OPEN ACCESS JOURNAL

BIOINTERFACE RESEARCH IN APPLIED CHEMISTRY

ORIGINAL ARTICLE

www.BiointerfaceResearch.com

ISSN 2069-5837

Volume 2, Issue 5, 2012, 409-416

Received: 31.05.2012 / Accepted: 19.09.2012 / Published on-line: 15.10.2012

Characteristic and controlled release of antiviral drug: A comparative study on preparative techniques and polymer affected parameter

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ABSTRACT

The aim of this work was to design and characterize microparticulate oral drug delivery system for an antiviral drug, Acyclovir for achieving sustained release. The present work encompasses microencapsulation techniques, core substances and other fundamentals involved in the preparation and characterization of microparticles. The microparticles were characterized for particle size, particle morphology, encapsulation efficiency, XRD and *in vitro* drug release. The release of drug from these microparticulate delivery systems was also compared, and a possible release mechanism was proposed using different kinetic models. The drug release from all the formulations followed anomalous diffusion mechanism and was best fit to Higuchi's kinetic model. Significant differences in percentage yield, entrapment efficiency, particle size and sustaining capacity were seen with microspheres prepared by two different methods. The *in-vitro* release of drug from microparticles was sustained over 17 days.

Keywords: *microencapsulation techniques, microparticles, kinetic models, sustained release*

1. INTRODUCTION

One of the most abundant organic polymers known to mankind is cellulose, a biopolymer [1] which has many biomedical applications [2]. Cellulose derivatives such as cellulose esters have a number of advantages including recyclability, reproducibility, biocompatibility, biodegradability, non toxicity, cost effectiveness and availability in a wide variety of forms [3, 4]. Cellulose esters including cellulose acetate (CA), cellulose acetate propionate (CAP), -butyrate (CAB), and -phthalate (CAPh) are well-known biopolymer derivatives having ability to form micro and nano particles [5].

Several techniques have been described in the literature for the preparation of microparticles, including solvent evaporation, phase separation, spray-drying and in situ polymerization [6-9]. Out of these microencapsulation techniques, the spray-drying process and solvent evaporation technique is widely used in the pharmaceutical industry [10]. Spray drying technique is of interest as an encapsulation technology because it is a one-step, easy to scale up and rapid process with reproducibility and versatility [11].

Due to the beneficial properties of cellulose esters, the focus of this study is at developing microparticulate formulation of an antiviral drug utilizing two different cellulose derivative (CAB and CAP) as matrix forming polymer. Acyclovir, an antiviral drug, was selected as a model drug as

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it is having short biological half life (2-3 h) [12] and low bioavailability (10–20%) [13]. Acyclovir microparticles are developed for their potential application in better control of chronic recurrent herpes infection. Moreover, this study, explores two different microparticle preparative methods such as novel solvent evaporation-matrix erosion [14] and spray drying technique [15]. The microparticles prepared by these two techniques are compared for their particle size, encapsulation efficiency and release kinetics. The effects of type and concentration of polymer on the release characteristics and physiochemical properties of the microparticles were investigated.

2. EXPERIMENTAL SECTION

2.1. Materials. Acyclovir was procured as a gift sample from Cadila Pharmaceuticals Ltd., (Ahmedabad, India). Cellulose acetate butyrate (Mn=12,000) and Cellulose acetate propionate (Mn=15,000), was purchased from Aldrich Chemical Company, Inc. (Milwaukee, WI, USA). Poly vinyl alcohol (Mw=20,000-30,000) and dichloromethane was purchased from S.D. Fine Chemicals (Mumbai, India). Dialysis membrane-110 was purchased from Himedia Laboratories Pvt. Ltd., Mumbai, India. Double-distilled water was used throughout the work. All other reagents and solvent used were of analytical grade.

2.2. Preparation of microparticles by spray drying technique. Drug loaded microparticles were prepared by loading drug into different concentrations to polymer weight ratios. The amounts of drug and polymer were different depending on the composition of the microparticles prepared.

