

FORMULATION AND IN VITRO EVALUATION OF SUSTAINED RELEASE MICROPARTICLES OF LOXOPROFEN SODIUM

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ABSTRACT

Background: Loxoprofen sodium is an essential NSAID which is 2-arylpropionoc acid derivative. It owns a very short half-life and principally used for treating osteoarthritis & rheumatoid arthritis. So far there is no loxoprofen sodium's modified release dosage form available and is commercialized as tablets and transdermal patches only. **Objective:** The aim of this study was to develop and characterize the sustained and prolonged release microparticles of loxoprofen sodium by utilizing HPMC as a bio-polymer. **Methods:** Coacervation-phase separation technique was used in this study. The prepared microparticles went through *in-vitro* evaluation to observe the outcome of various parameters (polymer to drug ratio, stirring speed, and stirring time) on the physicochemical behaviors of prepared micro particles. Prepared microparticles exhibited excellent encapsulation efficiency and percentage yield, i.e. up to 91.71% & 88.94% respectively. Micrometrics examination showed the evidence of good flow characteristics of microparticles. By using USP defined dissolution apparatus at pH 6.8 for 12 hours at $37 \pm 0.5^\circ\text{C}$ dissolution studies for the prepared formulations were performed. The accumulative drug release was retarded with the increase in drug to polymer ratio during observation. Most of the prepared batches were in pursuit of Higuchi model of release through Fickian diffusion model. The optimal batch F6 was selected for further assessment FTIR, SEM and XRD. **Results and Discussion:** FTIR results showed no interaction between polymer and drug. XRD proved the stability of the product. Scanning electron micrographs depict that the particles had, heterogeneous, irregular and porous surface. Mean diameter was calculated as 200 μm . Thus the microparticles were prepared successfully using best polymer and technique.

KEYWORDS: Formulation, in vitro evaluation, sustained release microparticles, Loxoprofen Sodium.

INTRODUCTION

Novel drug delivery system is replacing the conventional dosage form considerably for some years. Researchers and scientists have shown extraordinary interest in controlled/modified/sustained release dosage forms.^[1] The premier aspect that decides the fate of any dosage form is patient compliance. Non-compliance can be ascertained when patients are suffering from chronic illness, where they are administered multiple doses for a longer duration of time. Controlled/prolonged dosage form is the near way for countering these problems.^[2] The cardinal reason for formulation of controlled/sustained release dosage is to acquire the therapeutic concentration of drug in the plasma

continuing throughout the dosing frequency without the reduction in the peak concentration.^[3,4]

Consolidation of the chemicals within polymers is the fundamental method to achieve the controlled release. In this regard hydrophilic polymer matrices are utilized to develop a sustained dosage form to a large degree. Biodegradable polymers are being used as a carrier by almost all drug delivery systems available.^[5] The release of the active ingredient may be slow over a long period of time, it can be cyclic over a prolonged interval of time, or it can be elicited by the environment, or additional external effects. Among the varied ways of getting long term drug delivery, micro particles of

different polymers have been effectively used for decades for their positivity to encapsulate small particles that can interpenetrate through the barrier with accurately kinetics modeling, increased bioavailability, highly biocompatible and symptomatic of sustained release to the body tissues.^[5] Easy administration and patient compliance are the advantages of oral administration. So that's why this route of administration is getting importance and attention of the researchers in the case of physiological compartments, drug devices and also testing and designing of products with acknowledgments to sustained release systems.^[6]

During microencapsulation, particles of micro sized solid/liquid/gas are enclosed in inert micro sized shell that protects and isolates them from external environment.^[7] The obtained products through this method are called microspheres, microparticles and micro capsules.^[8] In the last three decades, pharmaceutical researchers and scientists have successfully controlled the process of drug release and absorption to get effective and enough levels of plasma drug in respect of prolonged duration of time by developing parental and oral microparticulate drug delivery system.^[9] They are small tiny particles of solid or small tiny droplets of liquid having diameter that ranges from 0.1µm to 200µm, surrounded by synthetic natural polymeric films with varying thickness & level of permeability, and they act as a substance controlling the release rate.^[10,11]

Within this context, Loxoprofen is a non-steroidal anti-inflammatory drug (NSAID), it is having antipyretic, anti-inflammatory and analgesic properties with a very low ability to cause gastric ulcers.^[12] It's hydrophilic in nature and is propionic acid derivative same as ibuprofen and naproxen.^[13] It has equal racemic mixture of four

isomers because of ophthalmic activity.^[14] In Pakistan it is also available under the brand names of Loxomac, Qizta and loxfen. Loxoprofen along with the other drug combination is not available.^[15] Transdermal patches and tablet form of Loxoprofen are available in markets but novel drug delivery formulation of loxoprofen is yet to be marketed.^[16] Keeping in view of the alterative dosage forms, the objectives of this study were to formulate the sustained release microparticles of loxoprofen sodium, using Hydroxypropyl methyl cellulose (HPMC) by coacervation method. Further, we observed the outcome of various parameters and carried out the in-vitro assessment of microparticles.

MATERIALS AND METHODS

Preparation of Loxoprofen sodium loaded Hydroxypropyl methyl cellulose (HPMC) microparticles.

The microparticles were formulated by coacervation non-solvent addition method.^[17,18] by, first dissolving Loxoprofen sodium 1gm at room temperature in 20 ml of methanol until clear solution was obtained, then HPMC (1, 2 and 3 gm) were dissolved at room temperature in 20 ml dichloromethane and clear solution was obtained. Afterwards drug and polymer solutions were mixed and stirred at room temperature. Different ratios of drug and polymer, in addition with other parameters such as stirring time and stirring speed with pre-determined differences for every formulation of different ratios of polymer were set (Table 1). The external phase containing polymer solution and drug was added slowly drop-by-drop with constant stirring to 50ml of liquid paraffin using magnetic stirrer for 2 hrs at 700 rpm. Fine microspheres obtained were filtered, washed thrice by n-hexane to remove liquid paraffin. Microparticles were dried overnight at room temperature.

Table 1: Formulations of Loxoprofen Sodium Microparticles Using Various Parameters.

