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Development and Characterization of Mucoadhesive Microspheres of Diltiazem by Using Emulsification Technique

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Abstract
Developed Diltiazem mucoadhesive microspheres prepared by emulsification technique due to the problems of frequent administration and variable bioavailability (40-60%) after oral administration of conventional dosage forms of Diltiazem can be attenuated by designing it in the form of mucoadhesive microspheres which would prolong the residence time at the absorption site to facilitate intimate contact with the absorption surface and thereby improve and enhance the bioavailability .The obtained microspheres were spherical in shape and the drug remained dispersed in the polymer matrix at amorphous state. The in-vitro drug release mechanism was non-fickian type controlled by swelling and relaxation of polymer.

Keywords
Diltiazem, emulsification technique, natural and synthetic polymers, microspheres, Franz diffusion cell, zero order kinetics.

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1.INTRODUCTION
The Novel Drug Delivery System started the alternative means of delivering the drug in the form of microspheres. [1] Novel drug delivery system development is largely based on promoting the therapeutic effects of a drug and minimizing its toxic effects by increasing the amount and persistence of a drug in the vicinity of a target cell and reducing the drug exposure of nontarget cells. [2] The term Microsphere is defined as a spherical particle with size varying with diameters in the micrometer range (typically 1µm to 1000µm (1mm), containing a core substance. The microspheres are characteristically free flowing powders consisting of proteins or synthetic polymers, which are biodegradable in nature, and ideally having a particle size less than 200 micrometer.[3] Diltiazem HCl is extensively used either alone or in combination therapy to tr eat hypertension, atrial fibrillation and flutter, paroxysmal supra ventricular tachycardia and for the treatment of stable and unstable angina pectoris. It has short half-life of 2-3 h and bioavailability of 33-44% as only 40% of the oral dose reaches to systemic circulation in an unchanged

form, mainly because of hepatic metabolism.[4] The emulsification-internal gelation technique of microencapsulation use an external oil phase and thereby may reduce the drug diffusion during encapsulation process and improve the drug entrapment efficiency.[5]

2. MATERIALS AND METHOD

2.1 MATERIALS

Diltiazem was collected as a gift sample from Hetero labs, Hyderabad, Ethyl cellulose, Eudragit, Carbopol 934 and other excipients were purchased from AR chemicals.

2.2 METHODODOLOGY[6,7]

FTIR studies

Active pharmaceutical ingredient and inactive ingredient compatibility was established by differentiate spectra of FT-IR analysis of Pure drug with that of different excipients used in the formulation.

Formulation Development

Table-1: Formulation development of Diltiazem microspheres

F.no	Polymer	Drug and polymer ratio	Stirring speed
F1	Eudragit	1:1	1000
F2	Eudragit	1:2	1000
F3	Eudragit	1:3	1000
F4	Carbopol 934	1:1	1000
F5	Carbopol 934	1:2	1000
F6	Carbopol 934	1:3	1000
F7	Ethyl cellulose	1:3	1000
F8	Ethyl cellulose	1:3	1000
F9	Ethyl cellulose	1:4	1000

Method of preparation

Diltiazem microspheres were prepared by using single emulsion technique. Briefly Diltiazem was dissolved in 5 ml distilled water. Polymers was dissolved in Dichloromethane at various drug - polymer ratios (1:1, 1:2 and 1:3). Then these drug and polymer solutions were mixed and emulsified using a Remi Lab Magnetic stirrer at 2000 rpm for about 10 min to form stable w/o emulsion. This stable w/o emulsion was slowly added to 200 ml aqueous solution containing 1 % PVA and stirred at 1000 rpm by a mechanical stirrer equipped with a three bladed propeller (Remi motors, India) at room temperature for 2 h to allow the solvent to evaporate completely. Microspheres were isolated by filtration and washed with distilled water several time to remove PVA. The produced microspheres were dried at room temperature for 24hrs and dried in vacuum chamber at 25 ^{0}C for 2hrs to remove any residual solvent.

Evaluation parameters[8,9,10]

The formulated microspheres were characterized for various parameters such as Yield of sustained microspheres, surface morphology of microspheres, drug entrapment efficiency, release rate of the drug, drug release kinetics and stability studies.