The w/o emulsion was prepared by adding aqueous solution of ACY (20%, dry weight of the polymer) to the oil phase consisting of CAB and CAP (1, 2 and 3 wt % in dichloromethane). The obtained solutions were fed to the nozzle (0.7 mm inner diameter) of advanced spray-drier, LU-222 (Labultima, Mumbai, India) having co-current flow, with peristaltic pump. Then the solution was atomized by the force of the compressed air, and blown together with a hot air to the chamber. The high temperature in the chamber evaporates solvent and the carrier polymer encloses the drug. The conditions of the spraying process for each preparation were: inlet air temperature 55°C; outlet air temperature, 40°C; and feed spray rate, 10 ml/min, and pressure bar at ~2 atm. The spray-dried microparticles were harvested from the apparatus collector weighed and stored under vacuum at room temperature for 48 h.

The compositions of various formulations along with formulation codes are summarized in Table 1. Each formulation was produced in triplicate.

2.3. Preparation of microparticles by w/o/w double emulsion solvent evaporation-matrix erosion technique. The modified w/o/w double emulsion solvent evaporation-matrix erosion technique was used to prepare microparticles containing ACY. CAB and CAP (1%-3% (w/w)) solution was prepared in 150 ml of dichloromethane. ACY (20% (w/w) of dry weight of polymer) solution was prepared by dissolving it in distilled water separately. The above two solutions were mixed and stirred thoroughly using a high-speed stirrer (Remi, Mumbai, India) to form a homogeneous solution. This mixture was dispersed in 1 wt% polyvinyl alcohol and was continuously stirred for the stipulated time under ambient conditions for about 3 h to form rigid spherical spheres. Microparticles were using Whatmann filter paper, washed with distilled water, and dried at dried at 40 ◦C overnight.

2.4. Determination of drug loading and encapsulation efficiency. To determine the average drug content, a sample of 100 mg accurately weighed drug loaded microparticles were crushed in porcelain mortar and then the powdered microparticles were suspended in 100 ml phosphate buffer solution (PBS) (pH 7.4). The solution was filtered and the filtrate was analyzed for drug content at the wavelength of 254 nm using a UV/VIS double beam spectrophotometer (SP-3000+, Tokyo,

Japan). All the experiments were carried out three times. The amount of drug loading and encapsulation efficiency of the drug loaded microparticles were calculated using the following equation (1, 2) [16]. Results are tabulated in table 1.

$$\% \text{ Drug loading} = \left(\frac{\text{Weight of drug in microspheres}}{\text{Weight of microspheres}} \right) \times 100 \longrightarrow (1)$$

$$\% \text{ Encapsulation Efficiency} = \left(\frac{\% \text{ Drug loading}}{\% \text{ Theoritical loading}} \right) \times 100 \longrightarrow (2)$$

2.5. Yield of microparticles. The yield of microparticles was calculated as a percentage of the total amounts of polymer and drug added during the preparation of microparticles.

2.6. Scanning electron microscopy. Scanning electron micrographs of polymeric drug loaded microparticles were taken to study its surface morphology & surface characteristics. A small amount of powder samples were spread over metal stubs after dispersing in water and allowed to dry in air under ambient conditions. The stub containing the sample was placed in the scanning electron microscope (SEM, JSM-5410, Jeol, Tokyo, Japan) chamber. The surface of samples were scanned using a gaseous secondary electron detector (working pressure: 0.8 torr, acceleration voltage: 30 kV) XL 30, Philips (Eindhoven, The Netherlands).

2.7. Particle size studies. Particle size of microparticles were determined by dynamic laser light scattering (Halos-BR, Symantec, Germany). Dry sample technique using a dry sample adapter was used. The completely dried particles were placed on the sample tray with an inbuilt vacuum under a compressed air system, which was used to suspend the particles. The laser obscuration range was maintained between 0.1-875 μm. The volume mean diameter (VMD) was recorded. Each batch was analyzed in triplicate.

2.8. X-ray diffraction Studies. X-ray diffraction (XRD) patterns were obtained using Philips X-ray diffractometer (X pert), with voltage of 40 kV, current of 40 mA and Cu α-radiation source in the range of 3° to 50° of 2θ with 1s of scan time. Pristine ACY and ACY-loaded microparticles were evaluated by powder XRD technique for their crystalline nature.

