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FORMULATION AND EVALUATION OF FELODIPINE LOADED MUCOADHESIVE MICROSPHERES FOR INTRANASAL DRUG DELIVERY

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Keywords:

Felodipine, intranasal drug delivery, emulsification cross-linking, mucoadhesive microspheres

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ABSTRACT

In this study, the use of chitosan as a polymer and natural mucilage as mucoadhesive agent for microencapsulation of Felodipine employing emulsification cross-linking method was investigated. Chitosan was selected as a polymer because it satisfied all the required parameters of intranasal drug delivery system. Chitosan posses numerous advantages such as it is biodegradable, biocompatible, non-toxic, non-irritant and has mucoadhesive property as well which are suitable for the preparation of intranasal formulation. Here, five different formulations were prepared by varing the various physical parameters such as quantity of polymer, quantity of glutaraldehyde and stirring speed of mechanical stirrer. From this it was observed the on increasing the quantity of polymer the mean particle size of microspheres increases, on increasing the quantity of glutaraldehyde along with time interval the mean particle size of microspheres increases while on increasing the stirring speed of mechanical stirrer the mean particle size of microspheres decreases. Then,the five batches of microspheres were characterised for drug content, percentage yield , particle size, entrapment efficiency,surface morphology. The effect of polymer on the in-vitro release of Felodipine was studied and observed that with increase in polymer quantity the rate of drug release decreases. Thereby, the results of above studies were discussed in the article.

1.0 INTRODUCTION

The most desirable and convenient method of drug administration is the oral route because of their ease of administration. However, in many instances oral administration is not desirable when the drug undergoes significant degradation via first pass effect in liver. Hence, lack of systemic absorption through the gastrointestinal tract led to research on alternate routes of drug delivery such as parenteral, intramuscular, subcutaneous, intranasal, transdermal, etc. Intranasal drug delivery is now recognized to be a useful and reliable alternative to oral and parenteral routes. The nasal mucosa has been considered as a potential administration route to achieve faster and higher level of drug absorption. This is due to large surface area of nose and the thin, porous, highly vascularized nasal epithelium which ensures high absorption and rapid transport of the absorbed substances directly into the systemic blood circulation avoiding drug metabolism in the liver. If drugs are absorbed in the olfactory region they will be absorbed directly into the central nervous system bypassing the tight blood brain barrier[1, 2, 3]. Intranasal delivery provide a viable and attractive option for administrating various therapeutic agents. Advantages of IN administration include a large surface area for delivery, rapid achievement of target drug level and avoidance of first pass metabolism; furthermore, this delivery route is non invasive, maximizing patient comfort and compliance. In addition IN dosing may facilitate transport of central nervous system (CNS) drugs into brain [2, 3]. Though great progress have been achieved regarding our understanding of the pathogenic basis of neurological disease, there are only a small number of effective drugs for treating these diseases. Every day new potentially active molecules are synthesized but they are discarded for their toxicity or low bioavailability. In fact, the main limitation for a drug active on the central nervous system (CNS) is precisely to carry it in the CNS, that as well known is over protected by the blood-brain barrier (BBB) that segregates the brain interstitial fluid from the circulating blood [4, 5, 6].

2.0 MATERIAL AND METHODS

Felodipine was procured as a gift sample from Scott Edil pharmaceuticals,baddi. Chitosan was purchased from Hi-media labs,Mumbai. Sources of natural mucilage such as vegetables and fruits were purchased from local market. Glutaraldehyde, acetic acid, span 80, acetone, petroleum ether were purchased from S.D. Fine Chemicals, Mumbai.

3.0 PREPARATION OF FELODIPINE LOADED MICROSPHERES

- **Extraction of mucilage**

Edible vegetables,fruits were collected from local market.

↓

Those were washed with distilled water to remove any adherent material and cut into small pieces

↓

Distilled water was added to it and heated at 60^0C for four hours on water bath

↓

Cooled and thick viscous solution was then passed through muslin cloth

↓

Filtrate was diluted with distilled water and kept undisturebed overnight in a refrigerator

↓

Upper clear solution were decanted and clear solution was concentrated at 60^0C on water bath

↓

Concentrate was cooled to room temperature and precipitated in acetone

↓

Precipitate was washed with acetone and dried at 50^0C in hot air oven. Dried mass was powdered and stored in desiccators till further use.[105]

- **Characterization of mucoadhesive agents**

1. **Measurement of pH**

   pH of 1% w/v aqueous solution of mucilaginous substances were measured by pH meter.

2. **Study of swelling index**

   20mg sample of mucilage was kept with 10ml of water in a measuring cylinder. After 24hours the mucilage was filtered through filter paper and weighed. Then the swelling index of samples were observed.

