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Piroxicam loaded polymer hybrid microspheres based tablets with modified release kinetics: Development, characterization and *in vivo* evaluation

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Abstract: Piroxicam (PC) is a non-steroidal anti-inflammatory drug characterized by poor aqueous solubility and reported to cause and impart crucial GIT irritation, bleeding, peptic and duodenal ulcer. Engineering of PC loaded microcapsules and its surface modification using different polymers has become the popular approach to address the said issues. The purpose of the study was to develop new PC loaded gastro-protective polymer hybrid microspheres (PHM) with subsequent conversion to tablet dosage form having modified dissolution rate and improved bioavailability. The crystallinity of the PC loaded PHM were established through powder X-ray diffraction. The optimised microspheres, PC-M1 with particle size 32±3.0µm, entrapment efficiency 83.78±2.5% and in vitro drug release 87.1±2.6% were further subjected to tablets development and in vivo evaluation. The in *vitro* drug release study conducted for PHM at pH media 1.2 and 6.8 demonstrated retarded and enhanced drug release rates (P<0.001) respectively. Both accelerated and real time stability studies confirmed stability of the PC loaded PHM based tablets. A substantial improvement in the drug plasma concentration 12.6±2.36 (P<0.001) was observed for the produced tablets compared to the marketed formulations.

Keywords: Piroxicam, microspheres, dissolution, bioavailability, stability.

INTRODUCTION

Development of the novel dosage forms build on modulating physicochemical properties of the drug compounds. Physiochemical attributes are the key parameters to modulate the pharmacokinetics (PK) and pharmacodynamics (PD) of the drug candidates (Hashida 2020). It takes ages to develop new drug molecules while addressing the issues with the current drug compounds. Therefore, advancement in drug delivery approaches to produce novel dosage forms has got a noticeable attraction for the drug delivery scientists (Zhao *et al*., 2019)

Due to the poor water solubility attributes majority of the drug candidates have become a difficult task for the drug delivery researcher to develop them as a dosage form. There are very potential drug candidates which belong to Biopharmaceutical Classification System Class II (BCS II). The erratic dissolution rate that is a rate limiting step for absorption of these compounds has grouped them as blockbuster drugs. (Mrsny 2012) There have been reported a range of approaches to improve physicochemical properties of these drug compounds with subsequent enhanced PK and PD.

These advanced strategies include solid dispersion, pellets, floating systems, liposomes, micro emulsions, liquid crystals, solid dispersions and nanoparticles/ nanocrystals (Goke *et al*., 2018; Khan *et al*., 2020). Piroxicam is a nonsteroidal anti-inflammatory drug, used in moderate to severe pain, rheumatoid arthritis, osteoarthritis, ankylosing spondylitis and fever (Ben Romdhane *et al*., 2019). PC belongs to BCS Class II

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which have poor water solubility and strong permeability across the cell membrane. However, its anti-inflammatory and analgesic actions are delayed due to slow and erratic absorption in gastrointestinal tract (Mohammadi-Samani *et al*., 2018). It is a non-selective Nonsteroidal anti-inflammatory (NSAID) drug with sever Gastrointestinal tract side effect. Irritation of the gastric mucosa, ulceration, blood perforation with peptic and duodenal ulcer are the fatal issues related to Piroxicam side effects (Bindu *et al*., 2020; Cooper *et al*., 2019).

To circumvent the aforementioned issues in connection to PC, several formulation approaches have been employed which include, matrix system, enteric coating, sustain released, floating drug delivery and lipid carrier systems (Lopes *et al*., 2016; Liu *et al*., 2017; Homayun *et al*., 2019). However, these drug delivery strategies have not significantly resolved the present issues. The composite polymeric/ polymer hybrid micro spheres is the smart and emerging approach in order to modify the drug release rate and to enhance its dissolution, bioavailability and protection (Hameed *et al*., 2020). The microsphere is a promising technology and have got a noticeable attraction in controlled release and drug targeting (Lengyel *et al*., 2019). Various pH dependent polymers have been developed for biomedical and in targeted drug delivery system with specific response at a particular pH environment (Vlachou *et al*., 2020). Eudragit L100 is meth acrylic acid and methyl meth acrylic acid copolymer of an anionic nature. The dissolution of Eudragit L100 is at a pH range of above 6, used for more effective, site specific and stable enteric coating with fast dissolution (Jain *et al*., 2020). In novel drug development, Eudragit L100 exhibits key role in numerous formulation such as microspheres, micro- sponges, nanoparticles, liposomes, liposomes, tablets etc. for different productions such as enteric coating, delayed release, permeation of insulin and to boost bioavailability (De Leo *et al*., 2020; Shepard *et al*., 2020).

To date, PHM based tablets for piroxicam to address the aforementioned key issues associated with this drug compound have not been reported. Therefore, the main objective of this research was to develop PC loaded PHM based tablets with modified drug release kinetics, enhanced dissolution and bioavailability attributes. The combination of Hydroxy propyl methyl cellulose (HPMC.E5) and surfactant Sodium lauryl sulphate (SLS) with Eudragit L100 (EUD) to develop the smart hybrid microspheres will be helpful to improve solubility and dissolution of the PC at the target site (small intestine). (Kini *et al*., 2011) The development of modified release PC loaded gastro-protective micro spheres-based tablets are believed to enhance drug safety, control release rate at a therapeutic concentration, to relief local irritation and tissue damage of GIT.

