Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

Pelagia Research Library

Der Pharmacia Sinica, 2015, 6(10):47-55

Der Pharmacia Sinica
ISSN: 0976-8688
CODEN (USA): PSHIBD

Formulation and *in vitro* evaluation of alginate-tamarind kernel polysaccharide microparticles

Bharati S. Patil[a,b]*, Hitendra S. Mahajan[c] and Sanjay J. Surana[c]

[a]*J. N. T. University, Kakinada, Andhra-Pradesh, India*
[b]*Sandip Institute of Pharmaceutical Sciences, Mahiravani, Nashik, Maharashtra, India*
[c]*R. C. Patel Institute of Pharmaceutical Education and Research, Shirpur, Maharashtra, India*

ABSTRACT

Felodipine alginate TKP microparticles were developed using sodium alginate and tamarind kernel polysaccharide (TKP) by ionic gelation technique using 5-level, 2-factor central composite experimental design. The concentration of sodium alginate (X_1) and concentration of calcium chloride (X_2) were selected as independent variables. % production yields (Y_1), % entrapment efficiency (Y_2) and % mucoadhesion strength (Y_3) were taken as response variables. Evaluation of microparticles was carried out in terms of shape, size, entrapment efficiency (EE), In vitro release, Scanning electron microscopy (SEM), and stability studies. The results revealed that concentration of alginate and calcium chloride had a significant synergistic effect on % entrapment efficiency and mucoadhesion strength.

Key words: Sodium alginate, Tamarind kernel polysaccharide, Ionic gelation method, Central composite experimental design, Microparticles.

INTRODUCTION

Polysaccharides show very attractive advantages in comparison to synthetic polymers. They are widely present in living organisms, are usually abundant and show a number of peculiar physicochemical properties; furthermore, these macromolecules are, in most cases, non-toxic, biocompatible and can be obtained from renewable sources. For these reasons, polysaccharides seem to be particularly suitable for different applications in the wide field of pharmaceutics [1]. Alginate is an anionic copolymer of 1, 4-linked β -D-mannuronic acid and α-L-guluronic acid, can be used for encapsulation of a wide range of drugs, with minimal use of organic solvents. Tamarind gum, derived from the seeds of Tamarindus indica. A common and most important tree of India and Southeast Asia belongs to the family Leguminosae. Tamarind gum is composed of xyloglucan polysaccharide which is (1, 4)-linked-β-D-glucan backbone chain, which has (1, 6)-linked -α-D-xylose branches that are partially substituted by (1, 2)-linked β-D-galactoxylose. For use in drug-release studies polymer is partially degraded by β-galactosidase to eliminate 44% of the galactose residues [2]. Residence time or mucoadhesion may be controlled by mixing two or more water-soluble polymers with different mucoadhesion [3]. Curcumin-loaded nano-microparticulate systems using naturally occurring polymers including chitosan, carrageenan, and alginate were developed and evaluated to improve the solubility of curcumin [4]. Co-grinding of hydrophobic drugs with a hydrophilic polymer improves the wettability of hydrophobic drugs, there by resulting in improvement of dissolution [5,6]. Different technological methods, such as micronization, formation of solvates, adsorbate, complexes, solid dispersions, and microspheres have been reported in order to enhance dissolution characteristics of drugs with low water solubility [7].

Due to poor water solubility attempts have been made to improve dissolution rate of felodipine such as solid dispersions with HPMC and surfactants [8] and encapsulation of micronized felodipine particles in poly-(ethylene glycol) 4000 [9]. Extended release tablets [10] and nanodispersions [11] of felodipine were also reported.

Mucoadhesive microspheres of felodipine for nasal delivery were developed to avoid extensive first pass metabolism and thereby improve the therapeutic efficacy of the drug [12].

Felodipine is a long-acting 1, 4-dihydropyridine calcium channel blocker. It acts primarily on vascular smooth muscle cells by stabilizing voltage-gated L-type calcium channels in their inactive conformation. Felodipine is used to treat mild to moderate essential hypertension [13].

The objective of the present study is to optimize the formulation of felodipine alginate TKP microparticles. As felodipine is poorly water soluble drug, microparticles were developed using natural (hydrophilic) polysaccharides like TKP and sodium alginate which may enhance its solubility. In this study the concentration of sodium alginate and calcium chloride was optimized using central composite design of response surface methodology to prepare microparticles having maximum production yield, entrapment efficiency and mucoadhesive strength.

