AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

Microencapsulation of a Mixture of Herbal Extracts by Non Solvent Addition Method

SeemanchalaRath*, Bijan Kumar Gupta, NripendraNath Bala

1. BDCA College of Pharmacy and Technology, Jessore Road (south), Hridaypur, Barasat, Kolkata – 700127, West Bengal, India

ABSTRACT

Stress is a biological response to aversive conditions that tend to threaten or perturb the homeostasis of the organisms. Stress is one of the basic factors in the etiology of number of diseases and stress has been postulated to be involved in pathogenesis of various diseases, such as psychiatric disorders like depression and anxiety, immune suppression, endocrine disorder like diabetes mellitus, impotency, cognitive dysfunction, peptic ulcer, ulcerative colitis and cardiovascular disorder like atherosclerosis and hypertension. So a mixture of herbal extracts like Arjuna, Ashwagandha, Brahmi and Shankhapushpi in equal ratios was microencapsulated using different types of wall polymers by non solvent addition method. Microcapsules were evaluated for their percentage yield, percentage actual drug content, percentage extract entrapment efficiency, flowability and drug release kinetics. Kollicoat SR 30 D and aluminium stearate were observed as effective in prevention of particle aggregation during phase separation. Microcapsules were shown controlled drug release pattern for 12 hours due to the presence of Eudragit RS 100 and Eudragit RL 100. Both these wall polymers are responsible for controlling the drug release from microcapsules through diffusion in phosphate buffer medium having pH 7.4.

Keywords: Aluminium stearate, Controlled release, Dissolution, Eudragit, Herbal extracts, Kollicoat SR 30 D.

*Corresponding Author Email: seemanchalarath@gmail.com

Received 25 May 2012, Accepted 14 June 2012

Please cite this article in press as: SeemanchalaRath *et al.*, Microencapsulation of a Mixture of Herbal Extracts by Non Solvent Addition Method. American Journal of PharmTech Research 2012.

INTRODUCTION

Now-a-days due to fast modern life & great increase in populations there is severe competitions for self sustenance. These are leading to stress. Stress is a biological response to aversive conditions that tend to threaten or perturb the homeostasis of the organisms. [1, 2]Stress is one of the basic factors in the etiology of number of diseases and stress has been postulated to be involved in pathogenesis of various diseases, such as psychiatric disorders like depression and anxiety; immune suppression; endocrine disorder like diabetes mellitus; impotency; cognitive dysfunction; peptic ulcer; ulcerative colitis; cardiovascular disorder like atherosclerosis and hypertension.[3] Neurological type of stress may be due to physiological abrasion leading to hypersympathetic activities centrally as well as peripherally. The neurotransmitters released does great harm to cardiovascular system and are the cause of several diseases and ultimately death. Stress has been shown to induce a marked rise in the brain levels of biogenic amines such as adrenaline, non-adrenaline and 5-hydroxytryptamine (Serotonin). These chemical substances are released in response to stress signals and are meant to assist the organisms to cope with stress. Hence stress, whether it is physical or neurological usually excites the sympathetic system to provide extra activation of body in the states of stress, which is called as "sympathetic stress response"[4]. So now stress management is an important factor without which man may disappear from the globe. That is why the international community is attracted to Indian Heritage of Yoga & Ayurveda Medicines. There are various herbal drugs like Arjuna, [5, 6, 7]Ashwagandha, [8, 9]Brahmi, [10, 11, 12] and Shankhapushpi[13, 14, 15, 16, 17]& many others which are known to control or prevent stress. Neurological type of stress may be due to physiological aberrations leading to hyper sympathetic activities as well as cardiovascular disorders. So in the present investigation an attempt has been made to develop a controlled drug delivery system containing a mixture of herbal extract like Arjuna, Ashwagandha, Brahmi and Shankhapushpi in equal ratios through microencapsulation using different types of wall polymers.The study also included effect of different types of polymers as well as different types of anti-aggregating agents on the dissolution profile of microcapsules.