2.9. Drug release study. The *in vitro* drug release study was performed using USP XXIV basket apparatus (Electrolab, TDT-06T, Mumbai, India) containing 900 mL of freshly prepared 0.1 mol L^{-1} HCl at pH 1.2 and phosphate buffer (pH 7.2) as a dissolution medium at 100 rpm. Microparticles equivalent to 100 mg of ACY were used for the test. At predetermined time intervals, a suitable volume of the sample was withdrawn using a sampling syringe. Then it was filtered promptly through a 0.45mm membrane filter disc and diluted suitably. The absorbance of the resulting solution was then measured with UV/VIS spectrophotometer (SP-3000+, Tokyo, Japan) at 254 nm. The volume withdrawn was replenished with an equal volume of fresh dissolution medium pre-warmed to 37 °C to maintain the sink conditions.

2.9.1. Release kinetics. In order to investigate the drug release mechanism from microparticles, the release data were analyzed by fitting the *in vitro* release data obtained in following mathematical models: zero order equation (equation 1), first order equation (equation 2) and Higuchi model (equation 3).

$$Q_t = k_0 t \qquad (3)$$

Where, k_0 is zero-order rate constant expressed in units of concentration/time and t is the time.

$$ln\ Q_t = ln\ Q_0 - k_1\ t \quad (4)$$

Where, k_1 is first order constant.

$$Q_t = k_h\ t^{1/2} \quad (5)$$

Where, k is the higuchi constant reflecting the design variables of the system.

The following plots were made; Q_t vs t (zero order kinetic model), log(Q_0-Q_t) vs t (first order kinetic model) and Q_t vs square root of t (higuchi model) [17], where Q_t is the total amount of drug released at time t and Q_0 is the initial amount of the drug present in the microparticles. The rate constants were calculated for the respective models. Further, to find out the mechanism of drug release, the first 60% drug release was fitted in Korsmeyer-Peppas [18] model (equation 4).

$$Q_t = k\ t^n \quad (6)$$

Where, k is the kinetic constant and n is the diffusion exponent.

3. RESULTS SECTION

3.1. Encapsulation efficiency, particle size and production yield. Table 1 lists the theoretical compositions, production yield, particle size and encapsulation efficiencies of microparicles obtained by different techniques.

3.1.1. Effect microencapsulation technique. The results indicated that the spray dried microparticles were produced with encapsulation efficiencies above 85% whereas encapsulation efficiencies ranged between 50% and 55% for the particles prepared with novel solvent evaporation-matrix erosion technique. Highest encapsulation (94.84 %) was observed for formulation F6.

The spray dried microparticles were in the size range of 4-10 µm for different formulations it may be because of lower viscosity of the spray solution, aspirator speed, and feed pump flow rate. However, modified novel solvent evaporation-matrix erosion technique produced the particle size in the size range of 19-20 µm, which is bigger than those of the spray dried microparticles.

The production yield was determined by taking the ratio of the mass of particles in the product, relative to the mass of the initial amount of drug and polymer solution of formulation per batch. Production yields of spray dried microparticles was found to be low, reaching a maximum of 55%, while from modified novel solvent evaporation-matrix erosion technique production yields were above 85%. Spray dryer yielded low production of microparticles because of its structure, microparticles adhere to the walls of cyclone which are difficult to collect [19].

3.1.2. Effect of polymer type. Encapsulation efficiency of the micrparticle prepared with cellulose acetate butyrate was higher (F1, F2, F3) than for the microparticle prepared with the cellulose acetate propionate (F4, F5, F6). Thus, spray drying yields a high active material encapsulation efficiency compared to that of solvent evaporation-matrix erosion technique (Table 1). Also CAB particles were smaller in size and production yield was slightly higher than that of CAP.

3.1.3. Effect of polymer concentration. The encapsulation of the drug and particle size increased due to increase in polymer concentration in a fixed volume of organic solvent [20]. This might be due to formation of thick wall of polymer around the drug due to higher polymer concentration. The increase in yield as a function of polymer concentration could be also observed, i.e. as the spray fluid feed polymer concentrations increases from 1% to 3%, a significant increase in yield was observed. [21]. Thus, microencapsulation technique, type and concentration of polymer have been shown to affect encapsulation efficiency, particle size and production yield.

