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Formulation and evaluation of polymeric microspheres of a poorly soluble drug celecoxib

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***Abstract*---**The aim of this present work was to formulate microspheres of BCS Class II drug Celecoxib. The drug was identified by melting point study and FT-IR spectroscopy. Therapeutic success of a drug is greatly depended on its bioavailability. Majority of the recently discovered drugs shows poor solubility (BCS Class II) which decreases the bioavailability upon oral administration. Microspheres are a novel technique to circumvent this obstacle, which can increase bioavailability of drugs significantly. Polymeric microspheres with Polyvinylpyrrolidone and Eudragit L-100, prepared by Emulsion Solvent Diffusion and Evaporation technique, showed a range of drug release profile at intestinal pH 6.8. Different batches of microspheres were prepared by varying the drug-polymer ratio. Microparticulate systems like microspheres improve absorption which increases the bioavailability, ultimately leading to more efficient drug therapy. The microsphere system also provides protection to the gastric mucosa from GIT-irritant drugs thus decreasing toxic effects and sustained

releases allows lower dosage frequency. Prepared microspheres were evaluated on various parameters like entrapment efficiency, %yield, particle size, scanning electron microscope analysis, and in-vitro drug release profile. Solid spherical microspheres were produced which showed good entrapment efficiency (43%-91%) and released 70-90% of the drug content in 10hrs. Optimized formulation showed prolonged activity without losing therapeutic activity of the drug.

***Keywords*---**celecoxib microspheres, emulsion solvent diffusion evaporation technique, Eudragit l100-PVP polymeric microspheres.

Introduction

Various novel techniques have been developed by scientist through research to overcome the barriers in providing an ideal oral dosage form. One such technique is microspheres, which essentially means spherical balls of polymer with sizes in the micrometer range. Microspheres offer many benefits like sustained release of drug, reduction in GI toxicity, controlled release, reduced dosing frequency etc(1). One major use of microspheres is in modulating solubility of poorly water soluble drugs, classified as BCS Class II drugs. Microspheres have been shown to greatly improve solubility of such drugs thus increasing their bioavailability in process. Moreover, this technique is easy to upscale and remains cost effective even in industry scales. Thus providing a novel and cheap technique for delivery of BCS Class II drugs (2). Celecoxib,4-[5-(4-Methylphenyl)- 3-(trifluoromethyl)-1H-pyrazol-1-yl] benzenesulfonamide, with the empirical formula C17H14F3N7O2S, is a selective COX II inhibiting Non Steroidal Anti- Inflammatory Drug (3). It is used in conditions like arthritis, rheumatoid arthritis, FAP etc. Recently many researchers have formulated celecoxib containing formulations for colonic delivery bypassing the stomach on oral intake, thus reducing GIT toxic effects. Side effects of the drug can be circumvented by formulating a sustained release formulation(4–6). The present work is proposed to study the influence of drug polymer ratio on microspheres loaded with Celecoxib, prepared by solvent evaporation technique. Eudragit l 100 was used to provide a sustain release drug delivery. Prepared microspheres were evaluated for drug entrapment efficiency, micromeritics characters, floating behavior and in vitro drug release.

The specific objective of the present study is detailed below

- Preformulation study of celecoxib and polymer compatibility
- Selection of polymers and other chemicals required in the process
- Formulation and development of microspheres
- Evaluation of the formulations

Materials

Celecoxib was generously gifted by Aarti Drugs Pvt. Ltd., Eudragit L 100 and PVP were obtained from Balaji Drug House Pvt. Ltd. Distilled water was used for all the experiments. Dichloromethane and ethanol were obtained from Merck Specialties Pvt. Ltd and Sigma Aldrich Chemicals Pvt. Ltd respectively. Polyvinyl

alcohol and Tween-80 was obtained from Rankem Pvt. Ltd. All other chemicals and reagents used were of AR grade, procured commercially and used as such without further purification.

