Formulation and Evaluation of Mucoadhesive Microspheres of Carvedilol for Nasal Delivery

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Abstract

Carvedilol having oral bioavailability is about 10-20% due to first pass metabolism. The formulation of Carvedilol in to nasal microsphere will improve bioavailability, to reduce the dosing frequency and to improve the patient compliance. These nasal microspheres were prepared by the ionic gelation method. The prepared microspheres were subjected to various evaluation parameters and in vitro release studies. Highest percentage of entrapment was obtained by increasing the amount of polymer with respect to uniformity of drug. The particle sizes of the prepared microspheres were determined by optical microscopy method and morphology by SEM analysis. The prepared microspheres have gained good spherical geometry with smooth surface as evidence by SEM studies. The entrapment efficiency for F4was found to be 58.15±2.16 % with maximum drug loading of 40 around. The best-fit release kinetics was achieved with Korsmeyer-Peppas plot followed by zero order and first order kinetics. The release of drug was influenced by the drug to polymer ratio and particle size and was found to be both diffusion and dissolution controlled. The study showed that Carvedilol microspheres of (F4 batch) got better sustained effect over a period of 12 hours. Finding of all this investigation conclusively demonstrate prolongation of drug release at a constant and controlled rate.

KEYWORDS: Carvedilol, sodium alginate, Entrapment efficiency, SEM, *in-vitro*profile.

INTRODUCTION[1,2]

The intranasal application of tobacco stuff, cocaine, and various hallucinogenic and psychotropic agents has been known for a long time. It is therefore surprising that only in the past decade the intranasal administration of drugs for the systemic use has attracted much attention. Recent reviews1-6 books and symposium proceedings 7-8 Show a strongly increasing interest in nasal delivery for systemic absorption as an alternative to the parenteral route.

Historically, the use of the nasal route for drug delivery has received attention of mankind since ancient times. Nasal therapy, also called “NASAYA KARMA”, has been recognized form of treatment in the Ayurvedic systems of Indian medicine.

For many years, drugs have been administered intranasal for their local effect on the mucosa

(e.g. Antihistamines, decongestant, vasoconstrictors and antibiotics). In more recent years many drugs have been shown to achieve a better systemic bioavailability by self-medicationthrough the nasal route than by oral administration. Some of them have been shown to duplicate the plasma profile as I.V. administration. More recently the intranasal

route has aroused increasing interest as means of the systemic administration of vaccine, hormones, peptides and certain other drugs.

There are situations in which a systemic medication is required but the parenteral administration may be either undesirable or impractical, whereas the oral administration may not be suitable due to some potential systemic bioavailability problems associated with stability and/or hepatic first pass metabolism.

Transdermal delivery has the advantage of providing direct entry of drug into the systemic circulation, as well as ease of administration. The nasal mucosa, unlike the skin, is not constructed form the highly keratinized stratum corneum, but form numerous microvilli underlined with rich vascularity. The nasal route, therefore, appears to be ideally suitable for nonparenteral administration of drugs intended for systemic medication.

Nasal drug delivery is a useful delivery method for drugs that are active in low doses and show no minimal oral bioavailability. The nasal route circumvents hepatic first pass elimination associated with the oral delivery: it is easily accessible and suitable for self-medication. Currently, two classes of nasally delivered therapeutics are on the market.

The first one comprises low molecular weight and hydrophobic drugs for the treatment of the nasal mucosa and sinus, including decongestants, topical steroids, antibiotics and other(OTC) products.

The second class encompasses a few drugs, which have sufficient nasal absorption for displaying systemic effects. Important candidates are the compounds, generally administered by injection and hardly absorbed after oral administration, due to their instability in gastrointestinal tract, poor absorption properties, and their rapid and extensive biotransformation111-13. Therefore, nasal delivery is promising alternative route for the administration of peptides and protein drugs in particular.

MATERIALS AND METHODS

Materials

Carvedilol was provided as gift sample from AbhinandanRasayana, Mumbai, Sodium alginate were obtained from Glenmark pharmaceuticals, (Nashik), Gellan gum were obtained from S.D Fine Chemicals, Aluminium silica 10%,were obtained from Research-Lab Fine Chem. Industry – Mumbai.

Method

Identification drug and polymers

Organoleptic properties:

Carvedilol was studied for its organoleptic characters such as appearance, colour, and odour.

Solubility:[3]

Solubility of carvedilol was checked in various solvents which include selection of suitable solvent to dissolve respective drug.

Melting point determination:[4]

Melting point was determined to check the purity of the drug. Melting point was determined by capillary method by using Thiele's tube containing Liquid paraffin.

UV Spectroscopy

Determination of λ max in phosphate buffer pH 6.8:
The standard solution (100µg/ ml) of pure drug (Carvedilol) was prepared in phosphate buffer pH 6.8

Infra-Red Spectroscopy (IR):
The IR spectra of dry sample of Carvedilolwas taken and analysed between 4000 cm^{-1} to 400 cm^{-1}

Infra-Red Spectroscopy of Drug and Polymer Mixture:
The FT-IR spectra of dry sample of sodium alginate was taken and analysed between 4000 cm^{-1} - 400 cm^{-1}.

Differential scanning calorimetry (DSC):

DSC interpretation of drug and polymer[5]
The DSC measurements were performed on drug, polymer, physical mixture of drug and polymer (1:1) and optimized formulation. The DSC measurement was performed on a Shimadzu DSC (DSC 60). The thermo grams were obtained at a scanning rate of 10 ºC /min over a temperature range of 30-400 ºC under an inert atmosphere flushed with nitrogen at a rate of 50ml/min.

