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FORMULATION AND EVALAUTION OF SUSTAINED RELEASED AZITHROMYCIN MICROSPHERE BY EMULSION SOLVENT EVAPORATION TECHNIQUE

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ABSTRACT

Azithromycin is macrolide antibiotic is effective against a wide variety of bacteria such as *Hemophilus influenzae*, *Streptococcus pneumoniae*, *Mycoplasma pneumoniae*, *Staphylococcus aureus*, *mycobacterium avium*, and many others. The aim of present work was to formulate sustained released azithromycin microsphere to overcome the problem related to drug like insoluble in water, bitter taste and gastrointestinal side-effects. Microspheres were prepared by varying drug polymer ratio using emulsion solvent evaporation technique. Characterization of microspheres was done by Differential Scanning Calorimetry (DSC), X-Ray diffractometry (XRD), Surface Morphology (SEM), Stability studies.

Keywords: Sustained Released Microsphere, Azithromycin, Solvent evaporation technique, Ethyl Cellulose, Ethyl Acetate

INTRODUCTION

The objective of any drug delivery system is to provide drug in therapeutic amount to the proper site in the body to achieve immediately and then maintain the desired drug concentration. These idealized objectives are achieved by appropriately developed sustained release drug delivery which also has diverse applicability and merits. There are certain drugs having large dose they may cause GI side effect so to avoid this drug can be formulated in sustained form to release slowly in lower GI tract.

The sustained release drug delivery includes the application of physical and polymer chemistry. These polymers slowly release the drug in bio-system and maintain drug blood level within therapeutic range for longer duration. Some of the products characterize the drug permeation through the appropriate biological membrane and any first pass metabolic effects prior to the entry of drug into systemic circulation. The fact that the absorption and release rate of the drug from the dosage form, is one of the interesting and most recent developments in pharmaceutical field[1, 2]. Microencapsulation is a process whereby relatively thin coating of polymers are applied to small particles of solid or droplets of liquid and dispersions. The microencapsulation processes produce small particles ranging in size from 1 to 1000 µm. There are different names for these particles: microparticle, microsphere, microcapsule and micromatrix. The microparticle system has become an indispensable part of the sustained drug delivery fields for the past few decades since it can readily be adapted for various administration methods[3, 4, 5].

MATERIALS AND METHODS

Procurement of drug and excipients:

The drug Azithromycin and polymer Ethyl cellulose (50 cps) were gifted from Zim Laboratories Ltd, Nagpur, where other AR grade excipients like Polyvinyl alcohol, Dichloromethane, Chloroform, Ethyl acetate,

Sodium hydroxide and Dibasic sodium phosphate were supplied by Loba chemical, Mumbai.

Microspheres were prepared by using mechanical stirrer of Remi Motors Ltd, Mumbai. Evaluation and characterization of microspheres was carried out by using equipments like USP Tablet dissolution apparatus Type II (TDT 08L), UV-Visible double beam Spectrophotometer (UV-1800), FTIR (8400SCCE), X Ray diffractometer (PW1700), DSC (61000), Scanning Electron Microscope (JSM 6380 A), Light Microscope and Stage Micrometer.

METHODS

Preliminary study [6, 7, 8]:

The Preformulation study like Preliminary identification test (melting point, solubility) characterization of drug and polymer and their interaction study were performed**.**

Spectroscopic studies [9, 10, 11]:

UV Spectroscopy studies involved Determination of λ max and preparation of standard curve of Azithromycin at 244 nm. The IR spectrum of the drug sample was recorded by IR spectroscopy. The drug polymer interaction was carried out using FT-IR Apparatus.

Formulation of microspheres:

The formulations were prepared by using solvents ethyl acetate at different drug: polymer ratio (1:1, 1:1.5, and 1:2).

Method of preparation [12, 13, 14]:

In the O/W emulsion solvent evaporation method, the polymer (ethyl cellulose) was dissolved in internal organic phase of ethyl acetate. Accurately weighed quantity 1g of Azithromycin was dispersed or dissolved in the polymer solution. This resulting mixture was poured slowly with stirring into 100 ml of a 0.015% w/v aqueous solution of polyvinyl alcohol. The emulsion was then stirred continuously at 700 rpm for 1 hr to evaporate the solvent. The microspheres were recovered by vacuum filtration, washed with 200 ml of deionized water and dried at room temperature.