MATERIALS AND METHODS

Materials

Hydroalcoholic (70% ethanolic) extract of the following herbs (Dry powder), such as Arjuna bark (*Terminalia arjuna*) (TA), Ashwagandha root (*Withania somnifera*) (TA), Brahmi whole plant (*Bacopamonnieri*) (BM) and Shankhapushpi whole plant (*Convolvulus pluricaulis*) (CP)

obtained as gift samples from EMAMI Ltd. Kolkata, India. Eudragit RS 100 (ERS) obtained as a gift sample from SUN PHARMA, Vadodara, Gujurat, India. Eudragit RL 100 (ERL), Kollicoat SR 30D (KSR) and Eudragit L 30 D-55 (ELD) were obtained as a gift sample from ALEMBIC Pvt. Ltd. Vadodara, Gujurat, India. Ethyl cellulose (EC), cellulose acetate (CA), hydroxy propyl methyl cellulose (HPMC), aluminium stearate, (AST), magnesium stearate (MS) and talc were purchased from LobaChemie. Sodium carboxymethylcellulose (CMC), toluene, petroleum ether (PE), potassium dihydrogen phosphate, sodium hydroxide pellets, concentrated hydrochloric acid were purchased from MERCK. Double distilled water (DDW) was prepared in the laboratory from demineralised water. All the reagents used were of analytical grade and were used as received.

Preparation of microcapsules

A mixture of 4 extracts in dry powder form such as TA, WS, BM and CP in equal ratios (ME) was considered as drug for the entire study. A non-solvent addition method was employed for the preparation of microcapsules of the herbal extracts using different wall polymers and different anti aggregating agents. Accurately weighed quantity of wall polymers were dissolved in 30 ml of boiled toluene. 1 gm of ME (250mg of each extract such as TA, WS, BM and CP i.e. all the extracts were in equal ratio) triturated with accurately weighed quantity of anti-aggregating agent and was dispersed in the above polymer solution with stirring by an electrical stirrer (REMI) at a speed of 500 rpm. 60 ml of petroleum ether (non-solvent) was added drop wise to the above dispersion at a speed of 1ml/minute from a fixed height (5cm). Then the microcapsules formed were washed thrice with 50ml portion of petroleum ether for rigidisation.[18] In case of M1, KSR was not mixed with the extracts, but dispersed in polymer solution.[19] Then the microcapsules were collected by decantation, filtration, air dried for 10 minutes and dried to a constant weight in a desiccator over silica gel self-indicating coarse and finally stored in another desiccator over silica gel self-indicating coarse for further studies. Total five different batches of microcapsules were prepared whose details were given in Table 1.

Percentage yield, % EEE and flowability

Prepared microcapsules were dried to a constant weight in a desiccator over silica gel self-indicating coarse for percentage yield determination.[20] Accurately weighed quantity (100 mg) of microcapsules were stirred with 0.1 M HCl in a magnetic stirrer for 48 hours in order to extract the entrapped drug completely and was diluted up to 250 ml with 0.1 M HCl. Then the solution was centrifuged at 4000 rpm for 10 minutes and the absorbance was measured at 275 nm under UV-Visible spectrophotometer (SHIMADZU 1700-JAPAN) against 0.1M HCl as reference

standard or blank. The Percentage extract encapsulation efficiency or percentage extract entrapment efficiency (% EEE) was calculated from actual percentage dug content (ADC) or practical % DC (Drug Content) and theoretical % DC (TDC) by multiplying 100 to the ratio of ADC to TDC[21]. Flow property of the microcapsules was studied by determining angle of repose. Angle of repose was determined by fixed funnel and free standing cone method, in which a funnel is secured with its tip at a fixed height above a graph paper that is placed on a flat horizontal surface. Microcapsules were carefully poured through the funnel until the apex of the conical pile just touches the tip of the funnel. The radius of the conical pile and angle of repose (AR) was determined.[22]