FORMULATION AND DEVELOPMENT

Formulation of mucoadhesive microsphere:[2,6]

Ionic gelation method was employed to prepare nasal microsphere of Carvedilol using, Sodium alginate, xanthan gum, gellan gum, and other excipient.

Preparation of Carvedilolmucoadhesive microsphere:[7, 8]
The composition of the various Carvedilolmucoadhesive microspheres formulations were mentioned table. Carvedilol and mucoadhesive polymers were individually passed through sieve ≠ 80. The required quantities of mucoadhesive polymers were dissolved in purified water to form a homogenous solution. Carvedilol was added to thepolymer solution , mixed thoroughly with magnetic stirrer at 400 rpm to form a homogeneous dispersion andresulting dispersion was sonicated for 30 min to remove entrapped air bubbles. For the formation of mucoadhesive microspheres homogeneous dispersion was then extruded manually drop wise into 10% crosslinking (aluminum
silicate) using syringe (needle size 24 G). The extruded droplets were cured in the aluminiumsulphate solution for30 minutes to complete the reaction and to produce spherical rigid microspheres [10]. The obtained ritonavir rmicrospheres were collected by decantation, washed continually with distilled water and dried at 45°C for12 hour.

Table no. 1: Composition of Carvedilolmucoadhesive microspheres

Batch	Drug(mg)	Sodium alginate(mg)	Xanthan gum(mg)	Gellan gum(mg)	Aluminium silica 10% (mg.)
F_1	50	25	12.5	-	5
F_2	50	25	25	-	5
F_3	50	25	37.5	-	5

F_4	50	25	50	-	5
F_5	50	25	-	12.5	5
F_6	50	25	-	25	5
F_7	50	25	-	37.5	5
F_8	50	25	-	50	5

OPTIMIZATION DATA ANALYSIS

The targeted response parameters were statistically analyzed by applying one- way ANOVA in Design-Expert. [9, 10, 11, 12, 13]

Evaluation of microspheres: [14, 15, 16]

Percentage yield:

The yield of microsphere was determined by comparing the whole weight of microsphere formed against the combined weight of the copolymer and drug.

$$\% \text{ Yield} = \frac{\text{Mass of microspheres obtained}}{\text{Total weight of drug and polymer used}} \times 100$$

Bulk Density:

Accurately weighed microspheres were carefully transferred into graduated measuring cylinder. The microspheres were then made uniform and the volume occupied by the microspheres was noted as per the graduation marks on the cylinder as mL. It is expressed in g/cm^3 and is calculated using the following formula.

$$\text{Bulk density} = \frac{\text{Weight of microspheres}}{\text{Bulk volume}}$$

Tap Density:

Accurately weighed microspheres were carefully transferred into graduated measuring cylinder. Care should be taken that a level surface of the microspheres is formed. The cylinder was then placed in the tapping apparatus and tapped until no further decrease in volume of microsphere takes place (100 taps). The final (tapped) volume occupied by the microspheres was noted as per the graduation marks on the cylinder as mL. Tap density is expressed in g/cm^3 and is calculated using the following formula.

$$\text{Tap density} = \frac{\text{Weight of microspheres}}{\text{Tap volume}}$$

Compressibility index (Carr's index) and Hausner's ratio:

Carr's index and Hausner'sratio measure the propensity of microspheres to be compressed and the flow ability of microspheres. Carr's index and Hausner's ratio were calculated using following formula.

$$\text{Carr's Index} = \frac{\text{Tapped density} - \text{Bulk density}}{\text{Tapped density}} \times 100$$

$$\text{Hausner's Ratio} = \frac{\text{Tapped density}}{\text{Bulk density}}$$

Angle of repose (θ):

The flow characteristics of microspheres were assessed by determining the angle of repose. Angle of repose is defined as the maximum angle possible between the surface of a pile of a powder and the horizontal plane. Sufficient quantity of microspheres was passed through a funnel from a particular height (1 cm) onto a flat surface until it formed a heap, which touched the tip of the funnel. The height (h) and the radius (r) of the heap were measured. The angle of repose was calculated by using the formula.

$$\text{Angle of repose } (\theta) = \tan^{-1} (h/r)$$

Entrapment efficiency:

100 mg equivalent of Carvedilol containing microspheres were dissolved in 100mL of phosphate buffer pH 6.8 for 12 hrs with continuous stirring. The samples were filtered and were analysed at 254nm by using UV spectrophotometer.

Particle size analysis:

Particle size of different batches of microspheres was determined by optical microscopy. The projected diameter of microspheres from each batch was determined using ocular micrometer and stage micrometer equipped with optical microscope. Analysis was carried out by observing the slide containing microspheres under the microscope. The average particle size of the microspheres was expressed as diameter.

Particle shape and surface morphology:

The morphology of the prepared microspheres was investigated by scanning electron microscopy (Diya Labs, New Mumbai). The microspheres were fixed on adequate supports and coated with gold under an argon atmosphere using a gold sputter module in a high-vacuum evaporator. Observations under different magnifications were performed at 15 kV.