Method

Emulsion-solvent diffusion evaporation technique (7) was opted for preparing microspheres. Polymer (PVP and Eudragit L 100) and drug (Celecoxib) in a 1:1 ratio were dissolved in a solvent mixture of dichloromethane and ethanol (1:1). Then, the drug-polymer solution was added drop-by-drop into 1.5% PVA solution containing 0.3% Tween-80. Resultant emulsion was stirred at 900 rpm using a propeller-type agitator for 3 hours or until the solvents are completely evaporated. Solid discreet microspheres were collected by filtration through Whatmann No. 1 filter paper under vacuum and washed 3 times with distilled water. The prepared microspheres were air dried in a desiccator for 24hrs.

Table 1: Optimized formulae for celecoxib microspheres

SL NO	Formulation (Drug : Polymer)	Drug (Celecoxib) (mg)	PVP (mg)	Eudragit L 100 (mg)
1	F_1 (1:1)	100	50	50
2	F_2 (1:2)	100	100	100
3	F_3 (1:3)	100	150	150
4	F_4 (1:4)	100	200	200
5	F_5 (1:1)	100	70	30
6	F_6 (1:1)	100	30	70

Evaluation (8,9)

Melting point determination

Melting point is the temperature at which matter changes state from solid to liquid. At this temperature, theoretically the matter is in equilibrium between solid and liquid state at a pressure of 1 atmosphere. The drug is filled in a one-side sealed glass capillary and dipped in the apparatus equipped with a magnification glass and thermometer, to precisely measure the temperature at which the transition from solid form to liquid occurs. The measurement was done in triplicate.

FT-IR Spectrophotometric analysis

FTIR (Buker α, Germany) spectra of the pure drug, drug polymer physical mixture and prepared formulation were studied to evaluated chemical interactions between the drug and polymer. The drug and KBr was dried for 1 hr at 70°C in a hot air drier. The dried KBr and drug was triturated finely in a pestle mortar and then converted in to a pellet by a hydraulic press. Pellets of pure KBr, and drug-polymer mixtures and prepared formulation were also made for studying chemical interaction.

Percentage yield

The percentage yield of microspheres was calculated by dividing the total weight of produced microspheres by theoretical quantity of microspheres produced.

% yield = (amount of microspheres produced / theoretical amount) x 100 %

Drug entrapment efficiency

A weighed amount of drug loaded microspheres (equivalent to 20 mg of drug) was extracted using 20 ml of methanol. The solution was suitably diluted, and the absorbance was taken at 254nm. Actual amount of drug in the microspheres were calculated using the calibration curve.

Drug entrapment efficiency = (actual drug content / theoretical drug content) x 100 %

SEM analysis

Scanning electron microscope (SEM) was used to study the microspheres visually and to examine the surface characteristic and mean diameter of the prepared microspheres. Average diameter of 100 particles was measured to calculate mean particle size for each formulation. The microspheres were mounted on the SEM sample stab using a double sided tape. The samples were then coated with gold for 5 minutes to increase the conductivity of the sample which improves the resolution of the picture. The coated microspheres were observed under the SEM at an accelerating voltage of 15 kV and photographed.

Particle size and zeta potential

Particle size and zeta potential of the prepared microspheres were checked using Zetasizer Ver. 7.11 (Malvern Instruments Ltd.). The samples were dispersed in double distilled water and taken into the cuvette for analyzing in the instrument.

In-vitro dissolution study

Celecoxib is BCS class II drug i.e. low solubility and highly permeability, thus making dissolution the rate controlling factor in in-vivo absorption process. In-vitro dissolution study was performed in a USP type II apparatus (Disso 2000, Lab India, Chennai.) at 50 rpm and 37± 0.5ºC. The dissolution medium of 450ml contained phosphate buffer pH 6.8 + 2% w/v SLS, addition of SLS is to maintain sink conditions.(10) Aliquots of 5ml were drawn from the medium at specific intervals and 5 ml fresh buffer solution was added immediately. The aliquots were filtered through Whatmann no. 1 filter paper, and then suitably diluted for UV spectrophotometer analysis at 254 nm.

Results and Discussions

Melting point

The melting point of the pure drug was found to be within the standard range.