MATERIALS AND METHODS

Materials

Tamarind kernel polysaccharide (TKP) was obtained from Hindustan Gums and Chemicals Pvt. Ltd. (Bhiwani, India). Felodipine was obtained as a gift sample from Cipla Ltd. (Mumbai, India) Sodium alginate and calcium chloride were obtained as gratitude samples from Thomas Baker Chemicals Pvt. Ltd (Mumbai, India) and poloxamer 188 (P188) from Mylan Laboratories (Nasik, India). Freshly excised rectal cavity was obtained from the local butcher shop (Nasik, India). All other chemicals were of reagent grade and were used as such.

Formulation of felodipine alginate TKP microparticles

Felodipine alginate TKP microparticles were prepared by ionic gelation method using calcium chloride as cross-linking agent. Different concentrations of aqueous solutions of sodium alginate (2-3%) mixed with 1% aqueous gel of TKP and felodipine (1%w/v). The resulting mixture was added drop-wise into aqueous solution of calcium chloride (5-10%) using 24 gauge needle. The added droplets were retained in $CaCl_2$ solution for 15 min to complete curing reaction and to produce rigid microparticles. The microparticles so prepared were collected by decantation technique and dried overnight at room temperature [14, 15, 16].

The preparation of Felodipine alginate TKP microparticles was carried out using systematic design of experiments employing Design Expert Software (version 8.0.3.1 Stat-Ease Inc.). A central composite design with α=1.41421 was employed as per standard protocol. In the present study, five level two factor central composite designs were employed for optimization of microparticles (Table1). The concentration of concentration of sodium alginate (X_1) and calcium chloride (X_2) were selected as independent variables. % production yields (Y_1), entrapment efficiency (Y_2) and % mucoadhesion strength (Y_3) were taken as response variables.

Table 1: Levels of process parameters used in experiments

Factors	Levels				
	+α	-α	0	+1	-1
Concentration of Sodium Alginate	3.21	1.79	2.5	3	2
Concentration of $CaCl_2$	11	3.96	7.5	10	5

Thirteen formulations were developed as shown in Table 2. The central point (0, 0) was studied in pentet in order to evaluate the experimental error. The statistical analysis of data was carried out by Design Expert software.

Characterization of felodipine alginate TKP microparticles:

Production Yield

Production yields were determined using following formula.

$$\text{Production Yield} = \frac{\text{Practical yield of microparticles after drying}}{\text{Theoretical yield}} \times 100$$

$$\text{(Total amt of drug and polymers added initially)}$$

Table 2: Central composite Design of Response Surface Methodology by Quadratic model

Formulation Code	Sodium alginate (X_1)	Conc. of $CaCl_2$ (X_2)	% Production Yield (Y_1)	% EE (Y_2)	% Mucoadhesion Strength(Y_3)
F1	2.5	7.5	91.61	93.7	90.87
F2	2	5	88.58	78.08	86.0
F3	3	5	89.51	89.96	90.82
F4	2.5	11	92.79	91.0	91.87
F5	2.5	3.96	85.47	82.03	89.33
F6	2	10	89.65	85.58	86.44
F7	2.5	7.5	89.88	93.44	89.67
F8	1.79	7.5	86.15	84.21	85.33
F9	2.5	7.5	90.79	91.85	91.0
F10	3.21	7.5	92.72	93.97	92
F11	2.5	7.5	89.66	92.44	91.33
F12	2.5	7.5	91.84	92.37	90.67
F13	3	10	93.28	94.73	94.67

Entrapment efficiency

The amount of felodipine in alginate TKP microparticles was determined by triturating microparticles in mortar and then extracted with ethanol, followed by filtration through membrane filter (45µm pore size) and after certain dilution with distilled water, analyzed spectrophotometrically at 360.4 nm [17]. Entrapment efficiency was calculated using the following equation:

$$\%EE = \frac{\text{Practical amt. of drug entrapped in microparticles}}{\text{Theoretical amt. of drug added in microparticles}} \times 100$$

***In vitro* Mucoadhesive strength of microparticles using Falling Liquid Film Technique**

A freshly excised goat rectal mucosa was obtained from local slaughterhouse and rinsed with normal saline. The tissue was pinned onto a polyethylene support inclined at an angle of 60^o. A 25 number (No) of counted felodipine microparticles were placed on the trough of the mucosal surface, were hydrated with water and allowed to interact for 15 min. A 50 ml volume of pH 6.8 phosphate buffer was allowed to flow over the tissue at the rate of 40 drops/min. The number of the microparticles remaining on mucosal surface (Ns) was counted. The adhesive strength was determined using formula given below [18].

