ISSN 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

Available online at: http://www.iajps.com *Research Article*

PREPARATION AND EVALUATION OF CIPROFLOXACIN DRY EMULSION CONTAINING SUSTAINED RELEASE BEADS BY IONTOPHORETIC GELLATION METHOD

Patil Bhagyashree Subhash*, M. A. Bhutkar , Tina Raju, Tanushree Sarkar, Dr. S. K. Mohite

Rajarambapu College of Pharmacy, Kasegaon. Dist-Sangli-415404.Maharashtra (India)

Abstract

The objective of the current investigation is to reduce dosing frequency and improve patient compliance by designing and systematically evaluating sustained release micro beads of Ciprofloxacin. Method: Ciprofloxacin-loaded microbeads were successfully prepared by ionotophoretic gellation and cross linking technique using sodium alginate as the hydrophilic carrier and calcium chloride as cross linking agent. Prepared beads were evaluated for particle size, swelling ratio, drying rate, drug entrapment, bio adhesion study, in vitro release kinetics. Differential scanning calorimetry, Fourier transform infrared spectroscopy were performed in order to determine the stability of the formulation and the presence of drug excipient interactions, Scanning electron microscopic studies were carried out to find the morphology of the microbeads. Particle size distributions of drug loaded formulations were measured by an optical microscope. stability study. Particle size distribution of both placebo and drug loaded formulations were measured by an optical microscope and particle size of optimized beads was determined by SEM. No significant drug-polymer interactions were observed in FT-IR studies. In-vitro drug release profile of Ciprofloxacin micro beads was examined in phosphate buffer pH 6.8.

Keywords: *Ciprofloxacin, HPMC, Chitosan, Dry Emulsion.*

Corresponding Author:

Patil Bhagyashree Subhash

Rajarambapu College of Pharmacy,

Kasegaon, Dist-Sangli-415404.

Please cite this article in press as Bhagya Shree et al. ***Preparation and Evaluation of Ciprofloxacin Dry Emulsion Containing Sustained Release Beads by Iontophoretic Gellation Method,*** *Indo American J of Pharm Sci 2015:2(1):552-558.*

INTRODUCTION

Drug delivery systems (DDS) that can precisely control the release rates or target drugs to a specific body site have had an enormous impact on the health care system. Carrier technology offers an intelligent approach for drug delivery by coupling the drug to a carrier particle such as Microbeads, Nanoparticles, Liposomes etc. which modulates the release and absorption characteristics of the drug microbeads constitute an important part of these particulate DDS by virtue of their small size and efficient carrier characteristics. However, the success of these Novel DDS is limited due to their short residence time at the site of absorption. It would, therefore, be advantageous to have means for providing an intimate contact of the DDS with absorbing membranes. The goal of any ideal drug delivery system is to provide a therapeutic amount of drug to the proper site in the body to achieve prompt response, and thus maintain the desired drug concentration. Such a conceptualized ideal drug delivery can be possible with intravenous infusion of drug at the site of action over a desired period of time [1, 2].