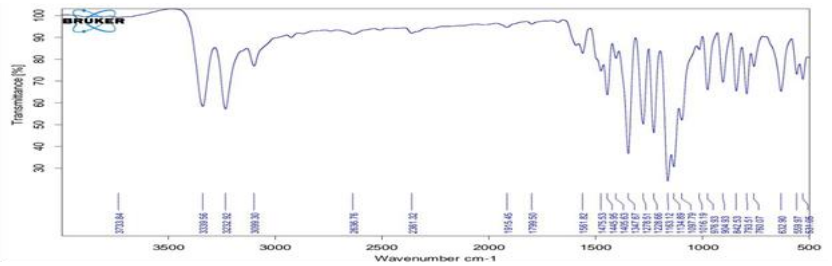
Table no. 2: Melting point

Sl. no.	Temperature °C
1	155-158
2	156-160
3	156-159

Practical melting point = 155-159°C
Theoretical melting point = 157-159°C

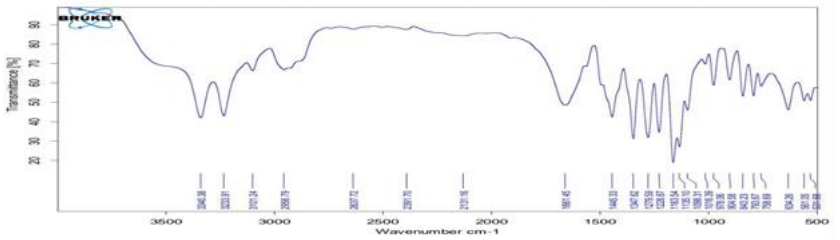
FT-IR Spectrophotometric analysis

Fourier transform infra red spectroscopy was used to further confirm the identification of the drug and check for drug-polymer interaction. A FT-IR spectrum is called the fingerprint of a molecule. Radiation at infrared range is capable of exciting the bonds between different chemical moieties. Each specific bond absorbs a specific wavelength of light and this property helps in identifying chemical compounds with great certainty. FT-IR spectrum of Celecoxib, Eudragit L 100 and PVP (Fig. 1- 4) were studied to confirm the chemicals and check for any chemical interaction between the drug and polymers. Celecoxib showed characteristic peaks at 3160 cm^{-1} and 3260 cm^{-1} corresponding to NH symmetric and asymmetric stretching and bands at 1150 and 1340 cm^{-1} shows symmetric and asymmetric stretching of the S=O group respectively. Peaks at 1560 cm^{-1} and 780 cm^{-1} correspond to NH bending and aromatic –CH bends respectively. Characteristic peaks for PVP are at 2925 cm^{-1} (C-H stretch) and 1652 cm^{-1} (C=O). Important peaks for identifying Eudragit L-100 are absorption bands at 1157, 1184, 1261 cm^{-1} attributed to ester vibrations. Peaks at 1701 and 1736 cm^{-1} are due to C=O stretching of the carboxylic acid and esterified carboxyl group. (11) Presence of the identifying peaks in the spectrum of the sample drug, drug polymer physical mixture and prepared formulation (F4) and no appearance or disappearance of peaks from the formulation F4 spectra, confirmed purity of drug and stability of drug-polymer combination.



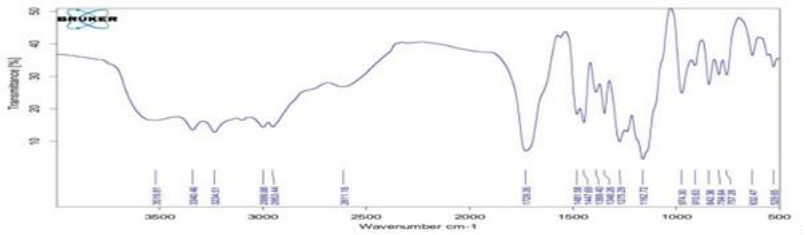
Pure Celecoxib

Fig. 1: FTIR spectra of pure celecoxib



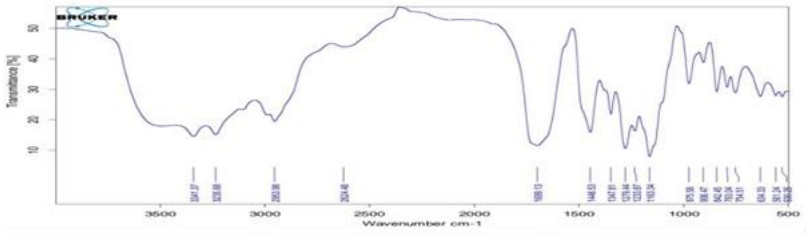
Celecoxib + PVP

Fig. 2: FTIR spectra of celecoxib and PVP mixture



Celecoxib + Eudragit L 100

Fig. 3: FTIR spectra of celecoxib and Eudragit L 100 mixture



Formulation F4

Fig. 4: FTIR spectra of Formulation F4

Percentage yield

The prepared formulation showed yield in the range of 58-89%. Formulation F1giving the lowest yield of 58% and F4 giving the highest yield. Yield increased with increasing polymer concentration which is in accordance with findings by other researchers.(12)

