

Formulation and Evaluation of sustained release losartan potassium microspheres

¹Dr. Vijaya Sabhavathu, ²Dr. Sunil Naik Kethavath, Dr. N Murali Krishna,
⁴Dr. Manchu Kalyani

¹Associate Professor, Department of Pharmacology, Rangaraya Medical College, Kakinada, Andhra Pradesh, India

²Associate Professor, Department of General Medicine, Government Medical College, Srikakulam, Andhra Pradesh, India

³Associate Professor, Department of Pharmacology, Prathima Relief Medical College, Warangal, Telangana, India

⁴Assistant Professor, Department of Community Medicine, Andhra medical College, Visakhapatnam, Andhra Pradesh, India

Corresponding Author:
Dr. Manchu Kalyani

Abstract

Oral drug delivery system using sustained release microparticles preparation is a nice option for the methodology expansion and for up gradation purpose. In this present work microspheres containing Losartan potassium with different synthetic and natural polymers, gums like HPMC, PVP, Xanthan gum, Guar gum and Sodium alginate were prepared by Ionic Gelation Method. The influence of formulation factors (drug-polymer ratio and type of polymer) on the morphology, particle size distribution, drug loading capacity, micromeritics properties and the *in-vitro* release characteristics of the microspheres were investigated. Physical characterizations of Losartan potassium microspheres were also carried out using Scanning Electron Microscopy, and FTIR Spectrophotometry. Microspheres were evaluated for different parameters like bulk density, tapped density, angle of repose and drug entrapment efficiency. *In vitro* dissolution studies were carried out in pH 7.4 Phosphate Buffer and 0.1N HCL buffer for about 12hrs and samples were examined by UV-Visible Spectroscopy. The results demonstrated that formulations (FA, FB, FC) showed Losartan potassium discharge speed in series of 91-94% when compared (FD, FE and FH) demonstrated a Losartan potassium discharge speed from 85. -94% to and those of (FG, FH and FI) which have displayed drug release rates in the range of 94.-98% up to duration of 12 hours. This denotes that if the quantity of rate retarding polymer is raised, then it leads to retard discharge of drug. The synergistic effect was observed when the HPMC was combined with Xanthan gum. Hence batch FH indicates the better results than batch FI. As formulation FH shown 97.78% cumulative drug release pattern when the HPMC is combined with the natural gums for retarding drug discharge. Formulation containing HPMC and Xanthan gum gave well >85%. FH batch gave 93.40%, other formulations gave little bit drug loading than this batch. It can be happened due to viscosity caused by used material best % recovery was obtained for batch FH-96.49%. Overall 0% recovery of microsphere obtained > 83%.

Keywords: Losartan potassium, microspheres, ionic gelation, entrapment efficiency, angle of repose

Introduction

Controlled drug delivery technique presents front line part of today's developed technique, in this includes many scientific approaches, serving for individual care. The drug deliverance technique having abundant advantages than existing conventional type of dosage, it involves enhanced effectiveness, minimized poisoning, enhanced consumer conformity also ease. This type of drug deliverance technique utilizes micro molecules, for caring drugs. As the varieties of forms for dosage are invented like microparticle as well as nanoparticles shown more significance [1].

Sustained release system

Advantages

Circum vent patient's compliance problem due to reduced frequency of dosing. Frequent administration of normal drug delivery system can be overcome by.

- Make use of a less total drug.
- Reduce or eradicate local or systemic side effects.
- Reduces drug accretion with chronic dosing.
- Obtain less latent of reduction in drug activity with chronic use.
- Better competence in treating patients.
- Treat situation more quickly.
- Bio availability of some drugs Enhanced.
- Utilizing specific consequence. E.g. treatment of arthritis.
- Cost-cutting measure.
- Overall, sustained release forms facilitated increased dependability of therapy.

Disadvantages

- Abridged potential for dose titration.
- Dose dumping may lead to toxicity.
- Enlarged potential for Hepatic Metabolism.
- Constancy problems.
- Requirement of additional patient education.
- Recovery of drug is not possible in case of toxicity.
- Unpredictable *in vitro-in vivo* correlation.