The solid carriers used to prepare dry emulsions are gelatin lactose, malto dextrin, mannitol, povidone, sucrose etc. Insoluble carrier like colloidal silica [3] can also be used. Dry emulsions can be prepared by spray drying [4], Lyophilization [5, 6] and rotary evaporation. The solid carrier may undergo partial or complete transformation into an amorphous state. Since the amorphous carrier exhibits a strong tendency to crystallize at a particular elevated temperature and relative humidity, physical stability problems may arise. Stability tests for amorphous solid carriers like lactose, malto dextrin, mannitol and sucrose will be carried out. To avoid stability problems water soluble polymers like hydroxyl propyl methyl cellulose, methyl cellulose and povidone used as solid carriers. The dry Emulsion formulation aim is to improve the bioavailability of drug substances and reduce their side effects [7, 8, 9]. Dry Emulsions are attractive because they are physically and microbiologically stable formulations. They represent a potential oral drug delivery system for lipophilic and low soluble drug substances [10]. Dry Emulsions are prepared by techniques like Lyophilization, Spray drying, and Rotary Evaporation. For preparing dry Emulsions the organic fillers used are Lactose, Mannitol, Malto-dextrins. Commonly used co-solvents are Polyethylene glycol, Propylene glycol, Glycerol etc. The thickening agents used are Natural and synthetic gums, Cellulose derivatives, colloidal silica. The sweetening agents used are Glucose, Aspartame, Sucrose etc. For preparing oil in water emulsions medium chain triglycerides are generally used as lipid phase so the preferred oils are sesame oil, olive oil and peppermint oil. Ciprofloxacin is a third generation Cephalosporin antibiotic; it has very low solubility in biological fluids. So to enhance the bioavailability and stability of drug it is formulated as a dry Emulsion After conducting preliminary studies on these water soluble polymers, it was concluded that dry emulsions with hydroxyl propyl methyl cellulose (HPMC) as solid carrier is most promising. For preparing dry emulsions lipidic solvents like fractionated coconut oil, Miglyol812, Capmul MCML–8, Phosal 53 MCT, sesame oil, Lecithin, almond oil etc., can be used. Water soluble polymer HPMC facilitated the emulsification of liquid o/w emulsions due to its ability to reduce the surface tension. As the concentration of HPMC in liquid O/W emulsions is increased a reduced droplet size distribution can be obtained.

MATERIAL AND METHODS

Ciprofloxacin was obtained as a gift sample from Micro lab, Mumbai LtD., Sodium Alginate was supplied by Loba chemie pvt. Ltd., Calcium chloride (CAS 10035-04-8) was supplied by Loba chemie pvt, Ltd. HPMC E5 obtained from On top pharmaceuticals, Bangalore. Olive oil, Sesame oil and Peppermint oil were obtained from Empire Scientific Company. Propylene glycol, Tween 80 and Span 80 were received from Karan scientific Company.

Preparation of Placebo Microbeads:

The microbeads were prepared by the ionotropic gellation technique. Microbeads were prepared by using sodium alginate alone and combination with coating polymers like gelatin and pectin and calcium chloride used as counter ion., 25 ml of a 2% w/v aqueous solution of sodium alginate was introduced drop wise from a glass syringe with a size-23 needle into 10 ml of an aqueous calcium chloride solution being stirred at 10 rpm. The concentration of $CaCl_2$ in the solution should be 2%w/v, allow the beads to be formed by running the stirrer for 15 min. Check the beads under microscope. Then rigidize the beads by adding 1ml of 25% solution of glutaraldehyde. Allow stirring for further 1 hour at 10 rpm. After stirring for one hour filter the solution and collect the beads. Obtained microbeads were washed with water and dried at 50ºC in an oven.

Preparation of Alginate–Gelatin Microbeads:

Four batches of drug loaded microbeads were prepared using optimized concentration of sodium alginate and gelatin (sodium alginate 1.75% and gelatin 0.25%) as a coating polymer. To 50ml of de-ionized water, gelatin was added and stirred with the

electric stirrer to form mucilage. Then sodium alginate was added to form uniform dispersion. Weighed quantity of ciprofloxacin was added and homogenized for 5 min. The resulting dispersion was dropped through syringe with needle into 10ml of 2%w/v aqueous calcium chloride solution and stirred at 10rpm. After stirring for 1 hour, the formed beads were separated by filtration, washed with distilled water, dried at 50 ^{0}C in an oven.