$$\%\ \text{Mucoadhesive strength} = \frac{Ns}{No} \times 100$$

***In vitro* release study of felodipine alginate TKP microparticles**

Accurately weighed felodipine alginate TKP microparticles (equivalent to 5 mg of felodipine) were subjected to release rate study in 500 ml of pH 6.8 phosphate buffer using USP dissolution test apparatus II (Model: Tablet Dissolution Test Apparatus, Labindia) with autosampler. Felodipine (5 mg) was used as control and subjected to release rate study. Samples were periodically withdrawn and replaced with same volume of fresh buffer solution, and assayed using a spectrophotometer at 360.4 nm [17].

Particle size

Microparticles dispersed in immersion oil were evaluated for the particle size using Motic DMW2-223 digital microscope (Motic Instruments Inc. Canada) equipped with a 1/399 CCD camera imaging accessory and computer controlled image analysis software (Motic images 2000, 1.3 version).

Surface Morphology by SEM

The morphology of felodipine alginate TKP microparticles (optimized batch) was studied using scanning electron microscopy (JSM 6390, JEOL).

Zeta potential study
The dispersion of microparticles in distilled water was filled in zeta cell and placed in the Zeta Sizer (Nano ZS90, Malvern Instruments, UK).

FTIR spectroscopy
The samples were subjected to FT-IR spectroscopy in a Fourier-transform infrared spectrophotometer (IR Affinity-1, Shimadzu, Japan) in range of 4500-500 cm^{-1} as KBr pellet.

Stability study
Stability studies were carried out for of optimized formulation of felodipine alginate TKP microparticles as per ICH guidelines at $40^0C \pm 2^0C$ /75% ± 5% RH for 3 months. % entrapment efficiency and % cumulative drug release were determined at 1, 2, and 3 months interval.

RESULTS AND DISCUSSION

Polymers with positively or negatively charged groups interact with molecules of opposite charges to form three-dimensional networks. Aqueous solutions of charged polysaccharides often gel after interacting with small ions of opposite charges. The reaction of sodium alginate and TKP , with multivalent cation like calcium chloride (cation crosslinker) allows the formation of bridges between the polymeric chains and results in inter-cross-linking (by electrostatic interaction) between the polymer molecules, have eventually resulted in formation of gel. In the present research, interaction of alginate and TKP with calcium ions has been explored to prepare felodipine alginate TKP microparticles. Ionic gelation method is simple, reproducible and free from use of organic solvents. Table 2 shows the results of 13 formulations developed using the design protocol. The data obtained was analyzed and fitted into various polynomial models. It was observed that the response % production yield (Y_1) fitted best into the linear response surface model. On the other hand entrapment efficiency (Y_2) and % mucoadhesion strength (Y_3) fitted best into the quadratic response surface model. The polynomial models showing the relationship between the independent variables and the responses Y_1, Y_2 and Y_3 can be expressed by the following Eqs.

$$Y_1=90.15+1.73X_1+1.90X_2$$

$$Y_2= 92.76 + 4.35X_1 +3.12X_2 - 0.68\ X_1X_2-2.01\ X_1^2-3.30\ X_2^2$$

$$Y_3=90.71+2.81X_1+0.99X2+0.85\ X_1X_2-1.06\ X_1^2-0.092\ X_2^2$$

The polynomial models were further analyzed by ANOVA analysis (Table 3) to estimate the significance of response surface models. For all responses 1) Model F-value implies that models were significant 2) Lack of Fit value implies that lack of fit is insignificant. 3) The predicted R^2 were in reasonable agreement with Adjusted R^2. 4) Adequate precision measures the signal to noise ratio. A ratio greater than 4 is desirable and indicates an adequate signal. All models can be used to navigate the design space. 5) The lower values of coefficient of variance (C.V.) indicate the reliability of experiments carried out.