Formulation code	Drug polymer ratio	Stirring time (h)	Stirring speed (rpm)
F1	1:1	2	700
F2	1:2	2	700
F3	1:3	2	700
F4	1:3	3	700
F5	1:3	4	700
F6	1:3	2	500
F7	1:3	2	900

In-vitro Evaluation of Formulations

Percentage Yield

The % age yield was calculated^[19] by the formula

$$\text{Yield (\%)} = P \div (W_D + W_P) \times 100$$

Where, P = weight of microparticles, Wpand WD are the weight of polymer and drug respectively which were used in the formulation of microparticles.

Determination of Encapsulation efficiency and Drug loading

25 mg accurately weighed microparticles were soaked in 25 ml of methanol, and left for 24 hrs., the solution was filtered and then examined for drug content on UV-spectrophotometer at 220 nm. Encapsulation efficiency and drug loading of all the formulations were determined by using following equations

$$\text{EE (\%)} = A \div T \times 100$$

A represents = actual drug loading and T= theoretical drug content. Yield was calculated using following equation. The experiments to find the EE were performed thrice.

Micromeritics studies

Tap density

Simple and conventional process was used to measure tapped density. Graduated cylinder of 10 ml was used and filled with the weighed microparticles after it was tapped 100 times. Calculations were done after using formula given.

$$\text{Tapped Density} = \frac{\text{Weight of microspheres}}{\text{Vol of microspheres after 100 tappings}}$$

Compressibility Index

The formula given was used to measure compressibility index;

$$\text{C.I} = \frac{\text{Initial vol} - \text{Final vol}}{\text{Initial Vol}} \times 100$$

Hausner's Ratio

Hausner's ratio, is an index for measuring flow properties of microspheres can be measured by the formula

$$\text{Hausner's Ratio} = \frac{\text{Vol before tapping}}{\text{Vol after tapping}}$$

Angle of Repose

Prepared Microparticles were passed through the funnel on a flat plain surface at a certain height and radius of the heap's base was measured then was put in the following formula to measure the angle of repose.^[20]

$$\theta = \tan^{-1} h / r$$

Drug Polymer Interaction Studies

Fourier Transform Infrared Spectroscopy

The possible chemical interaction between the polymer and drug was enumerated by FTIR (Fourier transform infrared spectroscopy). Individual spectrum of drug, polymer & microparticles recorded using KBr pellet press process at wavelength range of 500 to 4000 cm^{-1} .

X-ray Diffractometry

The X-ray Diffraction studies of drug, drug loaded microparticles and polymer were performed to analyze the affects of microencapsulation on the crystallinity of drug. Diffraction angle (2θ) for diffractometer was optimized between 10° - 80° with $0.05^\circ/\text{min}$ step angle, for scanning of specimen at 40 kV voltages, and 20 mA current and Cu-K α radiations.

Scanning electron microscopy

Scanning Electron microscopy was done to examine the surface characteristics and morphology of drug loaded

microparticles using Carbon sputter technique at the voltage of 5 kV.

In-vitro Drug Release of Microparticles

The in-vitro release study of loxoprofen from microparticles was assessed in simulated intestinal fluid at phosphate buffer pH 6.8. An accurately amount of microparticles equal to 100 mg of loxoprofen sodium was shifted to dissolution medium of 900 ml at 37°C and 100 rpm in a USP XXII basket procedure I (Dt70, pharma-test Germany). A sample of 5 ml was taken at 0.25, 0.50, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12.hrs and replaced with the same volume of corresponding fresh medium to maintain the sink condition. The samples were then filtered and examined on UV-spectrophotometer (CECIL 2041, 2000 series, Cambridge England) at 220 nm. Every batch of the loxoprofen was examined three times by the same method.

Release Kinetics

The drug release data from particles after duration of 12 hrs was fixed in different models of kinetics (Zero order, first order, and korsmeyer-peppas, Higuchi) for determining the release mechanism and kinetics. The model adapted is given as follows.

Equation of zero order is

$$C_t = k_0 \cdot t$$

Where 'Ct' is % age quantity of drug released at time 't' and 'k₀' is constant.^[21]

First order equation is

$$\ln(100 - Ct) = \ln 100 - k_1 \cdot t \quad [22,23]$$

Equation of Higuchi's model; $C_t = k_H \cdot t^{1/2}$ ^[24]

Korsmeyer-Peppas model's equation; $C_t / C = k_{kp} \cdot t^n$

If $n \leq 0.45$ in the case of tablets it represents CASE-1, which is Fickian diffusion release mechanism. When "n" is smaller than 0.89 but greater than 0.45 it depicts non-Fickian or Analogous release, for super release (case-2) n is more than 0.89 and for zero order $n=0.89$. This model was employed for the real understanding and mechanism.^[25] All analysis were done Microsoft office excel 2019.

RESULTS

In-vitro Evaluatio

Encapsulation efficiency, percentage yield and drug loading.

Encapsulation efficiency, %age yield and drug loading of the 7 formulations of loxoprofen sodium microparticles were analyzed and the outcome of different parameters i.e polymer to drug ratio, stirring time, and stirring speed were analyzed then compared. The table below shows results.

Table 3: Results encompassing %age yield, Theoretical leading %age, Actual loadin, Encapsulation efficiency, of seven different formulations of Loxoprofen sodium microparticles.

Formulation	Percentage yield %	Theoretical loading %	Actual loading %	Encapsulation efficacy %
F1	76.3	50	33.67	54.4
F2	81.7	33.3	24.92	72.3
F3	88.1	25	17.32	88.9
F4	88.9	25	17.64	88.3
F5	87.4	25	17.55	88.4
F6	83.4	25	16.61	91.7
F7	87.2	25	18.11	89.2

Micromeritics studies

Micromeritics studies that are, tapped density, hausner ratio, bulk density, angle of repose and compressibility index of all the prepared formulations were determined.

Table 4: Micromeritics studies of different batches of microparticles.