The compositions of Azithromycin microspheres using ethyl acetate as solvent were given in **Table 1.**

Evaluation of microspheres[15, 16]:

Angle of repose:

Angle of repose is defined as the maximum angle possible between the surface of pile of powder and horizontal plane. The angle of repose for the microspheres of each formulation was determined by the funnel method. The microspheres were allowed to flow out of the funnel orifice on a plane paper kept on the horizontal surface. It forms a pile of microspheres on the paper. The angle of repose was calculated by substituting the values of the base radius 'R' and pile height 'H' in the following equation

$$\theta = \tan^{-1} \frac{H}{R}$$

Bulk density of all batches of microspheres was determined by pouring gently 2 g of sample through a glass funnel into a 10 ml graduated cylinder. The volume occupied by the sample was recorded. Bulk density was calculated as per given formula:

$$\text{Bulk density} = \frac{\text{Weight of sample}}{\text{Volume occupied by the sample}}$$

Tapped Density:

The tapped density was determined by pouring 2 g of microspheres through a glass funnel into a 10 ml graduated cylinder. The cylinder was tapped from height of 2 inches until a constant volume was obtained. Volume occupied by the sample after tapping was recorded .The values for tapped density was calculated as per given formula:

$$\text{Tapped density (g/ml)} = \frac{\text{Weight of sample}}{\text{Volume occupied by the sample}}$$

Compressibility index:

The compressibility indices of the formulation blends were determined using Carr's compressibility index formula.

$$\text{Carr's Index} = \frac{\text{Tapped density} - \text{Bulk density}}{\text{Tapped density}} \times 100$$

Hausner Ratio:

It provides an indication of the degree of densification which could result from vibration of feed hopper. Lower the Hausner ratio better is the flowability. It was calculated as per given formula.

$$\text{Hausner ratio} = \frac{\text{Tapped density}}{\text{Bulk density}}$$

Determination of particle size [17]:

The particle size was determined using stage micrometer. The diameters of about 300 microspheres were measured and the average particle size was determined.

Estimation of drug loading[18, 19]:

For determination of drug content, microspheres equivalent to 100 mg were weighed and dissolved in 100ml of acetone. After suitable dilutions were with phosphate buffer (pH 6.0), the resulting solution was analyzed spectrophotometrically at 244 nm.

$$\text{Encapsulation efficiency (\%)} = \frac{\text{Actual drug content}}{\text{Theoretical drug content}} \times 100$$

$$\text{Drug loading (\%)} = \frac{\text{Weight of drug}}{\text{Weight of microspheres}} \times 100$$

***In-vitro* dissolution study[20, 21, 22]:**

The study was carried out using dissolution apparatus USP Type-I (Rotating Basket type).

Accurately weighed microspheres equivalent to 200 mg of Azithromycin were taken in muslin cloth and it was kept in baskets. Dissolution study was carried out in phosphate buffer pH 6.0 at 50 RPM at temp 37 ± 0.5°c. During dissolution study 10 ml of aliquot was withdrawn at a time intervals of 1 to 12 hr and same was replaced with equal volume of fresh medium. The withdrawn samples were filtered through Whatmann filter paper and absorbances were measured at 244 nm. Drug concentration in the samples was determined from the calibration curve.

Characterization of microspheres:

Differential Scanning Calorimetry [9, 10, 11, 27]:

The DSC measurements were performed on a differential scanning colorimeter with thermal analyzer. All accurately weighed samples (about 10 mg of Azithromycin, ethyl cellulose, physical mixtures, and formulation) were placed in a sealed aluminium pan, before heating under nitrogen flow (10 ml/min) at a scanning rate of 20 °c per min from 100 to 300 °c an empty aluminium pan was used as reference.

X-Ray diffractometry [13, 14, 15, 23]:

The powder X-ray diffraction pattern of Azithromycin and polymer were obtained using Phillips X-ray diffractometer with a Ni-filtered CuKα-radiation at a scanning speed of 10 °/min at 2θ.The graph was plotted in 2 theta angle Vs intensity count.

Surface Morphology [24]

The microspheres were coated with Platinum by ion sputtering using Autofine coater. The microspheres were kept on the sample holder and the scanning electron micrographs were taken.

Stability studies [35]

The study was carried out by storing the microspheres in glass bottle 40 °c and 75% RH for 30 days. These samples were collected on 7th, 14th, 21st, 28th day and analyzed for changes drug content and *in-vitro* dissolution studies.

RESULTS

Preformulation studies:

The melting point of azithromycin was found in the range of 119 – 121 °c. The wavelength of maximum absorbance (λ max) found was 244 nm.