MATERIALS AND METHODS

Materials

Piroxicam (B.No. Pram 073011415) and SLS (SLS; B.No.SLS-1313) were gifted by Navegal Laboratories Hattar Industrial State Haripur Pak. ALPE China provided us EUD-L100; Batch No: 150312. Ortho phosphorous acid and Methanol were purchased from Sigma-Aldrich. USA. ASPE China provided us HPMC E-5. Kollidone; Batch No: 304129 and Avicel were purchased from ASPE China. Aries Pharma Peshawar gifted us Talcum powder and magnesium stearate (Batch: 2291).

Methods

Preparation of PC loaded PHM

The PC loaded PHM were developed by modified oil/water emulsion solvent evaporation technique. The EUD-L100 solution was prepared by dissolving 1.0g of Eudragit in 25ml of ethanol. The drug solution was composed of 2% w/v PC in 25ml of methanol (25ml). The PC solution was added to the polymeric solution of EUD-L100 and made up volume with chloroform by increasing EUD-L100 ratio accordingly. The stirring of this mixture was continued until a clear and uniform drug polymer solution obtained. Polymeric stabilizer HPMC.E5 and surfactant 0.1% w/v (SLS) were added in water to prepare continuous aqueous phase. The organic phase containing PC and Eudragit L100 was incorporated in this continuous aqueous phase and stirred vigorously at 1000 rpm with magnetic stirrer at 35°C temperature for 06 hrs to completely evaporate the solvents. table 1 shows the formulation parameters. The engineered microspheres were washed with n-hexane. The hybrid microspheres were collected through vacuum filtration, dried and stored for future use (Al-Nasi and Al-Tahami 2016).

Solid State Characterization

Particle Size Analysis

Particle size distribution of PC microspheres was analysed by means of Sympatec laser diffraction particle size analyser with a dry dispersion unit (HELOS & RODOS). The samples (15-20 mg) in an air pressure of 4 bar at a rate of 30 mm/s were fed into the analyser.

Scanning Electron Microscopy

The morphological attributes of the raw and produced microspheres of PC were evaluated through SEM (scanning electron microscopy) using JSM5910 (S-570, Hitachi, Japan). The samples were sprinkled on a tape with double adhesion, fixed to an aluminium stub. The gold was coated on that stub using a gold sputter with high vacuum evaporator under an argon atmosphere. The micrographs were obtained at different magnifications from the coated sample to investigate the spherical nature and surface topography.

Drug - Polymer Compatibility Study

IR spectroscopy was executed by Fourier transformed infrared spectrophotometer (IR Prestige-21 Shimadzu,

Japan). The FTIR analysis of pure drug, physical mixture, polymers, microspheres and developed microspheres was conducted for determination of the interaction among formulation ingredients. The potassium bromide disc method was employed and the discs were prepared by compressing the powder into disc at a force of 5.2Ƭcm^{-2} on KBr –press for 3 minutes (Essa *et al*., 2015). The scanning range was in between 400-4000cm^{-1} with resolution of 4cm^{-1}.

Powder x-ray Diffraction (PXRD)

The polymorphic nature of the drug in the developed microspheres was determined by X-ray diffraction machine (JEOL Japan, JDX3532,) and compared with the unprocessed drug. The X-ray diffractograms were obtained at a scanning speed of 2°/min with radiation of Cu Kα1 having wavelength of 1.5106 Å, working at35 kW and 20 mA in the sequence of 2θ (5°to70°).

Entrapment Efficiency and Percentage Yield

Percent entrapment efficiency (%EE) of the developed PC loaded microspheres in different formulations (M2-M6) was analysed. The crushed microspheres of PC were taken in 25 ml volumetric flask and dissolved in small amount of methanol followed by making the volume up to the mark and adjusted the pH 6.8 using phosphate buffer. The resulted solution was stirred for 01 hours at 35˚C and filtered through Whatman filter paper Grade 602 h. Different dilutions were made from this filtrate using methanol and the absorbance was measured at 354nm by UV-spectrophotometer (IRMECO Model U2020, Germany). The entrapment efficiency, drug loading and the % yield of the microspheres were calculated by the formula given as follow:

$$\text{Drug Entrapment Efficiency (\%)} = \frac{\text{Practical drug content}}{\text{Theoretical drug content}} \times 100$$

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

$$\text{\% yield of microspheres.} = \text{Practical yield/ Theoretical yield} \times 100$$

Preparation of Microspheres based Piroxicam tablets

The optimised PC loaded polymer hybrid microspheres (PC-M1) were converted to tablets by direct compression method. The microspheres of 307.8 mg contained 20 mg of Piroxicam were accurately weighed followed by mixing with different concentration of the excipients which include Avicle pH-102 (251.02mg), Kolidone-30 (24mg), Talcum powder (5mg) and magnesium stearate (12mg). The microspheres and excipients were mixed followed by compression through ZP-17 machine to yield the desired tablets. The compression force in the range of 7-8kg/cm² was applied for 30 second with the die (size of 12 mm).

Evaluation of Modified Release PC loaded Polymer Hybrid Microspheres based tablets

Monsanto hardness tester (MH-1; Galvano scientific) was used to determine hardness of the microspheres loaded tablets. Thickness and diameter of the produced tablets were determined by the calibrated Vernier calliper; Y128 (China).

For Friability test, Roche friabilator (Navegal Laboratories, Pakistan) was operated at a speed of 25 rpm for 4 minutes. The tablets were taken out from friabilator, removed dust and weighed again. Weight variation test on twenty tablets from each batch was conducted. The content uniformity test was conducted on 20mg PC powder achieved by crushing the six tablets with the subsequent quantification for active ingredients using UV- spectrophotometer at 354nm.