Table 3: Statistical summary of response surface model

Response variable	Model									Lack-of-fit	
	F-value	Prob>F	R^2	Adjus. R^2	Pred. R^2	Adeq. Prec.	Std. Dev.	% CV	PRESS	F-value	Prob>F
Y_1	15.29	0.0009	0.7536	0.7043	0.5404	11.499	1.31	1.46	32.22	2.31	0.2191
Y_2	45.58	< 0.0001	0.9701	0.9488	0.8279	19.212	1.20	1.34	57.65	4.16	0.1011
Y_3	34.45	< 0.0001	0.9610	0.9331	0.8253	20.501	0.69	0.77	14.85	1.47	0.3489

Three dimensional response surface plots generated by the Design Expert software are presented in fig. 1, 2 and 3 for the studied responses i.e. % production yield, % entrapment efficiency and % mucoadhesion strength. Fig.1 depicts response surface plot of concentration of sodium alginate (X_1) and concentration of $CaCl_2$ (X_2) on % production yield. It indicates that increasing polymer concentration resulted in increase in % production yield which was more pronounced at higher crosslinker concentrations. In addition at higher values of polymer concentration increasing the crosslinker concentration did not result in any significant change in% production yield. These results may be attributed to the inadequate interaction between the polymer and crosslinker in solution rendered more viscous by increasing polymer concentration.

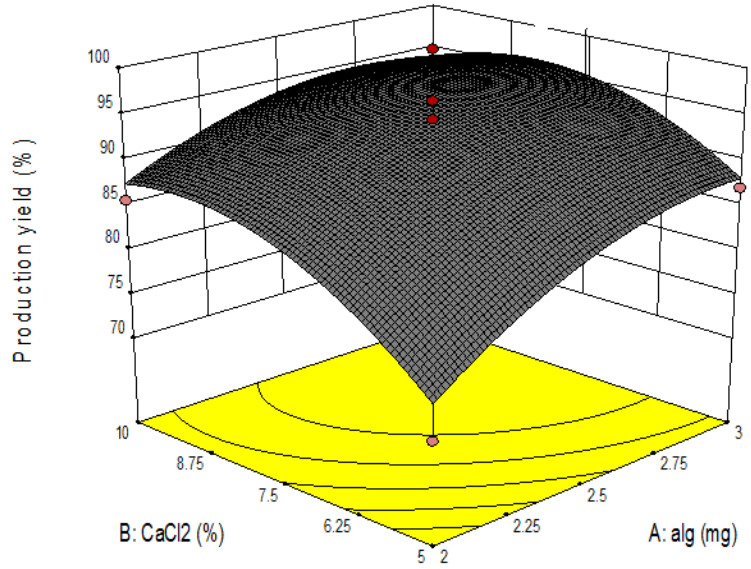

Fig.1. Response surface plots for the A) sodium alginate and B) calcium chloride on % Production yield

Fig. 2 and 3 represents response surface plot of the effect of alginate concentration and crosslinker concentration on % mucoadhesion and % entrapment efficiency strength respectively. This explains that the higher the amount of alginate more will be the % entrapment efficiency and % mucoadhesion strength. A numerical optimization technique with desirability approach was used to select the optimal concentrations of polymer and crosslinking agent under the constraints of maximizing production yield, entrapment efficiency as well as mucoadhesion strength. The optimal calculated parameters were concentrations of alginate 3.000% and calcium chloride 9.669%. The optimized batch of felodipine loaded microparticles was found to have 93.53% production yield, 94.73% EE and 93.98% mucoadhesion strength.

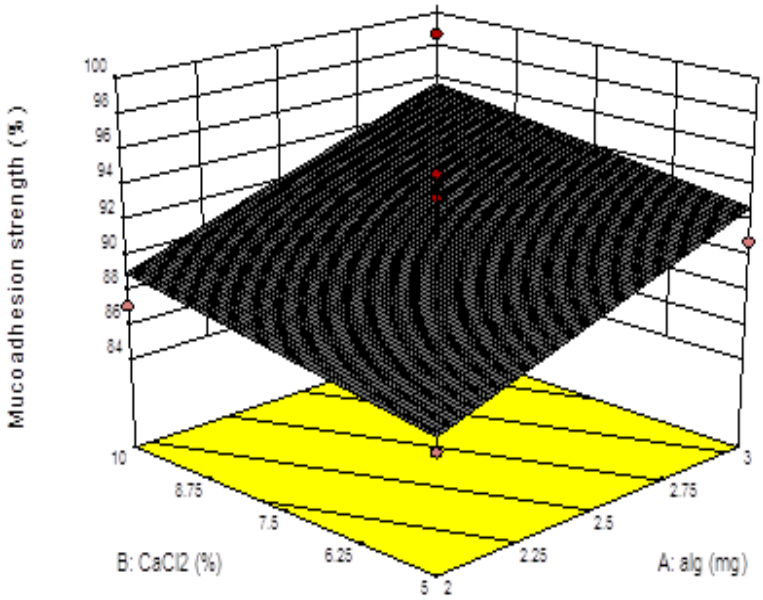

Fig.2. Response surface plots for the A) sodium alginate and B) calcium chloride on % mucoadhesion strength