Table no. 3: Yield percentage

Formulation number	% yield
F1	58.35
F2	65.3
F3	82.25
F4	89.51
F5	60.18
F6	68.5

Entrapment efficiency

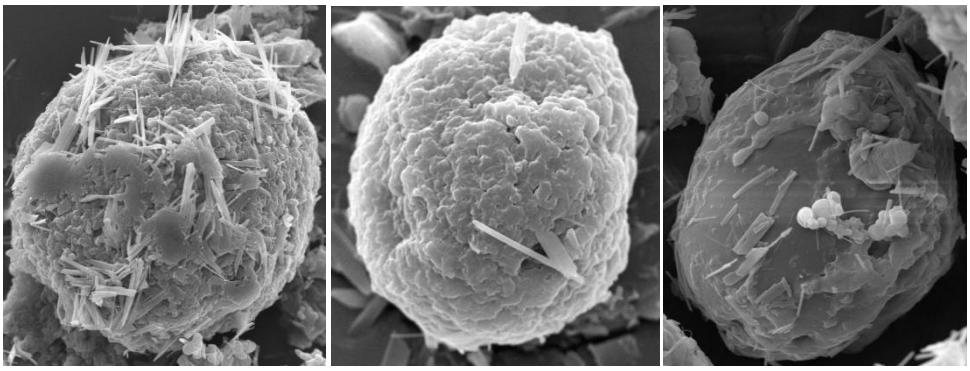
All formulation showed good entrapment of the API. Formulation F6 showed the lowest entrapment efficiency of 34.34% and F4 showed 91% entrapment. Increasing the polymer amount from 100 mg to 400mg resulted in increased entrapment of the drug from 48-91%. Higher concentration of Eudragit l 100 leads to decrease in entrapment efficiency, this may be due to the low drug carrying capacity of the polymer.(13)

Table no. 4: Entrapment efficiency

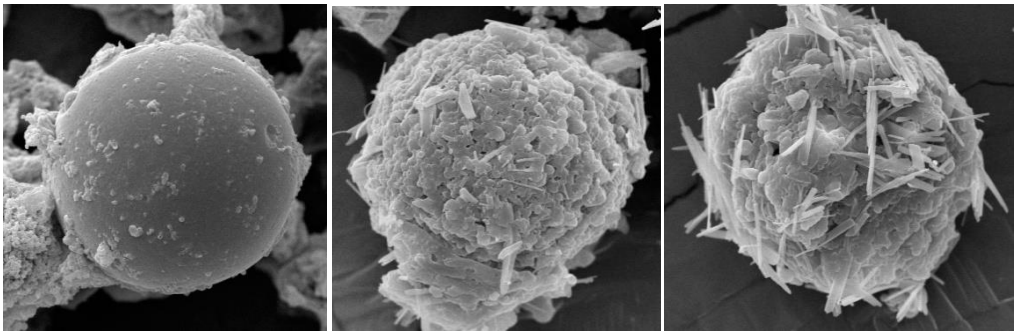
Formulation number	Entrapment efficiency %
F1	48.8
F2	62.12
F3	76.61
F4	91.28
F5	52.7
F6	43.32

SEM analysis

Scanning electron microscope provides a visual examination of the microspheres. SEM analysis (Fig. 5) showed solid spherical microspheres with drug bounded to the surface. Formulation F4 showed the best surface topology with smooth surfaces and minimum surface bound drug. All formulations were examined for mean particle size by checking mean diameter of 100 microspheres. Particle size increased with increasing polymer concentration. Formulation F1, F5 and F6 containing low amounts of polymers showed smallest microspheres with mean diameter of 25.60 ± 10.69, 23.82±8.55 and 26.85 ± 12.51 µm respectively. Highest mean diameter was recorded in Formulation F4, containing highest amounts of polymer. The particle sizes for all batches were within range of 23.82±8.55 - 65.34±13.92 µm