Stability Study

The stability study of the selected batch of the microspheres based tablet was carried out at refrigerated, room and accelerated temperature according to standard ICH (International conference on Harmonization) for zone III & IV. The stability protocol was designed and based on the ICH guidelines. The microsphere based tablets of optimized formulation samples were filled in amber coloured vials of 5ml capacity, stoppered with rubber closure, and crimped with an aluminium over-seal.. The microspheres occupied glass vials were placed at refrigerated condition (5±3C˚), room temperature (25±2˚C) and under accelerated condition of (40±2˚C) with relative humidity of (75±5%) in a stability chamber (Lab Tron/model#STCL-400L) for six months. The Microspheres based tablets of optimized ratio (1:4) were analysed for .0, 1, .2, 4 and 6 months and assessed for physical appearance, percent moisture contents analysis, hardness, friability drug release and finally % drug assay.

Dissolution and Drug Release Kinetics

In vitro drug release studies of the developed PC loaded microspheres (PC-M1-PC-M5) were carried out in different pH media including pH 1.2 and pH 6.8 using dissolution apparatus paddle type-II. Equal quantity of the microspheres was added to the dissolution medium of 900 ml. Temperature of the dissolution bath and rotation speed of peddles were set 37ºC and 100rpm respectively. The 5ml samples were withdrawn from the dissolution vessels at the time intervals of 0, 20,40,60,80,100 and 120 minutes and maintained the sink conditions by replacement of the 5ml fresh aliquots. The dissolution studies at pH 1.2 and pH 6.8 were conducted for 120 and 220 minutes respectively. Extended dissolution studies of the tablet dosage form produced from the optimised polymer hybrid microspheres (PC-M1) was carried out for 220minutes and compared with the marketed formulations and raw PC drug. For the first 01hr and 20 minutes, dissolution was carried out in the medium with pH 1.2 with the subsequent swapping to pH 6.8 adjusted with the phosphate buffer and stopped the dissolution on 220 min. Quantification of the active drug in the samples withdrawn at different time points was determined using UV spectrophotometer.

Table 1: Formulation composition and process parameters of PC Loaded polymer hybrid microspheres

Formulation Code	PC:EUD-L100	SLS % (w/v)	HPMC E5 (g)	Organic Phase/ml	Aqueous Phase/ml	Stirring (Rpm)	Temp ˚C
PC-M1TT	1:2	0.1	0.002	50	75	1000	35
PC-M2	1:3	0.1	0.003	75	100	1000	35
PC-M3	1:4	0.1	0.004	100	125	1000	35
PC-M4	1:5	0.1	0.005	125	150	1000	35
PC-M5	1:6	0.1	0.006	150	175	1000	35

Abbreviations: Temp; Temperature and EUD; Eudragit.

Table 2: Percentage yield& entrapment efficiency of different formulations

Formulations Codes	Entrapment Efficiency %	% Yield	Mean Particle Size (µm)	% Drug loading
PC-M1	76.62± 3.0	89.03±2.0	18.5±2.5	9.01±0.02
PC-M2	77.77± 3.5	92.01±2.5	25.6±1.5	6.5±0.12
PC-M3	83.78± 2.5	94.12±3.0	28.2±2.0	5.8±0.14
PC-M4	85.14±2.5	94.2±3.5	76.3±3.0	4.7±0.218
PC-M5	89.17±3.5	95.2±2.0	92.7±2.8	4.2±0.145

The data has been calculated as (SEM), n=3

Table 3: Evaluation of PC loaded PHM based tablets

Parameters	Accepted limit ± SD
Thickness(mm) N =10	4.1±0.50
Friability* (%) N =20	0.55±0.021
Hardness (kg/cm²) N =10	4.3±1.0
Weight variation (mg) N=20	609±2.08
Content Uniformity (%) N =10	90.1±2.43
Moisture%(w/w) N =10	0.11±0.01

N=no. of Tablets,

Table 4: Values of R^2 for various kinetic model

Formulation code	Zero Order (R^2)	First Order (R^2)	Higuchi (R^2)	Kors Meyers Peppas (R^2)	Release exponent (n)
PC-M1	0.886	0.980	0.991	0.935	0.577
PC-M2	0.944	0.974	0.972	0.911	0.676
PC-M3	0.938	0.987	0.9896	0.946	0.674
PC-M4	0.931	0.456	0.9801	0.937	0.676
PC-M5	0.956	0.367	0.9582	0.893	0.773

Abbreviations: PC, Piroxicam; PC-M, Piroxicam loaded microspheres

Table 5: Comparative Pharmacokinetic parameters of PC loaded gastro protective microsphere based Tablet of PC-MT1, PC- Marketed drug and PC-Control

Sample	Pharmacokinetic parameter			
	$T_{1/2}$ (h)	T_{max} (h)	C_{max} (µg/mL)	AUC_{0-t} (µg h/mL)
PC-Control	7.45±2.14	2.0±1.46	5.51±3.67	44.1±10.8
PC-Marketed drug	8.91±2.57	2.0±1.03	9.52±2.32*	91.8±10.4***
PC-MT1	11.7±1.04**	4.0±1.64**	12.6±2.36***	132±8.57***

Values are expressed as mean ± SD. ANOVA (one way) followed by post *hoc* Dunnett's test. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ as compared to PC-control.