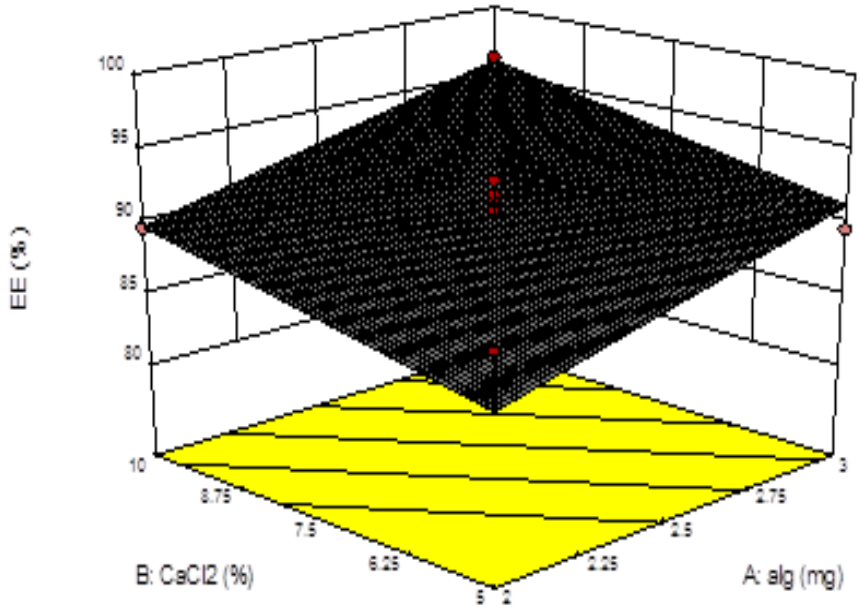

Fig.3. Response surface plots for the A) sodium alginate and B) calcium chloride on % entrapment efficiency

Entrapment efficiency of the microparticles is dependent on aqueous solubility of drug, the concentration of sodium alginate and concentration of aqueous $CaCl_2$ solution. It was observed that by increasing the concentration of sodium alginate and aqueous $CaCl_2$ solution, the entrapment efficiency of the microparticles also increases. As the quantity of sodium alginate increases, it results into the greater degree of cross linking with more available active calcium binding sites in the polymeric chains which increases the compactness of the insoluble matrices. If the concentration of polymer is further increased, a decrease in entrapment is observed, which may be due to higher viscosity of the polymeric solution which hinders diffusion of drug in the polymer. More intact matrix network is formed which slows down the diffusion of drug in aqueous $CaCl_2$ solution in which the microparticles are formed resulting in more drug entrapment [19, 20]. Moreover, as felodipine is water insoluble, less diffusion of drug in aqueous manufacturing vehicle takes place. Hence higher entrapment efficiency was achieved [21].

Mucoadhesion strength was determined to ensure the adhesion of formulation to the mucosa for a prolonged period of time at the site of administration.

