Asian Journal of Biomedical and Pharmaceutical Sciences
ISSN : 2249 - 622X

RESEARCH ARTICLE

Received on: 03-11-2014
Accepted on: 30-11-2014
Published on: 11-12-2014

Prasanth Kumar Acholu
Dr. Reddy's Laboratories Ltd, Hyderabad
Email: apkreddy02@yahoo.co.in

QR Code for Mobile users

Conflict of Interest: None Declared !

DOI: 10.15272/ajbps.v4i38.627

Ex-vivo and In-vivo Evaluation of Formulated Novel Felodipine Core in Cup Buccal Tablets

Prasanth Kumar Acholu,*[1] Sudhakar Yajaman,[2] K. N. Jayaveera[3]
[1] Dr. Reddy's Laboratories Ltd, Hyderabad.
[2]Department of Pharmacy, Government Polytechnic for Women, Kadapa.
[3] Former OTRI Director& Professor in Chemistry, JNTUA. Anantapur

Abstract

Novel buccal mucoadhesive tablets of felodipine as core in cup systems preparation and evaluation in-vitro of adhesive cups, core and novel tablets is studied and published in the earlier research paper. In this investigation the formulated novel buccal tablets which are optimized through *in-vitro* studies are selected and performed the *ex-vivo and in-vivo* studies on rabbits. Drug permeation study of felodipine buccal tablets was carried out using porcine buccal mucosa, which was procured from local slaughter house and placed in Krebs buffer pH 6.8. *In-vivo* experiments were conducted after approval of the protocol from Institutional Animals Ethics Committee. As per the protocol, optimized formulations were tested in Albino rabbits for pharmacokinetic studies simultaneously for both formulations. Studies were conducted in rabbits in the weight range of 1.5 to 2.5 kg. From the permeation data different permeation parameters like flux (Jss), permeation coefficient (kp), diffusion coefficient (D), amount drug permeated at 6 hr and release rate constant (k) were calculated. The flux obtained was 5478.04 to 8478.78 mcg/cm2/h with permeability coefficient of 0.038 to 0.058 cm/h. The peak plasma concentration (Cmax) felodipine in the pure drug and its complex were 233.75±5.40 and 517.25±23.92 ng/mL, while AUC0-6hr and AUC0-∞ were found to be 1867.5±42.07, 2293.04±82.13 ng.hr/mL, 3116.99±116.46 and 36970.91±4044.98 ng.hr/mL respectively. These values indicated maximum plasma concentration and area under the curve were achieved by felodipine complex formulation. C_{max} value was 2.3 times and AUC0-12hr was 1.56 times higher for complex than felodipine. The relative percent bioavailability (Frel) of felodipine complex observed was 130.58% indicated enhanced oral bioavailability of felodipine complex.

Keywords: buccal, *ex-vivo*, felodipine, flux, *in-vivo*, permeation.

Cite this article as:
Prasanth Kumar Acholu, Sudhakar Yajaman, K. N. Jayaveera. *Ex-vivo and In-vivo* Evaluation of Formulated Novel Felodipine Core in Cup Buccal Tablets. Asian Journal of Biomedical and Pharmaceutical Sciences; 04 (38); 2014, 35-40.

INTRODUCTION

Among the various routes of drug delivery, the oral route is perhaps the one mostly preferred by patients and clinicians. Based on our current understandings of biochemical and physiological aspects of absorption and metabolism, many drugs, cannot be delivered effectively through the conventional oral route, because after administration are subjected to pre-systemic clearance extensively in liver, which often leads to a lack of significant correlation between membrane permeability[1], absorption and bioavailability. The buccal mucosa has been investigated for local and systemic delivery of therapeutic peptides and other drugs that are subjected to first-pass metabolism or are unstable within the rest of the gastrointestinal tract[2]. Buccal delivery offers a safer mode of drug utilization, since drug absorption can be promptly terminated in cases of toxicity by removing the dosage form from the buccal cavity.

The research work carried out on formulation of felodipine novel buccal tablets as core in cup systems. The dissolution studies of various formulations prepared with sodium alginate, sodium carboxymethyl cellulose, chitosan, carbopol were performed and from the results obtained after the bio adhesive strength and other physical parameters the optimized formulations were selected and studied for *ex-vivo* and *in-vivo* estimations in the porcine buccal mucosa and albino rabbits respectively.