The dissolution data were then fitted to various kinetic models (zero order, first order Higuchi, Kors Meyers Peppas). The co-relation coefficient value (R^2) and n-value of (Kors Meyers Peppas) were attained by regression analysis of the plots for linear curves determination .(Maurya, Gupta *et al*., 2011)

Oral Bioavailability Study

Healthy rabbits weighing 2±0.3kg of either sex were used in the experiment. The in vivo study was carried as per regulations and bye-laws-2008 approved by the Departmental research and ethical committee constituted by the University of Malakand (SP, Issue-1). Before dosing the animals were kept fasted for 12 h. The drugs were administered orally to various groups, with each groups contained six animals. (Giunchedi, Conte *et al*., 1999)

The Piroxicam control, PC-MT4 formulations and the marketed tablets (PROXIM, Novartis) were administered through oral route (20 mg/kg). At various time intervals (0 to 24 hrs), about 0.5mL of blood samples were collected and placed in heparin tubes. The blood samples were centrifuged for 10 min at 3,000 to 4,000 rpm and plasma was separated. (Joseph, Lakshmi *et al*., 2002) The collected plasma was stored at -20˚C until use. The plasma samples were analysed for drug quantification by HPLC (Millipore HPLC 501pump water/484 tuneable absorbance Detector model#M.45). A mobile phase consisting of methanol: buffer (60:40) was prepared. A retention time of 4.8 min with a flow rate of 2mL/min was selected. The HPLC column consisting of µ-Bondapak C_{18} column having dimensions of 300 cm length, 3.9 mm in width and 10µm particle size. The plasma samples were mixed with acetonitrile and then centrifuged. The supernatant (20µL) was then was then injected into the HPLC system having a UV detector set at a max of 354 nm (2016). The pharmacokinetics parameters determined include maximal plasma concentration (C_{max}), time to reach maximal plasma concentration (T_{max}), half-life ($t_{1/2}$), and the area under the concentration-time curve (AUC).

STATISTICAL ANALYSIS

The collected data was analysed in triplicate with mean and standard deviation. In addition, other imperative statistical analyses including ANOVA (one way analysis of variance) and t-test (two tailed) considering the p value <0.05 as a significant were carried out as well. The PK parameters in the plasma were determined using the pharmacokinetic software WinNonLin (v 4.0; Pharsight Software, Mountain View, CA, USA).

RESULTS

Development of Microspheres and the Influence of Method Conditions

In the development process for the PC loaded PHM, It was observed that high concentration of EUD-L100 and HPMC.E5 resulted in large particle size of PC microspheres. The mean diameters of the particle size of PC loaded PHM were dependent on polymer concentration dispersed in the organic phase (table2). The optimised process conditions including adequate concentrations of EUD-L100 (1:2) and HPMC.E5 (0.002gm), appropriate mixing ratio of organic and liquid phases (50:75/ml) and high stirring rate (1000rpm) produced smaller microspheres (18.5±2.5µm) with high % yield (89.03±2.0) and high drug loading capacity (9.01±0.02) (table 2). In addition, it can be seen from the table 2, that increase in polymer concentration resulted in high % yield, high entrapment efficiency but yielded the microspheres with large particle size (92.7±2.8µm).

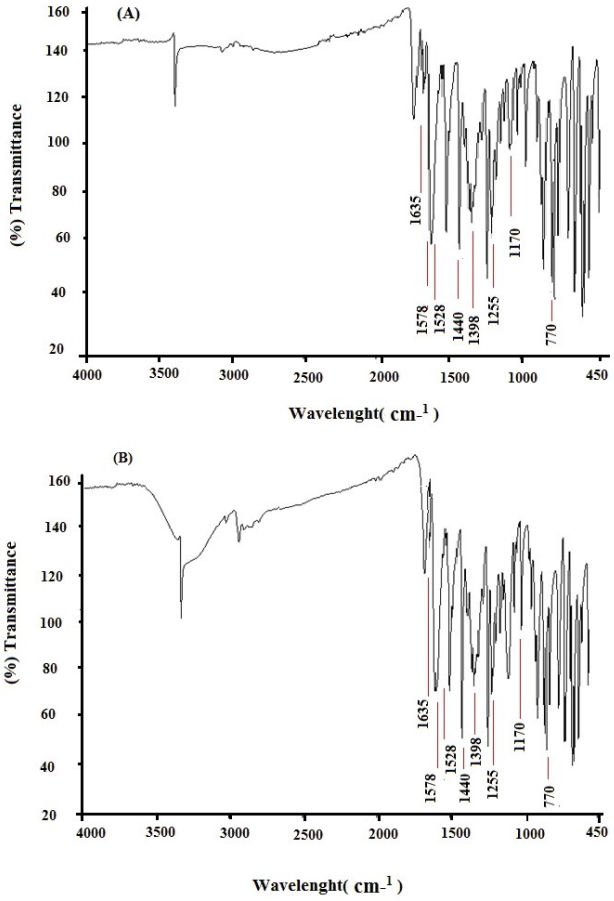

Fig. 1: (A) FTIR of pure PC (B). FTIR of PC loaded microspheres

Drug Polymers Compatibility and Thermal Analysis

The chemical interactions between the drug and polymers were determined using FTIR spectroscopy. Fig. 1 clearly shows that there has not been observed any chemical interaction among the formulations components during development of hybrid micro spheres. The specific peaks of PC (unprocessed) and PC loaded micro spheres were showed at (-NH and -OH stretching) which stalemates 1635 cm-^{1},1398, (N-H-CO_3 stretching vibration) bend at 1528 cm-^{1}, (secondary NH_2 stretching) at 1440 cm-^{1},(CH_3

and Ar C=C stretching) curve at 1355 cm-^{1} (sym.-CH_3) at 1155cm-^{1} and (-SO_2-N) ties at 770 cm-^{1}.