Evaluation of microspheres:

All the formulations show angle of repose value in the range of 19.72 to 20.42. The values for bulk density were found in the range of 0.352 to 0.450. The values for tapped density were found to range from 0.414 to 0.510. The compressibility index values were found in the range of 11.76 to 14.99 respectively. Hausner's ratio was ranging from 1.13 to 1.17. The average particle size of microcapsules is found to be within 151.263 to 171.342 µm. The % encapsulation efficiency is

found to be in the range of 73.50 to 75. 60%. *In – vitro* dissolution study, % cumulative drug release from formulation in 12 hrs was decreased from 80.13 to 69.98 % represented in **Table 2.**

The dissolution data for formulations C1, C2, and C3 was fitted to various drug release kinetic models like Zero order, First order, Higuchi Matrix and Korsemeyer Peppas, Hixon-Crowel model. Kinetic Treatment to Dissolution Data proved important information about rate constants (K), correlation coefficients (R) obtained for various models were tabulated in **Table 3.**

Characterization of microspheres:

The FT-IR spectra of pure drug - Azithromycin, ethyl cellulose, physical mixture and formulation (C1) shows major functional groups of Azithromycin (methyl substituted nitrogen on lactone ring) in spectra of individual drugs as well as in spectra of physical mixture and formulation (C1**).** FT-IR of Formulation C1 presented in **Fig 1.** DSC curve presented in **fig 2** of Azithromycin shows a single endothermic peak at 121 $^{\circ}$c, due to melting of the drug. Optimized formulation C1 which contains Azithromycin and ethyl cellulose, the thermogram indicates characteristic peaks for melting of Azithromycin at 121 $^{\circ}$c.

The XRD pattern presented in **fig 3** of formulation exhibited halo pattern with less intense and denser peaks compared to plain Azithromycin, Scanning Electron Microscopy results were presented in **fig 4a and 4b**. Accelerated stability studies (AST) was carried for optimized batch (C1) by exposing it to 40°c/75% RH for one month and analyzed the samples at the interval of 7,14,21,28 days and the samples was analyzed for drug content and *in-vitro* dissolution study. The stability studies show drug content after 28 day was 74.14 where percent cumulative drug release 80.55.

DISCUSSION

The XRD scan of Azithromycin showed intense peaks of crystallinity. Diffractogram of azithromycin showed high intensity peaks between 2θ of 10-20^{0} values demonstrating the crystalline nature of drug. No intense peaks were observed in diffractogram of ethyl cellulose which indicates amorphous nature. Thus, preformulation studies indicated that there is no interaction between Azithromycin and ethyl cellulose.

Physical evaluation of microsphere formulation for angle of repose, Compressibility index and Hausner's ratio showed that they had good flow properties.

The microsphere formulated using ethyl acetate as internal organic phase or solvent in ratio1: 1 (C1) showed better encapsulation efficiency than other formulations. All the formulations showed diffusion exponent (n) varying from 0.524 to 0.668. From the n values of all formulations it can be concluded that as the concentration and viscosity of polymer was increased the value of diffusion exponent also increases. From the dissolution profile of formulations C1, C2 and C3, It can be concluded that formulation C1 shows better drug release (80%in 12 hrs) than C2 and C3. Formulations C1 to C3 showed retardation of Azithromycin as concentration of ethyl cellulose increased. Total release in 12 hrs was decreased from 80% to 70%.

The IR data of Azithromycin loaded ethyl cellulose microsphere also showed the peaks same as that the peaks in IR spectrum of pure drug, this suggested that there was no chemical interaction between ethyl cellulose and Azithromycin. The DSC results also support IR spectrometry results indicating absence of drug - polymer interactions. The XRD pattern of formulation C1 exhibited halo pattern with less intense and denser peaks compared to plain Azithromycin. This indicated that Azithromycin is dispersed at the molecular level in the blend of polymeric matrix.

The SEM photographs showed that the microspheres were spherical in nature and had a smooth surface. SEM photographs revealed the absence of crystals of drug on the surface of microspheres and uniform distribution of the drug within the microspheres.

The stability study conducted for observing effect of temperature on the formulation showed that there is no significant change in drug content and *in-vitro* drug release of optimized formulation.

CONCUSION

From the investigation carried out and results obtained, it was observed that the % of drug released from microspheres was sustained. As the GI side effects of Azithromycin are related to its high dose which can be minimized by formulating as microspheres. It would also helps to improve bioavailability because of absorption window and microencapsulation helps to mask the bitter taste of drug. Hence from this investigation, we can propose that the objective of the study is achieved.