Table 1: Composition of Ciprofloxacin Microbeads

Formulation code	Sodium alginate	HPMC	Chitosan	Calcium chloride	Cross linking agent	Curing time
S	2.4%	-	-	3%	1%	1hrs
H1	1.4%	2%	-	3%	1%	1hrs
H2	1.65%	1.86%	-	3%	1%	1hrs
H3	1.74%	0.8%	-	3%	1%	1hrs
H4	1.88%	0.46%	-	3%	1%	1hrs
C1	1.4%	-	1.5%	3%	1%	1hrs
C2	1.65%	-	0.842%	3%	1%	1hrs
C3	1.74%	-	0.62%	3%	1%	1hrs
C4	1.88%	-	0.42%	3%	1%	1hrs

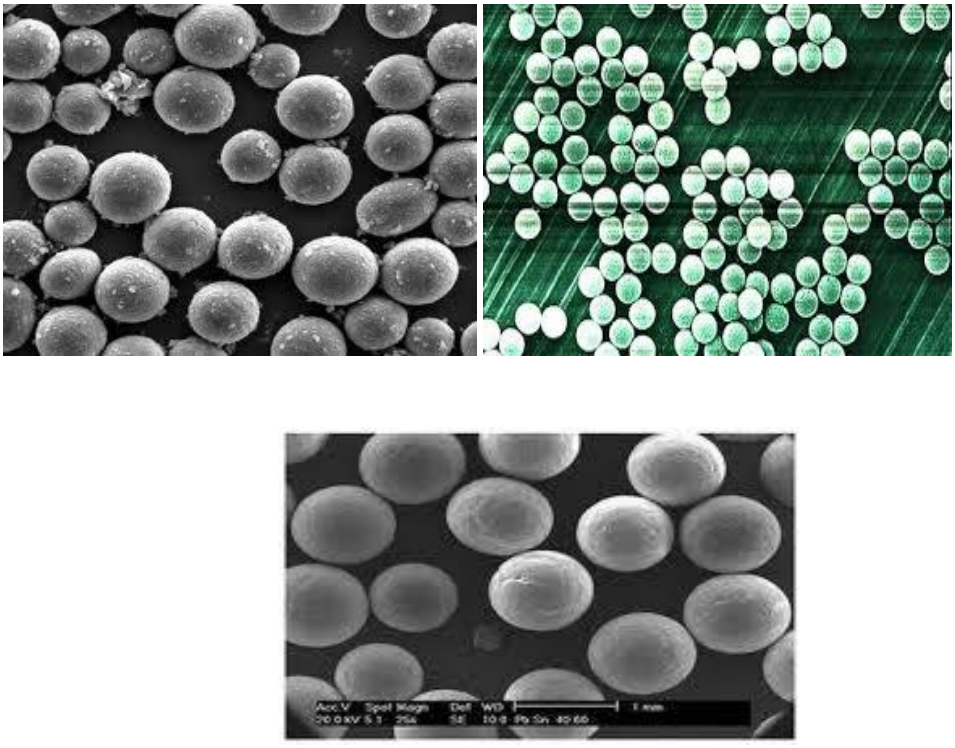

Fig.1 SEM Scanning images of Microbeads.

Evaluation of Drug Loaded Micro beads

Estimation of drug content and encapsulation efficiency

Ciprofloxacin content in the microbeads was estimated by a UV-Spectrophotometric method. Accurately weighed 50mg of microbeads were suspended in 100ml of phosphate buffer pH 6.8.The resulting solution was kept for 24hrs. Next day it was stirred for 15min. The solution was filtered, after suitable dilution, Ciprofloxacin content in the filtrate was analyzed at 235nm using Shimadzu 1201 UV-Visible spectrophotometer. The obtained was determined using following relationship;

$$\% \text{ Drug entrapment efficiency} = \frac{\text{Actual drug content}}{\text{Theoretical drug content}} \times 100$$

Absorbance was plotted on the standard curve to get the exact concentration of the drug. Calculating this concentration with dilution factor we get the percentage of actual drug content and entrapment efficiency.

Granulometric study:

The particle size has significant effect on the release profile of microbeads. Size distribution determined by sieve analysis was carried out on mechanical sieve shaker, using different meshes (#12, #16, #22, #30) of American society of testing materials (ASTM).