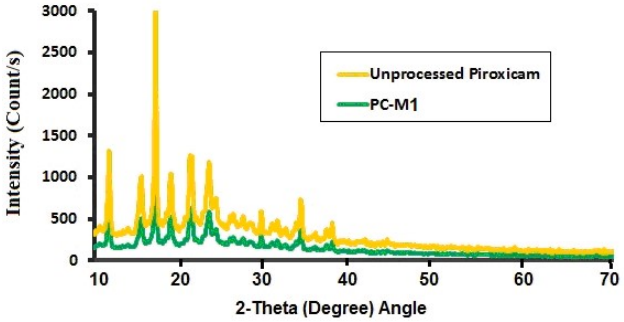

Fig. 2: P-XRD spectra of unprocessed PC and PC-M1

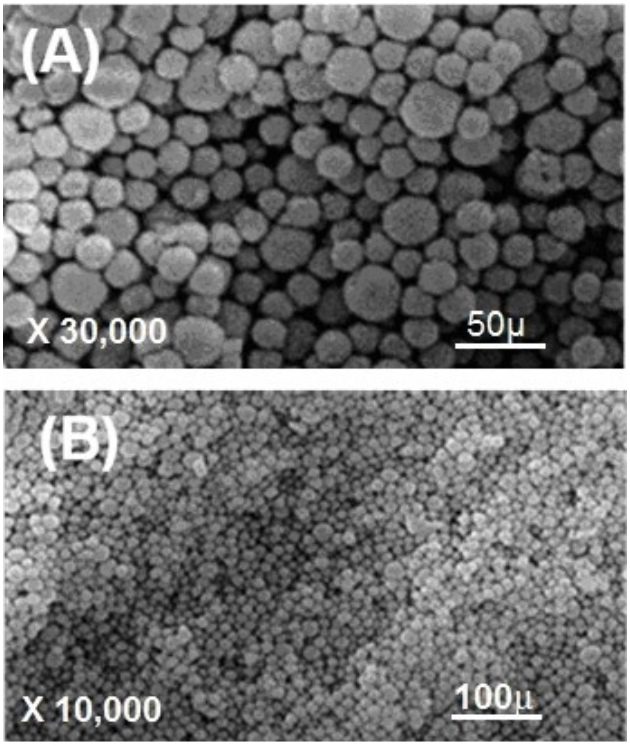

Fig. 3: (A) Scanning electron micrographs of PC-M4 at magnification of 30,000 and 15KV (B) Scanning electron micrographs of PC-M1 at magnification of 10,000 and 15KV

Powder X-ray Diffraction and SEM Studies

The X Ray diffraction patterns of the unprocessed PC and optimised microspheres showed that the processed PC maintained its crystalline nature in polymer hybrid microspheres. There were observed sharp x ray diffraction peaks for both of the unprocessed and processed samples (fig. 2). However, XRD peaks for hybrid microspheres were found with small intensities compared to the unprocessed PC particles.

The SEM photomicrographs of the developed micro spheres indicated that the produced microspheres were discrete in shape, spherical, uniform and no crystal traces were found on the surfaces. Microspheres with higher quantity of polymer ratios presented much more smooth surfaces than those microspheres formulated from lower quantity of polymers. The micrographs were recorded at magnification of 10,000 and 30,000 x and accelerating voltage of 15 KV. A cross-sections SEM of the microspheres indicated as a single and discrete unit without getting compact (fig. 3).

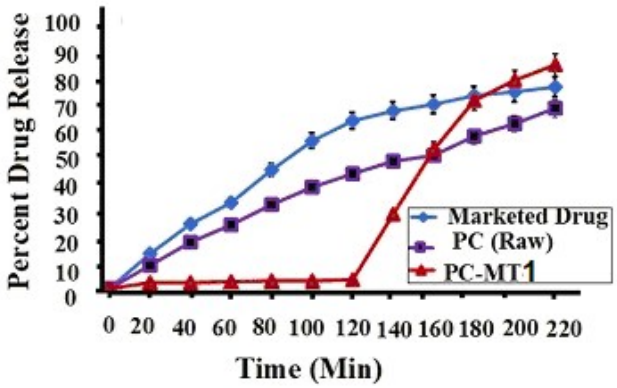

Fig. 4: Comparative Dissolution profile of PC (Marketed Drug), PC(Raw) and optimised hybrid microspheres; PC-M1 at pH1.2 and pH 6.8

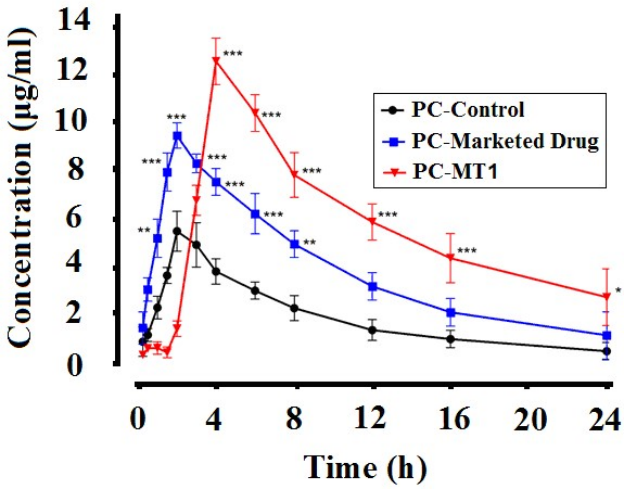

Fig. 5: PK profile of Piroxicam, PC-MT1 formulation, and PC- marketed drug. Plot of plasma concentration (µg/mL) *vs* time (h). Data are presented as mean ± SEM. Data are presented as mean ± SEM. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ compared to controlled animal group.