   Swelling index = [(W2-W1)/ W1] X100

   Where,W1= weight of mucoadhesive agent before swelling W2=weight of mucoadhesive agent after swelling

3. **Loss on drying**

   20mg sample was taken in petridish and dried in an oven at 105^0C until a constant weight was obtained.

   Loss on drying(%)=[(W1-W2) /W1] X 100

   Where,W1=original weight of sample(gm), W2=constant weight of sample attained(gm)[106]

- **Method: Emulsification cross-linking**

Microspheres were prepared using **emulsion cross linking method**.100mg chitosan was dissolved in distilled water and 1ml glacial acetic acid was added to chitosan solution and kept for overnight stirring on a mechanical stirrer(REMI).The 5mg drug was dissolved in ethanol and mixed well in the polymer solution, and then aqueous solution of mucilage(10ml) was added into it.10 ml of the above resultant mixture was then injected through a syringe (no. 20) into 50 ml of oil phase containing span 80 (0.4ml) and stirring was performed by mechanical stirrer at 1500 rpm to form w/o emulsion. Oil phase was edible sunflower oil. After 60 min of homogenization period glutaraldehyde(1ml) was added to it. It was then left for stabilization and cross-linking for a period of 2hours. Microspheres obtained were centrifuged at 1000rpm.The sediment was then washed with acetone thrice, and then dried in a hot air oven at 50°C.

4.0 CHARACTERISATION AND EVALUATION OF MICROSPHERES

All formulation having different ratios of drug:polymer:mucilage were subjected to following evaluation tests.

- **Particle size**

The size of microspheres was measured using a microscope with the help of optical microscope, and the mean particle by means of a calibrated stage micrometer with eye piece micrometer [80].

Procedure

Calibrate the eye piece micrometer using stage micrometer and find out the length of one division of eye piece micrometer. Prepare the slide by using a small quantity of treated microspheres and mount a drop of glycerin and cover with cover slip. Replace the stage micrometer with the prepared slide. Measure the diameter of the microspheres by observing the no. of divisions covered by microspheres [107].

Calculation for calibration of eye piece micrometer:

Focus stage micrometer and eye piece micrometer and find out the coincidence and do the measurement. 85th division of eye piece micrometer coincides with 10th division of stage micrometer [107]. So;

$$= \frac{\text{No. of divisions of stage micrometer}}{\text{No. of divisions of eye piece micrometer}}$$

= 10/85

= 0.0117mm.

- **Surface morphology**

Surface morphology and surface appearance of microspheres before and during degradation studies were examined by scanning electron microscope (FESEM, S-4300, Hitachi, Japan). Samples for SEM were freeze dried, mounted on metals with double sided tape. Pictures were taken and examined for surface morphology and surface appearance of microspheres [107].

- **Percentage yield (% production yield)**

The yield was calculated as the weight of the microspheres recovered from each batch divided by total weight of drug and polymer used to prepare that batch multiplied by 100.

All the experimental units were analyzed in triplicate (n=3) [108].

The production yield (PY) was calculated by the equation,

$$PY = (Pc / Tc) X 100$$

Where:

Pc - Practical content,

Tc - Theoretical content [81,82].

- **Determination of drug content and entrapment efficiency**

Accurately weighed 100 mg of microspheres were crushed in glass mortar pestle then the crushed microspheres were suspended in 100ml of methanol. After 12 hour the solution was filtered and the filterate was analysed for drug content using UV spectrophotometer at 237nm. The drug loading and entrapment efficiency (EE) were calculated as follows:

Drug content(%) = W_D / W_T X 100

Where, W_D = weight of drug loaded in microspheres

W_T = total weight of microspheres

Entrapment efficiency = M_{actual} /$M_{theoratical}$

Where, M_{actual} = weighed quantity of microspheres

$M_{theoratical}$ = theoretical quantity of drug and polymer in microspheres

- **Degree of swelling**

Accurately weighed amount of microspheres(10mg) was placed on Millipore filter paper wetted with phosphate buffer pH 6.8 in a petridish for 6 hours. Than the microspheres were weighed and the degree of swelling(α) was calculated using the following formula:

$\alpha = W_S - W_O/W_O$

Where, W_O = Weight of microspheres before swelling

Ws = Weight of microspheres after swelling

- ***In vitro* drug release studies**

In vitro drug release studies were carried out for prepared microspheres by using USP dissolution rate test apparatus-II (37±0.5°C). An accurately weighed amount of microspheres equivalent to 10 mg of the drug were placed in basket separately. The dissolution medium was phosphate buffer as stimulated (SIF) (900ml, pH 6.8) for 12 hrs. 5 ml of the samples were withdrawn at specified time intervals (0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 hrs.) and equal volume of fresh medium was replaced immediately. After a suitable dilution, samples were analyzed by UV spectrophotometry. All the studies were carried out in triplicate [109].