3.2. Surface morphology. The shapes and surface characteristics of the microparticles using SEM revealed that spray dried microparticles appear roughly spherical and had rugged surface (Figure 1a) whereas microparticles obtained from novel solvent evaporation-matrix erosion technique were more smother (Figure 1b) for both the polymers CAB and CAP.

Table 1. Composition, production yield, mean particle size and encapsulation efficiency of different formulations

Formulation Code	Cellulose Acetate Butyrate (%, w/w)	Cellulose Acetate Propionate (%, w/w)	Acyclovir (wt %)	Production yield (M±SD) (%)	Volume mean particle size (*M*±SD) (µm)	Encapsulation Efficiency (%)
F1	1	-	20	42.19±0.62	4.8±0.50	90.38
F2	2	-	20	46.58±0.36	5.2±0.35	92.42
F3	3	-	20	49.78±0.54	5.9±0.37	94.84
F4	-	1	20	50.48±0.34	6.7±0.65	83.20
F5	-	2	20	53.73±1.08	8.5±0.54	86.56
F6	-	3	20	55.45±0.75	10.1±0.58	90.64
F7[a]	3		20	91.36±1.57	19.4±0.63	53.15
F8[a]	-	3	20	89.32±1.44	20.4±0.45	50.05

[a]Microparticles prepared by novel solvent evaporation-matrix erosion technique.

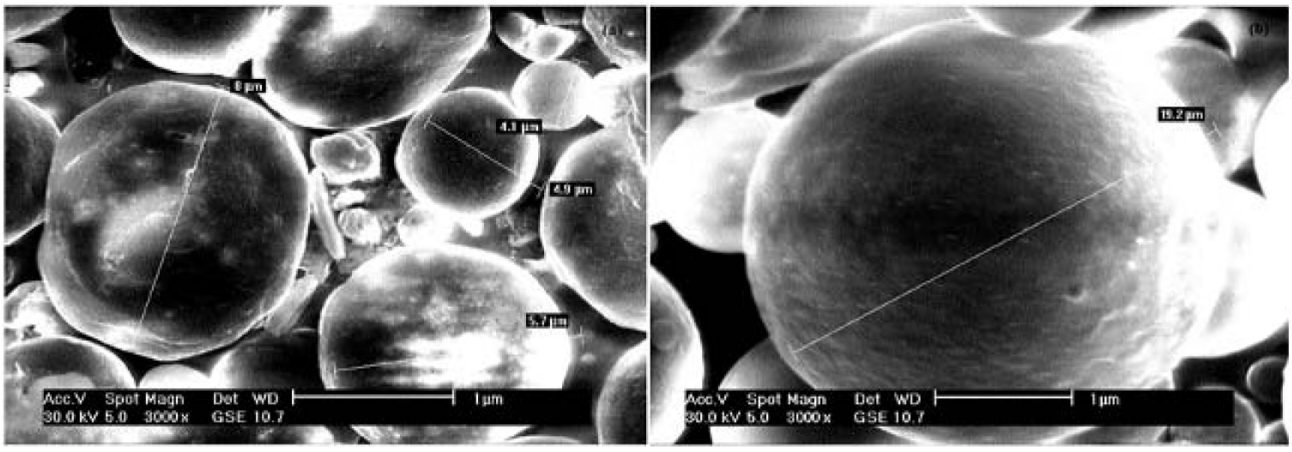

Figure 1: Scanning electron micrographs of Acyclovir loaded microparticles prepared by (a) Spray drying technique method (b) novel solvent evaporation matrix erosion method.

3.3. X-ray diffraction. XRD analysis of microparticles and its individual components was carried out to find out any change in the crystallinity of drug during microencapsulation. XRD diffractograms of (a) pristine ACY, (b) CAB, (c) CAP and (d) ACY loaded microparticle are presented in Figure 2. It can be seen from figure, the XRD diffractograms of ACY and drug loaded microparticles were showing different peaks. It indicates that ACY underwent a transition from a crystalline to an amorphous state. Here, the characteristic intensities of ACY have been overlapped with the noise of the coated polymer confirming the amorphous nature of ACY in the formulated microparticles [16].