Particles that passed through one sieve but were retained on the other were collected and weighed and the distribution was analyzed based on the weight fraction on each sieve.

Loose Surface Crystal Study (LSC)

This study was conducted to estimate the amount of drug present on the surface of the micro beads which showed immediate release in dissolution media. 100mg of micro beads were suspended in 100ml of phosphate buffer (pH 6.8), simulating the dissolution media. The samples were shaken vigorously for 15min in a mechanical shaker. The amount of drug leached out from the surface was analyzed Spectro photometrically at 235nm. Percentage of drug released with respect to entrapped drug in the sample was recorded.

***In-vitro* release studies**

In-vitro release studies of prepared microbeads were carried out using phosphate buffer (pH 6.8) using USP- basket type apparatus. Accurately weighed quantity of 250 mg of prepared micro beads put into the basket rotated at a constant speed at 100rpm and maintained temperature 37±5ºC in 900ml of the dissolution medium (phosphate buffer pH6.8).The sample was withdrawn at 0.25hrs, 0.5hrs,1hrs, 2hrs, 3hrs, 4hrs, 5hrs, 6hrs, 7hrs,8hrs, 9hrs, 10hrs, 11hrs, 12hrs, 14hrs,18hrs and 24hrs. Each time interval 5 ml of sample was withdrawn, at the same time 5 ml of fresh dissolution media was added to maintain sink condition. The withdrawn samples were suitably diluted and measure the absorbance at 235 nm spectrophotometrically. Then calculate the cumulative percentage drug release at regular time intervals.

Drug content and entrapment efficiency

The result of drug content indicated that drug is uniformly dispersed in formulation. It was observed that, due to water insoluble nature of ciprofloxacin almost all the drug is entrapped in the polymer matrix resulted in higher drug content and encapsulation efficiency. Accurately weighed 50mg of drug-loaded microbeads were suspended in 100ml of simulated intestinal fluid of pH 7.2±0.4. The resulting solution was kept for 24 h. Next day it was stirred for 5 min and filtered. After suitable dilution, Ciprofloxacin content in the filtrate was analyzed Spectro photometrically at 235 nm using a Shimadzu 1201 UV-Vis spectrophotometer.

Size Distribution of Microbeads:

Size distribution of the microbeads was determined using standard test sieves (Filterwel, Mumbai, India). Particles that passed through one sieve but were retained on the other were collected and weighed. Distribution of particles was analyzed based on weight fraction on each sieve.

Swelling Properties:

Thirty beads were placed in a beaker containing 100ml of acidic buffer solution and then stirred with a magnetic stirrer at a speed 50 RPM. After 1h interval the equilibrium swollen beads were observed and measured under optical microscope. The magnitude of swelling was presented by ratio of the mean diameter of swollen beads to the mean diameter of the dried beads.

RESULTS AND DISCUSSION

Pre-formulation Studies:

Drug excipient compatibility studies: Before formulation of drug substances into a dosage form, it is essential that it should be chemically and physically characterized. Pre-formulation studies give the information needed to define the nature of the drug substance and provide a framework for the drug combination with pharmaceutical excipients in the fabrication of a dosage form. In this Compatibility studies one of the requirements for the selection of suitable polymers or carriers for pharmaceutical formulation is its compatibility.