- **Release models and kinetics**

Release kinetics can be studied by putting the value in all the equations which are given in the (Table 1)

Table 1: Equations for different model

Zero order	$Q_t = Q_0 + K_0 t$
First order	$\ln Q_t = \ln Q_0 + K_1 t$
Higuchi	$Q_t = K_H \sqrt{t}$
Korsmeyer-Peppas	$Q_t / Q_\infty = Kt^n$

- **Stability of Felodipine loaded microspheres**

The stability of the felodipine encapsulated in polymer chitosan was evaluated in a period for 45 days aiming to establish the formulation durability. The microspheres powder were put in to a bottle stored for 3 months at 25° C, 35° C and 40°C respectively. The physical properties and drug content were examined periodically. The drug content was assessed by the above mentioned UV-VIS spectrophotometric method. [110]

5.0 RESULTS AND DISCUSSIONS

The present research was executed in two phases. In the first phase, preformulation study was carried out to characterize the drug and to study drug-excipients compatibility and mucilage was also characterised. In the second phase, microspheres were prepared to optimize the polymer and its concentration.

Table 2: pH of 1 % solution of natural mucoadhesive agents

Mucilage source	pH
Calendula officinalis	6.46
Trapa natans	6.24
Brassica oleraceae	5.85
Carica papaya	5.71
Musa acuminate	6.06
Chitosan	3.23

Table 3: Swelling index of different natural mucoadhesive agents

Mucoadhesive polymer	Weight before swelling(mg)	Weight after swelling(mg)	Swelling index(%)
Calendula officinalis	20	25.01	25.05
Trapa natans	20	38.55	92.75
Brassica oleraceae	20	36.82	84.10
Musa acuminate	20	29.24	46.20
Chitosan	20	39.03	95.15

Table 4: Loss on drying of different natural mucoadhesive agents

Mucoadhesive polymer	Weight before LOD(mg)	Weight after LOD(mg)	Loss on drying(%)
Calendula officinalis	20	19.01	4.95
Trapa natans	20	19.05	4.75
Brassica oleraceae	20	19.11	4.45
Musa acuminate	20	18.94	5.21
Chitosan	20	19.33	3.35

Table 5: Ingredients used for drug loaded Chitosan microspheres

Sr.no	INGRDIENTS	F1	F2	F3	F4	F5
1.	Felodipine (mg)	5	5	5	5	5
2.	Chitosan(mg)	50	100	150	200	250
3.	Glacial acetic acid(ml)	1	1	1	1	1
4.	Sunflower oil(ml)	50	50	50	50	50

5.	Aqueous mucilage(ml)	10	10	10	10	10
6.	Span 80(ml)	0.4	0.4	0.4	0.4	0.4
7.	Isopropyl acohol(ml)	20	20	20	20	20
8.	Acetone (ml)	20	20	20	20	20

Table 6: Physicochemical parameters of microspheres

Formulation code	Drug:Polymer:mucilage ratio	Production yield	Entrapment efficiency	Particle size	Cumulative % drug release*
F1	1:10:2	69.3±0.81	84.8±0.46	24.76±0.49	80.12
F2	1:20:2	88.38±0.70	96.92±0.65	25.77±0.34	81.27
F3	1:30:2	69.22±0.49	80.1±0.44	27.91±0.66	80.24
F4	1:40:2	82.14±0.85	87.46±0.62	28.5±0.53	76.04
F5	1:50:2	72.15±0.47	78.28±0.35	29.18±0.62	72.65

Table 7: In-vitro drug release models for different Felodipine microspheres formulation

Formulation code	Zero order	First order	Higuchi model	Koresmeyer peppas model	
	R	R	R	R	'n'
F1	0.967	0.966	0.994	0.967	1.47
F2	0.973	0.971	0.980	0.965	2.01
F3	0.970	0.969	0.977	0.966	1.46
F4	0.968	0.967	0.968	0.924	1.40
F5	0.965	0.978	0.980	0.933	1.43

Table 8: Stability studies of formulation F2 at 40 ± 2^0 /75RH

Sampling intervals (days)	Storage condition (40 ± 2^0C/75RH)
	Drug content*
0	96.92±0.65
07	96.86±0.62
15	96.44±0.54
21	96.18±0.38
30	96.02±0.24
45	95.98±0.18
60	95.92±0.04