Formulation	Bulk density gm/ml	Tapped density g/ml	Compressibility index	Hausner ratio	Angle of repose
F1	0.154	0.194	29.4	1.243	18.4
F2	0.206	0.269	26.1	1.4209	19.2
F3	0.237	0.292	21.8	1.385	21.5
F4	0.224	0.284	22.1	1.347	20.9
F5	0.232	0.297	22.3	1.334	21.2
F6	0.302	0.357	24.7	1.286	23.6
F7	0.221	0.288	22.3	1.128	20.1

FTIR Studies

The array of unadulterated HPMC gives peaks of C-O-C an C-O absorption bands around 1004.77 cm^{-1} are of alcohol, meanwhile CH, CH₂, and CH₃ absorption peaks are found in $1250\text{-}1600\text{cm}^{-1}$ and also at $2820\text{-}2990\text{cm}^{-1}$ regions.^[26] The array of pure loxoprofen sodium gives peak of (C=O) expansion of carboxylic group at 1535.98 cm^{-1} and the next peak expanding of (C=O) of cyclo pentane group at 1719.86 cm^{-1} . The integral peak can be seen at 3689.44 cm^{-1} .^[27] FTIR array of formulated loxoprofen sodium loaded microparticles show the integral peaks of HPMC and loxoprofen sodium, however some of the peaks have been cloaked. This confirms the undamaged position of the drug in the microparticles, it is best to assume that no chemical change in the drug has taken place during the whole process.

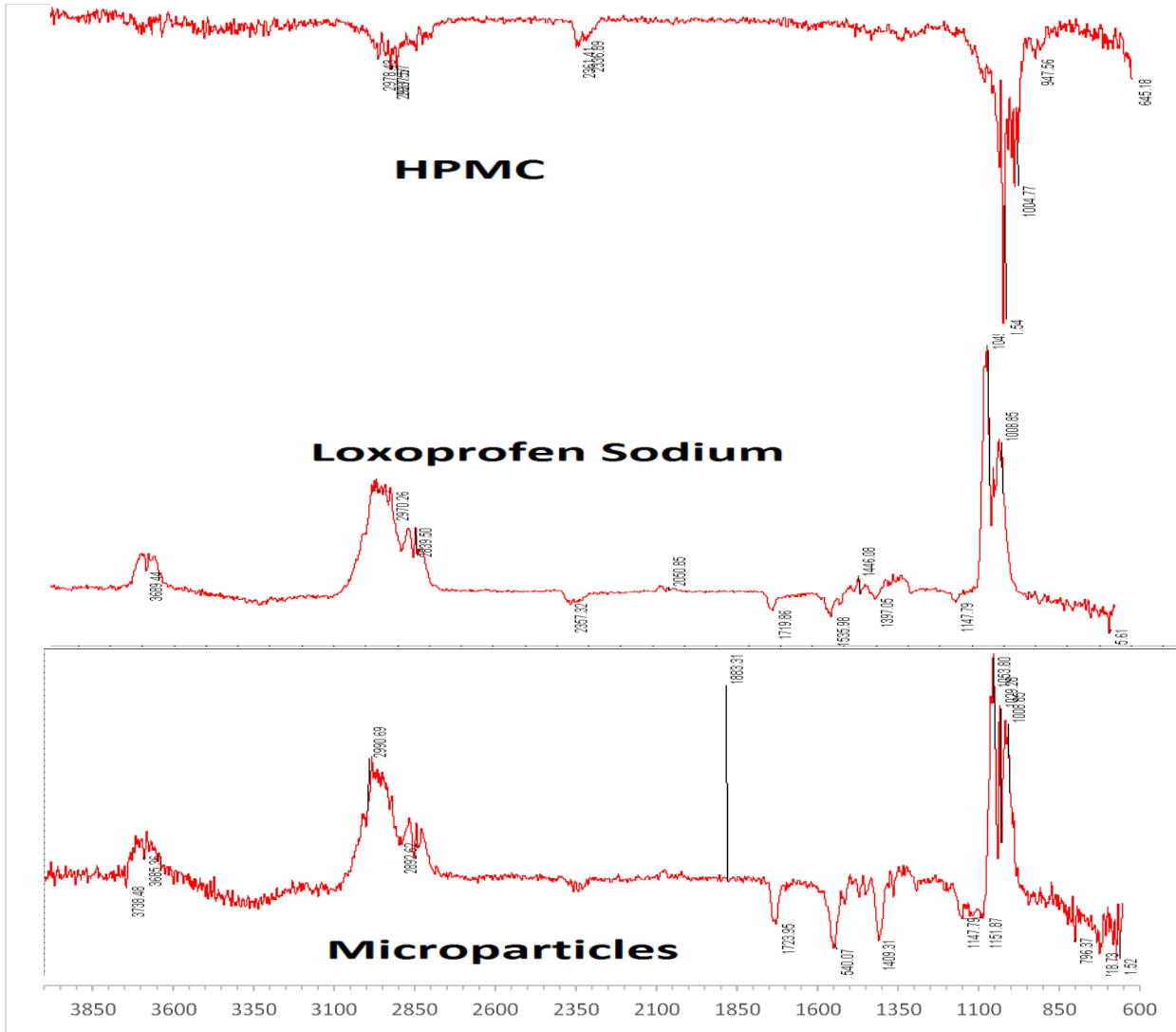


Figure 1: FTIR spectrum of HPMC, Loxoprofen sodium and Microparticles loaded with Loxoprofen.

X-ray Diffractometry (XRD)

Loxoprofen sodium, Hypermellose, and loxoprofen loaded microparticles were analyzed through XRD, and their pattern is illustrated in the following figure (Figure 2). The diffractograms containing peaks for drug and polymer of microparticles are also exhibited, with certain peaks having different location and intensity. Although it does not indicate the change in the crystallinity of the drug but it could suggest change in sample size and preparation, as microparticles and polymer were triturated prior to analyses. Sample preparation and size can affect the XRD pattern of the microparticles. Change in the crystallinity of the microparticles was not observed during the procedure.