Formulation F1 Formulation F2 Formulation F3



Formulation F4 Formulation F5 Formulation F6

Fig no. 5: SEM images of microspheres

Table no. 7 Particle size

Formulation Number	Mean Diameter ± S.D. (µm) (n=100)
F1	25.60 ± 10.69
F2	38.29 ± 13.49
F3	57.15 ± 11.62
F4	65.34 ± 13.92
F5	23.82 ± 8.55
F6	26.85 ± 12.51

Particle size and Zeta potential

Particle size and zeta potential (Fig. 6 and 7 respectively) were examined by Malvern Zetasizer for formulation F1. Polydiversity index (PDI) was found to be 1 which infers high degree of agglomeration of the microspheres. Zeta potential of -6.37 mV confirms high degree of agglomeration of particles. Due to such agglomeration of particles, good particle size measurements were not possible by Dynamic Light Scattering (DLS) technique used by the instrument. (14)

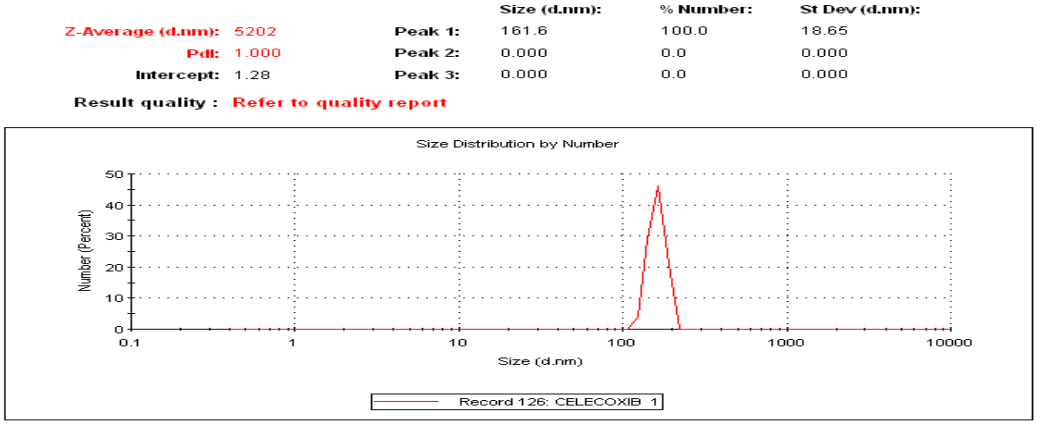


Fig. no. 6: Particle size

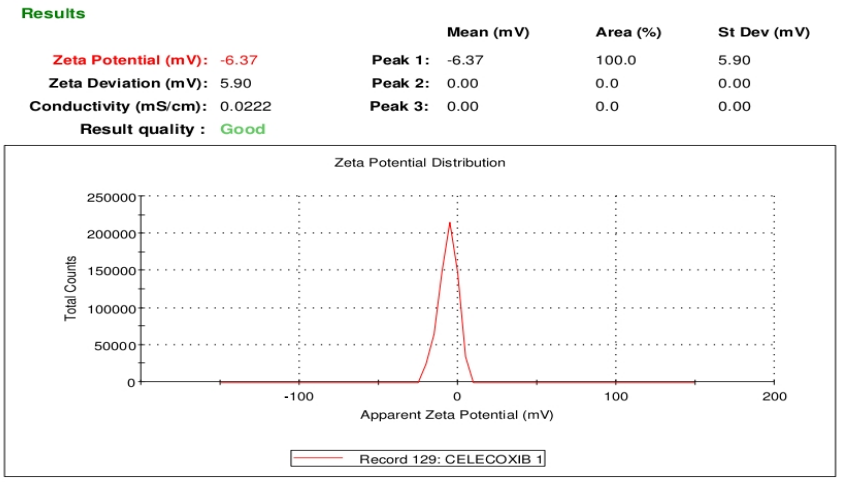


Fig. no. 7: Zeta Potential

In–vitro dissolution study

Low solubility drugs like celecoxib shows dissolution limited drug release, which makes in-vitro dissolution study highly crucial in determining in-vitro in-vivo correlation. Low solubility of the drug in water and phosphate buffer solutions makes achieving sink condition impossible. To remedy the problem 2% w/v SLS was added to the dissolution medium of phosphate buffer pH 6.8. (10) Dissolution was found to be depended on the concentration of polymer used in the formulation. %CDR vs. Time shows a burst release of 27-45% in all batches due to surface bound drug as seen in SEM study. Such burst release is in accordance with findings by other researchers.(15) The microsphere showed sustained release pattern which can be attributed to Eudragit L-100 polymer. Formulation F4 with high amounts of Eudragit L-100 showed the best sustained release while the highest release rate was seen in formulation F5 containing higher concentrations of PVP. Formulation F4 released 72% of the drug content in 10hrs with a sustained release pattern after an initial burst release of 27% due to surface bound drug. All formulation showed good release from 72% to 93% over 10hrs. Formulation F1, F5, F6 released 90+ % drug release while F2 and F3 released 87% and 80% respectively in 10 hrs.