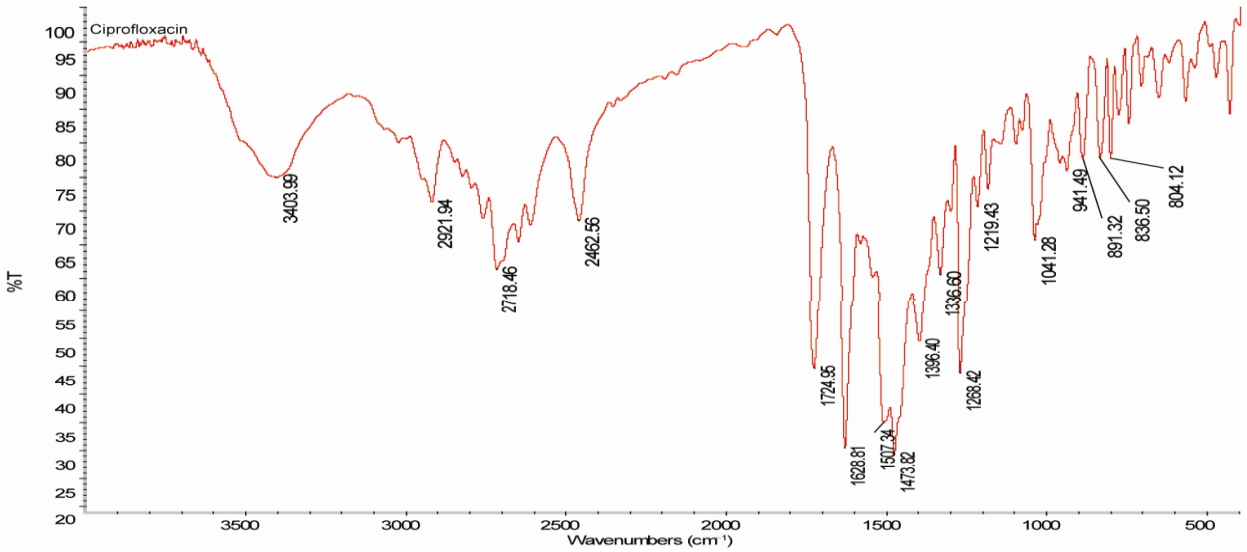

Fig 2: Infrared spectrum of Ciprofloxacin.

FTIR study: FTIR studies were performed by Potassium bromide pellet method.

Determination of melting point: Melting point of Ciprofloxacin was determined by capillary method.

Solubility: The solubility of ciprofloxacin was determined by adding excess but measured amount of drug in 100ml volumetric flask containing 7.2 phosphate buffer and kept under agitated conditions at 370c± 0.5 in water bath shaker for 2hrs. The dispersions were filtered through Whatmann filter paper and analyzed for the quantity of drug dissolved.

Ciprofloxacin pure drug analysis: The absorbance of the prepared solutions was checked using a UV spectrophotometer at 253 nm. pH 7.2 Phosphate buffer was used as the blank.

Characterization of Dry Emulsion

Drug content: Dry emulsion equivalent to 10 mg of drug is weighed accurately and dissolved in suitable solvent. Filter through Whattman filter paper no. 41. The stock solutions are diluted suitably. The drug content is analyzed by U V spectrophotometer.

Scanning electron microscopy:

SEM photomicrographs are taken by analytical scanning electron microscope for studying surface morphology.

Globule size determination:

Microscopic examination of the emulsion before and after reconstitution is observed.

Density:

The density of the dry emulsions was determined by helium pycnometry. For one determination each sample was measured seven times. A Pascal 140equipped with a dilatometer type CD3P was applied to determine the density of the dry emulsions by mercury porosimetry.

Moisture content:

Moisture content was determined by Thermo Gravimetric Analysis (Appro Adley Lima AN, 2008) approximately 15.00 –20.00 mg. samples were placed in the sample pan and the effluent gas was dry nitrogen. The scanning rate was 100C /min in the scan range 50-2000C. The moisture content can be determined as the weight loss between 50 and 1200C.

Surface characterization:

Scanning Electron Microscopy (SEM)is used to examine the outer macroscopic structure of the dry emulsion. Prior to microscopy samples were coated with gold/ palladium by sputtering for 300 Seconds in a Bio Rad, E5200 Auto Sputter coater. The samples were scanned at a voltage of 15 KV.