Sustained release system

Sustained release systems attain sluggish discharge of drugs long time period and in this drug are originally made available in amount to cause the desired pharmacological effect in body [2-5].

Microparticles

These are particulate dispersed and or firm particles having diameter 01-1000 μm . The drug is embedded or covered in polymer or dissolved in polymer. The microspheres or microcapsules can be obtained it is depends on the method of preparation, In Microcapsules active constituent is restricted to a hollow cover of polymeric material. In microspheres drug is equally distributed. The procedure of preparation allows managing microparticle diameter. It is important for various applications [6].

Applications of microsphere

- Controlled or Sustained release is probable.
- Taste covering in case of oral delivery.
- Fortification of drug or active component from moisture and/or oxygen and/or light.
- Allows grouping of incompatible constituents.
- Enhances flow properties of drug or active component.
- Simplicity of formulation.
- Solid microsphere is broadly used in reflective traffic paint [7-10].

Materials and Methods

Preformulation studies

The preformulation study like characterization drug sample which includes physical characterization and analytical methodologies, drug interaction studies (compatibility study) by FTIR spectroscopy and evaluation of microsphere including determination of micromeritics characters were performed for drug as well as natural gums.

Preparation of buffers and reagents

Preparation of SGF solution

SGF was prepared by dissolving 2 gms of sod chloride along with 7 ml of HCL in distilled water and volume was made upto 100ml. The pH was found to be 1.2 ± 0.1 .

Preparation of SIF solution

Disodium hydrogen phosphate (2.38 gms), 0.19 gm of potassium dihydrogen phosphate and 8.0 gm of sodium chloride were dissolved in distilled water and made up to 1000 ml with distilled water to get phosphate buffer saline pH (7.4).

Analytical method for estimation of Losartan potassium

A spectrophotometric method based on the measurement of extinction at 236 nm in 3 different dissolution fluids like Distilled water, SGF of pH 1.2 and SIF of pH 7.4 were used for the estimation of Losartan potassium.

Preparation of standard stock solution

Accurately weighed 100 mg of DH dissolved in 100 ml Dw/SGF.

Losartan potassium

SIF to give concentration of 1000 μ g/ml.

Scanning of losartan potassium

The standard stock solution was scanned in UV spectrophotometer in a wave length range of 200-300 nm. The maximum absorbance was found at 205 nm. Hence the same was used for estimation of Losartan potassium.

Preparation of calibration curve for Losartan potassium

From the standard stock solution 1 ml was pipetted out and diluted to 100 ml with DW/SGF/SIF to give stock solutions of 10 μ g/ml. Aliquots of 2, 4, 6, 8 and 10 ml of stock solutions were pipette out into different 10 ml volumetric flasks. The volume was made upto 10 ml to obtain a concentration of 2, 4, 6, 8 and 10 μ g/ml. The absorbance was measured at 205 nm using U.V. Spectrophotometer (Hitachi) against the blank. The procedure was performed in triplicate to validate the calibration curve.

Analytical method for estimation of losartan potassium

A spectrophotometric method based on the measurement of extinction at 205 nm in 3 different dissolution fluids like Distilled water, SGF of pH 1.2 and SIF of pH 7.4 were used for the estimation of Losartan potassium.

Preparation of standard stock solution

Accurately weighed 100 mg of Losartan potassium dissolved in 100 ml SIF to give concentration of 1000 μ g/ml.

Scanning of losartan potassium

The standard stock solution was scanned in UV spectrophotometer in a wave length range of 200-300 nm. The maximum absorbance was found at 205 nm. Hence the same was used for estimation of Losartan potassium.

Preparation of calibration curve for losartan potassium

From the standard stock solution 1 ml was pipetted out and diluted to 100 ml with SIF to give stock solutions of 10 μ g/ml. Serial dilutions were carried out so as to get different concentration of 5, 10,15,20,25 and 30 μ g/ml. The absorbance of solution was measured at 205 nm. against the suitable blank. The procedure was performed in triplicate to validate the calibration curve. A calibration graph was plotted by taking conc. on x-axis and absorbance on y-axis ^[11-14].