Table no. 8: %CDR with time

Time (hours)	% Cumulative Drug Release					
	F1	F2	F3	F4	F5	F6
0.5	34.27941176	30.70588	27.52941	27.264	45.26471	41.29412
1	38.89558824	37.79706	29.95294	33.30735	52.25294	51.67941
1.5	46.86764706	43.77206	36.76765	37.51176	63.41618	59.39559
2	63.12941176	51.7941	43.6544	44.6705	70.9911	70.1029

		2	1	9	8	4
3	72.68382353	63.07647	51.67206	48.46324	83.80441	79.33382
4	82.73382353	69.84559	59.11176	54.675	89.73676	86.27647
5	87.59264706	74.3	67.81912	58.83235	92.15147	87.99265
6	89.86470588	78.45441	72.47206	62.67794	93.15294	89.12647
7	91.05	82.83971	74.76618	65.09265	93.16176	91.21618
8	91.89411765	84.95735	76.87647	68.54706	94.10735	92.45147
9	92.7	86.48088	79.46324	72.22647	94.18971	93.18824
10	93.28088235	87.23676	80.13824	72.99559	94.40147	93.70441

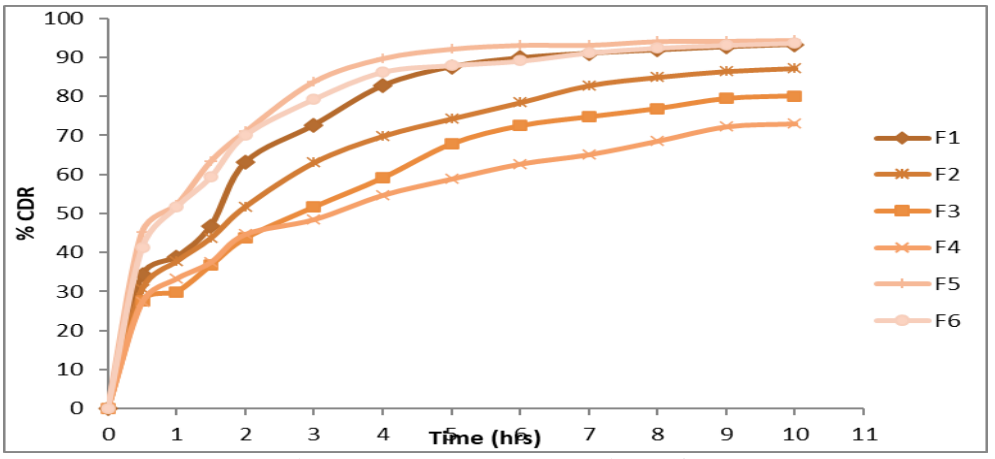


Figure no. 8: %CDR vs. Time plot

Conclusion

The aim of the study was to prepare and evaluate polymeric microspheres containing a poorly soluble drug celecoxib. Emulsion solvent diffusion technique was used considering ease of methodology to prepare microspheres of PVP and Eudragit L 100 in different ratios. The study data shows that polymeric microspheres with Eudragit L 100 and PVP, ethanol and dichloromethane as solvents, tween 80 as surfactant can be formulated. FTIR spectra showed no interaction between drug and polymers. Amount of polymer affected the yield and particle size i.e. a gradual increase in particle size (23.82±8.55 - 65.34±13.92 µm) and yield (58-89%) with increasing polymer amounts. Prepared microspheres had surface bound drug as confirmed by SEM analysis causing an initial burst release (27-45%), after which sustained release patterns were seen. In conclusion, optimized formulation F4 microspheres of celecoxib which showed satisfactory physicochemical properties and in-vitro drug release was successfully formulated.