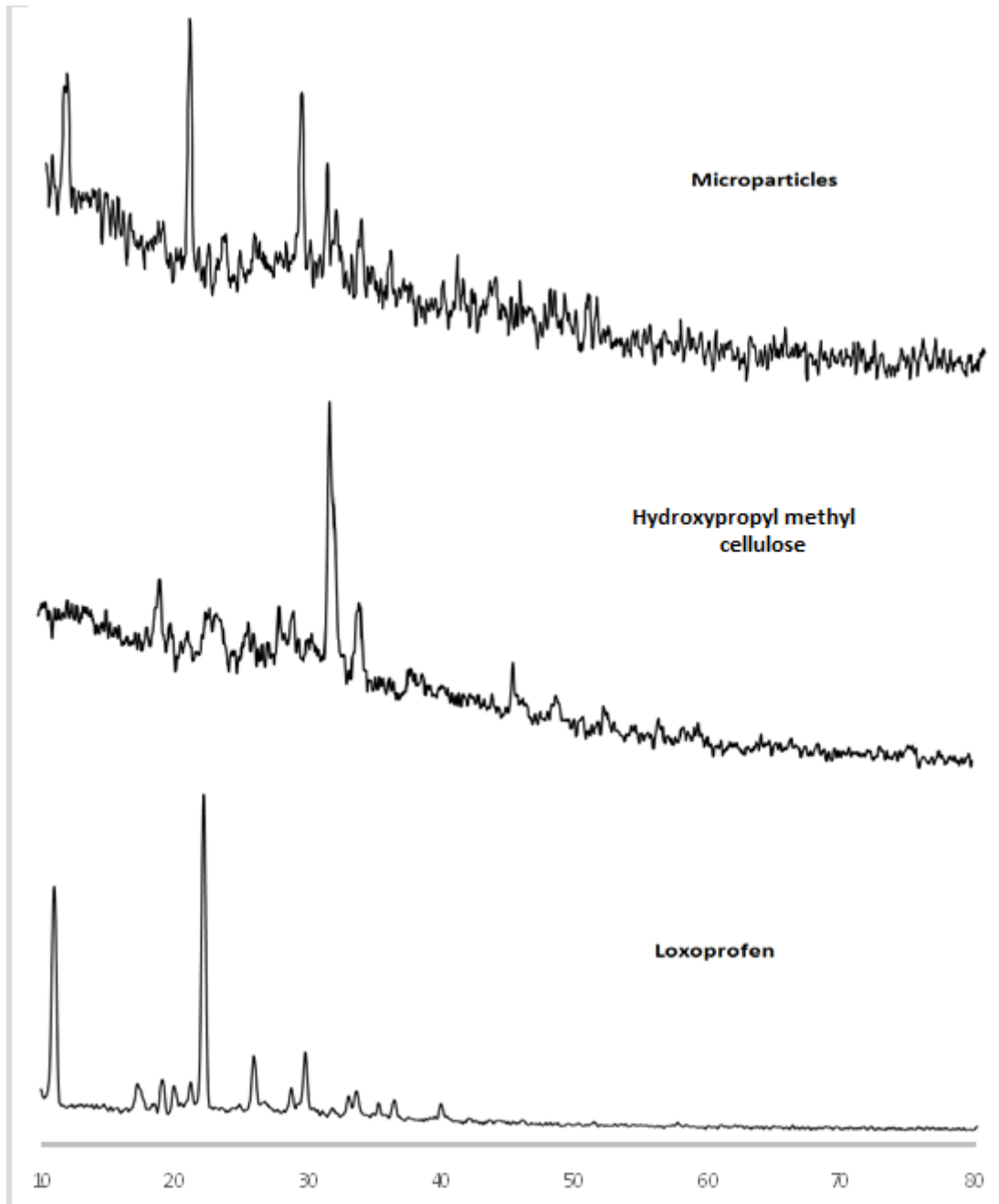


Figure 2: X-ray diffractometry of Loxoprofen sodium, Hypermellose, and loxoprofen loaded microparticles.

Scanning electron microscopy

Scanning Electron microscopy of loxoprofen loaded microparticles were taken at 10.1mm x 27 and 9.8mm x 1.50k magnification as shown in figure. Microparticles are irregular in shape, porous and heterogeneous with rough surfaces. The mean diameter of the microparticles is 190um.

Drug release in In-vitro

The dissolution studies in *in-vitro* were performed for all seven prepared formulations of loxoprofen sodium

loaded microparticles to check the pattern of release. This study was done for 12 hrs at $37 \pm 0.5^\circ\text{C}$ in simulated pH 6.8. The cumulative drug release of various formulations in 5 hrs was 50.94 - 98.12%. Formulation F1, F2, F3, F4, F5, F6 and F7 showed 98.12%, 87.34%, 68.53%, 86.76%, 95.27%, 50.25%, and 76.37% drug release respectively after 5 hrs (Figure 3). The outcome of different parameters, polymer to drug ratio, stirring time and stirring speed were examined.