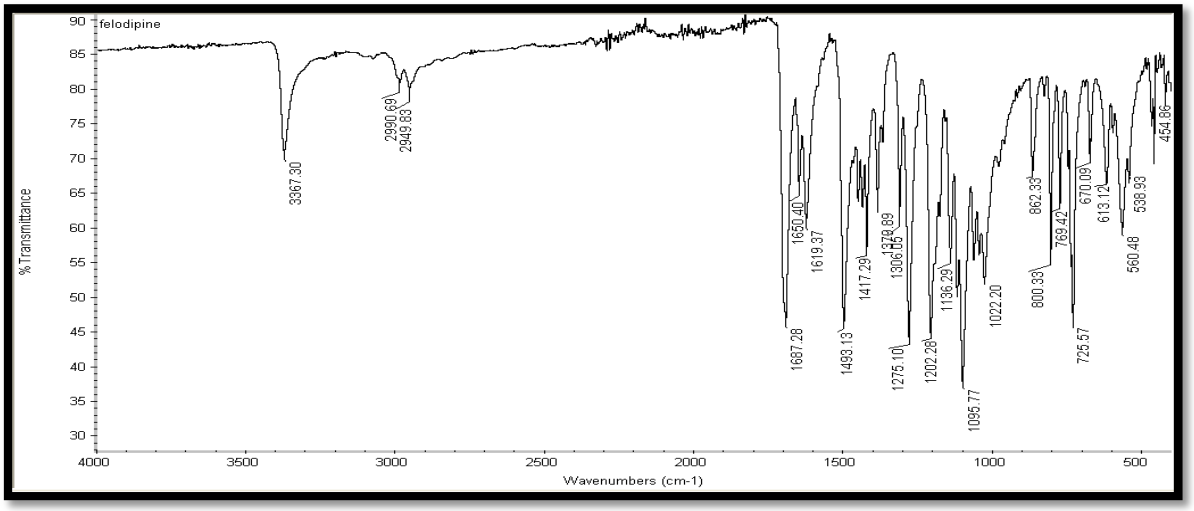


Figure 1: FTIR spectrum of Felodipine

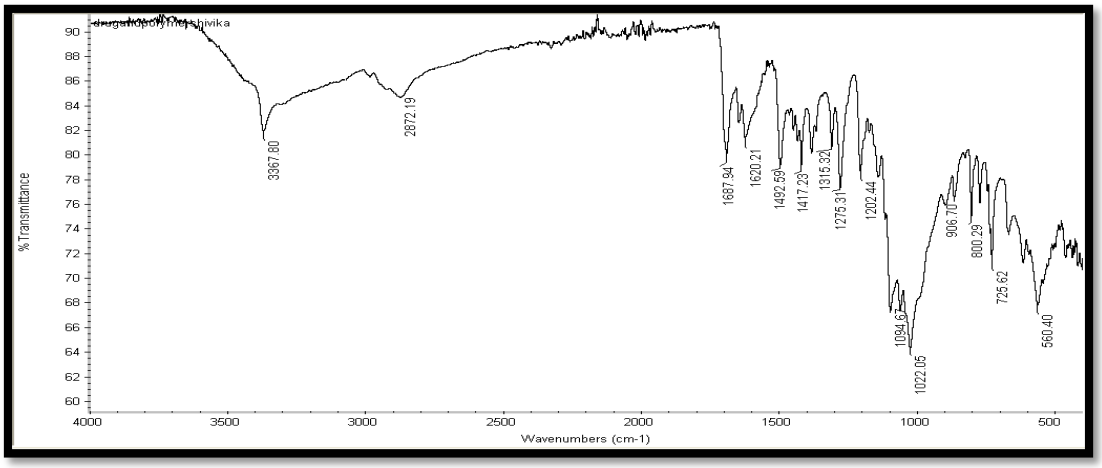
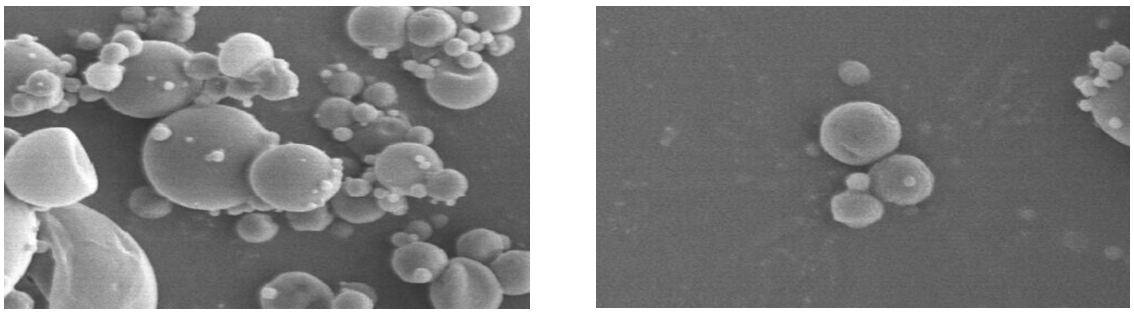


Figure 2: FTIR spectrum of Felodipine + Chitosan



(a) **(b)**

Figure 3: SEM images of Felodipine loaded chitosan microspheres.