***In vitro* drug release study:**

The in-vitro drug diffusion test of microsphere was performed using a glass-Franz diffusion cell apparatus, which consistence of donor and receptor compartment. A dialysis member was use to keep the microsphere(5 mg) on the donor side ,which allowed free diffusion of PCPM to the receptor compartment containing 25 ml phosphate buffer solution pH 6.4 that was with in the pH 6.4 that was with in pH of nasal cavity the temperature was maintain at 37= 1^0C using circulating water bath. The receptor compartment was stirred with a magnetic stirrer. At scheduled time intervals, aliquots (1ml) with same volume of fresh per warmed buffer solution. The sample was assayed

spectrophotometric ally at 234 nm. All experiment were carried out in triplicate, and average values were calculated.

Degree of swelling:[17]

The swelling ability of microsphere in physiological media was determined by swelling them in the phosphate buffer pH 6.8. microsphere were suspended in 5 ml of phosphate buffer pH6.8,the increase in partical size of microspheres was noted up to 8 hours and the swelling index was calculated.

Stability Study: [18, 19]

The mucoadhesive nasal microsphere were subjected for a period of three months as per ICH guideline at the 40 ^{0}C temperature and relative humidity 75% RH. The samples were withdrawn at 7 days, 15 days, 1, 2, 3 months for given temperature condition. The formulations were evaluated mainly for drug content at the predetermined intervals.

RESULT AND DISCUSSION

Organoleptic properties:

Carvedilol were given in Table no.2. The result shows the details of organoleptic properties of Carvedilolpowder complying with the description that is found in the literature.

Table no.2: Organoleptic Properties of Carvedilol

Drug	Properties	Observed Results
Carvedilol	Appearance	Fine powder
	Colour	White
	Odour	Slight odour

Melting point determination:

Melting point of Carvedilol was given in Table no.3. The melting point of the drug matches with the values found in literature.

Table no.3: Melting point of Carvedilol against reported value

Melting Point (°C)	
Literature	**Practical**
114-115 ^{0}c	115-117 ^{0}c

Ultraviolet-Visible Spectroscopy Study

Determination of λ_{max} of Carvedilol

The UV spectrum of Carvedilol phosphate buffer (pH 6.8) solution (10µg/ml) exhibited wavelength of absorbance maximum at 234nm.

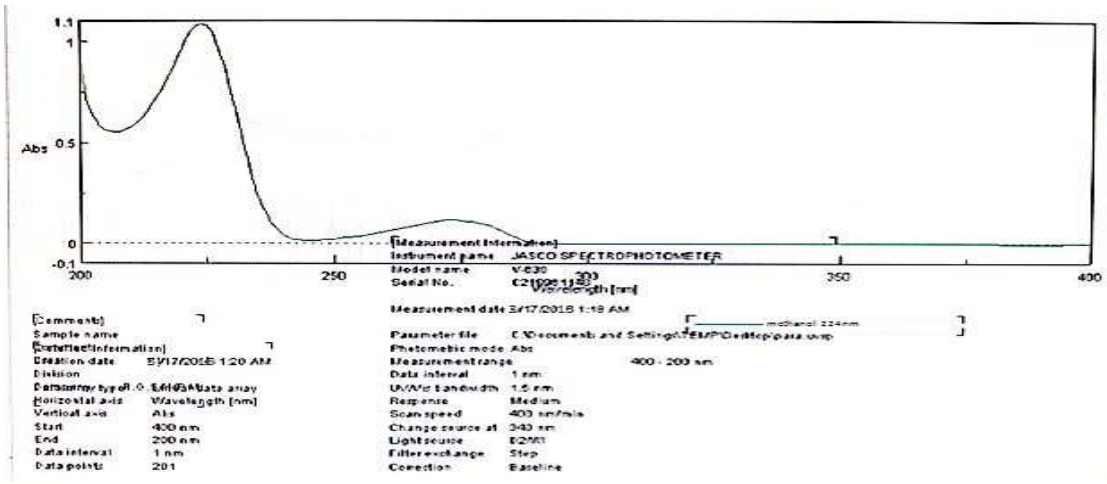

Fig.1: UV visible spectrum of carvedilol in phosphate buffer pH 6.8

IR Spectroscopy:

Infra-red spectrum of Carvedilol is shown in Fig.2 the absorption bands shown by Carvedilol are characteristics of the groups present in its molecular structure. The presence of absorption bands corresponding to the functional groups present in the structure of Carvedilol confirms the identification and purity of gifted Carvedilol sample