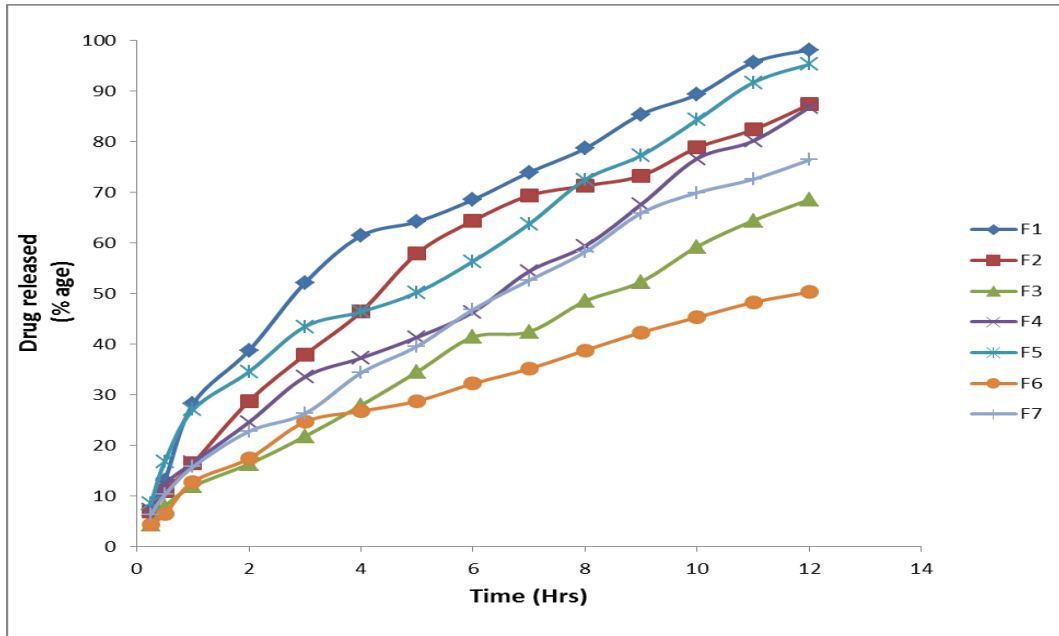


Figure 3: The in-vitro release profile of all Microparticles loaded with loxoprofen sodium.

Effect of Drug Polymer Ratio on Drug Release

The release pattern of drug was checked by changing the drug polymer ratio (Loxoprofen sodium: hydroxypropyl methylcellulose). The prepared formulation designated

as F1, F2, and F3 have the drug polymer ratio of 1:1, 1:2, and 1:3 showed 98.12%, 87.34%, and 68.53% respectively in 12 hrs study.

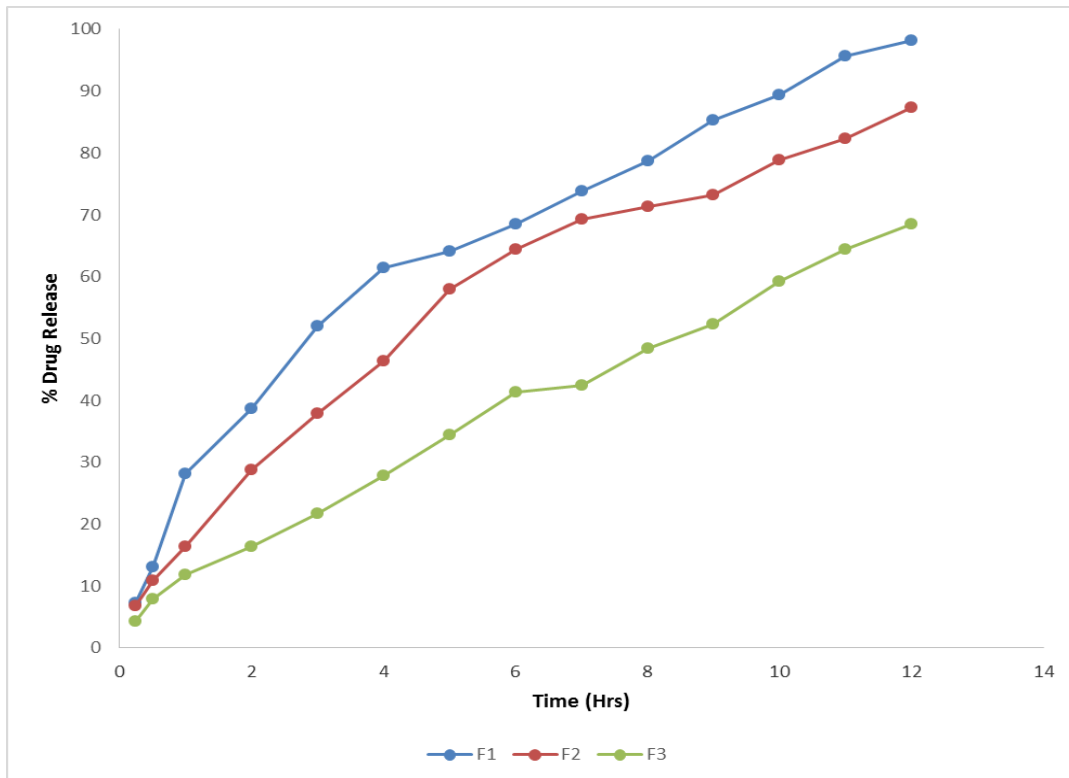


Figure 4: Effect of drug polymer ratio on the loxoprofen release pattern.

Effect of stirring time on drug release

The release pattern of all the formulations in question was checked which were formulated on different stirring

time that is 2 hours, 3 hours, and 4 hours for the formulation F3, F4, and F5, they showed release of 68.53%, 86.76% and 95.27% respectively.