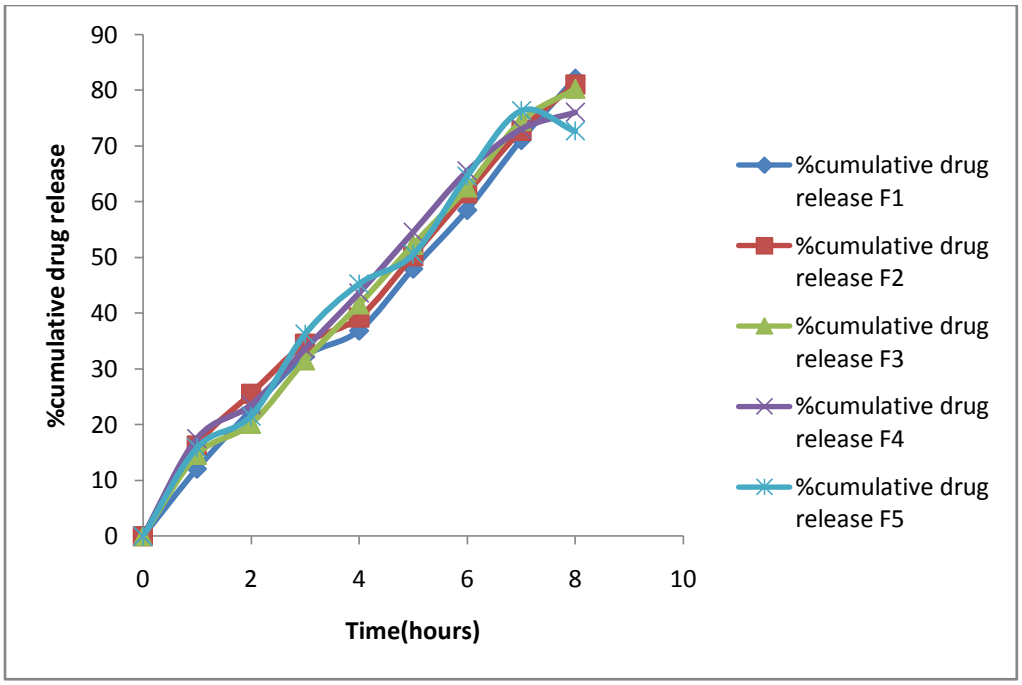


Figure 4: *In vitro* release rate profiles of felodipine from Chitosan microspheres in Phosphate buffer at 37 ± 0.5°C.