Preparation of microsphere

Formulation of sustained release microsphere procedure

The microsphere of Losartan potassium was prepared by using Ionotropic Gelation technique by varying the polymer and crosslinking agent concentration and keeping the other variables such as concentration of sodium alginate and rpm constant. In this method Losartan potassium dispersed into solution of sodium alginate (2% w/v) 50 ml stirred at 500 rpm using mechanical stirrer. To the resultant dispersion 2% aerosil was added and polymers (HPMC, Xanthan gum and combination) in required concentration (1:1,1:2,1:3 w/w)and stirred for 1 hr., then above bubble free dispersion transferred by 5 ml hypodermic needle with 24 gauze in CaCl₂ 127 solution 100 ml (4%, 6% and 8% w/v) and agitated at 600-800 rpm. Obtained microspheres removed, cleaned with water and dried at 40 °C.

Evaluation of microspheres

Sustained release microspheres thus prepared were evaluated for following parameters.

Particle size determination

The diameter of microsphere was found using an optical microscope as well as polarized light near about 50-100 microspheres were examined and average diameter was calculated using ocular micrometer.

Determination of bulk density

It is ratio of weight by volume. It was resolute by utilizing mark off cylinder, the precisely measured quantity of product microspheres inserted to cylinder and three times tapped. Noted the level, and calculated bulk density using formula.

Formula: $\rho_b = M/V$

ρ_b = Density Where, m = mass of sample v = volume of sample

Tapped density

The sample of about 10 cm³ was carefully introduced in 25 ml glass cylinder. The cylinder tapped at 1 inch height, with intervals of 2-3 second on a rough wood surface three-four times. Density of Bulkiness calculated by using equation below.

$D_o = M/V_p$

Here, D_o = Tap density M = samples wt (gm) V_p = final material volumes (cm³)

Angle of repose

It was carried out, using funnel, at sufficient height funnel was fixed and, microcapsules were added through it until the pile touched at bottom of funnel. Pile height as well as radius measured and using formula angle of repose calculated [Lachman *et al.*, 2003] and [M. Ganeshan *et al.*, 2000].

$\tan\theta = h/r$ Here, h = height r = is radius of pile.

In vitro Release profile study of formulated microspheres

The dissolution studies executed utilizing (type II) XXIV USP dissolution rate test apparatus in 0.1 N HCl for 2 hrs followed by pH 7.4 900ml dissolution media, at 50 rpm and 37^oC temperature upto 12 hrs. Using ELICO-164 U.V. Spectrophotometer double beam, 5ml of samples taken at different time gaps and 5ml of same dissolution medium added to uphold sink condition. Withdrawn aliquots diluted and analyzed spectrophotometrically at 205nm^[15-20].

Results

Table 1: Calibration Curve values of Losartan Potassium

Concentration (μg)	Absorbance	
	0.1N HCL	pH 7.4 Buffer
0	0.00	0.00
10	0.12	0.09
20	0.23	0.17
30	0.33	0.26
40	0.45	0.33
50	0.56	0.43

Table 2: Evaluation of losartan potassium Microsphere

Batch	Average microsphere	Bulk Density	Tap Density	Angle of Repose
FA	291.5	0.298	0.522	25 ⁰
FB	323.5	0.542	0.654	26 ⁰
FC	356.9	0.526	0.636	25 ⁰
FD	263.9	0.430	0.508	30 ⁰
FE	327.7	0.483	0.529	25 ⁰
FF	356.3	0.516	0.616	31 ⁰
FG	406.7	0.452	0.572	30 ⁰
FH	426.5	0.468	0.506	27 ⁰
FI	432.7	0.534	0.622	30 ⁰