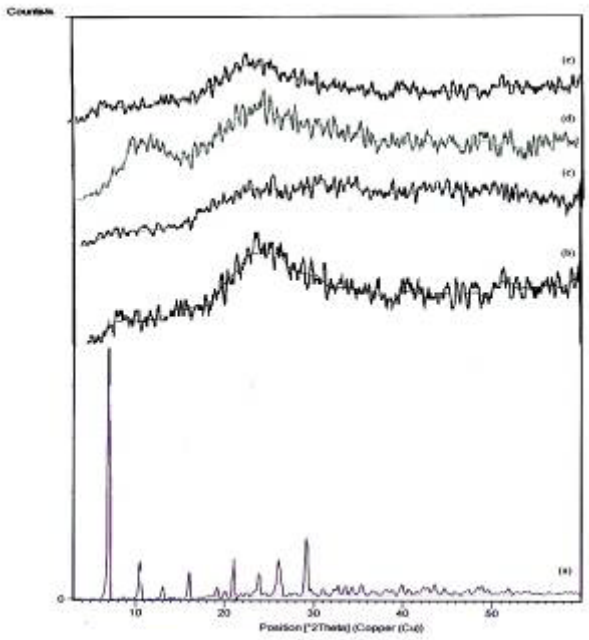

Figure 2. XRD spectra of (a) pristine acyclovir (b) CAB placebo microparticles, (c) CAP placebo microparticles (d) drug-loaded microparticles (CAB) and (e) drug-loaded microparticles (CAP).

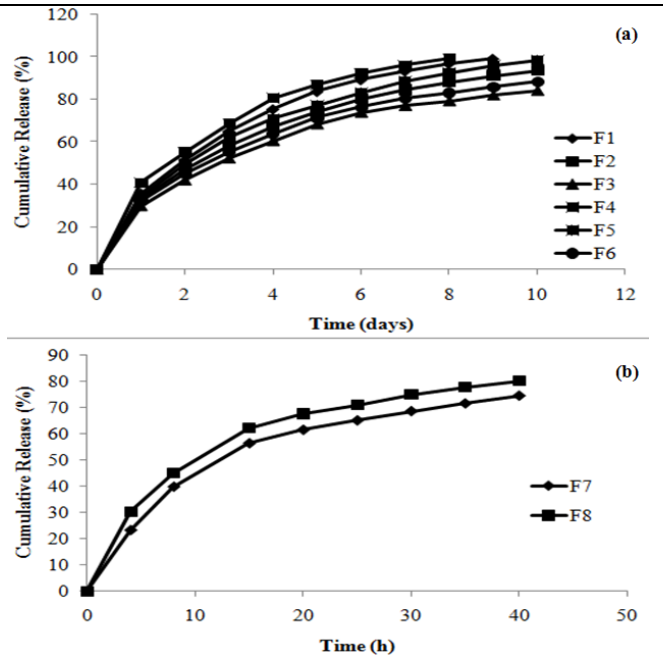

Figure 3. Dissolution profiles of different formulations (a) by spray drying method (b) by novel solvent evaporation-matrix erosion method

3.3. Drug release study. The effect of microencapsulation technique, type and concentration polymer on *in vitro* release was studied.

In vitro ACY release in the gastrointestinal tract was simulated by suspending a sample of particles in simulated gastric fluid (pH = 1.2) for 2 h. The particles were then resuspended in simulated intestinal fluid (pH = 6.8). The results in Figure 3 were presented as cumulative ACY release. The dissolution media (phosphate buffers) were gradually warmed to 37℃ to avoid erroneous results due to sudden temperature changes. At acidic pH, the drug release behavior of the different batches of microparticles is quite similar, independently on their polymeric composition. The total release in the gastric fluid was approximately 20% of the total ACY present in the particles. The release of ACY from cellulose derivatives CAB and CAP microparticle illustrated the rate of drug release from the microparticles depended on the polymer concentration, which indicates that the release rate decreases significantly with increasing the amount of polymer from 1 % to 3 % and the ACY release was sustained for longer period of time [22]. Results also showed that the microparticles having CAB matrix, release ACY for longer period compared to that of microparticles having CAP matrix. Thus results showed that cellulose derivatives, CAB was found to be the better carrier for ACY. Furthermore, spray dried microparticles showed maximum extended release of ACY within 17 days compared to this, microparticles prepared with modified novel solvent evaporation-matrix erosion technique showed lesser sustained drug release reaching of ACY within 60 h. The formulation F3 exhibits maximum release period compared to all the formulations.