Felodipine is a calcium channel blocker used as antihypertensive and antianginal drug. According to biopharmaceutical classification system, felodipine is class II drug, i.e., low solubility and high permeability. Felodipine has poor water solubility and hence poor dissolution and bioavailability after oral administration. In view of poor solubility and poor bioavailability of felodipine, in present study an attempt was made to study the bio-availibility enhancement through *in-vivo* studies of prepared novel novel buccal mucoadhesive tablets.

MATERIALS

Felodipine was received as a gift sample from Aurobindo Pharma Ltd., Hyderabad. Sulfobutyl ether β-Cyclodextrin (SBE-β-CD) was gifted by Cydex Pharma Inc., USA. Carbopol, Sodium carboxy methyl cellulose (SCMC), Sodium alginate, Guar gum, Hydroxypropyl methyl cellulose (HPMC) and Hydroxy ethyl cellulose were utilized from institution resources. Methanol, talc, microcrystalline cellulose procured from Karnataka fine Chem. Industries, Bangalore. Porcine buccal mucosa, for determining buccoadhesive strength and ex-vivo permeation studies, was procured from a local slaughter house. All other materials used were of analytical grade. Porcine buccal mucosa, for determining buccoadhesive strength and ex-vivo permeation studies, was procured from a local slaughter house. The in vivo study was carried out in 10 to 12 weeks of old healthy white rabbits (2-2.5 kg, male, *Orytolagus cuniculuss*) purchased from Banglore, India.

METHODS

Formulation of Novel Buccal Mucoadhesive Tablets

Novel buccal mucoadhesive tablets were prepared in a three-step process involving preparation of adhesive cups, core tablets and Novel buccal mucoadhesive tablets. Finally, novel core tablets in adhesive cups were prepared by inserting core tablets into the respective cups manually and compressed with little force using 4.5 mm flat faced punches.

Ex-vivo permeation study

Drug permeation study of felodipine buccal tablets was carried out using porcine buccal mucosa, which was procured from local slaughter house and placed in Krebs buffer pH 6.8. Tissue was prepared as procedure explained earlier. This study was done using Franz's diffusion cell[4]. It consists of upper cylindrical compartment open from above and containing the porcine buccal mucosa at its base. Lower compartment was in form of a closed cylinder having the sampling port and had Teflon coated magnetic needle at the base. The junction connecting two compartments was intended in such a way that the buccal membrane did not move from its place. The donor compartment was filled with 10 ml of phosphate isotonic buffer pH 6.8 containing 20% of methanol. The buccal tablet was located in such a manner that it must be fixed on the mucous membrane. The drug receptor partition contains 20 ml isotonic phosphate buffer having 20% methanol to maintain sink conditions[5]. The whole assembly was maintained at 37±1^{0C}. 5 ml of samples were collected from receptor compartment and restored with the same volume of fresh medium. The withdrawn samples were then diluted suitably and assayed spectrophotometrically at respective wavelength.

In-vivo studies on rabbits

As per the protocol, optimized formulations were tested in Albino rabbits for pharmacokinetic studies simultaneously for both formulations[6]. Studies were conducted in rabbits in the weight range of 1.5 to 2.5 kg. After habitation of ten days they were exposed to drug sample (Drug solution, optimized buccal formulations in serial one after other with wash out period of seven days (more than seven half lives). Rabbits were not given feed for 24h prior to drug administration and anaesthetized with pentobarbital (25 mg/kg). Intravenous dosing was performed using isoptin ampoule (2.5 mg/ml) (The dose decreased in

this case to prevent cardiac arrest of rabbit which may lead to death). For oral administration, measured dose drug in aqueous solution was administered by oral feeding needle to all four rabbits and blood volumes were collected from subsidiary ear vein using insulin syringe at specific intervals. For the blood withdrawal, rabbits were restrained in restrainers and 1 ml of blood was taken from subsidiary ear vein. Withdrawn blood was mixed gently with a drop of heparin solution (Inhep®) and centrifuged for 15 minutes at 4,000 rpm at 10°C. Centrifugation gives sedimentation of blood component and supernatant plasma. Plasma was separated and processed as mentioned earlier. Extracted drug was quantified in validated HPLC method. After a washout period of seven days, optimized formulations were positioned just over the incisor tooth (one it her side of the mouth) and held firmly in place with a finger over the lip for 30 seconds to ensure adhesion.