Percentage recovery (i.e. Yield) of microspheres

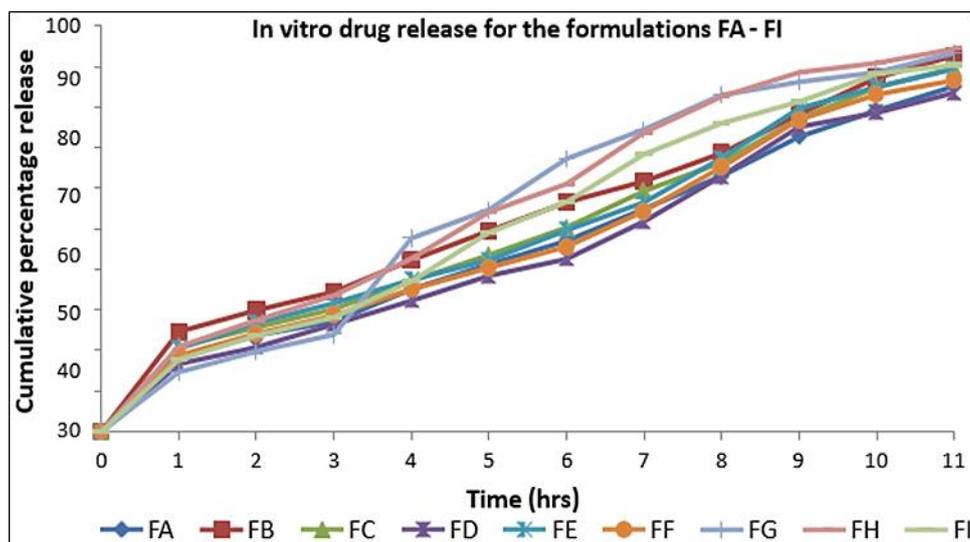
Best % recovery was obtained for batch FH - 96.47 %. Overall % recovery of microsphere obtained > 82%.

Estimation of losartan potassium

Formulation containing HPMC and Xanthan gum gave well >84%. FH batch gave 92.30%, other formulations gave little bit drug loading than this batch. It can be happened due to viscosity caused by used material.

Table 3: Percentage yield and Percent drug entrapment of microspheres

Batch	% Yield	% Drug Entrapment
FA	84.2	82.8
FB	86.5	85.2
FC	90.3	86.4



In vitro drug release for formulations FA-FI

The results demonstrated that formulations (FA, FB, FC) showed Losartan potassium discharge speed in series of 91-94% when compared (FD, FE and FH) demonstrated a Losartan potassium

discharge speed from 86. -93% to and those of (FG, FH and FI) which

have displayed drug release rates in the range of 93.-97% up to duration of 12 hours. This denotes that if quantity of rate retarding polymer raised, leads to retard discharge of drug. The synergistic effect was observed when the HPMC was combined with xanthan gum. Hence batch FH indicates the better results than batch FI. As formulation FH shown 97.78% cumulative drug release pattern, this was according to the Acceptance Table of Test 2 given in USP-NF 2007 time duration of 12 hrs. When the HPMC combined with the natural gums is used for retarding drug discharge.

Drug Release Kinetic Studies-The drug dissolution data was checked to discharge kinetics to check basis for medicament release by microspheres.

Table 4: *In vitro* drug release kinetics

Formulation n	<i>In vitro</i> drug release kinetics			
	Zero order	First order	Higuchi matrix	Korsmeyer-Peppas
FA	0.9886	0.9314	0.9381	0.9985
FB	0.9776	0.9693	0.9631	0.9978
FC	0.9858	0.9087	0.9431	0.9983
FD	0.9841	0.9264	0.9221	0.9988
FE	0.9808	0.9022	0.9381	0.9983
FF	0.9822	0.9133	0.9291	0.9985
FG	0.9605	0.9539	0.9371	0.9994
FH	0.9769	0.9533	0.9235	0.9988
FI	0.9886	0.9536	0.9532	0.9982