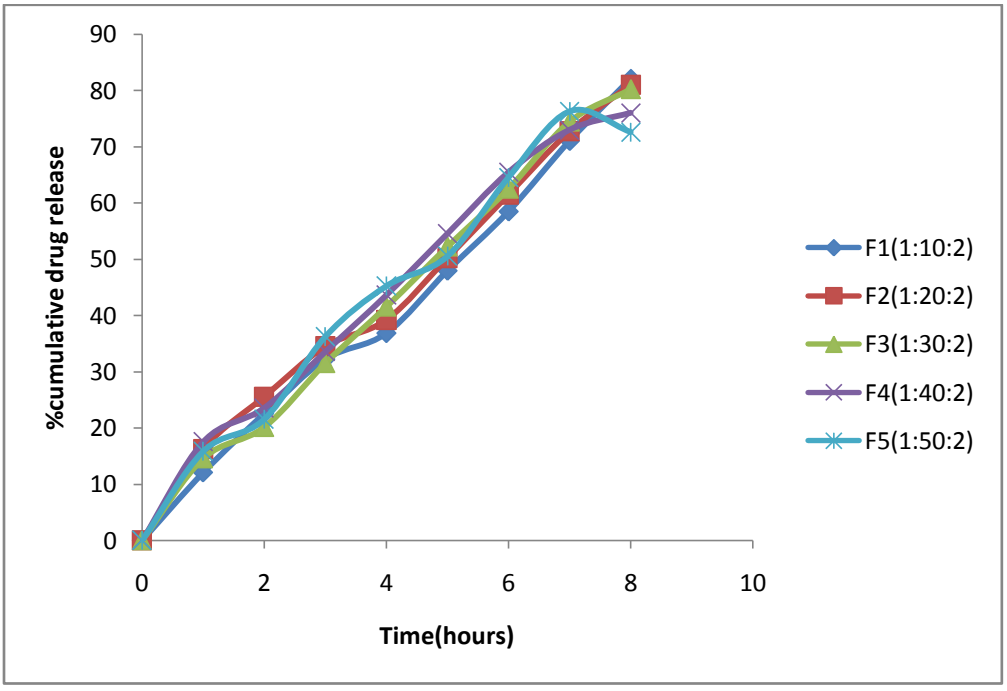


Figure 5: Zero order plots of different formulations of Felodipine microspheres

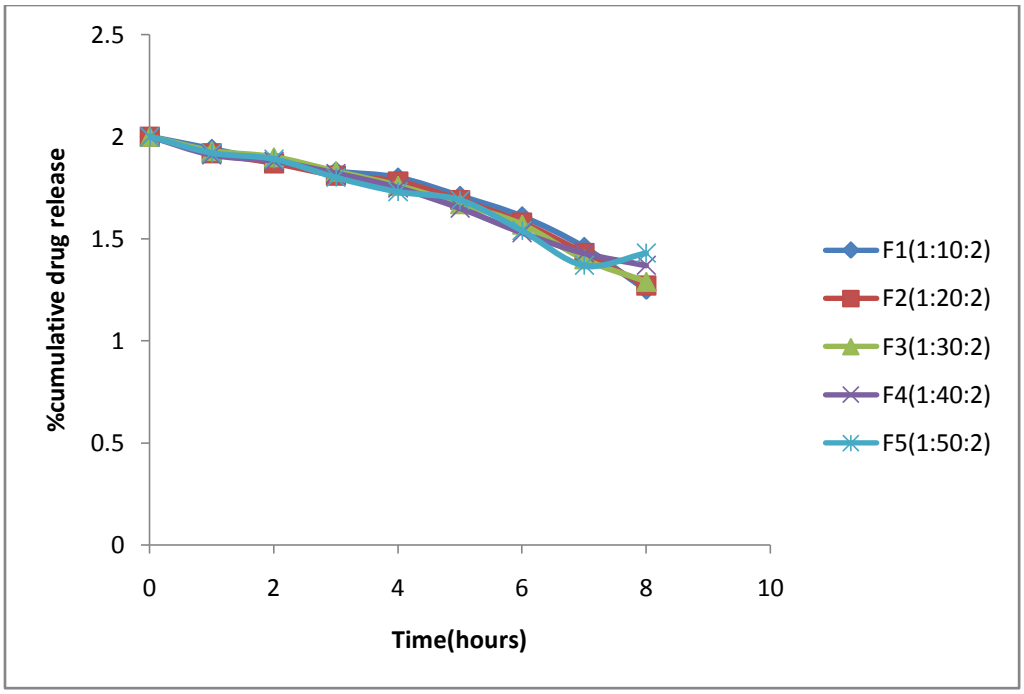


Figure 6: First order plots of different formulations of Felodipine microspheres.

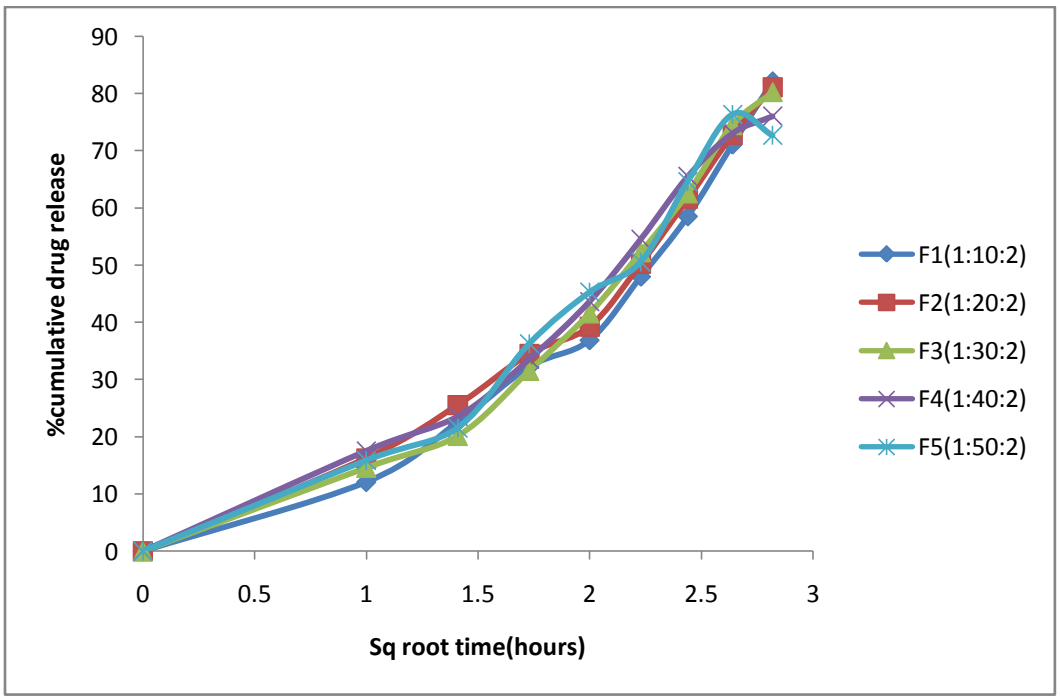


Figure 7: Higuchi model plots of different formulations of Felodipine microspheres.