In-vitro dissolution study and mechanism of drug release

Equivalent weight of microcapsules containing 200 mg ME was taken for in-vitro dissolution testing. Dissolution was carried out in 900 ml of 0.1 M HCl (acidic medium) for 2 hour followed by 10 hour in 900 ml of phosphate buffer having pH 7.4 (PBS) (alkaline medium) at 60 rpm at a temperature of 37 ± 0.5°C.[23]First sample was taken at 30 minute interval and subsequent samples were taken at an interval f 1 hour from the starting of dissolution. (Sampling interval was 0.5, 1, 2, 2.5, 3, 4, 5, 6, 7 and so on up to 12 hours from the start of dissolution). For the first three samples5 ml was withdrawn and diluted upto 25 ml with 0.1 M HCl and analyzed for absorbance measurement at 275 nm under UV-Visible spectrophotometer (SHIMADZU 1700-JAPAN) against 0.1M HCl as reference standard or blank. For the rest of the samples 5 ml was withdrawn diluted up to 25 ml with PBS and analyzed for absorbance measurement at 278 nm under UV-Visible spectrophotometer (SHIMADZU 1700-JAPAN) against PBS as blank. Each time after sample withdrawn 5 ml of respective dissolution medium was also replaced. In-vitro dissolution data were fitted into different mathematical models like Zero order,[24] First order,[25] Higuchi,[26] Hixson Crowell[27] and Korsmeyer Peppas model[28]and their correlation coefficient (R^2) values were used as an indicator of the best fitting for each of the models. Release exponent (n) value[29] of the Korsmeyer Peppas model was used to identify the mechanism of drug release.[30]

RESULTS AND DISCUSSION

Percentage yield, % EEE and flowability

Percentage yield of all the 5 batches of microcapsules were reported in **Table 2** as average ± SD (n=3). Percentage yield was more in case of M1 and least in case of M3, because M3 contains HPMC, which may not act as an anti aggregating agent. But KSR was proven to be a better anti-aggregating agent, because it prevents particle aggregation due to sticky nature of wall polymers

and given maximum yield in case of M1. After M1 maximum yield was observed in case of M2, which contains AST as an anti-aggregating agent. So KSR and AST were considered as having better anti-aggregating property than talc and MS, because M4 was having % yield less than M1 and M2. % EEE was more in case of M1 (94.6863 %) as it contains KSR as anti-aggregating agent. Next to M1 % EEE of M2 was 93.0533 %, because M2 contains AST. But M3 have least % EEE like percentage yield, because it contains HPMC, which may not effectively prevent particle aggregation. So KSR and AST were considered as better anti-aggregating agents for microencapsulation than talc, MS and HPMC. % yield as well as % EEE of M5 was also less though it contains AST which may be due to the sticky nature of CA. Angle of repose was less than 25 degrees in all the batches (excellent flowability) indicated that after microencapsulation flow property of ME was improved considerably, because before microencapsulation ME has AR value 35.4819 ± 0.7759 degrees (passable flow). Again for the angle of repose KSR and AST were responsible as evidenced from M1 and M2 **(Table 1).**

Table 1- Formulation details for microencapsulation

Ingredients (gm)	M1	M2	M3	M4	M5
Extract	1	1	1	1	1
ERL	1.5	2	1	1.5	0.5
ERS	-	-	-	-	0.5
EC	-	-	1	1.5	-
ELD	1.2 (4ml)	-	-	-	-
CA	-	-	-	-	1
KSR	0.6 (2ml)	-	-		-
AST	-	0.5	-	-	0.5
HPMC	-	-	0.5		-
Sod CMC	-	-	-	0.2	-
MST	-	-	-	0.2	-
TALC	-	-	-	0.2	-
TOTAL	4.3	3.5	3.5	4.6	3.5