Development and Evaluation of PC loaded Polymer Hybrid Microspheres based PC tablets

The optimised hybrid microspheres (PC-M1) due to their improved physicochemical attributes, were further developed into tablet dosage form. The developed tablets were further evaluated by conducting different physical parametric tests (table 3). The produced microspheres based tablets complied with the USP limits for all the conducted tests including friability and tensile strength (0.55±0.021), contents uniformity (90.8±2.43) and

moisture uptake (0.11±0.01-0.16±0.012), signifying minimum variation amongst different batches of developed formulations. By increasing the concentration of EUD-L100 there was a significant effect on tablet hardness (4.3±1.0-4.4±0.08), friability and tensile strength. The weight variation test also resulted in the acceptable range (609±2.08).

Dissolution and drug release kinetics

Fig. 4 represents the comparative dissolution profiles of the PC loaded micro spheres-based tablets (PC-MT1), raw PC and marketed counterpart ((PROXIM, Novartis) at different pH values. This study demonstrated that dissolution rate of the optimized formulation PC-M1 was very less (<4%) at pH1.2 in the first120 min as compared to the PC Marketed (70%) and PC (Raw) (40%) (fig. 4). Hence, by adjusting pH media there was observed an incredible increase in the dissolution rate (>90.0%) of PC-MT1 at pH 6.8. The PC (Raw) dissolution was comparatively less than that of PC Marketed at both pH media. In contrast to the PC Marketed formulation, the PC-MT1 showed significant improvement in dissolution at pH 6.8 as well (fig. 4).

The kinetics mechanism of drug release of PC-M followed Higuchi model and Anomalous transport ($0.5<n<1.0$) of Kors Meyer Peppas models with release exponent "n" value >0.5 (table 4). Mostly modified release formulations exhibit Higuchi model with interpretation of diffusional mechanism of erosion, diffusion and swelling from the polymeric matrices.

Stability Study

The optimised PC loaded microspheres based tablets were subjected to accelerate and real time stability studies. The important stability parameters investigated for 06 months did not show any significant deviation from the samples observed at the first day of the designed study. The key stability parameters which include % drug contents (99±3.6%), hardness (3.1±0.36kg/cm^3), friability (0.52%), physical appearance and % (w/w) moisture contents (0.19±0.03%) showed that PC-MT were stable at different storage conditions after 180 days. There was not observed any statistically significant differences (ANOVA, $p<0.05$) in the above parameters for samples stored at accelerated conditions (40°C±2°C/75 ±5RH).

Oral Bioavailability Study

Fig 5 depicts comparative bioavailability profiles of PC-MT1, raw PC and marketed formulations. The PK parameters, including area under the concentration-time curve (AUC), maximal plasma concentration (C_{max}), time to reach maximal plasma concentration, and biological half-life, are reported in (table 5). Administration of PC at a dose of 20mg/kg showed a mean elimination phase from 16 to 24h with an elimination half-life of 7.45 h with a clearance of 453.29mL/h. The distribution phase was observed from 8 to 12h with an observed volume of distribution of 4875.8mL. The absorption phase was noted from 0.3h to 6h. The C_{max} was observed as 5.51 µg/mL at 2h. The AUC from time zero to 24 h was 44.1 µg h/mL. The resulted important pharmacokinetics parameters (table 5) for the marketed PC drug include as HF (half life: 8.91h), C_{max} 9.52µg/mL ($P<0.05$ as compared to PC-Control, T_{max} (2.0h), AUC (91.8 µg h/mL ($P<0.001$) and a volume of distribution of 2802.9 mL. An elimination phase of 16h to 24h was observed with a clearance of 217.9mL/h. The distribution phase was noted as 8h to 12h, while the absorption phase was occurred as 0.3h to 6h. Noticeable fluctuations in the PK of PC-MT1 formulation was detected. When administered as microspheres, an increase in the plasma concentration of PC was noted throughout the study. The microspheres have resulted in a decreased elimination rate of PC as reflected from a significant increase ($P<0.01$) in the half-life of 11.7h (elimination phase: 16-24 h), as compared to the PC-control treated group. In addition, there was observed a significant increase for both the plasma concentration (12.6µg/mL) and time required to achieve Cmax (4hr) with $P<0.001$. Furthermore, the *in vivo* study resulted in 2571mL as volume of distribution with distribution phase from 8h to 12h. The microspheres also knowingly increased ($P<0.001$) the plasma exposure of PC as reflective from AUC monitoring from time 0 to 24 h (132µg h/mL) as shown in (table 5).