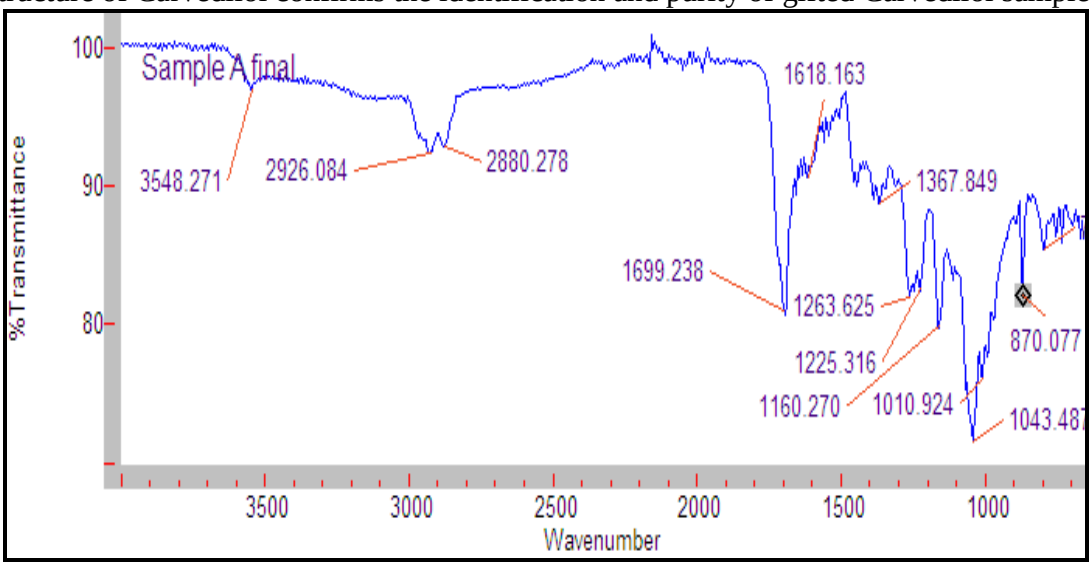

Fig.2: IR spectrum of Carvedilol

Infra-red Spectroscopy of drug and polymer mixture

Infra-red spectra of drug and polymers showed matching peaks with the drug spectra. The characteristic peaks of drug were also present in the spectra of all drug- polymer combinations is shown in Fig. 3.

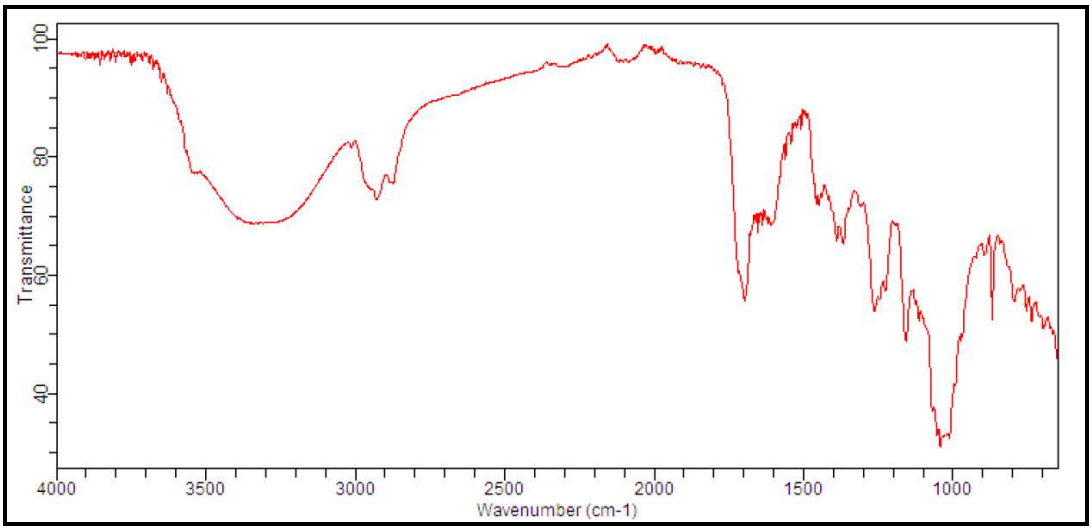

Fig.3: Infra-red spectrum of Drug and Polymer mixture

The major peaks of drug in Infra-red spectra indicate that, there is no interaction of drug with polymer

Differential scanning calorimetry (DSC) of physical mixture

One of the most classic applications of DSC analysis is the determination of the possible interactions between a drug entity and the excipients in its formulation. Figure.4 illustrates DSC profiles of physical mixture (Carvedilol and excipients.)

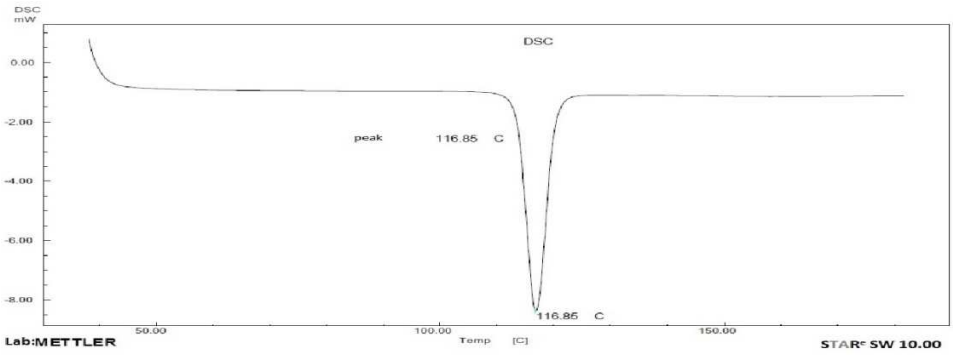

Fig.4: Differential scanning calorimetry (DSC) of physical mixture

Differential scanning calorimetries were carried out on pure drug Carvedilol and Carvedilolloaded microspheres usinga Shimadzu DSC 60 to evaluate any possible Carvedilolmucoadhesive polymers interaction. Samples (4mg each) were accurately

weighed into aluminum pans and sealed. DSC run were conducted over a temperature range 40-300 °C at a heating rate of 10 °C / min under nitrogen atmospheres.

Dissolution Study:

***In vitro* drug release:** Dissolution studies were performed for each of formulations, the percent cumulative drug releases of different formulations (F1-F8) are shown in Table no.4 carried out phosphate buffer pH 6.8 solutions using USP dissolution apparatus II.