Table 2- % Yield, TDC, ADC and %EEE of different batches

Code	% Yield (Average ± SD)	% TDC	% ADC (Average ± SD)	% EEE (Average ± SD)	AR in degrees (Average ± SD)
M1	92.1085 ± 1.6669	23.2558	22.0200 ± 0.6557	94.6860 ± 2.8197	20.0266 ± 0.6530
M2	84.3905 ± 2.3258	28.5714	26.5867 ± 0.8021	93.0533 ± 2.8073	21.1357 ± 0.7436
M3	78.7524 ± 1.9814	28.5714	24.6867 ± 0.7024	86.4033 ± 2.4583	22.0104 ± 0.5753
M4	83.3696 ± 2.1334	21.7391	20.1867 ± 0.5686	92.8587 ± 2.6157	22.4197 ± 0.4730
M5	83.0952 ± 2.3043	28.5714	25.5200 ± 0.7550	89.3200 ± 2.6424	22.2890 ± 0.5139

In-vitro dissolution study and mechanism of drug release

Percentage drug released (% DR) was minimal from M1 both after 2 hr and 12 hr (43.9473 % and 69.3403 % respectively) as compared to other formulations. Because M1 contains ERL and

ELD. ELD is an enteric polymer[31]and soluble at pH above 6 whereas ERL[32] is soluble at pH below 7. So initially more than 40 % DR was observed from all the formulations at 2 hr. All the formulations had shown a controlled release pattern for 12 hours. M2, M3, M4 and M5 had % DR more than 50 % after 2 hr, since they did not contain any enteric material. On the other hand M5 contains both ERL and ERS (both the polymers are insoluble at physiological pH, but permeable at pH below 7) resulting 63.2093 5 % DR after 2 hr and 95.7378 % DR after 12 hr. % DR was maximum from M5 because it contains fewer amounts of wall polymers. So ERL, ERS and ELD were considered as better wall polymers for controlling the drug release from microcapsules. M2 and M3 have shown somewhat similar release pattern both after 2 hr as well as after 12 hr also. Release profile of M2 and M3 were almost same with each other **(Figure 1)** because both contain 2 gm of coating polymer (M2 contains 2 gm of ERL and M3 contains 1 gm of ERL and 1 gm of EC). M1 provided controlled drug release pattern for 12 hr, due to the presence of ERL, ELD and KSR. Though M5 contains both ERS and ERL provided higher release rate than M1, since it contains smaller amount of ERS and ERL (0.5 gm each) and does not contain KSR. So it was observed that KSR acts as a better anti-aggregating agent and also as a better rate controlling polymer.[33]Dissolution data of all the five different batches of microcapsules were fitted to different mathematical models. Linear regression equation of different models was determined by least square method using Microsoft Excel 2007. R^2-values of different models were used as an indicator of the best fitting. In case of all the formulations R^2-values of Korsmeyer Peppas model were largest (ranging from 0.8164 to 0.9129), that is closest to 1 as compared to other models. So the dissolution profiles of all the formulations were best fitted to Korsmeyer Peppas model. Release exponent (n) value or slope of the Korsmeyer Peppas model was found to be from 0.2040 to 0.2784, which indicates that drug release was occurred through Fickian diffusion in all the five formulations. R^2-values and release exponent of different mathematical models are given in **Table 3**. Dissolution rate constants of different mathematical models such as Zero order, First order, Hixson Crowell and Higuchi were determined from their slope, whereas rate constant of Korsmeyer Peppas model was determined from its Y-intercept value. In case of fitted zero order model dissolution rate constant (K_0) was varied from 1.5590 to 3.6247 % hr^{-1}. Zero order dissolution rate constant was minimum in case of M1 because it contains ERL, KSR and ELD. ERL controls the drug release in PBS and ELD in 0.1 M HCl. All the mathematical models shown that dissolution rate constants were maximum in case of M5 and minimum in case of M1. Dissolution rate constants of various mathematical models are given in **Table 4.**