Estimation of drug in Plasma by HPLC Method

The plasma samples were estimated by means of a high performance liquid chromatographic in the mode of reversed phase (HPLC) method[7-8]

Instrumentation

The HPLC instrument consists of Agilent packed in Liquid C 1210 pump and injection device for sampling of Rheodyne set, integral by a 20 µl sample disk. In this detector has adjusted to working at a wavelength of 343 nm. ODS (Octadecylsilane) C18 column (10 µm, 249x4.8 mm) integral by a protection column was used for partition. The running solvent composed of acetyl nitrile as well methyl alcohol in the fraction of 7.4:2.4. The pH was maintained at 1.2 by ethelenetriamine. By using membrane filter (Sartorius USA) the mobile phase filtered and then removed gas by ultra sonication. Study was carried at a flow rate of 1.0 ml/min and quantification was done by length of peak[9].

Estimation of drug in plasma

For determination of drug samples in plasma, initially a standard curve was plotted for estimating plasma samples consisting known quantities of drug samples.

The sequence of test samples consisting 0.5 milliliter of plasma in all, 0.1 milliliter active substance dispersion consisting 1, 2, 4, 6 and 8 microgram of drug samples were placed then stirred. In to each test tube one milliliter of acetyl nitrile was placed, agitated vigorously and subjected for centrifugation at 6000 rpm for 15 minutes. The acetonitrile was evaporated and organic portion (0.5 ml) was collected by a dry pipe. To the above filtrate 0.45 milliliter of mobile solvent was incorporated and stirred for reformation. Afterward 20 µl was introduced by the column in support of chromatography examination[10].

Determination of Pharmacokinetic Parameters

a. Estimation of C_{max} and T_{max}

The data obtained from the time Vs concentration in plasma curve, maximum plasma concentration (C_{max}) and time at which maximum concentration obtained (T_{max}) were noted.

b. Estimation of k_{el} and $t^{1/2}$

On a semi logarithmic graph paper, time versus plasma concentration data was plotted. The rate constant for elimination (K_{el}) was estimated through the slope obtained from linear line during elimination phase (the "finest match" regression linear line from concentration positions in the time of elimination was plotted through technique of minimum squares). The respective $t^{1/2}$ estimated by the formula,

$t^{1/2}=0.693/Kel$

c. Estimation of absorbed drug percentages and ka

Drug absorbed percentages and Ka were estimated through the plasma drug quantity information explained by Wagner and Nelson. Determination of absorption rate from blood the fallowing equation used,

d"A" /dt=V"d" dC"b" /dt+K"el." C"b"

Where,

dA/dt= rate of absorption, Vd = apparent volume of distribution, Kel= rate constant for elimination

Here AT=quantity of absorbed drug at time T, CT = amount in blood on time T as well the amount below the basic signal is the AUC of blood Vs time plot among the given range. While the consecutive standards of AT/Vd are estimated, a maximum asymptotic digit [AT/Vd]∞ can be attained. The greatest asymptomatic digit is separated by consecutive digits of AT/Vd to give % absorption value i.e.,

(AT/Vd)/([AT/Vd]∞)×100

A plot of log non absorbed percentage verses time is a straight plot, the slope would be equivalent to –Ka/2.303 through this Ka can be determined.

d. Estimation of Area under the curve and bioavailability

The drug concentration in plasma Vs area under the curve plot for 12h time was determined, from an arithmetical graph of concentration in plasma Vs time from the method of trapezoidal rule. The remained area starting 12 h to ∞ time was estimated with help of the following equation,

$$[AUC]_{12}^{\infty} = \frac{concentration\ at\ 12th\ hour}{K_{el.}}$$

Then

$$[AUC]_{0}^{\infty} = [AUC]_{0}^{12} + [AUC]_{12}^{\infty}$$

Bioavailability (F) was determined according to the equation:

F=(AUC buccal × dose(IV))/(AUC IV × dose(buccal))

f. Determination of Mean Residence Time (MRT)

MRT can be used to for measuring the nature of drugs and metabolites to stay in the body. Excretion of drug is

at all times in equilibrium with drug in plasma while the drug in the organs and the MRT is the mean time spend by active substances in the whole system prior to excreted (on steady clearance circumstances). If it assumed that time period of active substance in blood is statistical distribution plot, this could be written as;

MRT=AUMC/AUC

RESULTS AND DISCUSSION

Drug permeation study for prepared buccal tablets was performed using bovine buccal mucosa. From the permeation data different permeation parameters like flux (Jss), permeation coefficient (kp), diffusion coefficient (D), amount drug permeated at 6 hr and release rate constant (k) were calculated. The flux obtained was 5478.04 to 8478.78 mcg/cm2/h with permeability coefficient of 0.038 to 0.058 cm/h. Chitosan get protonated in the aqueous solution and able to interact tight junction of epithelium of mucus membrane there by allowing paracellular permeation of hydrophilic drugs by enhancing the permeability coefficient. The increase in amount of chitosan enhances the flux and permeability coefficient thereby enhancing the permeation rate which was due to enhanced effect of chitosan. The mucosal membrane stand as an obstruction to passage of drug and it is exist in middle of epidermis as well gut in its permeation properties. The drug permeation study of optimized formulations by buccal mucosa data showed in the table 1 & 2.