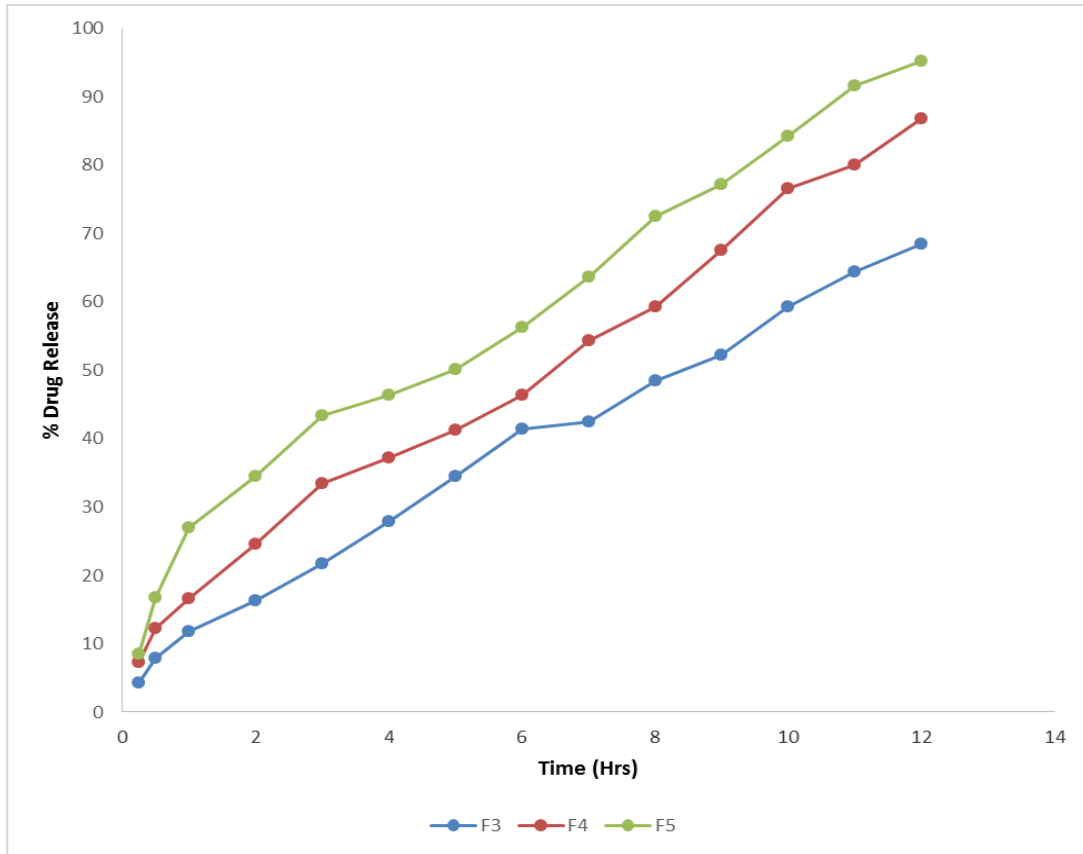


Figure 5: Effect of stirring time on release pattern of loxoprofen sodium.

Effect of stirring rate on drug release

The release pattern of the drug was also checked by varying the stirring speed that was 700 rpm, 500 rpm,

and 900 rpm, for the formulations F3, F6, and F7 they showed the release of 68.53%, 50.25%, and 76.37% respectively.

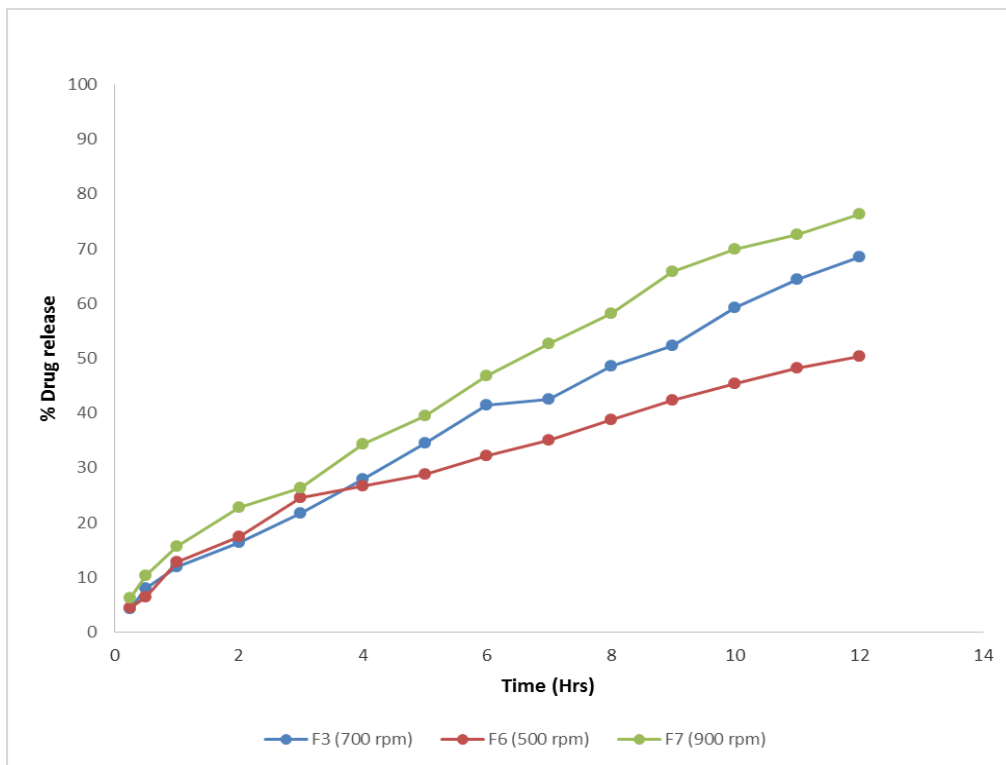


Figure 6: Effect of stirring rate on release pattern of loxoprofen sodium.

Release Kinetics

The drug release data of all the microparticles was fixed in various kinetic models to assess the kinetics and mode of drug release from microparticles. Zero order, First order, Higuchi model and korsmeyer-peppas, were used (figure 3.9- 3.15) to compare of R square and rate constant (k) for release was made in appraisal.

All the formulation follows zero order drug release except F1, R^2 value ranges from 0.98-0.99. F1 followed Higuchi model of drug release and its R^2 value was 0.99. As described by Korsmeyerpeppas all the prepared formulations strictly followed non-Fickian diffusion as the exponent (n) value is greater than 0.45. These formulations released the drug through the combination of diffusion and erosion mechanism.

Table 5: Kinetic modeling of drug release data of all the formulations of loxoprofen sodium loaded microparticles.

Formul-ation	Zero order Kinetic		First order Kinetics		Higuchi Model		KorsmeyerPeppas' Model	
	K_0 (h^{-1})	R^2	K_0 (h^{-1})	R^2	K_0 (h^{-1})	R^2	R^2	n
F1	17.69	0.973	-0.707	0.894	0.493	0.993	0.992	0.660
F2	12.96	0.995	-0.602	0.766	0.376	0.976	0.701	0.638
F3	9.218	0.989	-0.242	0.886	0.323	0.962	0.982	0.665
F4	10.67	0.993	-0.317	0.852	0.357	0.977	0.989	0.665
F5	10.95	0.993	-0.387	0.783	0.366	0.974	0.986	0.656
F6	12.38	0.993	-0.378	0.845	0.390	0.977	0.991	0.698
F7	11.08	0.993	-0.491	0.664	0.369	0.969	0.989	0.646

DISCUSSION

In the previous studies HMPC was successfully used to prepare sustained release microparticles of many drugs like Nimsulide, Oxaprozin, and Zidovudine.^[17,28,29]