Table no.4: Percentage cumulative drug release of 8 batches

Batch / Time	F1	F2	F3	F4	F5	F6	F7	F8
0	0.0	0.0	0.0	0.0	0.0	0.0	00	0.0
1	1.789±0.05	3.551± 0.18	3.223± 0.42	3.379± 1.05	4.83±0. 67	4.92±1. 13	4.73±0. 82	4.94±0. 72
2	5.025±0.49	11.86± 0.47	11.37± 1.00	8.934± 0.97	11.87± 0.81	11.84± 2.13	11.61± 0.56	11.69± 1.28
3	14.83±0.47	20.27± 1.27	20.38± 1.03	15.77± 0.60	20.35± 0.93	21.50± 0.98	21.39± 0.50	21.43± 1.32
4	22.32±0.20	26.16± 1.20	27.05± 1.03	22.36± 2.17	24.66± 0.90	27.17± 2.06	25.68± 1.36	25.89± 1.07
5	30.55±0.86	29.08± 0.47	36.67± 0.87	27.77± 2.43	28.95± 0.63	34.11± 1.79	32.43± 0.62	33.25± 2.25
6	40.18±0.23	36.59± 0.47	44.55± 1.34	34.01± 2.29	36.39± 1.68	44.35± 0.65	41.66± 0.59	42.66± 0.79
7	49.63±0.22	48.51± 0.63	48.20± 1.39	43.90± 1.03	48.17± 1.68	48.54± 2.37	48.36± 0.51	47.26± 1.27
8	57.75±0.25	55.59± 0.54	55.50± 1.37	53.63± 0.92	56.17± 0.84	54.13± 0.95	55.13± 2.01	53.11± 1.66
9	66.40±0.49	64.34± 0.78	65.12± 0.74	63.58± 1.50	64.11± 0.80	61.89± 1.05	64.50± 0.78	56.94± 2.26
10	71.03±0.52	72.35± 0.92	72.18± 0.73	73.34± 0.91	72.20± 1.02	65.58± 1.07	72.17± 0.54	64.28± 1.33
11	76.22±0.44	78.88± 1.15	79.97± 0.45	82.54± 1.09	79.80± 0.56	74.56± 1.09	81.44± 0.61	73.77± 0.78
12	86.01±0.88	88.05± 1.06	91.52± 1.49	96.09± 0.92	89.33± 0.70	85.51± 1.36	91.10± 0.62	87.32± 0.52

The results were tabulated in table no.4 and combine release graph of 8 formulations showed in fig.5all the formulations were subjected to in vitro release studies.

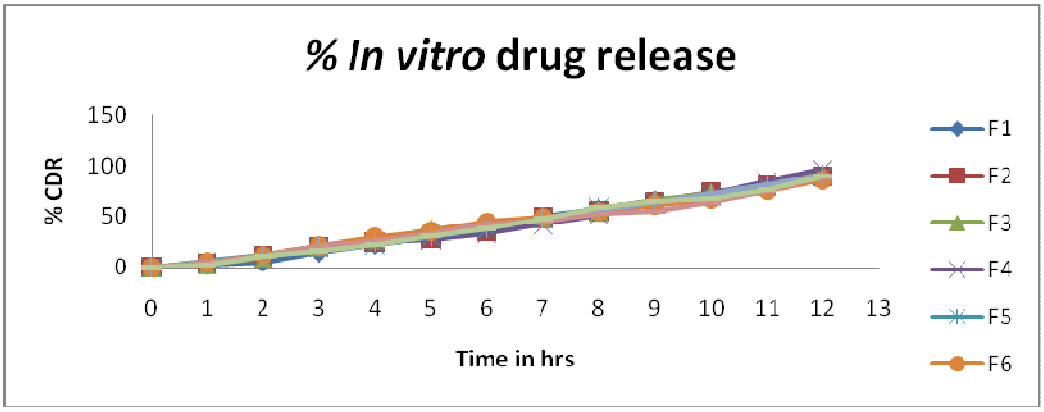

Fig.5*: In-vitro* drug release of formulation F1 to F8

Amongst all formulation F4 containing sodium alginate and xanthan gum showed maximum drug release of 96.09% after 12 hrs of study and also showed better contact with biological membrane. As compared to formulations prepared from combinations of sodium alginate and gellan gum.

Swelling studies:

Swelling properties of the spray dried microspheres are expressed as degree of swelling as shown in table no.5.

Table no.5: Swelling study of formulations

Hrs / Batch	1	2	3	4	5	6	7	8
F1	19.3±1.52	27.6±2.08	34.6±3.05	42.3±1.52	50.3±2.51	58±1.73	65.3±2.88	73±3
F2	22±2.64	27.6±2.08	34.6±2.51	42.3±1.52	50.3±2.51	58±1.73	66.3±2.08	76.6±2.51
F3	18.3±2.51	27±1	36.6±2.51	42.3±1.52	50.3±2.51	57.6±1.52	66±1.73	78.6±2.08
F4	15.6±3.21	25.6±1.52	36.6±2.51	47±2	50.3±2.51	57.6±1.52	66±1.73	73±2
F5	19.3±1.52	25.6+1.52	35.6±2.08	46±1	50.3±2.51	58±1.73	66.3±2.08	79.3±79.3
F6	11.3±3.05	20.3±2.08	29.3±3.05	39±2	48.3±3.05	57±2	65±2	75.6±75.6

F7	13.3±2.08	22.3±1.52	32±2	40.6±1.52	50.6±1.52	60.3±1.73	68.3±2.3	77.3±1.52
F8	15.6±1.52	15.3±1.52	34±1	43±1	51±2	59.6±2.08	69.3±3.21	79.3±1.52