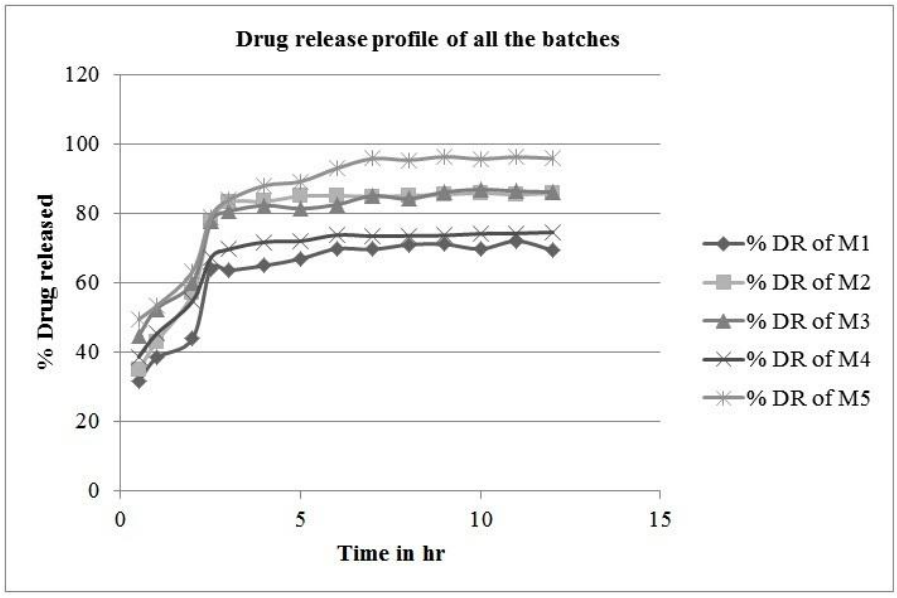

Figure1- Drug release profile of all the batches

Table 3- R^2-values and Release exponent of different mathematical models

R^2-values and Release exponent	M1	M2	M3	M4	M5
R2-value of Zero Order model	0.6045	0.5143	0.6019	0.5682	0.6953
R2-value of First Order model	0.7223	0.5870	0.7212	0.6331	0.8849
R2-value of Higuchi model	0.7385	0.6731	0.7497	0.7261	0.8329
R2-value of Hixson Crowell model	0.6859	0.5622	0.6800	0.6110	0.8311
R2-value of KorsmeyerPeppas model	0.8380	0.8164	0.8644	0.8563	0.9129
Release Exponent (n) value of KorsmeyerPeppas model	0.2153	0.2784	0.2077	0.2040	0.2308

Table 4- Dissolution rate constants of different mathematical models

Dissolution rate constants #	M1	M2	M3	M4	M5
K_0 (% hr^{-1})	1.5590	3.2769	2.8242	2.3394	3.6247
K_1 (hr^{-1})	0.0431	0.1110	0.1085	0.0626	0.2462
K_H (% $hr^{-1/2}$)	9.4480	16.2830	13.6900	11.4870	17.2320
K_{HX} (mg $hr^{-1/3}$)	0.0469	0.1412	0.1205	0.0793	0.2170
K_{KP} (hr^{-n})	0.4281	0.4898	0.5587	0.4888	0.5845

K0, K_1, K_H, K_{HX} and K_{KP} are dissolution rate constants for Zero order, First order, Higuchi, Hixson Crowell and KorsmeyerPeppas model respectively.