ACKNOWLEDGEMENT

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Table 1: The compositions of Azithromycin microspheres with veering drug: polymer ratio

Sr. No.	Ingredients	C1	C2	C3
1	Azithromycin	1g	1g	1g
2	Ethyl Cellulose (50cps)	1g	1.5 g	2 g
3	Ethyl Acetate	10 ml	10 ml	10 ml
4	Water (external Phase)	100 ml	100 ml	100 ml
5	Polyvinyl alcohol	0.015% w/w	0.015% w/w	0.015% w/w

Table 2: Representing % Cumulative drug release ± SD in *In – vitro* dissolution study

Sr. No.	%Cumulative drug release ± SD			
	Time (Hrs)	C1	C2	C3
1	1	5.13 ± 0.84	5.01 ± 0.57	4.98 ± 0.75
2	2	6.01 ± 0.35	5.91 ± 0.28	5.21 ± 0.36
3	3	6.96 ± 0.68	6.13 ± 0.34	5.99 ± 0.63
4	4	15.09 ± 0.46	6.84 ± 0.44	6.11 ± 0.22
5	5	23.22 ± 0.92	13.05 ± 0.25	8.54 ± 0.95
6	6	31.35 ± 0.46	18.11 ± 0.55	13.12 ± 0.31
7	7	39.49 ± 0.24	20.15 ± 0.36	17.21 ± 0.65
8	8	47.61 ± 0.57	30.86 ± 0.24	21.54 ± 0.24
9	9	55.74 ± 0.64	41.40 ± 0.57	30.18 ± 0.65
10	10	63.87 ± 0.24	54.14 ± 0.42	42.15 ± 0.96
11	11	72.05 ± 0.65	65.09 ± 0.74	56.18 ± 0.38
12	12	80.13 ± 0.24*	76.19 ± 0.24	69.98 ± 0.75

Table 3: Values of rate constants (K) and correlation coefficients (R) for release of Azithromycin microsphere in kinetic study

Batch	Zero Order		First Order		Matrix		Korsemeyer Peppas			Hixon- Crowel	
Code	(K)	(R)	(K)	(R)	(K)	(R)	(K)	(R)	(n)	(K)	(R)
C1	9.244	0.891	-0.206	0.985	27.14	0.996	25.75	0.998	0.524	-0.050	0.993
C2	8.511	0.931	-0.166	0.991	24.84	0.993	19.69	0.998	0.615	-0.043	0.995
C3	7.633	0.968	-0.133	0.994	22.08	0.978	15.51	0.997	0.668	-0.036	0.994

n = Diffusion exponent

Figure 1: FT-IR of Formulation C1

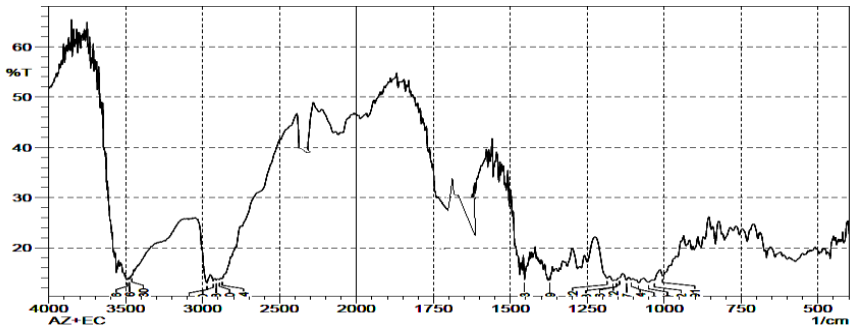


Figure 2: DSC Thermogram of Microspheres (C1)

