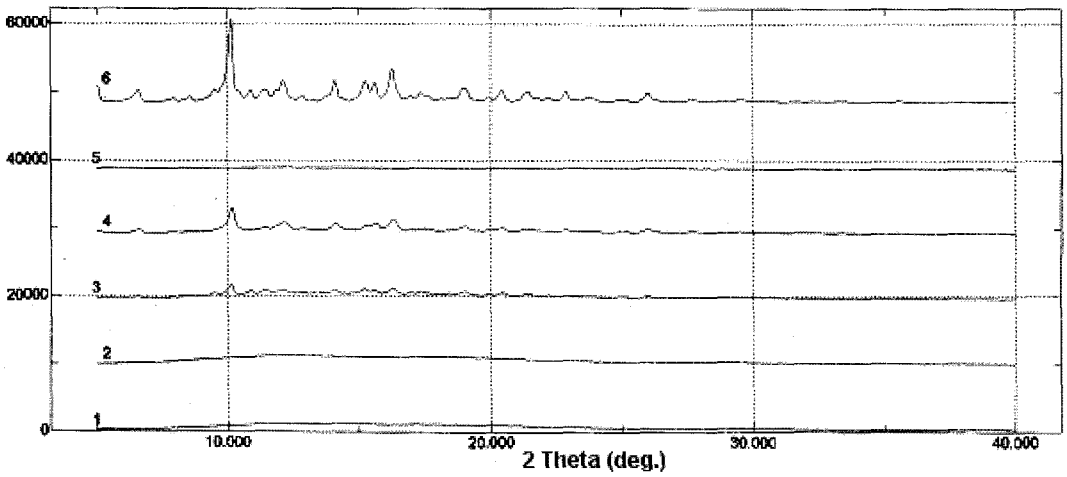

Fig. 2. X-ray diffraction patterns of CL (6), Eudragit RL100 (5), F5 (4), F6 (3), F7 (2), and F8 (1) formulations

The thermal property of mixtures of a drug and an excipient is of important interest in the pharmaceutical technology and this can normally be processed by the information obtained such as melting, recrystallisation, decomposition, out-gassing, or a change in heat capacity. This information can help to ascertain the physicochemical status of the entrapped drug in the excipient, to assess the interaction amongst different components during the fabrication process and to explain relevant properties of *in vitro* release. Fig. 3 and Fig. 4 demonstrate the DSC thermograms of pure material, Eudragit *RS 100*, Eudragit *RL 100* and microparticle formulations. The endothermic peak of melting of CL is at about 230°C. However, excipients did not show any endothermic peak. This shows that the excipients and the preparation methods did not affect the properties of CL (Yonemochi *et al.*, 1999; Mu *et al.*, 2005).

One of the important objectives of the pharmaceutical technology is to secure the stability and the effectiveness of the pharmaceutical products. Crystallinity is known as