In-vitro Drug Entrapment Studies:

The formulations of dry emulsions were subjected to evaluation of drug entrapment. The % drug entrapment of dry emulsion formulation with peppermint oil (Formulation 4) was 99.3%, and with olive oil (Formulation 3) was found to be 98.5%, with Sesame oil using single surfactant (Formulation 1) was 97.1% and with combination of surfactants (Formulation 2) was 97.9%.this shows that ciprofloxacin dry emulsion formulation with peppermint oil shows high drug entrapment efficiency.

In-vitro drug release studies:

On comparing the in vitro drug release studies of dry emulsion formulations prepared with the three oils, the dry emulsion formulation prepared with peppermint oil showed an immediate release of the drug enhancing its bioavailability. Then the *in vitro* release of this peppermint dry emulsion formulation was compared with marketed products like cefixime

dry suspension and tablet and with pure cefixime. When compared to other dosage forms like dry suspension and tablet the dry emulsion formulated with peppermint oil showed immediate release of drug. Thus the objective of the study was met.

The cumulative % drug release for formulations F1, F2, F3 and F4 at the end of 8hrs is 39.13, 97.44, 29.14 and 85.62 respectively. At the end of 5 hrs formulation F6 showed cumulative % drug release of 86.742. At the end of 4hrs formulation F2 showed cumulative % drug release of 90.15 and at the end of 2 hrs formulations F4 showed cumulative % drug release of 98.126. Stability studies were conducted for a period of 3 months.

.

Table 2: Formulation of Dry Emulsion

Ingredients	F1	F2	F3	F4
Ciprofloxacin	3gm	3gm	3gm	3gm
Peppermint oil	-	-	-	9ml
Olive oil	-	-	12ml	-
Sesame oil	15ml	15ml	-	-
Propylene Glycol	5ml	5ml	5ml	5ml
Tween 80	5ml	4ml	4ml	4ml
Span 80	-	3ml	3ml	3ml
HPMC	-	-	1gm	4ml
Mannitol	12gm	12gm	12gm	12gm
Sucrose	3gm	3gm	3gm	3gm
Purified Water	Q.S to 100ml	Q.S to 100ml	Q.S to 100ml	Q.S to 100ml

Table 3: In-Vitro Drug Entrapment Studies

Formulation code	Entrapment efficiency
F1	88.44%
F2	90.24%
F3	92.56%
F4	94.78%

Table 4: Comparison studies of Cumulative % Drug Release data

Time (min)	Cumulative % Drug Release Data			
	F1	**F2**	**F3 (Pure Drug)**	**F4 (Dry Emulsion)**
15	8.22%	6.78%	5.38%	12.43%
30	12.44%	10.34%	7.24%	16.36%
45	14.74%	12.64%	9.24%	20.64%
60	20.42%	42.44%	10.34%	46.86%
120	22.66%	56.23%	12.42%	52.56%
180	24.86%	84.22%	14.32%	62.74%
240	26.64%	94.20%	18.65%	72.44%
300	30.46%	-	20.66%	84.62%
360	32.52%	-	24.62%	-
420	32.82%	-	28.82%	-
480	34.64%	-	30.53%	-