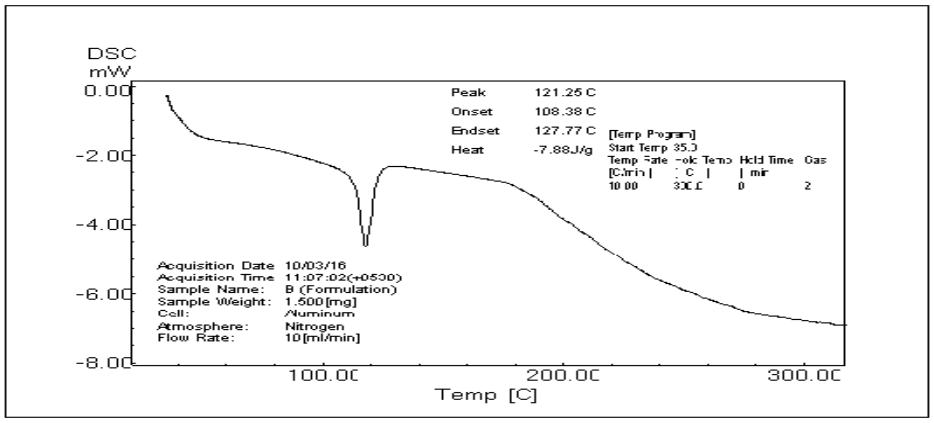


Figure 3: XRD pattern of microspheres (C1)

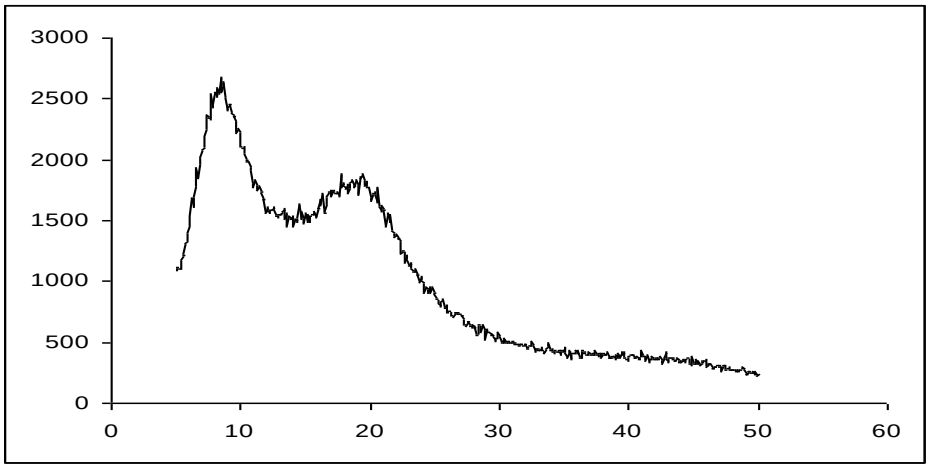


Fig 4a: **Scanning Electron Microscopy of group of microsphere formulated with Ethyl Acetate of C1 Formulation.**

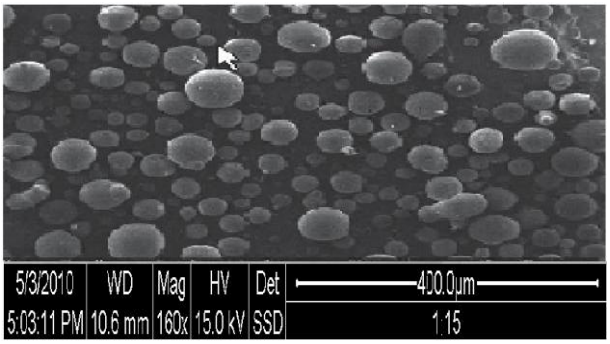
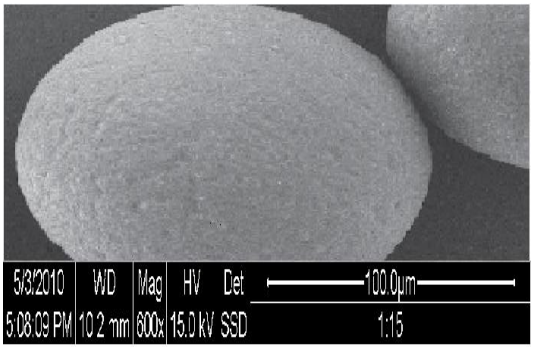