CONCLUSION

Herbal extracts were successfully microencapsulated using different types of wall polymers by non solvent addition method. Kollicoat SR 30 D and aluminum stearate were proven to be

having better anti-aggregating property as compared to talc, magnesium stearate and hydroxypropylmethyl cellulose. These anti-aggregating agents were effective against preventing particle aggregation during phase separation. So considerable percentage extract entrapment efficiency and percentage yield was also obtained. Flowability of the microcapsules were improved to excellent flow as compared to mixture of extracts. Eudragit RS 100, Eudragit RL 100 and Eudragit L 30 D 55 were having better rate controlling property. A controlled drug release pattern was obtained for 12 hour. Drug release mechanism was fickian diffusion from the different batches of microcapsules as revealed from the release exponent value of Korsmeyer Peppas model.

ACKNOWLEDGEMENT

Authors would like to thank BCDA Governing body members and BCDA Members Benevolent Trust.

REFERENCES

1. Bhattacharya K, Ghosal S. Experimental evaluation of the anti-stress activity of a herbal formulation, zetress. Journal of Natural Remedies 2000;1: 1-7.
2. Piazza PV, Lemoal M. The role of stress in drug self-administration. Trends Pharmacological Sciences 1998; 19: 67-74.
3. Hoffman BB. Catecholamines, sympathomimetic drugs, and adrenergic receptor antagonist. In, Hardman JG, Limbird LE, Gillman AG (eds). Goodman and Gillman's the pharmacological basis of therapeutics. 10th ed. New York: Tata McGraw-Hill; 2001; 235-238.
4. Subarnas A, Tadaro T, Nakahata N, Arai Y, Kinemuchi H, Oshima Y, Kisara K, Ohizuri Y. A possible mechanism of antidepressant activity of beta-amyrinpalmitate isolated from Lobelia inflates leaves in the forced swimming test. Life sciences 1993; 52: 289-296.
5. Bharani A, Ganguli A, Mathur LK, Jamra Y, Raman PG. Efficiency of Terminaliaarjuna in chronic stable angina: a double blind, placebo controlled cross over study comparing Terminaliaarjuna with isosorbidemononitrate. Ind Heart J 2002; 54(2): 170-175.
6. Kumar DS, Prabhakar YS. On the ethnomedical significance of the Arjun tree, Terminaliaarjuna (Roxb.). Weight and Arnot. J Ethnopharmacol 1987; 20(2): 173-190.
7. Dwivedi S, Jauhari R. Benefidcial effects of Terminaliaarjuna in coronary artery disease. Ind Heart J 1997; 49(5): 507-510.

8. Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy. 34th ed., Pune: Nirali Prakashan; 2006: 260-261, 518-520.
9. Grandhi A, MujumdarAM, Patwardhan B. A comparative pharmacological investigation of Ashwagandha and Ginseng. J. Ethnopharmacol 1994; 44(3): 131-135.
10. Sairam K, Dorababu M, Goel RK, Bhattacharya SK. Bacopamonnieri in experimental models of depression in rats. Phytomedicine 2002; 9(3): 207-211.
11. Singh HK, Dhawan BN, Neuropsycho pharmacological effects of the Ayurvedic nootropic Bacopamorrieri Linn. (Brahmi). Ind J Pharmacol 1997; 29 (5): 5359-5365.
12. Malyszko J, Urano T, Takada Y, Takada A. Stress and/or tranylcypromine treatment affects serotonergic measures in blood and brain in rats. Neurosci Res 1994; 19 (4): 365-371.
13. Sinha SN, Dixit VP, Madnawat AVS, Sharma OP. The possible potentiation of cognitive processing on administration of Convulvulusmicrophyllus in rats. Indian Med 1989;1(3): 1-6.
14. Nahata A, Patil UK, Dixit VK. Anxiolytic activity of Evolvulusalsinoides and Convulvuluspluricaulis in rodents. PharmaceutBiol 2009; 47(5): 444-451.
15. Nahata A, Patil UK, Dixit VK. Effect of ConvuluspluricaulisChoisy. On learning behavior and memory enhancement activity in rodents. Nat Prod Res 2008; 22(16): 1472-1482.
16. Mirzaev YR, Aripova SF. Neuro and psychopharmacological investigation of the alkaloids convolvine and atropine. Chem Nat Comp 1998; 34(1): 56-58.
17. Sairam K, Rao CV, Goel RK. Effect of ConvulvuluspluricaulisChois on gastric ulceration and secretion in rats. Ind J ExpBiol 2001; 39(4): 350-354.
18. Nixon JR, Meleka MR. The preparation and characterization of ethylcellulose-walled theophylline microcapsules. J Microencapsulation 1984; 1(1): 53-64.
19. Donbrow M, Hoffman A, Benita S. Phase separation modulation and aggregation prevention: mechanism of the non-solvent addition method in the presence and absence of polyisobutylene as an additive. J Microencapsulation 1990; 7(1): 1-15.
20. Shabaraya AR, Narayanacharyulu R. Design and evaluation of chitosan microspheres of metoprolol tartrate for sustained release. Ind J Pharm Sci 2003; 65(3): 250-252.
21. Mathew ST, Devi SG, Sandhya KV. Formulation and Evaluation of Ketorolac Tromethamine-loaded Albumin Microspheres for Potential Intramuscular Administration. AAPS Pharm Sci Tech 2007; 8(1): Article 14 (http://www.aapspharmscitech.org).