References

1. Babu GVMM, Shankar VG, Sankar KH, Seshasayana A, Kumar NK, Murthy KVR. Development of dissolution medium for a poorly water soluble drug celecoxib. Indian J Pharm Sci. 2002;64(6):588–91.
2. Bohr A, Kristensen J, Stride E, Dyas M, Edirisinghe M. Preparation of microspheres containing low solubility drug compound by electrohydrodynamic spraying. Int J Pharm [Internet]. 2011;412(1–2):59–67. Available from: http://dx.doi.org/10.1016/j.ijpharm.2011.04.005
3. Estevez, A. G., Espinosa, A. H. R., Rodríguez, D. L., & Leyva, T. F. (2019). Current approaches and controversies: legalization and non-legalization of drugs. *International Journal of Health & Medical Sciences*, *2*(1), 26-32. https://doi.org/10.31295/ijhms.v2n1.85
4. Ipsen. Product Monograph Therapeutic Product Monograph. 2015;1–17. Available from: www.prodoc.qc.ca (accessed on March 20, 2019)
5. Jitendra G, Govind M, L. P, Reena G. Emulsion solvent diffusion evaporation technique: Formulation Design Optimization and investigation of Aceclofenac Loaded Ethyl Cellulose Microspheres. Int J Drug Dev Res. 2013;5(4):336–49.
6. Kaity S, Maiti S, Ghosh AK, Pal D, Ghosh A, Banerjee S. Microsponges: A novel strategy for drug delivery system. J Adv Pharm Technol Res. 2010;1(3):283–90.
7. Kaur G, Paliwal S. Formulation and Evaluation of Etoricoxib Microbeads for Sustained Drug Delivery. Univers J Pharm Res. 2019;37–41.
8. Khoshnevisan K, Barkhi M. Zeta potential. 2015.
9. Kumar BS, Kumar KLS, Anand DCP, M.Saravanakumar, R.Thirumurthy. Formulation and Evaluation of Celecoxib Microspheres by Using Ethylcelluose and Eudragit S-100 in Colon Drug Delivery. 2010;2(5):322–8.
10. Pandya VM, Patel DJ, Patel JK, Patel RP. Formulation, characterization, and optimization of fast-dissolve tablets containing celecoxib solid dispersion. Dissolution Technol. 2009;16(4):22–7.
11. PR R, Moidutty L, H BC. Preparation and Evaluation of Delayed Release Aceclofenac Microspheres Title. Res J Pharm Tech. 2008;1(3):270–2.
12. Saxena A, Gaur K, Singh V, Singh RK, Dashora A. Floating Microspheres as Drug Delivery System. Am J Pharm Pharm Sci. 2014;1(2):27.
13. Shahzad MK, Ubaid M, Murtaza G. Formulation and optimization of celecoxib-loaded microspheres using response surface methodology. Trop J Pharm Res. 2012;11(5):695–702.
14. Suryasa, I. W., Rodríguez-Gámez, M., & Koldoris, T. (2021). Health and treatment of diabetes mellitus. *International Journal of Health Sciences*, *5*(1), i-v. https://doi.org/10.53730/ijhs.v5n1.2864
15. Thakkar H, Sharma RK, Mishra AK, Chuttani K, Murthy RSR. Celecoxib incorporated chitosan microspheres: In vitro and in vivo evaluation. J Drug Target. 2004;12(9–10):549–57.
16. Trivedi P, Verma A, Garud N. Preparation and characterization of aceclofenac microspheres. Asian J Pharm. 2008;2(2):110.
17. Varde NK, Pack DW. Microspheres for controlled release drug delivery. Expert Opin Biol Ther. 2004;4(1):35–51.