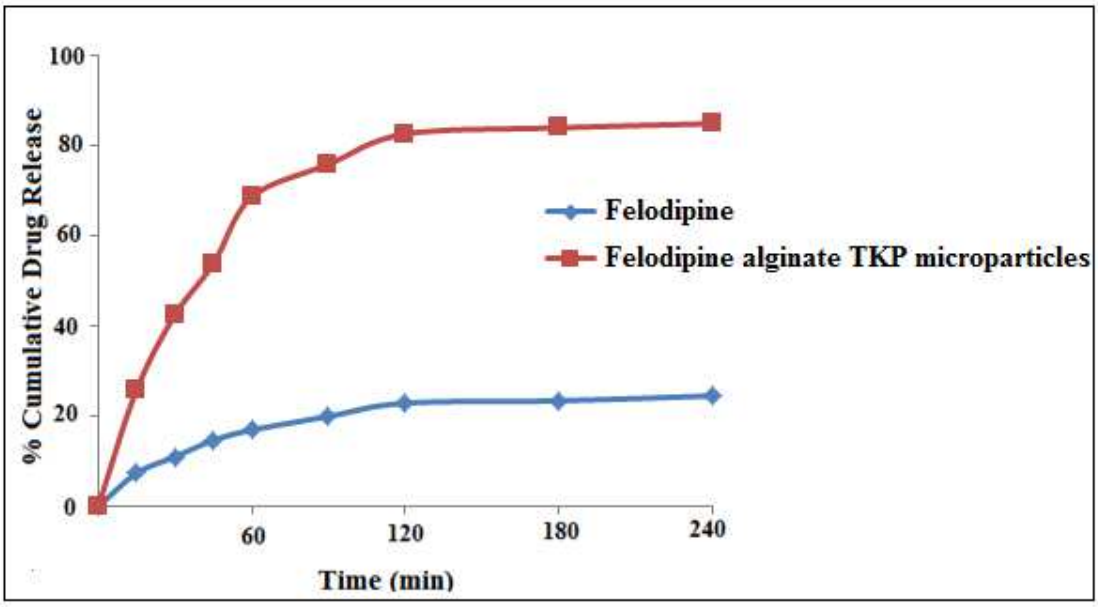

Fig 4. Release profiles of (A) Felodipine (B) Felodipine alginate TKP microparticles

From *in vitro* release study (Fig.4), as compared to felodipine, felodipine alginate TKP microparticles showed enhanced release of felodipine. The increased dissolution rate of felodipine alginate TKP microparticles as compared to plain drug may be attributed to the increased drug wettability, conversion to amorphous form and solubilization of the drug due to hydrophilic carrier. Size analysis of optimized batch of microparticles was done by using Motic DMW2-223 digital microscope. Size ranges from 521.17 - 686.76 µm. SEM analysis showed irregular shaped microparticles with rough and intact surface (Fig.5). The smooth texture of the microparticles surface leads to weak bioadhesive properties, while the coarser surface texture improves the adhesion through stronger mechanical interactions.

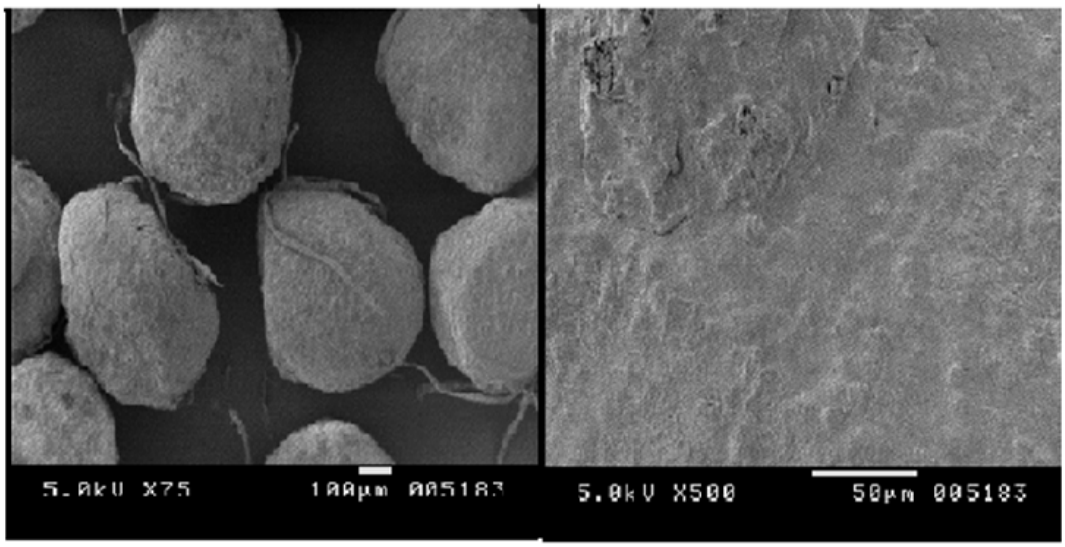

Fig.5. SEM images of felodipine loaded microparticles

The zeta potential of felodipine alginate TKP microparticles and blank microparticles were -6.98 and -10 respectively. Microparticles are negatively charged, indicating the presence of anionic polymer at the surface of all microparticles. Increase in zeta potential observed from blank to drug loaded microparticles i.e. from -10 to -6.98. Due to interaction between anionic moiety of TKP and alginate with cationic moiety of felodipine during microparticle formulation, increase in zeta potential may observed in drug loaded microparticles. Anionic charge on microparticles attributes to good mucoadhesive strength of microparticles.