22. Banker GS, Anderson NR. Tablets. In, Lachman L, Lieberman HA, Kanig JL (eds). The theory and practice of industrial pharmacy. 3rd ed., Bombay: Varghese Publishing House; 1990; 317.
23. Prakash K, Raju PN, Shanta KK, Lakshmi MN. Preparation and characterization of lamivudine microcapsules using various cellulose polymers. Trop J Pharm Res 2007; 6 (4): 841-847.
24. Shoaib MH, Tazeen J, Merchant HA, Yousuf RI. Evaluation of drug release kinetics from ibuprofen matrix tablets using HPMC. Pak J Pharm Sci 2006; 19(2): 119-124.
25. Donbrow M, Samuelov Y. Zero order drug delivery from double-layered porous films: release rate profiles from ethyl cellulose, hydroxypropylcellulose and polyethylene glycol mixtures. J Pharm Pharmacol 1980; 32 (7): 463–470.
26. Higuchi, T. Mechanism of sustained-action medication. Theoretical analysis of rate of release of solid drugs dispersed in solid matrices. J Pharm Sci 1963; 52 (12): 1145–1149.
27. Sinko PJ. Martin's Physical Pharmacy and Pharmaceutical Sciences. 5th ed., New York: Lippincott William's & Wilkins Publishers; 2006: 342.
28. Korsmeyer RW, Gurny R, Doelker EM, Buri P, Peppas NA. Mechanism of solute release from porous hydrophilic polymers. Int J Pharm 1983; 15: 25-35.
29. Avachat A, Kotwal V. Design and Evaluation of Matrix-Based Controlled Release Tablets of Diclofenac Sodium and Chondroitin Sulphate. AAPS PharmSciTech 2007; 8: 51–56.
30. Peppas NA. Analysis of Fickian and non-Fickian drug release from polymers. Pharm ActaHelv. 1985; 60: 110-111.
31. Blanco-Prieto MJ, Delie F. Particulate systems for oral drug delivery. In, Benita S (ed). Microencapsulation methods and industrial applications. 2nd ed., New York: Taylor and Francis Group; 2006; 533-545.
32. Benita S, Tamilvanan S. Lipid and polymeric colloidal carriers for ocular drug delivery. In, Benita S (ed). Microencapsulation methods and industrial applications. 2nd ed., New York: Taylor and Francis Group; 2006; 612-614.
33. Dashevsky A, Wagner K, Kolter K, Bodmeier R. Physicochemical and release properties of pellets coated with Kollicoat SR 30 D, a new aqueous polyvinyl acetate dispersion for extended release. Int J Pharm 2005; 290 (1-2): 15-23.