DISCUSSION

The polymeric microspheres were prepared by O/W Emulsification technique to modify the PC release in small intestine for gastric protection. Optimization and suitable control of all these parameters were significant for the development of PC microspheres that were hybrid in nature having discrete and spherical surface attributes. The organic phase viscosity stimulated methanol diffusion and emulsification due to the concentration of polymer inside (EudragitL100), while keeping stirring rate constant (1000 rpm). The fast stirring rate (1000rpm) was found the key factor to effectively mix and disperse the PC solution into the aqueous phase resulting into small droplets in emulsion (Hameed *et al*., 2020). On the other hand, increasing the stirring rate above 1000rpm was found less productive for microspheres yield, that might be due to rupturing of the droplets caused by high mixing speed. (Patel and Amin 2011) The adequate concentration of the surfactant; SLS (%1) exhibited a dominant role in emulsion formation and tended towards PC loaded PHM formation with smaller particles with more release from Eudragit L100 matrices and significantly enhanced the dissolution/solubility. The particle size of PC microspheres were in accordance with the higher concentrations of Eudragit L100 produced larger sized microspheres due to the presence of a higher polymer quantity in the adjusting volume (Diaf, Elbahr *et al*., 2020). The higher concentration of polymers sometimes leads to self-aggregation/micelle formation and

consequently particles with large sizes are produced. FT-IR spectrum of the PC loaded hybrid microspheres showed that there was no important shifting of the peaks consequently, it reveals that the PC was companionable with EUD-L100, HPMC and SLS. The decrease in the peak intensities of x rays diffraction of the hybrid PC microspheres can be due to the small particle size and reduction in crystallinity of the PC in hybrid microspheres (Shah *et al*., 2016). Furthermore, the other reason for the small peaks of PC in microspheres can also be due to the small angle reflection of the x-rays which can potentially lead to disappearance of some peaks and reduction intensities x-rays diffraction peaks fig. The PC in other microsphere forms has also been reported with reduced crystallinity.[26]There was not observed any changes in the stored PC microspheres for 180 days, that confirms the stable nature of PC in polymer hybrid microspheres loaded dosage form at accelerated conditions (Tyagi and Kori 2014). Stability study demonstrated the adequate selection of the HPMC, EUD and SLS was useful to protect the PC from the external factors including temperature and humidity. The suitable types and optimised concentration of the polymers have been reported the key factors to maintain integrity of the loaded drugs during storage conditions (Mahale and Saudagar 2019).

The drug release from microspheres was consistent with the pH dependent solubility of the polymer (Zou *et al*., 2015). In aqueous medium and digestive fluids, the EUD-L100 has no solubility but showed a very splendid release in alkaline media of small intestine. To defend the active ingredient from the gastric juices and to progress, improve and enhance drug effectiveness, the pH dependent Eudragit L 100 was preferred choice as coating polymer for gastro-protection. The use of HPMC.E5 along with Eudragit L100 with different proportions delayed the release of PC from microspheres based tablet at respective site and allowed PC to rapidly release from the microspheres at higher pH (6.8) due to Eudragit L100. This was accredited to acid resistivity of polymer (Eudragit L100), which dissolves solitary at pH > 6. Though, quicker PC release after the acidic stage (1.2 pH) could be attributed to ionization of the PC and particle reduction to micron level. The kinetics study suggested PC diffusion through pores channels with slight or minor influence of the swollen polymer on the release kinetics.

CONCLUSION

The polymer hybrid microspheres of PC were successfully produced by controlled solvent evaporation approach. It has become evident from this study that combination of EUD-L100, HPMC and SLS in the hybrid drug delivery system effectively controlled the drug release rate of PC at acidic and basic conditions. In contrast to the raw PC and marketed formulation, the developed PC loaded microspheres based tablets resulted in retarded release of the drug at stomach conditions (pH1.2) and enhanced the drug release at pH 6.8 (small intestine conditions). The PK study also confirmed the controlled release of PC from the hybrid microspheres system. This drug delivery system can be extended to other NSAIDs to effectively address their GIT issues.

REFERENCES

Al-Nasi AA and Al-Tahami KA (2016). Preparation, characterization, and in vitro release of ketoprofen loaded polymeric microspheres. *Int. J. Pharm. Tech. Res.,* **9**(4): 313-321.

Ben Romdhane H, Ammar H, Ben Fadhel N, Chadli Z, Ben Fredj N, Boughattas NA, Chaabane A and Aouam K (2019). Piroxicam-induced fixed drug eruption: Cross-reactivity with meloxicam. *Contact Dermatitis*, **81**(1): 24-26.

Bindu S, Mazumder S and Bandyopadhyay U (2020). Non-steroidal anti-inflammatory drugs (NSAIDs) and organ damage: A current perspective. *Biochem. Pharmaco.,* 114147.

British Pharmacopoeia (2016). London (UK), **2**: 1508.

Cooper C, Chapurlat R, Al-Daghri N, Herrero-Beaumont G, Bruyere O, Rannou F, Roth R, Uebelhart D and Reginster JY (2019). Safety of oral non-selective non-steroidal anti-inflammatory drugs in osteoarthritis: what does the literature say? *Drugs & Aging,* **36**(1): 15-24.

De Leo V, Di Gioia S, Milano F, Fini P, Comparelli R, Mancini E, Agostiano A, Conese M and Catucci L (2020). Eudragit S100 entrapped liposome for curcumin delivery: Anti-oxidative effect in Caco-2 cells. *Coatings,* **10**(2): 114.

Diaf K, Elbahr Z and Besbes N (2020). Piroxicam based microspheres: an overview of new formulations for the drug controlled release. *Moroccan. J. Heterocycl. Chem,* **20**(1): 20-21, 2040-2048.

Essa EA, Elkotb FE, Eldin EEZ and El Maghraby GM (2015). Development and evaluation of glibenclamide floating tablet with optimum release. *J. Deliv. Sci. Technol.,* **27**(1): 28-36.