DATA TREATMENT

Comparative evaluation of zero order model kinetics:

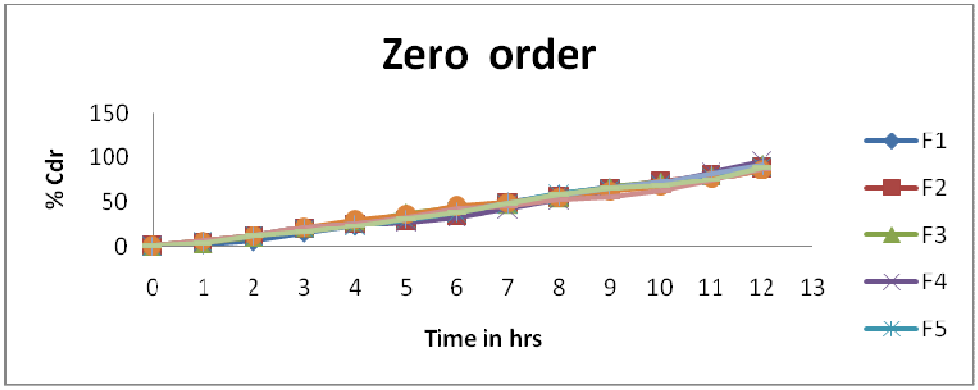

Fig.6: zero order release kinetic graph of formulations F1 to F8

Table no.6: r^2 values of zero order of formulations F1 to F8

formulation code	F1	F2	F3	F4	F5	F6	F7	F8
r^2	0.994	0.991	0.995	0.985	0.992	0.994	0.995	0.984

The r^2 value for the formulations F1 to F8 are in range of 0.984 to 0.995.

Comparative evaluation of first order model kinetics:

Fig.7: first order release kinetic graph of formulations F1 to F8

Table no.7: r^2 values of first order of F1 to F8 formulation

formulation code	F1	F2	F3	F4	F5	F6	F7	F8
r^2	0.813	0.869	0.804	0.899	0.913	0.843	0.880	0.868

The r^2 value for formulations F1 to F8 are in range of 0.804 to 0.913.

Comparative evaluation of higuchi model kinetics:

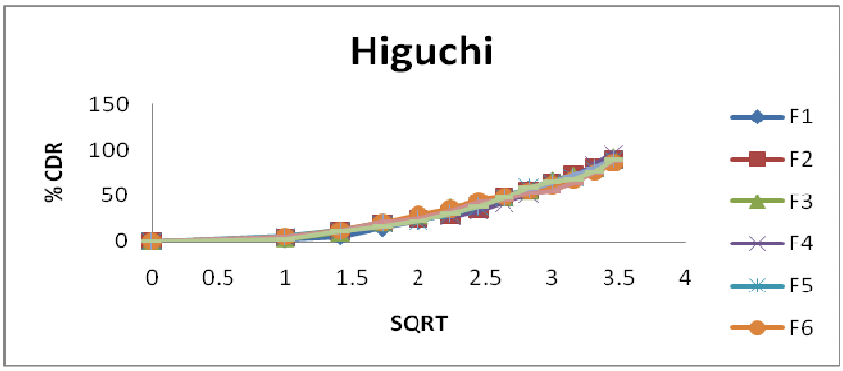

Fig.8: higuchi release kinetic graph of formulations F1 to F8

Table no.8: r^2 values of higuchi for formulations F1 to F8

formulation code	F1	F2	F3	F4	F5	F6	F7	F8
r^2	0.973	0.950	0.972	0.919	0.950	0.979	0.956	0.954

The r^2 value for the formulations F1 to F8 are in range of 0.919 to 0.979

Comparative evaluation of korsemayer'speppas model kinetics

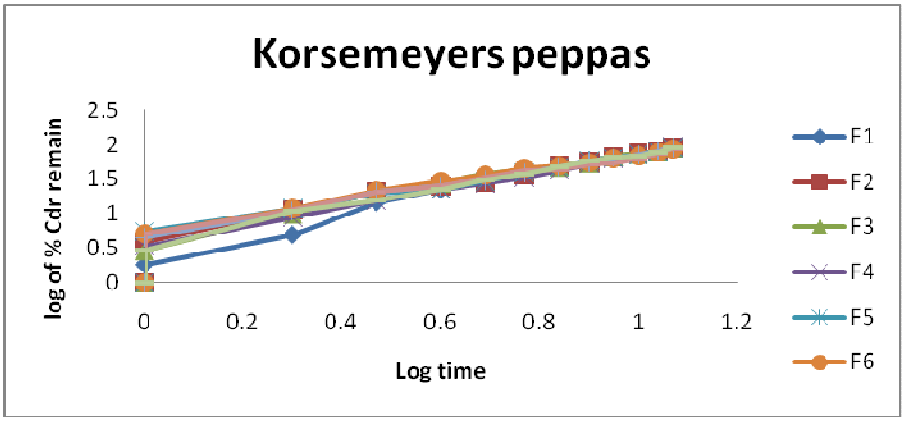

Fig.9: korsemayer'speppas kinetic graph of formulations F1 to F8

Table no.9: r^2 and n values of korsemeyer'speppasof formulations F1 to F8

formulation code	F1	F2	F3	F4	F5	F6	F7	F8
r^2	0.982	0.991	0.979	0.998	0.994	0.991	0.996	0.993
N	0.697	0.737	0.704	0.912	0.738	0.618	0.577	0.546

The r^2 value for the formulations F1 to F8 are in range of 0.779 to 0. 998.