Fig.4b: Whole image of microsphere



REFERENCES

1. Brahmankar DM, Jaiswal SB. Biopharmaceutics and pharmacokinetics a treatise. 1st ed. New Delhi: Vallabh Prakashan Publications; 1995. p. 50-57.
2. Vyaas SP, Khur RK. Targeted and controlled drug delivery- novel carrier systems, 1st ed. Delhi: Cbs Publishers & Distributers; 2006. p. 414-458.
3. Jackson LS, Lee K. Microencapsulation and the food industry. 1st ed. Lebensmittel-wissenschaft technologie 1991; 2;23.
4. Ming L, Olivier R, Denis P. Microencapsulation by solvent evaporation: state of the art for engineering approaches. Int J Pharm 2008;1:363.
5. Jain NK, Jain S, Bhandra D. Advances in controlled and novel drug delivery, 1st ed. Delhi: Cbs Publishers and Distributors; 2001. P. 426- 449.
6. Chien YW. Novel drug delivery system. 2nd ed. New York: Marcel Dekker Inc; 1992. P. 161-172
7. Julian BL, Leah EA, Scott MH, Thombre AG. Formulation design and pharmaceutical development of a novel controlled release form of azithromycin for single-dose therapy. Drug Dev And Ind Pharm 2009;35:1522-29.
8. The Indian Pharmacopoeia Commission, Ghaziabad. Indian Pharmacopoeia. 1996;2:246, 1:477- 78, 2:912- 913.
9. Mendham J, Denney RC, Barnes JD, Thomas MJK. Vogel's textbook of quantitative chemical analysis. 6th ed , New Delhi: Pearson Education; 2006. p. 503
10. Beckett AH, Stenlake JB. Practical pharmacutical chemistry. 4th ed. Nodia: Cbs Publishers And Distributers; 2007; (Pt 2): P. 383, 275
11. Skoog DA, Holler FJ, Crouch SR, Instumental anaiysis. 2nd ed. New Delhi: Cengage Learning India Private Ltd; 2008. p. 343, 982.
12. Behera BC, Sahoo SK, Dhal S, Barik BB, Gupta BK. Chracterization of glipizide-loaded polymethacrylate microspheres prepared by an emulsion solvent evaporation method. Trop J Pharm Research 2008;7(1):879-85
13. Deore BV, Mahajan HS, Deore UV. Development and characterization of sustained release microspheres by emulsion solvent diffusion method. Int J Pharm 2009;3:634-42.
14. Gao Y, Fu-De C, Guan Y. Preparation of roxithromycin-polymeric microspheres by the emulsion solvent diffusion method of taste masking. Int J Pharm 2006;318:62-9.
15. Hadkar UB. A handbook of practical physical pharmacy and physical pharmaceutics, 1st ed. Pune: Nirali Prakashan; 2004. p. 68-69.
16. Sinko PJ. Martin's physical pharmacy and pharmuceutical sciences. 5th ed. New Delhi: Wolters Kluwer Health (India) Pvt Ltd; 2007. p. 4, 533, 540, 546, 553, 558.
17. Lachman L, Libermann HA, Kanig J. The theory and practice of industrial pharmacy, 3rd ed. Bombay: Varghese Publication; 1991. p. 412- 428.
18. Benita S, Benoit LP, Puisieux F, Thics C: Characterization of drug-loaded poly (d,llactidc) microspheres. J Pharm Sci 1984;73:1721.
19. Suhagia BN, Shah SA, Rathod IS. Determination of azithromycin in pharmaceutical dosage form. Int J Pharm 2006;68:242-45.
20. Abazinge M, Jackson T, Yang Q, Owusu-Ababio G. Comparison of *in-vitro* and *in-vivo* release characteristics of sustained release drug delivery. Xenobiotica 2000;7 (2):77-81
21. Sultana N, Arayne MS, Hussain F. Degradation studies of azithromycin and its spectrophotometric determination in pharmaceutical dosage forms. Pak J Pharm 2006;19:94-98
22. United States Pharmacopoeial Convention, Rockville. Usp Nf 30. 2007; p. 2350.

23. Zhang ZY, Ping QN. Microencapsulation and characterization of tramadol- resin complexes. Int J Pharm 2000;66:107-113.
24. Patil.VB, Varsha BP. Preparation and evaluation of sustainedrelease nimsulide microspheres prepared from sodium alginate. Indian J.Pharm.Sci.2001:63(1);15-19.
25. Schimdt P. Secondary electron microscopy in pharmaceutical technology. In: Swarbrick J, Boylon, JC, editors. Encyclopedia of pharmaceutical technology. 3rd ed. New York:Marcel Dekker, Inc; 2007. p. 3217- 325.
26. Jens TC, Rhodes CT. Drug stability principles and practices. 3rd ed. New York:Informa Heathcare, 2005. p. 314-316, 322.

ABBREVIATION

IR: Infrared spectroscopy
DSC: Differential Scanning Calorimetry
XRD: X-Ray diffractometry
SEM: Surface Morphology
UV: Ultraviolet Visible double beam Spectrophotometer
K: Rate constants
R: Correlation coefficients

AST: Accelerated stability studies