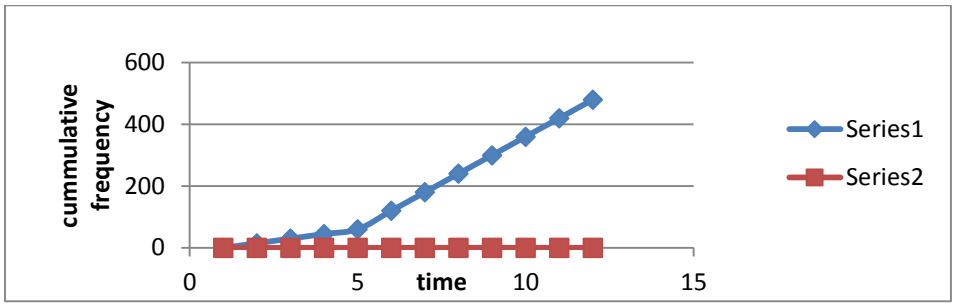

Fig.3 Comparison between Time Vs Pure Drug

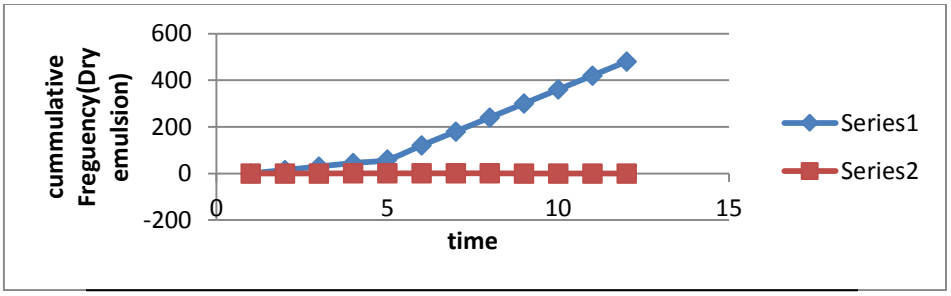

Fig.4 Comparison between Time Vs Dry emulsion

CONCLUSION

By formulating Ciprofloxacin as dry Emulsion its solubility and dissolution rate had been enhanced. The dry emulsion formulation was analyzed for the stability studies for 3months at 45 ºC with 75% RH The emulsion was analyzed for drug entrapment and cumulative % drug release till a period of 3 months, no variations in results were observed. After three months the dry emulsion was reconstituted and the emulsion formed was stable with desired consistency and viscosity and without any signs of instability. On comparison of dissolution rate of ciprofloxacin formulations it was found that, Pure ciprofloxacin < Dry Emulsion. From the above study it can be concluded that the dry Emulsion formulation (F4) showed an immediate release of drug when compared with pure ciprofloxacin.

REFERENCES

1.Adley Lima AN, Jose Sobrinho LS, Roberto AC, Correa JR, & PedroRolim Neto J, Alternative technologies to improve solubility of poorly water soluble drugs, Lat Am J Pharm, 27 (5), 2008, 789-797.

2. Chiou WL, Riegelman S, Pharmaceutical applications of Dry emulsion systems, J Pharm Sci, 60(9), 1971, 1281-1302.

3. Corveleyn S, Remon JP, 1998a, Formulation of a lyophilized dry emulsion tablet for the delivery of poorly soluble drugs, Int J Pharm, 166, 1998a, 65–74.

4. Corveleyn S, Remon JP, Bioavailability of hydrochlorothiazide: conventional versus freeze-dried tablets, Int J Pharm, 173, 1998b, 149–155.

5. Corveleyn S, Remon JP, Stability of freeze-dried tablets at different relative humidities, Drug Dev Ind Pharm, 25, 1999, 1005–1013.

6. Faldt P, Bergenstahl B, Fat encapsulation in spraydried food powders, J Am Oil Chem Soc, 72, 1995, 171–176.

7.Faldt P, Bergenstahl B, Changes in surface composition of spray-dried food powders due to lactose crystallisation, L.W.T. 29, 1996a, 438–446.

8. Faldt P, Bergenstahl B, Spray-dried whey protein: lactose:soybean oil emulsions; Surface composition and particle structure. Food Hydrocoll, 10, 1996b, 421–429.

9. Faldt P, Bergenstahl B, Spray-dried whey protein: lactose:soybean oil emulsions; redispersability, wettability and particle structure, Food Hydrocoll, 10, 1996c, 431–439.

10. Ford JL, Thermal analysis of hydroxypropylmethylcellulose and methylcellulose: powders, gels and matrix tablets. Int. J. Pharm. 179, 1999, 209–228.