Evaluation of microspheres:

Percentage yield:
The percentage yield of different batches was determined by weighing the microspheres after drying. The percentage yields of different formulations were found to be in range of 82.22 % to 93.75 % as shown in Table no.6

Table no.10: Percentage yield of different batches of microspheres

Batch No.	Total weight of microsphere	weight of drug +polymer	% yield
F1	198	225	88
F2	228	250	91.20
F3	230	240	93.75
F4	276	300	92
F5	185	225	82.22
F6	210	250	84
F7	249	275	90.54
F8	271	300	90.33

Flow properties of microspheres:
The prepared microspheres were evaluated for the flow properties like angle of repose, bulk density andHausner's ratio. Results obtained are given table no.7

Table no.11: Flow properties of different batches of microspheres

Batch No.	Bulk density (gm/cm^3)	Tapped density (gm/cm)	Carr's Index (%)	Hausner's ratio	Angle of repose(^{0}C)
F1	0.92±0.532	0.934±0.03	0.92±0.042	1.009±0.018	17.29±1.89
F2	1.011±0.01	1.021±0.01	0.979±0.011	1.009±0.07	15.94±1.44
F3	1.09±0.03	1.1±0.07	0.909±0.161	1.009±0.110	17.35±1.01
F4	1.014±0.02	1.02±0.04	0.588±0.281	1.005±0.031	18.98±1.30
F5	0.963±0.021	0.973±0.02	0.1±0.499	1.01±0.033	15.32±1.53
F6	0.984±0.023	0.994±0.02	0.9456±0.031	0.93±0.038	15.16±1.60
F7	1.068±0.09	1.079±0.09	1.019±0.089	1.01±0.090	16.33±2.34
F8	1.08±0.06	1.098±0.06	1.639±0.346	1.016±0.063	17.27±0.60

*Each value represents mean ± S.E. (n=3)

The microspheres for all eight formulations were evaluated. The angle of repose was found to be of all batches in range of 15.16 ± 1.60 to 18.98 ± 1.30, also bulk density was found to be in range of 0.92±0.532 to 1.09±0.03 (gm/cm^3). The outcome of tapped density was in range of 0.934±0.03 to 1.1±0.07 (gm/cm), while Carr's index was in range of 0.588±0.281% to 1.639±0.346. The Hausner's ratio was found in the range of 0.93±0.038 to 1.016±0.063. As per the micromeritic a study results indicated that, the microspheres showed good flow properties.

Entrapment efficiency:

The drug entrapment efficiency of different batches of microspheres was found in the range of 51.69±2.55% to 58.15±2.16%. The maximum percent of drug entrapment efficiency was found to be in the formulation batch (F4). The minimum percent of drug entrapment efficiency was found to be in the formulation batch (F5). The drug entrapment efficiency of different batches of microspheres was as shown in table no.8.

Table no.12: Entrapment efficiency of different batches of microspheres

Sr. No.	Formulation code	Entrapment efficiency (%)
1	F_1	54±1.21

2	F_2	55.88±0.59
3	F_3	55.41±1.99
4	F_4	58.15±2.16
5	F_5	51.69±2.55
6	F_6	53.45±0.55
7	F_7	51.96±1.35
8	F_8	53.45±1.22

The %EE was found to be in the range between 51.69±2.55% to 58.15±2.16%. The %EE showed dependence on drug loading, amount of cross-linking agent and time of cross-linking. The formulations loaded with higher amount of drug exhibited higher entrapment efficiencies. The entrapment efficiency, however, showed an inverse relationship with increase in cross link density, which will reduce the free volume spaces within the polymer matrix and hence, a reduction in entrapment efficiency.

Particle size analysis:

The particle size of microspheres varied among the formulation due to variation in the composition of formulations. The mean particle size of microspheres formulation was in the range of µm. Formulation F_4 showed relatively large size i.e. 143.33 µm and formulation F_6showed relatively small size i.e. 116 µm. The table 8.10 shows mean particle size of various batches.

Table no.13: Particle size of different batches of microspheres

Sr. no.	**Formulation code**	**Mean Particle size (µm)**
1	F_1	128.66
2	F_2	142
3	F_3	130
4	F_4	143.33
5	F_5	122.66
6	F_6	116
7	F_7	120.66
8	F_8	123.66

Preliminary studies showed that as the concentration of polymer was increased, the particle size also proportionally increased. Low polymer concentrations resulted in clumping of microspheres, whereas high polymer concentration resulted in formation of discrete microspheres with size 143.3µm.This could be attributed to an increase in the relative viscosity at higher concentration of polymer and formation of lager particles during emulsification. The size of microspheres was increased with an increased with an increase drug loading. This can be attributed to the corresponding increase in viscosity of drug polymer dispersion comprising the internal phase of the emulsion. A similar increase in the size of microspheres was also observed with increase in calcium chloride concentration as well as cross-linking time. The addition of higher amount of Ca^{2+} will result in relatively more cross-linking of the unitsof sodium alginate, thereby leading to formation of larger microspheres. Similarly increasing the cross linking time will increase the extent of cross-linking and thereby increase the particle size.

Particle shape and surface morphology:

Morphological analysis of the Carvidilolmicrosphere was carried out by optical microscopy. The microspheres were found to be discrete and spherical in shape.