F. code	Zero order	First order	Higuchi model	Korsmeyer-Peppas-rel. mechanism	Best fit model
FBT3	0.996862	0.94107	0.956853	1.072259	Zero order
FBT7	0.971426	0.88345	0.973952	0.943963	Higuchi
FBT9	0.997483	0.94203	0.964179	1.008679	Zero order
FBT11	0.999023	0.95485	0.960071	0.996792	Zero order
FBT12	0.998774	0.94449	0.954091	1.08190	Zero order
FBT18	0.997349	0.94149	0.964189	1.013667	Zero order

Table 1: Correlation coefficients (R2) and exponent for release mechanism values in ex-vivo permeation studies of felodipine buccal tablets

F. Code	Amt permeated Q12 (mcg/cm²)	Flux JSS (µg/cm²/hr)	Rel. rate constant K(µg/cm²/h$^{0.5}$)	Per. Coef. k_p(cm/h)	Dif. coef. D (cm²/h)
FBT3	6532.72	1054.87	4085.09	0.040	5.36
FBT7	8256.36	895.07	5025.32	0.051	6.45
FBT9	5458.98	907.32	4028.78	0.038	5.78
FBT11	8478.78	1229.46	6364.69	0.058	6.65
FBT12	7501.45	1125.15	5548.04	0.050	6.02
FBT18	5478.04	845.06	5006.39	0.052	6.14

Table 2: Drug permeation data of felodipine formulations in ex-vivo permeation studies

Time (hr)	Plasma concentration (ng/mL)				
	Subject A	Subject B	Subject C	Subject D	Mean ± S.D. (n=4)
0.5	93.5	43.1	51.3	47.2	58.775±20.25
1	118	120	115	132	121.25±6.45
2	145	150	138	156	147.25±6.60
3	230	235	228	242	233.75±5.40
4	180	175	176	192	180.75±6.75
5	151	147	148	155	150.25±3.11
6	69	62.3	72	85.6	72.22±8.48

Table 3: Plasma concentration following oral administration of felodipine in rabbits

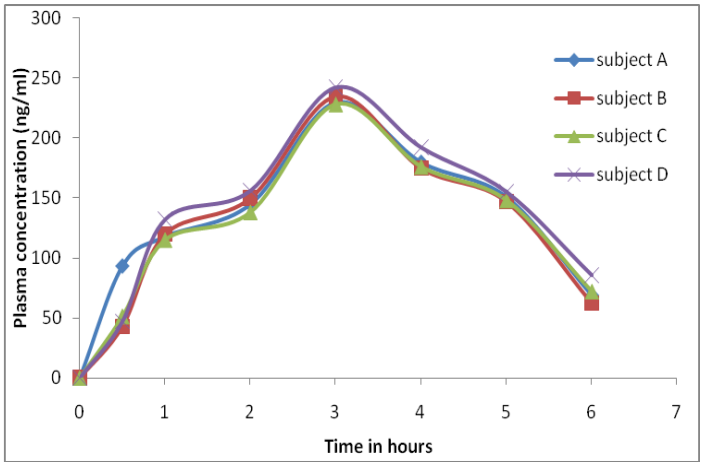

Fig: 1. Plasma concentration time profile following oral administration of felodipine in rabbits

Time (hr)	Plasma concentration (ng/mL)				
	Subject A	Subject B	Subject C	Subject D	Mean ± s.d. (n=4)
0.5	47.5	66.2	44.5	76.3	58.62±13.16
1	71.5	55.4	50.6	81.4	64.72±12.35
2	145	158	151	112	141.5±17.64
3	172	141	252	185	187.5±40.52
4	552	525	496	496	517.25±23.92
5	230	254	210	197	222.75±21.53
6	105	139	128	146	129.5±15.53

Table 4: Plasma concentration following buccal administration of felodipine sulfobutyl ether β-Cyclodextrins in rabbits