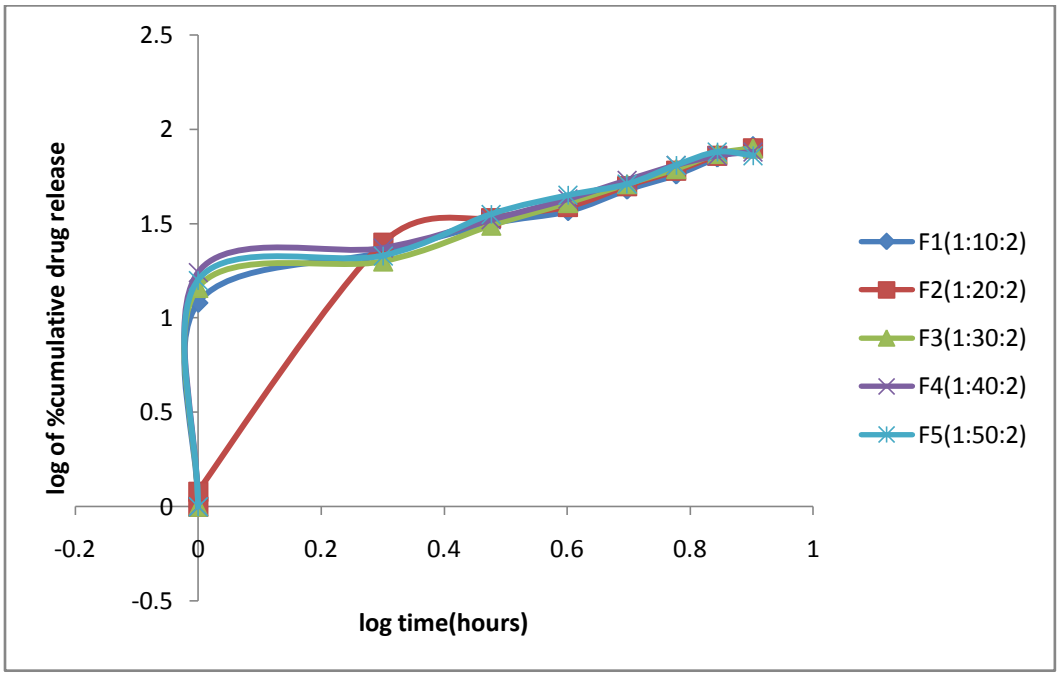


Figure 8: Koresmeyer peppas plot of different formulations of Felodipine microspheres.

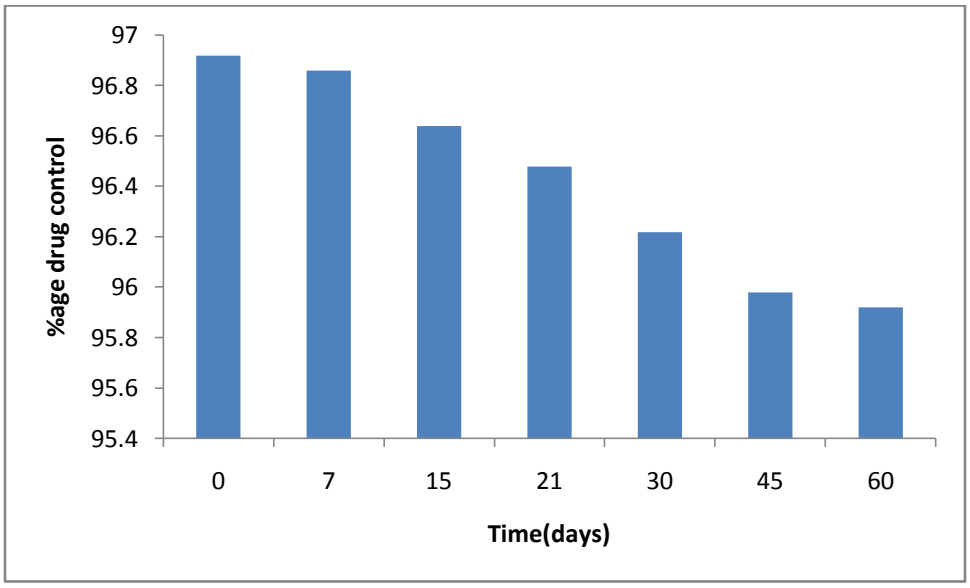


Figure 9: Stability studies of %age drug content for F2 formulation(40±2^0 C/75RH)

6.0 EVALUATION OF MICROSPHERES

1. Particle size distribution

Polymer quantities influenced the particle size of mucoadhesive Felodipine chitosan microspheres. We investigated the effect of polymer quantity on the size of the microspheres with the amount of glutaraldehyde fixed at 1 ml, and the stirring speed fixed at 1500 rpm for 2hours. The mean size of the microspheres increased as the quantity of polymer increased from 50 mg to 250 mg. The particle size was found to be between 24.76-29.18µm for F1 - F5. It was concluded that the mean size of the microspheres increased as the quantity of the polymer increased from 50 mg to 250 mg.