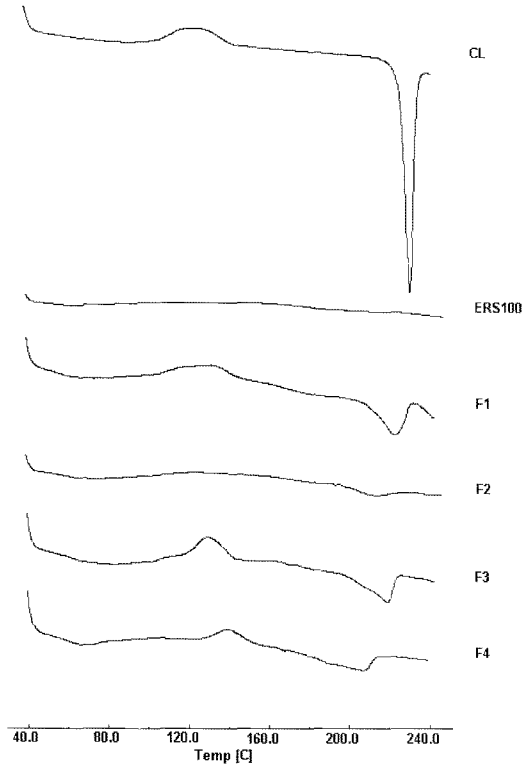

Fig. 3. DSC thermograms of CL, Eudragit RS 100 , F1, F2, F3, and F4 formulations

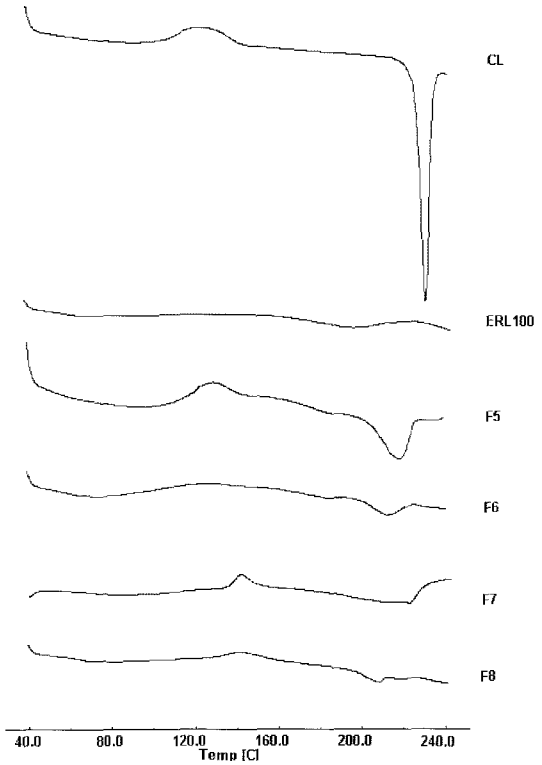

Fig. 4. DSC thermograms of CL, Eudragit RL100, F5, F6, F7, and F8 formulations

one of the important physicochemical properties of drug substances, to affect physicochemical stability, solubulity and absorption of drug substances as well as compression properties in the manufacturing process. It is known that the amorphous form of a drug substance is produced by the grinding of a single component, grinding it with some excipients, mixing it with porous substances, freeze-drying, compression, desorption of water of crystallization or spray-drying. The dissolution behavior and the bioavailability of water insoluble drugs could be improved by producing them in an amorphous form, since the amorphous form is more soluble than the crystalline form (Yonemochi *et al.,* 1999). For detecting the amorphous state of materials, infrared, X-ray absorption and DSC can be employed. Powder X-ray diffractometry is also useful to investigate the crystalline and amorphous characteristics. Our results suggested that the ground amorphous CL could have imperfections in the crystalline lattice. The amorphous states of CL prepared by both casting and spray-drying methods were different in physicochemical properties, dissolution and solubility parameters. It is generally accepted that drug release from the microparticles is strongly dependent on polymer crystalline behavior and drug dispersion state (Yonemochi *et al.,* 1999; Li *et al.,* 2005).

CL is stable in aqueous solutions of pH 5.0-8.0. At pH 2.0, the degradation half-life was 1.3±0.05 h. In gastric juice samples of pH 2.0, the degradation half-life was 1.0± 0.04 h (Erah *et al.,* 1997). The solubilitiy of amorphous CL in the phosphate buffer solution at 37°C decreased with increasing pH. In the acidic solution, decomposition of amorphous CL obeyed the pseudo-first order kinetics. In pH 1.39, amorphous CL degraded with a half-life of 17 min (Nakagawa *et al.,* 1992). Depending on these data, 0.1 M sodium acetate buffer (pH 5) was used in the dissolution tests. Prolonged release profile was obtained with microparticle formulations when compared to pure CL (Fig. 5 and Fig. 6). When the dissolution profiles were examined, no significant variation was found between the two methods of preparation. Influence of the vehicle type on the dissolution profile of CL can not be clearly seen. Equal volumes of glass beads and microparticles were put in the cells because loose powder bed due to homogeneous mixture of glass beads with microparticles enables the dissolution medium to easily pass through the bed and by the particles. This promotes the homogenous contact and wetting of each particle.

Conclusively, all the formulations showed prolonged release when compared to pure CL. While more rapid release of amorphous CL from small particle sized spray-dried microparticles is normally expected, the opposite was observed and the microparticles prepared by spray-drying method showed a slower release compared to the

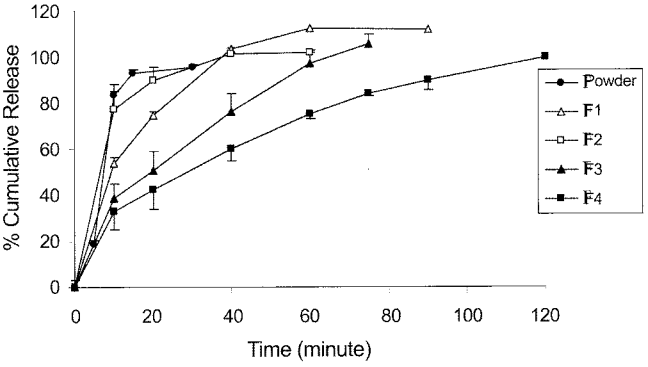

Fig. 5. Dissolution profiles of pure CL (powder), F1, F2, F3, and F4

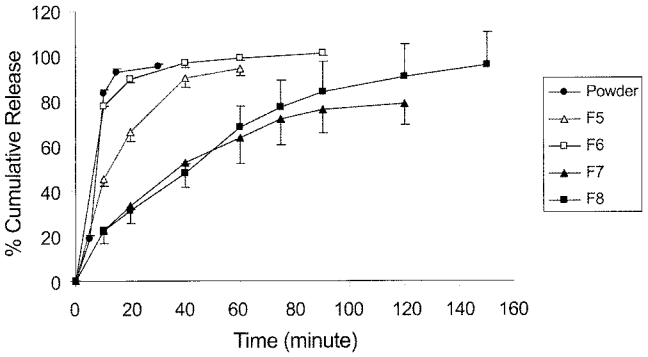

Fig. 6. Dissolution profiles of pure CL (powder), F5, F6, F7, and F8

casting-drying method. This may be attributed to the removal of CL from the vehicle during the steps involved in the casting-drying technique. The spray-drying method seems to be a more suitable method to prepare microparticles for prolongation in release.

CONCLUSION

All the dissolution profiles from the microparticles obtained in this study indicated a prolonged release of CL. Microparticles prepared by the spray-drying method had a slower release compared to the casting-drying method. The spray-drying method seems to yield microparticles for prolonged release of CL.

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