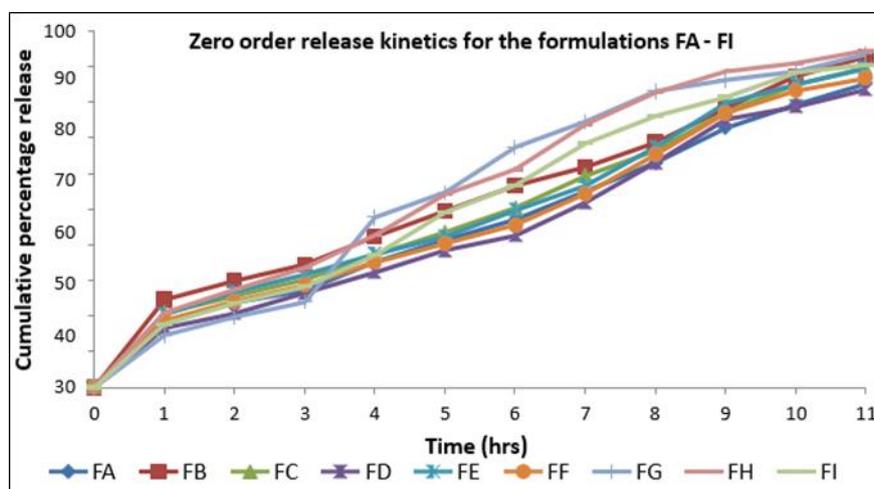


Fig 1: Zero order release kinetic for the formulations FA-FI

SEM of microsphere of optimized batch

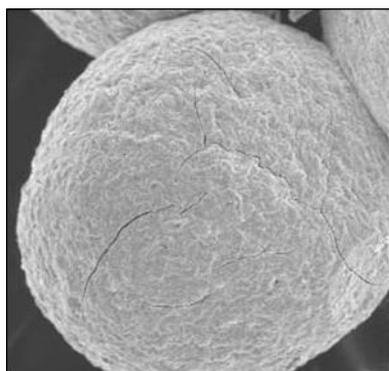


Fig 2: SEM-1 Shows size range of microspheres

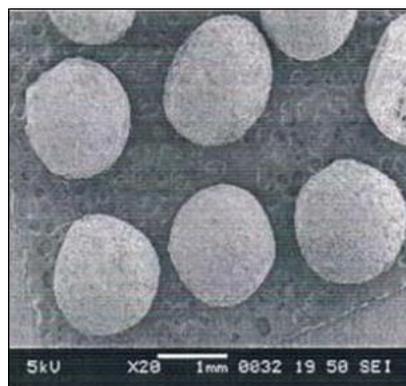


Fig 3: SEM-2 Shows size range of microspheres



Fig 4: SEM-3 shows cross section view of microspheres at X50

Discussion

The results of the density of bulkiness and density of tapping were mentioned in table. Bulkiness values were lies in 0.297 to 0.542 g/cm³ and density of tapping values lies in 0.508 to 0.654 g/cm³ i.e. less than 1.2, indicates good packing. The values of Average particle size and angle of repose were lies in between 291.4 to 432.6 and 25° to 30°, respectively indicates acceptable particle size, flow property and also good packing ability. Oral sustain drug delivery systems have received much attention of the researchers these days. The rationale for developing a sustain release formulation is to enhance its therapeutic benefits, reducing its side effects and improving the management of diseased condition. A number of strategies have been developed to obtain sustain release of the drug in the body. These include a simple microspheres to more technologically sophisticated products which are introduced to the market place. Studies have been carried out for developing sustain release microspheres of Losartan potassium by using polymeric materials like HPMC, PVP, Xanthan gum, Guar gum and sodium alginate. These were selected as microspheres forming polymers for controlled release as these were reported to have microspheres forming properties. The sustained release solid dispersed microparticles can resolve the problem of shorter biological half-life drugs by sustaining the effect of drugs or its action, by the mechanism of slow discharge of Losartan potassium from dosage form. The prepared microspheres were evaluated for particle size, bulk density, tapped density, angle of repose, drug entrapment efficiency (DEE) and FTIR. The microspheres were then evaluated by *in vitro* dissolution studies.