Giunchedi P, Conte U, Chetoni P and Saettone M (1999). Pectin microspheres as ophthalmic carriers for piroxicam: Evaluation *in vitro* and *in vivo* in albino rabbits. *Eur. J. Phrm. Sci.,* **9**(1): 1-7.

Göke K, Lorenz T, Repanas A, Schneider F, Steiner D, Baumann K, Bunjes H, Dietzel A, Finke JH and Glasmacher B (2018). Novel strategies for the formulation and processing of poorly water-soluble drugs. *Eur. J. Pharm. Biopharm.,* **126**: 40-56.

Hameed HA, Khan S, Shahid M, Ullah R, Bari A, Ali SS, Hussain Z, Sohail M, Khan SU and Htar TT (2020). Engineering of naproxen loaded polymer hybrid enteric microspheres for modified release tablets: Development, characterization, *in silico* modelling and *in vivo* evaluation. *Drug Des. Devel. Ther.,* **14**: 27.

Hashida M (2020). Role of pharmacokinetic consideration for the development of drug delivery systems: A historical overview. *Adv. Drug Deliv. Rev.,* **157**: 71-82

Homayun B, Lin X and Choi HJ (2019). Challenges and recent progress in oral drug delivery systems for biopharmaceuticals. *Pharmaceutics,* **11**(3): 129.

Jain SK, Jain AK and Rajpoot K (2020). Expedition of Eudragit® polymers in the development of novel drug delivery systems. *Curr. Drug Deliv,* **17**(6): 448-469.

Joseph N, Lakshmi S and Jayakrishnan A (2002). A floating-type oral dosage form for piroxicam based on hollow polycarbonate microspheres: *in vitro* and *in vivo* evaluation in rabbits. *J. Control Release,* **79**(1-3): 71-79.

Khan MA, Khan S, Shahid M, Raza A, Khan N, Khan J, Khan Z and Aziz HC (2020). Clarithromycin loaded lipid polymer hybrid nanoparticles: Fabrication, *in vitro* and *in vivo* evaluation. *Pak. J. Pharm. Sci.,* **33**(3): 1303-1313.

Kini AG, Dixit M and Kulkarni P (2011). Enhancement of solubility and dissolution rate of poorly water soluble drug by spray drying using different grade of chitosan. *Int. J. Pharm. Pharm. Sci.,* **3**(2): 231-235.

Lengyel M, Kállai-Szabó N, Antal V, Laki AJ and Antal I (2019). Microparticles, microspheres, and micro-capsules for advanced drug delivery. *Scientia Pharmaceutica.,* **87**(3): 20.

Liu L, Yao W, Rao Y, Lu X and Gao J (2017). pH-Responsive carriers for oral drug delivery: Challenges and opportunities of current platforms. *Drug Delivery,* **24**(1): 569-581.

Lopes CM, Bettencourt C, Rossi A, Buttini F and Barata P (2016). Overview on gastroretentive drug delivery systems for improving drug bioavailability. *Int. J. Pharm.,* **510**(1): 144-158.

Mahale MM and Saudagar R (2019). Microsphere: A Review. *J. Drug Deliv. Ther.,* **9**(3-s): 854-856.

Maurya AK, Gupta V, Gupta N and Shrivastav V (2011). Development and evaluation of stable microsphere of Diltiazem hydrochloride an antihypertensive drug. *Int. J. Pharm. Life Sci.,* **2**(2): 583-589.

Mohammadi-Samani S, Zojaji S and Entezar-Almahdi E (2018). Piroxicam loaded solid lipid nanoparticles for topical delivery: Preparation, characterization and in vitro permeation assessment. *J. Drug Deliv. Sci. Technol.,* **47**: 427-433.

Mrsny RJ (2012). Oral drug delivery research in Europe. *J. Contro. Release,* **161**(2): 247-253.

Patel MM and Amin AF (2011). Process, optimization and characterization of mebeverine hydrochloride loaded guar gum microspheres for irritable bowel syndrome. *Carbohydr. Polym,* **86**(2): 536-545.

Shah SMH, Ullah F, Khan S, Shah SMM, De Matas M, Hussain Z, Minhas MU, Abd El-Salam NM, Assi KH and Isreb M (2016). Smart nanocrystals of artemether: Fabrication, characterization and comparative *in vitro* and *in vivo* antimalarial evaluation. *Drug Des. Devel. Ther.,* **2016**(10): 3837.

Shepard KB, Adam MS, Morgen MM, Mudie DM, Regan DT, Baumann JM and Vodak DT (2020). Impact of process parameters on particle morphology and filament formation in spray dried Eudragit L100 polymer. *Powder Technol,* **362**: 221-230.

Tyagi LK and Kori ML (2014). Stability study and *in-vivo* evaluation of lornoxicam loaded ethyl cellulose microspheres. *Int. J. Pharmaceut. Sci. Drug Res.,* **6**(1): 26-30.

Vlachou M, Geraniou E and Siamidi A (2020). Modified release of furosemide from Eudragits® and poly (ethylene oxide)-based matrices and dry-coated tablets. *Acta. Pharmaceutica.,* **70**(1): 49-61.

Zhao Z, Ukidve A, Krishnan V and Mitragotri S (2019). Effect of physicochemical and surface properties on *in vivo* fate of drug nanocarriers. *Adv Drug Deliv Rev,* **143**: 3-21.

Zou X, Zhao X, Ye L, Wang Q and Li H (2015). Preparation and drug release behavior of pH-responsive bovine serum albumin-loaded chitosan microspheres. *J. Ind. Eng. Chem.,* **21**: 1389-1397.