3.3.1. Release kinetics. The *in vitro* release kinetics was carried out by fitting the release data to models representing zero-order, first-order, and Higuchi's square root of time. The correlation coefficient of different kinetic models for all the formulations containing various polymers is tabulated in Table 2. It was found that all the formulations were best fit into Higuchi's square root

release model. It confirmed that the drug is very slowly diffusing out of the polymer matrices and showing a much sustained release.

To examine the mechanism of release the data were fitted to Korsmeyer-Peppas model. According to Korsmeyer-Peppas, a value of the exponent, n=0.5, 0.5<n<1, n=1.0 indicates Fickian diffusion, non-Fickian diffusion and Case II transport, respectively. Studies revealed that for all the formulations, n values lesser than 0.5 indicating a fickian release.

Table 2: Correlation coefficient (r), reaction rate constants (k) and diffusion exponent (n) of the model equations applied to the release of acyclovir from microparticles.

Formulation Code	Zero Order		First Order		Higuchi		Korsmeyer-Peppas Model	
	r	k_0	r	k_1	r	k_h	r	n
F1	0.902	2.345	0.984	-0.080	0.994	11.68	0.987	0.286
F2	0.928	2.292	0.990	-0.074	0.996	10.52	0.992	0.258
F3	0.946	2.217	0.992	-0.071	0.998	9.87	0.995	0.247
F4	0.854	2.601	0.981	-0.089	0.984	13.45	0.982	0.388
F5	0.861	2.454	0.984	-0.088	0.990	12.82	0.984	0.347
F6	0.884	2.372	0.987	-0.084	0.994	12.24	0.988	0.298
F7[a]	0.798	3.772	0.977	-0.092	0.969	14.82	0.980	0.443
F8[a]	0.782	4.102	0.972	-0.097	0.965	15.23	0.978	0.452

4. CONCLUSIONS

The present study demonstrated that there is no significant difference in terms of the physical properties of all the microparticles prepared by spray drying and the solvent evaporation-matrix erosion technique. However, comparing both the techniques, spray drying is the most appropriate technique to prepare acyclovir formulation because it produced microparticles with improved mean particle size, excellent entrapment efficiency, controlled drug release behavior, reproducibility, rapidness and simplicity. Among the cellulose derivatives, CAB was found to be the best polymer for acyclovir formulation. All the results suggested the potential application of spray drying technique and CAB for formulating acyclovir formulation as controlled release dosage form with enhanced bioavailability and reduced dose frequency for improved patient compliance.

5. ACKNOWLEDGMENT

Authors would like to thank SICART, Vallabh Vidyanagar, Anand, Gujarat, India for analytical characterization. We are grateful to Charutar Vidyamandal (CVM) and Pradip S. Patel, Directer, ARIBAS, New Vallabh Vidyanagar, for providing all the necessary research facilities.

6. REFERENCES

[1] Siro I., Plackett D., Microfibrillated cellulose and new nanocomposite materials: a review, *Cellulose,* 17, 459-494, **2010**.

[2] Thummler K., Fischer S., Feldner A., Weber V., Ettenauer M., Loth F., Falkenhagen D., Preparation and characterization of cellulose microspheres, *Cellulose,* 18, 135-142, **2011**.