The following conclusions were drawn from the results

- Microspheres of Losartan potassium obtained utilizing orifice ionic gelation technique using HPMC and Xanthan gum and their combination as a polymer with various ratios.
- The prepared microcapsules were free flowing, non-sticky.

- All the formulations showed satisfactory results.
- The obtained results stated that the natural polymers can be used for sustaining the release of drug.
- In the above view of findings it can be suggested that HPMC when combined with the hydrophilic natural gums shows the synergistic effects and hence can be utilized to prolong the release of Losartan potassium.
- The PVP polyvinylpyrrolidone has proved an excellent solubility enhancer for Losartan potassium.
- Among the different combinations of natural polymers and drug many combinations were shown optimum results.
- The release retardant materials are cheap, readily available, safe, having wide regulatory acceptance and easy to handle for economic point of view.
- This study also explored polymers and gum in different combination and demonstrated the ability to retard drug release.
- It could be cost effective formulation that does not require the use of costly polymers and solvents.
- It may be beneficial to adopt such simple technology for the commercial manufacture of persistent release microspheres.

The results demonstrated that formulations (FA, FB, FC) showed Losartan potassium discharge speed in series of 91-95% when compared (FD, FE and FH) demonstrated a Losartan potassium discharge speed from 86. -94% to and those of (FG, FH and FI) which have displayed drug release rates in the range of 93.-98% up to duration of 12 hours. This denotes that if quantity of rate retarding polymer raised, leads to retard discharge of drug. The synergistic effect was observed when the HPMC was combined with Xanthan gum. Hence batch FH indicates the better results than batch FI. The batch FH followed the zero order and Non Fickian drug release except batch FH, it follows the Hixson Crowel model Non-Fickian drug release. Best % recovery was obtained for batch FH-96.48%. Overall o% recovery of microsphere obtained > 83%. Formulation containing HPMC and Xanthan gum gave well >85 %. FH batch gave 94.30 %, other formulations gave little bit drug loading than this batch. It can be happened due to viscosity caused by used material. Losartan potassium, the first of a new class of potent and specific AT1-selective, non-peptide, angiotensin II antagonists, significantly reduces mean 24-h ambulatory blood pressure and tough clinic sitting blood pressure, and is well tolerated.

Drug release from all the microcapsules was studied in acid buffer for initial two hours and phosphate buffer for a period of next ten hours. The release pattern was slow and extended for twelve hours. Log percentage of drug remaining versus time curves exhibited straight line for all the formulations and confirmed that the release rates followed first order kinetics. Cumulative percentage of drug release versus square root of time curves showed linearity and it proves that all the formulations follows Higuchi model, suggesting that diffusion may be the mechanism of drug release. (Figure: 42.). Log cumulative percentage of drug release versus log time curves shows high linearity and it proves that all the formulations follow Korsmeyer-Peppas model.

Conclusion

In this study, the drug release data for microcapsules of all formulations containing different co polymers except FH showed good fit into first-order with the highest correlation coefficient ($r > 0.98633$), the rate constants were calculated from the slopes of the respective plots. High correlation was observed with the first-order plots rather than Higuchi and zero- order model. The data obtained were also put in Korsmeyer-Peppas model in order to find

out n' value, which describes the drug release mechanism. The formulations FA to FG showed good linearity ($r=0.983604$ to 0.999489) with slope (n) values ranging from 0.460464 to 0.769428 , indicating that diffusion is the dominant mechanism of drug release with these formulations. This indicates a first order release controlled by non Fickian diffusion. The diffusion exponent of release profile (slope) has a value of ($n>0.5$), which indicates a zero order release controlled by non Fickian diffusion. The analysis of regression values of Higuchi plot and Korsmeyer-Peppas plot and "n" values of Korsmeyer-Peppas model shows a combination of diffusional and dissolutional mechanism indicating the drug release from the formulations was controlled by more than one process.

Conflict of interest: None.

Funding support: Nil.