Different parameters including polymer drug concentration, stirring speed, stirring time & their outcomes on the formulations were acknowledged.^[30]

Different ratios of drug and polymer were used i.e 1:1, 1:2, & 1:3 for preparing microparticles.^[17] Stirring speed was varied for the formulation to check the effects on the formulation which were 500, 700, and 900 rpm, as well as the stirring time which was 2, 3, and 4 hours used in selective formulations of microparticles to analyze its outcome on their release patterns.^[30]

It was observed that with the increase in the polymer concentration there was increase in the percentage yield, decrease in drug loading and increase in the encapsulation efficacy as in case of.^[17] It was observed that the micromeritics for the microparticles such as density, angle of repose, hausner ratio, were increased with the increase in drug polymer ratio which indicate the good flow properties.^[31]

After earlier physicochemical & micromeritics studies, *in vitro* dissolution studies were carried out. All the formulation showed sustained release properties as shown in (fig3.5) but F6 was seen to be the most efficient formulation having drug polymer ratio of 1:3, F6 released the least amount of drug for a prolonged period of time among all. By increasing the polymer ratio there was decrease in the release of drug from the microparticles thus it indicates the good sustained release property. This is because of increase in polymer density and consequently it leads to the length of drug diffusional path, as claimed by.^[32-35] Furthermore,

Change in stirring time showed no significant changes in percentage yield, drug loading and encapsulation efficacy, this phenomena has been excessively observed in highly hydrophilic drugs.^[36,37] There was a slight change seen in the micromeritics properties of microparticles with change in time. Slight increase in density with increase in time was observed. There was increase in the drug release from the microparticles with the increase in time. In addition, increase in stirring rate caused a slight increase in drug loading and percentage yield, encapsulation efficacy was decreased with the increase in stirring rate, this is due to drug movement to the external phase because of small particle size and larger surface area.^[30,38] Micromeritics studies showed slight decrease in density and angle of repose this is because of the particle size reduction by increasing speed.^[30] As far as the effect of stirring rate on drug release from the microparticles is concerned, it was established through results that increase stirring rate was directly proportional to drug release from the microparticles.

The drug release data of all the prepared formulations was then incorporated in various kinetic models and outcomes established that all the formulation were following the zero order drug release kinetics except F1, which followed higuchi model. According to Korsmeyerpeppas model the prepared formulations followed non-Fickian diffusion as expected, with the exponent (n) value was greater than 0.45. These formulations released the drug through the combination of diffusion and erosion mechanism.

The formulation F6 was found to be the optimum formulation among all batches based on the outcomes of physicochemical, micromeritics and *in vitro* dissolution studies. F6 has 1:3 drug polymer ratio, 500 rpm stirring

speed with 2 hours of stirring time. F6 was further analysed through FTIR, XRD and SEM to study the morphology and stability of drug in the formulation. SEM indicates that the mean size of microparticles is 190µm, round & oval in shape with rough surfaces. Drug maintained its integrity, was found stable and no chemical reaction took place at any stage between drug and polymer, confirmed by FTIR & XRD.

CONCLUSION

Sustained release microparticles of the drug loxoprofen sodium were successfully attained and evaluated using the technique of coacervation phase separation non-solvent addition method. Coacervation non-solvent addition technique turned out to be the most suitable method of developing of sustained/extended release microparticles & HPMC demonstrated as one of the best polymer for encapsulation of bioactive materials particularly for controlled release delivery system. The microparticles prepared showed excellent release of drug for about 9 hours. Drug polymer ratio, stirring speed and time showed good effects in reducing release of drug and vice versa.

REFERENCES

1. Parashar T, Singh V, Singh G, Tyagi S, Patel C, Gupta A. Novel oral sustained release technology: a concise review. *International Journal of Research and Development in Pharmacy & Life Sciences*, 2013; 2(2): 262-269.
2. Shahzad M, Ubaid M, Murtaza G. Formulation and optimization of celecoxib-loaded microspheres using response surface methodology. *Tropical Journal of Pharmaceutical Research*, 2012; 11(5): 695-702.
3. Rafiq M, Wahab A, Hussain A, Muhammad S. In vitro and in vivo evaluation of two sustained release formulations of diltiazem HCl. *African Journal of Pharmacy and Pharmacology*, 2010; 4(9): 678-694.
4. Jantzen GM, Robinson JR. Sustained-and controlled-release drug delivery systems. *Drugs and The Pharmaceutical Sciences*, 1996; 72: 575-610.
5. Lin L-C, Chang SJ, Chen SF, Chou YJ, Kuo SM. Effects Of Ph Of Na 5 P 3 O 10/Naoh Reaction Solution On The Properties Of Chitosan Microspheres. *Biomedical Engineering: Applications, Basis and Communications*. 2006; 18(04): 167-177.
6. Wani M. Controlled Release System-A Review, 2008, 6 (1). Available on www.pharmainfo.net/review, 2008.
7. Ghosh S, Blumenthal HJ, Davidson E, Roseman S. Glucosamine Metabolism: V. enzymatic synthesis of glucosamine 6-phosphate. *Journal of Biological Chemistry*, 1960; 235(5): 1265-1273.
8. Jyothi NVN, Prasanna PM, Sakarkar SN, Prabha KS, Ramaiah PS, Srawan G. Microencapsulation techniques, factors influencing encapsulation efficiency. *Journal of microencapsulation*, 2010; 27(3): 187-197.
9. Sipos P. *Formulation optimization of ammonio methacrylate copolymer based sustained release microspheres*. Szte, 2008.
10. Wise DL. *Handbook of pharmaceutical controlled release technology*. CRC press, 2000.
11. Phadtare D, Phadtare G, Nilesh B, Asawat M. Hypromellose—a choice of polymer in extended release tablet formulation. *World J Pharm Pharm Sci.*, 2014; 3(9): 551-566.
12. Terada A, Naruto S, Wachi K, Tanaka S, Iizuka Y, Misaka E. Synthesis and antiinflammatory activity of [(cycloalkylmethyl) phenyl] acetic acids and related compounds. *Journal of medicinal chemistry*, 1984; 27(2): 212-216.
13. Fine DH, Johnson B, Vasquez G. Systems and devices for generating nitric oxide. Google Patents, 2013.
14. Manka JS, Wang Y. Optically active functional fluid markers. Google Patents, 2014.
15. Jun HW, Kang L. Composition and method for enhanced transdermal absorption of nonsteroidal anti-inflammatory drugs. Google Patents, 2002.
16. Yoshikawa S, Murata R, Shida S, et al. Evaluation of correlation between dissolution rates of loxoprofen tablets and their surface morphology observed by scanning electron microscope and atomic force microscope. *Chemical and pharmaceutical bulletin*, 2010; 58(1): 34-37.
17. Khan S, Ahmad M, Murtaza G, et al. Formulation of nimesulide floating microparticles using low-viscosity hydroxypropyl methylcellulose. *Tropical Journal of Pharmaceutical Research*, 2010; 9(3).
18. Chudiwal P, Pawar P, Nagaras M, Mandlik S, Pandya S, Wakte P. Statistical evaluation and optimization of influence of viscosity and content of polymer on floating microspheres of clarithromycin. *Int J PharmTech Res.*, 2009; 1: 1366-1372.
19. Horoz BB, Kiliçarslan M, Yüksel N, Baykara T. Influence of aluminum tristearate and sucrose stearate as the dispersing agents on physical properties and release characteristics of Eudragit RS microspheres. *Aaps Pharmscitech*, 2006; 7(1): E111-E117.
20. Shariff A, Manna P, Paranjothy K, Manjula M. Entrapment of andrographolide in cross-linked alginate pellets: I. Formulation and evaluation of associated release kinetics. *Pakistan journal of pharmaceutical sciences*, 2007; 20(1): 1-9.
21. Najib N, Suleiman M. The kinetics of drug release from ethylcellulose solid dispersions. *Drug development and industrial pharmacy*, 1985; 11(12): 2169-2181.
22. Gibaldi M, Feldman S. Establishment of sink conditions in dissolution rate determinations. Theoretical considerations and application to nondisintegrating dosage forms. *Journal of pharmaceutical sciences*, 1967; 56(10): 1238-1242.
23. Wagner JG. Interpretation of percent dissolved-time plots derived from in vitro testing of conventional