[3] Samir M. A. S. A., Alloin F., Dufresne A., Review of recent research into cellulosic whiskers, their properties and their application in nanocomposite field, *Biomacromolecules,* 6**,** 612-626, **2005**.
[4] Nishio Y., Blends of poly(e-caprolactone) with cellulose alkyl esters: effect of the alkyl side-chain length and degree of substitution on miscibility, *Cellulose,* 4, 131-145, **1997**.
[5] Edgar K. J., Cellulose esters in drug delivery, *Cellulose,* 14, 49-64, **2007**.
[6] Choudhury P. K., Kar M., Controlled release metformin hydrochloride microspheres of ethyl cellulose prepared by different methods and study on the polymer affected parameters, *Journal of Microencapsulation,* 26, 46-53, **2009**.
[7] Zhang J. X., Zhu K. J., Chen D., Preparation of bovine serum albumin loaded poly (D, L-lactic-co-glycolic acid) microspheres by a modified phase separation technique, *Journal of Microencapsulation,* 22, 117-126, **2005**.
[8] Raval J. P., Naik D. R., Patel P. S., Spray-dried cefixime encapsulated poly(lactide-coglycolide) microparticles: in vitro evaluation and release kinetics with antibacterial activity, *Drying Technology,* 30, 865-872, **2012**.
[9] Jeong W. J., Kim J. Y., Choo J., Lee E. K., Han C. S., Beebe D. J., Seong G. H., Lee S. H., Continuous fabrication of biocatalyst immobilized microparticles using photopolymerization and immiscible liquids in microfluidic systems, *Langmuir,* 21, 3738-3741, **2005**.
[10] Mujumdar A. S., Research and developments in drying: Recent trends and future prospectus, *Drying Technology,* 22, 1-26, **2004**.
[11] Barot B. S., Parejiya P. B., Patel T. M., Parikh R. K., Gohel M. C., Development of directly compressible metformin hydrochloride by the spray-drying technique, *Acta Pharmaceutica,* 60, 165–175, 2010.
[12] Gupta S. K., Gupta U., Omray L. K., Yadav R., Soni V. K., Preparation and characterization of floating drug delivery system of acyclovir, *International Journal of Applied Pharmaceutics,* 2, 710-714, **2010**.
[13] Sweetman S. C., Martindale, The Complete Drug Reference (33th ed.) London: Pharmaceutical Press, 612, **2002**.
[14] Muniyandy S., Boddapati A., Development and evaluation of ethylcellulose floating microspheres loaded with ranitidine hydrochloride by novel solvent evaporation-matrix erosion method, *Carbohydrate Polymers,* 85, 592-598, **2011**.
[15] Raval J. A., Patel J. K., Patel M. M., Formulation and in vitro characterization of spray dried microspheres of amoxicillin, *Acta Pharmaceutica,* 60, 455–465, **2010**.
[16] Raval J. P., Naik D. R., Amin K. A., Patel P. S, Controlled-release and antibacterial studies of doxycycline-loaded poly(ε-caprolactone) microspheres, *Journal of Saudi Chemical Soceity,* http://dx.doi.org/10.1016/j.jscs.2011.11.004, **2011**.
[17] Higuchi T., Mechanism of sustained-action medication. Theoretical analysis of rate of release of solid drugs dispersed in solid matrices, *Journal of Pharmaceutical Sciences,* 52, 1145-1149, **1963**.
[18] Korsmeyer R.W., Gurny R., Doelker E. M., Buri P., Peppas N. A., Mechanism of solute release from porous hydrophilic polymers, *International Journal of Pharmaceutics,* 15, 25-35 **1983**.
[19] Martinac A., Filipovic-Grcic J., Voinovich D., Perissutti B., Franceschinis E., Development and bioadhesive properties of chitosan-ethylcellulose microspheres for nasal delivery, *International Journal of Pharmaceutics,* 291, 69-77, **2005**.
[20] Raval J. P., Naik D. R., Patel P. S., Preparation and evaluation of cellulose acetate butyrate microspheres containing diclofenac sodium, *International Journal Drug Formulation and Research,* 2, 247-259, **2011**.
[21] Prinn K. B., Costantino H. R., Tracy M., Statistical modeling of protein spray drying at the lab scale, *AAPS PharmSciTech*, 3, 1-8, **2002**.
[22] Srivastava A. K., Ridhurkar D. N., Wadhwa C. S., Floating microspheres of cimetidine: formulation, characterization and in vitro evaluation, *Acta Pharmaceutica,* 55, 277-285, **2005**.