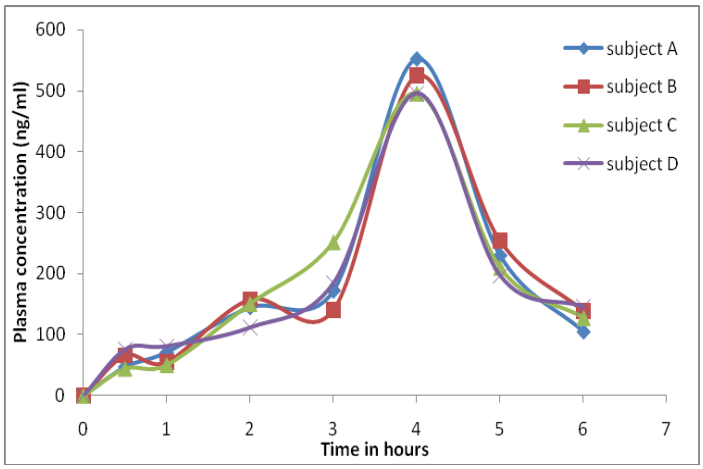

Fig: 2. Plasma concentration time profile following buccal administration of felodipine sulfobutyl ether β-Cyclodextrins in rabbits

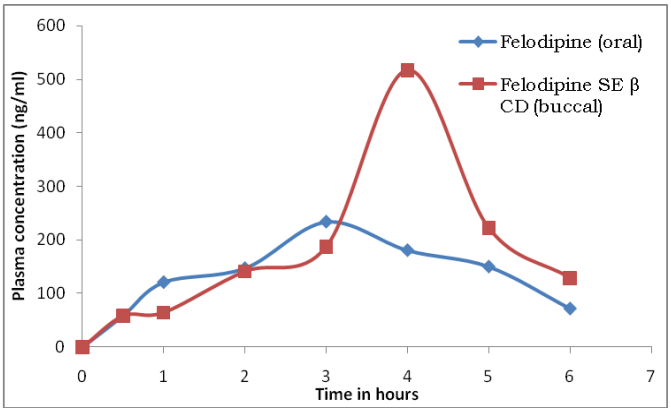

Fig. 3. Comparative mean felodipine plasma concentration-time profile

In-vivo studies on rabbits

Optimized tablets were chosen and characterized for evaluation of pharmacokinetics by means of HPLC in reverse phase mode. Standard curve for the determination of drug in plasma by HPLC method and it shows y=43895x+1651.6 and regression value (r^2) of 0.9989.

All the calculated pharmacokinetic parameters such as C_{max}, T_{max}, AUC0-6hr, AUC 0-∞, AUMC 0-∞, MRT, Ke, t½, V d/F and Cl/F for each subject following single oral administration of felodipine and its SE β-CD complex and mean, S.D., values of pharmacokinetic parameters for each subject are given in table 3 & 4. Bar diagrams of AUC0-6hr for all subjects with both felodipine and its complex are shown in fig.4 & 5.