The study of IR spectra of felodipine (Fig. 6) demonstrates that the characteristic absorption bands for N-H stretching vibration of secondary amine, C=O stretching vibration and C=C stretching vibration, C-N, C-O-C and C-C stretching vibration appeared at 3369, 1693, 1620, 1276, 1307 and 1138cm^{-1}, respectively. The almost identical absorption bands with lower intensity were observed from felodipine alginate TKP microparticles, but absent in blank alginate TKP microparticles. The peak attributed to the -CH2 groups present at 2924 cm^{-1} in sodium alginate and TKP and is also observed in blank and Felodipine alginate TKP microparticles. In sodium alginate some distinct peaks such as carboxyl group showed strong absorption bands at 1653 cm^{-1}, 1411 cm^{-1} and 1276 cm-1, due to carboxyl anions asymmetric and symmetric stretching vibrations disappearing or becoming weak at 1427 cm^{-1} in the microparticles. In the spectrum of sodium alginate band at 1656 cm^{-1} is attributed to the absorption band of the carbonyl (-HC=O) stretching but disappearing in drug loaded microparticles. The other band at 1022 cm^{-1} that was assigned to the stretching vibration of (CH-OH) in TKP appeared at 1641 cm^{-1} and 1026 cm^{-1} for the drug loaded microparticles. As a result, it can be concluded that all the components which were used to form the microparticles are present.