Evaluation of optimized batch

SEM: Morphology of the optimized batch F4was studied by SEM analysis.

According to SEM studies, the microspheres were found to be discreate, almost spherical and free flowing. The surface was rough and porous which indicated fickian diffusion.

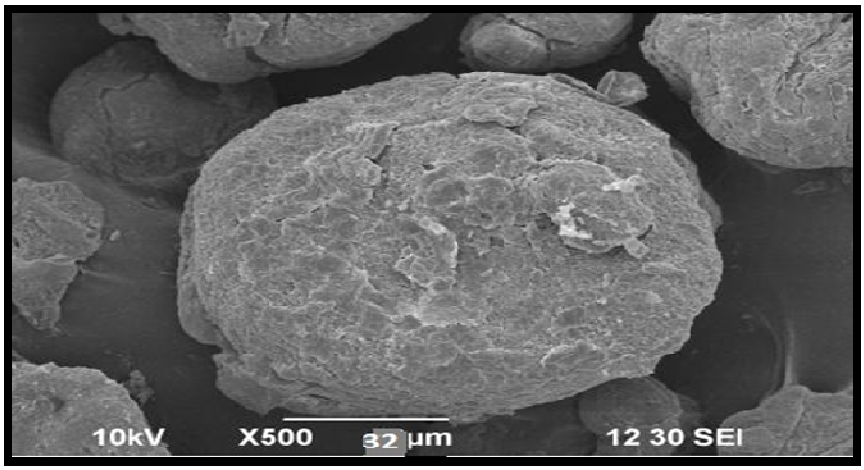

Fig.10: SEM image of optimized batch

REFERENCES

1. Lohani A, Chaudhary GP. Mucoadhesive microspheres: A novel approach to increase gastroretention. Chron Young Sci. 2012;3:121-128.
2. Rania HF. Statistical approach for assessing the influence of calcium silicate and HPMC on the formulation of novel alfuzosin hydrochloride mucoadhesive-floating beads as gastroretentive drug delivery systems. AAPS PharmSciTech. 2012; 13(3): 990–1004.
3. European Pharmacopoeia, 6th Edition, 2011, Volume 1, page no. 31.

4. Shah V, Sharma M, Parmar V, Upadhyay U. Formulation of sildenafil citrate loaded nasal microsphers: An *in vitro*, ex vivo characterization. Int J Drug Deliv. 2010; 2:213-220.
5. Samirkumar P, Chand T, Talsania M. Formulation development and evaluation of microspheres containing duloxetine hydrochloride. IJRPBS. 2013:4(2):568-572.
6. Schaefer MJ, Singh J. Effect of isopropyl myristic acid ester on the physical characteristics and *in vitro* release of etoposide from PLGA microspheres. AAPS PharmSciTech. 2000:13:1(4):E32.
7. Wotton PK, Wade G, Moreton RC. Excipients in international pharmaceutical product registration - aspects of quality, safety and efficacy. In: Cartwright AC, Matthews BR. (Editors.), London, UK: Ellis Horwood. 1994;5:148-171.
8. Chaudhari A, Jadhav K R, Kadam V J, An over view: microspheres as a nasal drug delivery system, International Journal of Pharmaceutical Sciences Review and Research.2010;5(1):8-17.
9. Girish B, Pasha I, Gowda DV. Formulation and evaluation of sustained release matrix tables of Flurbiprofen using Guar gum. International Journal of Pharmacy and Pharmaceutical Sciences, 2012(5)4:120-123.
10. Chandrashekhar Y, Senthil S. Formulation and In vitro evaluation of Didanosine sustained release matrix tablets using natural gums. International Journal of Research in Pharmaceutical and Biomedical Sciences. 2011(1)2:245-251.
11. Jain S, Yadav SK. Preparation and evaluation of sustained release matrix tablet of Furosemide using natural polymers. Research Journal of Pharm and Tech, 2008(4)1:374-376.
12. Hingmire LP, Sakarkar DM. Formulation and development of sustained release matrix tablet using natural polymers. Indian Journal of Research in Pharmacy and Biotechnology, 2013(5) 1: 761-764.
13. Dash S, Murthy PN, Nath L. Kinetic modelling on drug release from controlled drug delivery systems. ActaPoloniac Pharmaceutica-Drug Research, 2010(3): 217-223.
14. Morath L P, Microspheres as nasal drug delivery systems,Advanced Drug Delivery Reviews. 1998; 29:185–194.
15. Alagusundaram M, MadhuSudana Chetty.C, UmashankariK, Attuluri B, Lavanya.C and Ramkanth S, Microspheres as a Novel Drug Delivery System - A Review,International Journal of ChemTech Research.2009;1(3):526-534.
16. Vyas S. P, Khar R K, Targeted& Controlled drug delivery, 1st edition, CBS publishers & distributors. 2004: 317-354.
17. Aliyapareen,Lizhar., Ahmed Syed. Formulation and evaluation of mucoadhesive microspheres of Lamivudine. International journal of pharmaceutical sciences and drug research. 2014;6(2):102-108
18. Surender V. Vipin K.Jyoti.et.al.Formulation, evaluation and optimization of mucoadhesive microspheres of carvedilol. Bulletin of pharmaceutical research. 2014;4(1):14-20.
19. Rajesh K,Pravallica P. Venkata T.et.al. Formulation and evaluation of controlled release microspheres of carvedilol. International journal of innovative pharmaceutical research.2011;2(1):98-101.