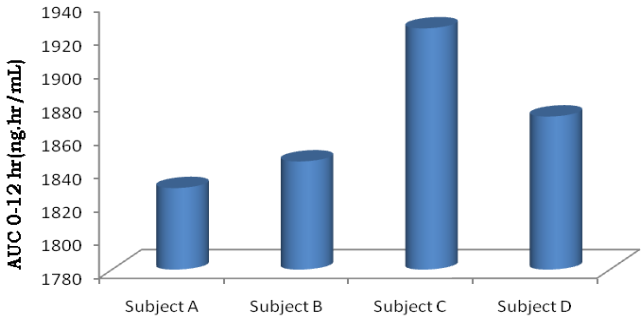

Fig: 4. AUC 0-6hr for felodipine in rabbits

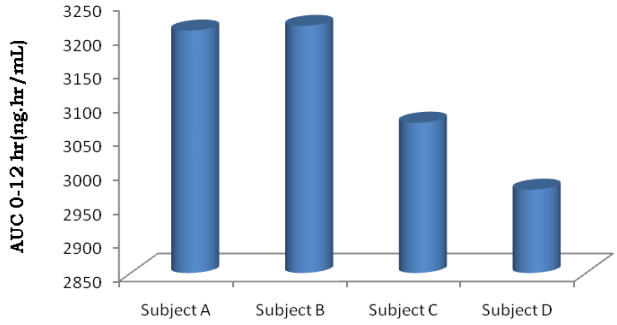

Fig: 5. AUC 0-6hr for felodipine SE β-CD in rabbits

The peak plasma concentration (C_{max}) felodipine in the pure drug and its complex were 233.75±5.40 and 517.25±23.92 ng/mL, while AUC0-6hr and AUC0-∞ were found to be 1867.5±42.07, 2293.04±82.13 ng.hr/mL, 3116.99±116.46 and 36970.91±4044.98 ng.hr/mL respectively. These values indicated maximum plasma concentration and area under the curve were achieved by felodipine complex formulation. C_{max} value was 2.3 times and AUC0-12hr was 1.56 times higher for complex than felodipine. The relative percent bioavailability (Frel) of felodipine complex observed was 130.58% indicated enhanced oral bioavailability of felodipine complex.

The T_{max} values were unchanged and found to be 3 hr for felodipine and 4 hr for felodipine complex groups. The elimination rate constant was found to be in the range of 0.0152 to 0.178 hr^{-1} and t½ for felodipine was found to be 2.84±0.21 hr and for feldipine complex it was 3.69±0.21 and is nearer to the reported values. For pure drug, the Vd/F and Cl/F were found to be 26.83±1.69 L and 4.36±0.158 L/hr respectively and for its complex, 14.92±1.56 L and 2.59±0.09 L/hr respectively.

CONCLUSION

The variability in oral pharmacokinetics of felodipine could also be explained by its limited solubility. Because the water solubility of felodipine is negligible, its dissolution is dependent on the pH and the inherent solubilisation capacity of the buccal cavity. In rabbits receiving felodipine drug, a small variation of these factors could lead to a big difference in dissolution properties and subsequently in the plasma drug concentration. However, in rabbits receiving SE β-CD, could solubilise felodipine, the impact of such factors became non determinative and thus, the oral kinetics in rabbits receiving SE β-CD complexes showed less variability than that in rabbits receiving felodipine drug. It was noticed that there was a primary peak at 0.5 hr and a secondary peak at about 4 hr (T_{max}) after dosing in the concentration vs. time graph for rabbits given the felodipine drug where as such a phenomenon was not observed for the rats receiving SE β-CD complexes. Earlier literature on felodipine also reported similar type of double peak absorption phenomenon as observed in the present investigation.

REFERENCES

1. Andrews G.P., Laverty T.P., Jones D.S., Mucoadhesive polymeric platforms for controlled drug delivery, Eur. J. Pharm. Biopharm . 2009; 71: 505-518.
2. Gilles P., Ghazali F.A., Rathbone J. Systemic oral mucosal drug delivery systems and delivery systems, in: M.J. Rathbone (Ed.), Oral Mucosal Drug Delivery , Vol. 74, Marcel Dekker Inc, New York, 1996; 241-285.
3. Nakhat PD, Kondawar AA, Babla IV, Rathi LG, Yeole PG, Studies on buccoadhesive tablets of terbutaline sulphate Ind J Pharm Sci. 2000; 69 (4): 505-510.

4. Koks C. H. W., Rosing H., Meenhorst P. L., Bult A., Beijnen J. H. High-performance liquid chromatographic determination of the antifungal drug fluconazole in plasma and saliva of human immunodeficiency virus-infected patients. J. Chromatogr. B. 1995; 663:345–351.
5. Perioli L., Ambrogi V., Rubini D., Giovagnoli S., Ricci M., Blasi P., Rossi C. Novel mucoadhesive buccal formulation containing metronidazole for the treatment of periodontal disease. J. Control. Rel. 2004; 95:521–533.
6. V. Kumar, S. R. Cotran, L. S. Robins. Obstructive lung diseases. Basic Pathology, 5th edition, WB Saunders Company, Philadelphia, 1992, 5: 389-392. [2] L. Borgstrom, L. Nyberg, S. Jonsson, et al. Pharmacokinetic evaluation in man of terbutaline given as separate enantiomers and as the racemate. B. J. Clin Pharmacol. 1989, 27: 49-56.
7. Kamath KR, Park K (1994). Mucosal adhesive preparation In: Swarbrick J, Boylan JC (Eds.) Ency. Pharm. Tech. 10: 133.
8. Higuchi T (1963). Mechanism of sustained action medication: Theoretical analysis of the rate of release of solid drug dispersed in solid matrices. J. Pharm. Sci. 52:1145-1149.
9. Attama AA, Adikwu MU, Nnamani PO (2003). Delivery of diclofenac sodium via non-disintegrating bioadhesive tablets of paraffin wax. STP Pharm. Sci. 13: 147-150.