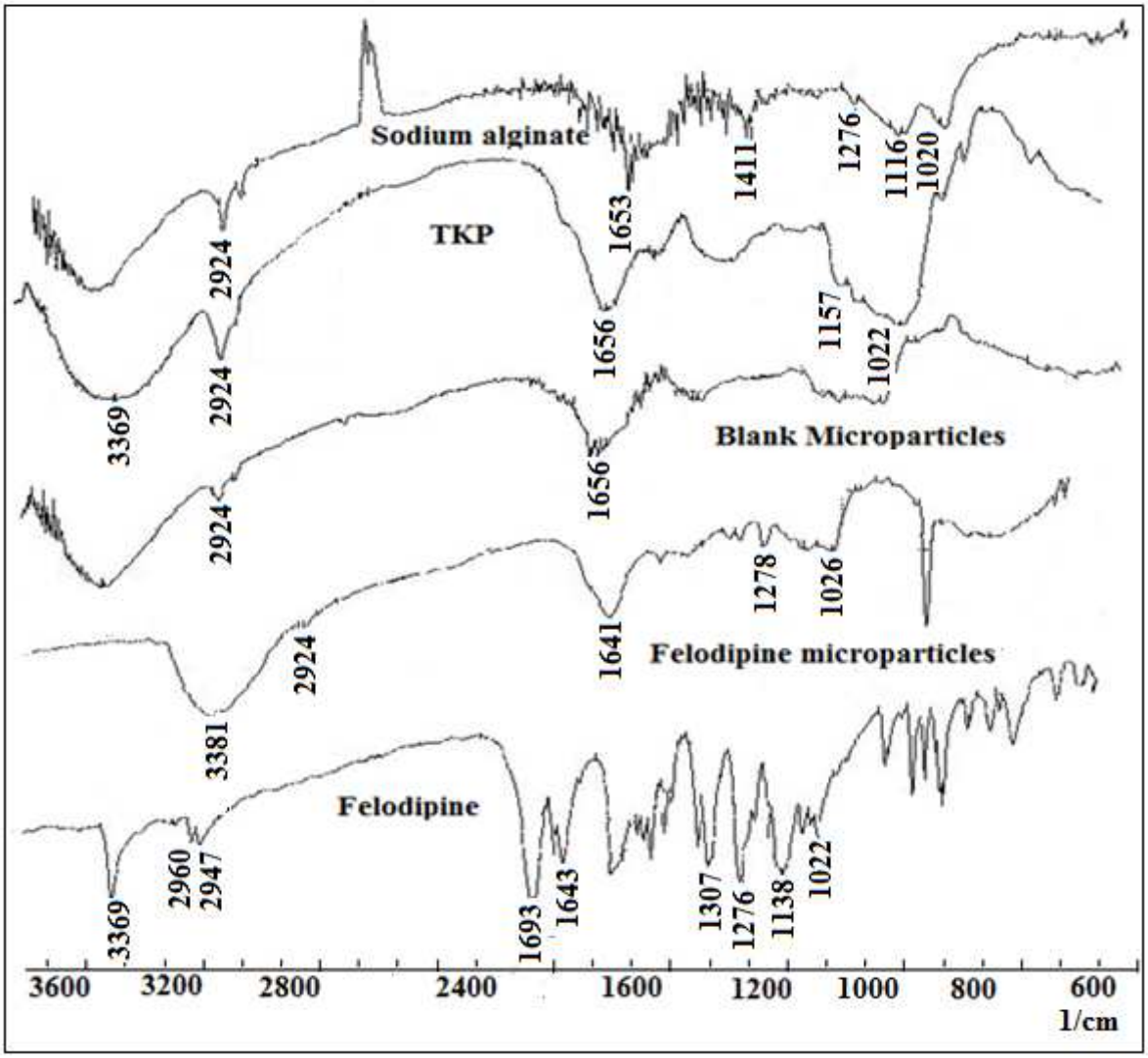

Fig.6. IR Spectra of Felodipine, Felodipine alginate TKP microparticles, Blank microparticles, TKP and sodium alginate

The entrapment efficiency of optimized batch of microparticles after 3 months (91.449 ± 0.409) indicated that the drug was retained within the microparticles throughout the stability period. The formulation was found stable even after exposing to stress conditions of temperature and moisture.

CONCLUSION

In this research, felodipine was entrapped into alginate TKP microparticles matrix using an ionic gelation method with calcium ions. Results from the present study suggest that the low oral bioavailability of felodipine could be well circumvented by ionic gelation method. Entrapment of felodipine in TKP and alginate matrix significantly improved the dissolution rate of felodipine through a number of factors such as drug amorphization and increased drug solubility. In future study, felodipine alginate TKP microparticles can be administered rectally to avoid first pass metabolism.

REFERENCES

[1] Kulkarni D, Dwivedi A.K, Sarin J.P.S., Singh S. *Indian J. Pharm. Sci.***1997**, 59, 1-7.
[2] Ruel-Garie E., Leroux J C. *Eur. J. Pharm. Biopharm.* **2004**, 58, 409-426.
[3] Nakamura F., Ohta R., Machida Y., Nagai T. *Int. J. Pharm.***1996**,134,173-181
[4] Smyth HDC, Villanueva D.G., Ibrahim M. Sherbiny E., Herrera-Ruiz D. BioMed Research International, Article ID 724763, **2013**, 9, http://dx.doi.org/10.1155/2013/724763
[5] Liversidge G.G., Cundy K.C. *Int. J. Pharm.* **1995**,125, 91-97.
[6] Kubo H., Osawa T., Takashima K., Mizobe M. *Biol. Pharm. Bull.* **1996**,19,741-7 [DOI] PMid:8741587

[7] Kim C.K., Park J.S. *Am. J. Drug Del*. **2004**, 2, 113–130.
[8] Won D H, Kim M S, Lee S, Park J S, Hwang S J. *Int. J. Pharm*. **2005**, 301, 199–208.
[9] Chiou A H, Cheng H, Wang D. J. *Microcapsul*. **2006**.
[10] Wingstrand K., Abrahamsson B., Edgar B. *Int. J. Pharm*. **1990**, 60, 151-156.
[11] Karavas E., Georgarakis E., Bikiaris D. *Int. J. Pharm*. **2006**, 313,189–197.
[12] Mishra S., Patel N.S., Kumar M., Pathak K. *Drug Deliv. Letters* **2014**, 5, 3.
[13] Sweetman S.C. Martindale. The complete drug reference, Cardiovascular Drugs, 34th ed. London: Pharmaceutical Press; **2005**, pp.914.
[14] Das M.K, Senapati P.C., *Acta Pol Pharm.* **2007**, 64, 253-62.
[15] Belgamwar V., Shah V., Surana S.J., *Curr. Drug Deliv*. **2009**, 6, 113-121.
[16] Zhang J., Xu S., Zhang S., Zhaoli D, *Iranian Polymer Journal* **2008**, 17, 899-906.
[17] Won D., Kim M., Lee S., Park J., Hwang S., *Int. J. Pharm*.**2005**, 301,199–208.
[18] Carvalho F.C., Bruschi M.L., Evangelista R.C., Gremiao MPD, *Brazilian J. Pharm. Sci.* **2010**,46, http://dx.doi.org/10.1590/S1984-82502010000100002
[19] Takka S, Ocak OH, Acarturk F, *Eur J Pharm Sci*.**1998**, 6, 241-6.
[20] Mirghani A, Idkaidek NM, Salem M S, Najib NM, *Drug Dev. Ind. Pharm.* **2000**, 26, 791-5.
[21] Aslani P, Kennedy RA, *J. Controlled Release*. **1996**, 42, 75- 82.