2. Surface Morphology

The Scanning Electron Microscopy (SEM) was used to determine the shape and surface morphology of microspheres. SEM images of the formulation F2 shown in figure no. 3 revealed that the microspheres were spherical in shape. The surface morphology reveals that the microspheres were porous which may be due to rapid escape of the volatile solvents during formulation.

3. Percentage yield

The production yield was found to be between 69.3±0.81-88.38±0.70 from F1-F5. The highest production was found in formulation F2 (1:20:2) 88.38%. The yield of other formulation F1,F3,F4,F5 was less due to flocculation and aggregation of microspheres as the viscosity increase with increase in quantity of polymer as shown in table no. 2. [10]

4. Drug content and Encapsulation efficiency

It can be concluded that the highest entrapment efficiency of drug loaded microspheres was found to be 96.92±0.65 for formulation F2(1:20:2).The entrapment efficiency was found to be 84.8%,96.92%,80.1%,87.46%,78.28% for F1,F2,F3,F4,F5 formulations respectively. The entrapment efficiency of formulation F3, F4 and F5 (80.1±0.44,87.46±0.62 and 78.28±0.35) were found to be less as compared to F2 formulation(96.92±0.65). This may be due to increased amount of Chitosan in the formulationas it may decrease interfacial tension between the drug and the aqueous phase and motivate its apparent solubility in aqueous phase resulting in loss of drug.

5. *In vitro* drug release studies

The different microspheres formulations were subjected to *in- vitro* release studies using USP dissolution rate test apparatus-II (37 ± 0.5°C). It was observed that for each formulation the drug release decreased with increase in the amount of polymer as shown in table no. 2. This may be due to the fact that the release of drug from the polymer takes place after complete swelling of the polymer and as the amount of polymer in the formulation increases the time required to swell also increases.

6. Drug release kinetics

The regression coefficient values of different microspheres formulations namely F1-F5 was found to be between 0.924-0.994, respectively for zero order model; 0.965 – 0.973,respectively for first order model and 0.966–0.978, respectively for Higuchi model 0.968-0.994 and similarly for korsemeyer peppas model 0.924-0.967.The R values were much closer to one for the Higuchi kinetics. From the correlation coefficient values it was concluded that the drug release form the different microspheres formulations follow Higuchi model. Higuchi model explained the matrix diffusion mechanism of drug release. The correlation coefficient values for Higuchi model confirmed that drug release followed matrix diffusion mechanism or Higuchi pattern release which was due to the fact that no swelling or no erosion of microspheres takes place during drug release experiments.[112]

8.0 CONCLUSION

The study conducted so far on "Formulation and evaluation of Felodipine loaded mucoadhesive intranasal chitosan microspheres using natural mucilage" reveals following conclusions:

1. Biodegradable and mucoadhesive intranasal microspheres of drug Felodipine can be prepared by employing emulsification cross-linking method using natural mucilage. The method of preparation was found to be simple, mucilage and can be effectively blended with chitosan to form microspheres.
2. The SEM study shows that Chitosan-microspheres containing Feloipine were spherical in shape with a smooth and non-porous surface. The FTIR studies confirmed that no chemical interaction took place during encapsulation process.
3. The mean particle size of microspheres was in range of 25.19-49.5 µm depending upon the concentration of polymer used. The average particle size of microspheres ranged from 25–

50 mm, and such particles are considered to be suitable for nasal administration. It was also noted that with the increase in the drug:polymer ratio there was a slight increase in the size of microspheres.

4. Entrapment efficiency was in range of 78.28 to 96.92%. A higher encapsulation efficiency of drug are obtained at a 1:20:2 drug:polymer:mucilage ratio. Amount of polymer affected the entrapment efficiency as amount of polymer increased entrapment efficiency was also increased.

5. *In -vitro* release of Felodipine was found to be slow and prolonged. To prevent rapid drug release, C microspheres were cross-linked with chemical cross-linking agent glutaraldehyde. The study indicated that the amount of drug release decrease with an increase in the polymer concentration.

6. So it was cocncluded that the formulation F2 was best with 31.53 µm particle size and 81.27% drug release.

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