- tablets and capsules. *Journal of Pharmaceutical Sciences*, 1969; 58(10): 1253-1257.
24. Higuchi T. Mechanism of sustained-action medication. Theoretical analysis of rate of release of solid drugs dispersed in solid matrices. *Journal of pharmaceutical sciences*, 1963; 52(12): 1145-1149.
 25. Vueba M, De Carvalho LB, Veiga F, Sousa J, Pina M. Influence of cellulose ether polymers on ketoprofen release from hydrophilic matrix tablets. *European Journal of Pharmaceutics and Biopharmaceutics*, 2004; 58(1): 51-59.
 26. Weiss P, Lapkowski M, Legeros RZ, Bouler JM, Jean A, Daculsi G. Fourier-transform infrared spectroscopy study of an organic–mineral composite for bone and dental substitute materials. *Journal of Materials Science: Materials in Medicine*. 1997/10/01, 1997; 8(10): 621-629. doi:10.1023/A:1018519419539.
 27. Zaman M, Adnan S, Saeed MA, et al. Formulation and in-vitro evaluation of sustained release matrix tablets of cellulose based hydrophilic and hydrophobic polymers loaded with loxoprofen sodium. *Indo American J Pharma Res.*, 2013; 3(9): 7389-7398.
 28. Gudas G, Karedla RR, Annadi Chiranjeevi TK. Formulation and evaluation of hpmc microspheres of oxaprozin for sustained release. *Indo American Journal of Pharmaceutical Research*, 2012; 2(7): 806-810.
 29. Phalguni Y, Venkateshwarlu B, Gudas GK, Debnath S. HPMC microspheres of zidovudine for sustained release. *International journal of pharmacy and pharmaceutical sciences*, 2010; 2(4): 41-43.
 30. Kashif PM, Madni A, Ashfaq M, et al. Development of Eudragit RS 100 Microparticles Loaded with Ropinirole: Optimization and In Vitro Evaluation Studies. *AAPS PharmSciTech*, 2016; 1-13.
 31. Rios M. Developments in powder flow testing. *Pharmaceutical technology*, 2006; 30(2).
 32. Rastogi R, Sultana Y, Aqil M, et al. Alginate microspheres of isoniazid for oral sustained drug delivery. *International journal of pharmaceutics*, 2007; 334(1): 71-77.
 33. Sultana Y, Mall S, Maurya D. Formulation and in vitro evaluation of sustained release microspheres of diltiazem hydrochloride prepared by emulsification-internal gelation method. *Journal of dispersion science and technology*, 2009; 31(1): 138-147.
 34. Singh M, Vijay V. Formulation and characterization of mucoadhesive microspheres using verapamil hydrochloride as model drug. *International Journal of Pharmaceutical Sciences and Research*, 2011; 2(12): 3260.
 35. Akifuddin S, Abbas Z, Marihal S, Ranadev A, IH SK, Kulkarni R. Preparation, characterization and in-vitro evaluation of microcapsules for controlled release of Diltiazem hydrochloride by Ionotropic gelation technique. *Journal of Applied Pharmaceutical Science*, 2013; 3(4): 35.
 36. Alex R, Bodmeier R. Encapsulation of water-soluble drugs by a modified solvent evaporation method. I. Effect of process and formulation variables on drug entrapment. *Journal of microencapsulation*, 1990; 7(3): 347-355.
 37. Bilati U, Allémann E, Doelker E. Poly (D, L-lactide-co-glycolide) protein-loaded nanoparticles prepared by the double emulsion method—processing and formulation issues for enhanced entrapment efficiency. *Journal of microencapsulation*, 2005; 22(2): 205-214.
 38. Abdallah MH, Sammour OA, El-ghamry HA, El-nahas HM, Barakat W. Development and characterization of controlled release ketoprofen microspheres. *Journal of Applied Pharmaceutical Science*, 2012; 2(3): 6.