6. References

1. Chien YW Novel Drug Delivery Systems, ed. by Chien YW, Marcel Dekker, Inc., New York, U.S.A., 1992, 139-196.
2. Rang and Dale, Hormones, Text Book of Pharmacology, 5th Edition, edited by Laurence Hunder, 2004, 385.
3. Khan GM, Zhu JB. Controlled release co precipitates of ibuprofen and carbopol preparation, characterization and *in vitro* release. Sciences. 2001;1:355-360.
4. Mehta KA, Kislaloglu MS, Phuapradit W, Malick AW, Shah NH. Release performance of a poorly soluble drug from a novel Eudragit-based multi-unit erosion matrix. Int. J Pharm. 2001;213:72.
5. Liu J, Zhang F, McGinity JW. Properties of lipophilic matrix tablets containing phenylpropanolamine hydrochloride prepared by hot-melt extrusion. Eur. J Pharm Biopharm. 2001;52:181-Y190.
6. Rowe RC. Molecular weight dependence of the properties of ethyl cellulose and hydroxypropyl methylcellulose films. Int. J Pharm. 1992;88:405-408.
7. Akbuga J. Furosemide-loaded ethyl cellulose microspheres prepared by spherical crystallization technique: morphology and release characterization. Int. J Pharm. 1991;76:193-198.
8. Eldrige JH, Hommond CJ, Meulbroek JA, Staas JK, Gilley RM, Tice TR. Controlled vaccine release in the gut-associated lymphoid tissues. Part I. orally administered biodegradable microspheres target the payer's patches. J Control. Rel. 1990;11:205-214.
9. Janselijak I, Nicolaidou CF, Nixon JR. Dissolution from tablets prepared using ethyl cellulose microcapsules J Pharm. Pharmacol. 1977;29:169-172.
10. Martin A, Micromeritics, In: Physical Pharmacy. 4th ed. Philadelphia: Lippincott Williams & Wilkins, 2001, 423-52.
11. Banker GS, Anderson NR. Tablets. In: Lachman L, Lieberman HA, Kanig JL. The Theory and Practice of Industrial Pharmacy. 3rd ed. Philadelphia: PA: Lea & Febiger, 1986, 293-45.
12. Raghuram RK, Srinivas M, Srinivas R. Once-daily sustained-release matrix tablets of nicorandil formulation and *in vitro* evaluation, AAPS Pharma Sci. Tech. 2003;4(4):E61.
13. Guidance for Industry, Dissolution Testing of Immediate Release Solid Oral Dosage Forms, U.S. Department of Health and Human Services Food and Drug Administration Center for Drug Evaluation and Research (CDER), [Cited 2009 August 8]. Available from: <http://www.fda.gov/downloads/Drugs/>.
14. Higuchi T. Mechanism of sustained action medication, theoretical analysis of rate of release of solid drugs dispersed in solid matrices, J Pharm Sci. 1963;52:1145-1149.

15. Bourne W. Pharmacokinetics, In: Banker GS, Rhodes CT, Eds., Modern Pharmaceutics, 4th ed, New York, NY; Marcel Dekker Inc., 2002, 67-92.
16. Hadjiioannou TP, Christian GD, Koupparis MA. Quantitative Calculations in Pharmaceutical Practice and Research, VCH Publishers Inc., New York, NY, 1993, 345-348.
17. Peppas NA, Analysis of Fickian and non-Fickian drug release from polymers, Pharm Acta Helv. 1985;60:110-111.
18. Edube NK, Hikal AH, Christy MW, Beer DC. Effect of drug formulation process variables on granulation and compaction characteristics of heterogenous Matrices part 1, HPMC and HPC systems. Int. J Pharm. 1997;156:49-57.
19. Basak SC, Jayakumar Reddy BM, Lucas Mani KP. Formulation and release behaviour of sustained release ambroxol hydrochloride HPMC matrix tablet, Indian J Pharm Sci., 2006, 594-597.
20. Dakkuri A, Schoeder HG, Delva PP. Sustained Release from Matrices II, effect of surfactants on Tripeleminamine Hydrochloride Release. J Pharm Sci., 1987a, 352-354.