Yield of sustained microspheres

The Diltiazem microparticles was intended from the required amount of microspheres obtained divided by the total amount of all non-volatile components.

$$\% \text{ Yield} = \frac{\text{Actual weight of the microspheres}}{\text{Total weight of the microspheres}} \times 100$$

Particle size

Microspheres of particle was determined by optical microscopy. The eyepiece micrometer was calibrate utilizing a stage micrometer. The microspheres were spread over a slide and imagine under an optical microscope using an eyepiece micrometer.

Surface morphology of the microspheres

The surface morphology of the pure drug, surface of microspheres, drug loaded mucoadhesive microspheres and cross section of microsphere was examined by scanning electron microscopy. The samples were mounted directly onto the SEM sample holder using double-sided sticking tape and coated with a thin layer of gold using sputter coater unit atmosphere in order to make them conductive. SEM images were recorded at the different magnification at the acceleration voltage of 15 kV.

Drug entrapment efficiency (DEE)

The required quantity of drug entrapped was estimated by crushing 50 mg of Diltiazem microspheres by using mortar and pestle. This microspheres powder sample was poured in to a 100 ml volumetric flask and add the 6.8 phosphate buffer. After that the solution was taken into a beaker and sonicated in a bath sonicator for 2 hours. The solution was filtered, and absorbance was measured after suitable dilutions spectrophotometrically at 262 nm against blank.

Formula:

$$DEE = \frac{\text{Amount of drug actually present}}{\text{Theoretical drug load expected}} \times 100$$

Swelling index

The swelling index of microspheres was evaluated by a known weight (100 mg) of drug loaded microspheres which were placed in 100 mL of simulated intestinal fluid (SIF, pH 7.4 phosphate buffer) and allowed to swell for required period of time. The excess surface adhered liquid drops were removed by blotting with filter paper and swollen microspheres were weighed using microbalance. The degree of swelling was calculated from the difference between the initial weight of the microspheres and the weight at the time of determination using the formula:

$$\text{Swelling index} = \frac{Wf - Wi}{Wi} \times 100$$

where,

Wi = initial weights of microspheres

Wf = final weights of microspheres.

Mucoadhesive study

The mucoadhesive property of the microspheres was studied using mucoadhesion method. In this method, freshly excised piece of goat intestinal mucosa (2 × 3 cm) was mounted onto glass slides with elastic bands. About 100 microspheres were spread onto the wet rinsed intestinal mucosa and there after the support was hung onto the arm of a USP tablet disintegrating test machine. The disintegration machine containing tissue specimen was adjusted for a slow and regular up and down moment in a test fluid at 37˚C taken in a beaker. At the end of 1 h and later at hourly intervals up to 8 h, the machine was stopped and the number of microspheres still adhering onto the tissue was counted and the percentage of mucoadhesion was calculated. The test was performed in pH 1.2 HCl buffer and pH 6.8 phosphate buffer.

$$\% \text{ Mucoadhesion} = \frac{\text{No. Of microspheres attached to mucosa after washing}}{\text{Total microspheres applied}} \times 100$$

SEM Analysis

The surface characteristic of prepared crystal was studied by SEM (ZEISS Electron Microscope, EVO MA 15). Powder samples was mounted onto aluminum stub using double sided adhesive tape and sputter coated with a thin layer of gold at 10 Torr vacuum before examination. The specimens were scanned with an electron beam of acceleration potential of 20 kV and the images were collected as secondary electron mode.

***In vitro* drug release study**

In vitro drug release studies were carried out for all formulations in Franz diffusion cell. Microspheres equivalent to 10 mg of Diltiazem was placed in donor compartment. Aliquots 5ml were withdrawn at a predetermined intervals and equal volume of dissolution medium was replaced to maintain sink to maintain constant receotir phase volume. The necessary dilutions were made with 6.8 pH buffer and the solution was analysed for spectrophotometrically using UV-Visible spectrophotometer (Lab India) at 262 nm against an appropriate blank. Three trials were carried out for all formulations. From this cumulative percentage drug release was calculated and plotted against function of time to study the drug release.

Mechanism of drug release [11,12]

The obtained dissolution data was fitted into various kinetic models to understand the pattern of the drug release from sustained microspheres. The models used were zero order (equation 1) First order (equation 2) and Higuchi model (equation 3) and Koresmeyer Peppas model (equation 4).

***i) zero order release kinetics*:**

$R = K_o t$ -- (1)

R=cumulative percent drug release

Ko=zero order rate constant

ii) First order release kinetics

$\log C = \log C_o - K_1 t / 2.303$ -- (2)

where

C = cumulative percent drug release

K_1 = first order rate constant

iii) Higuchi model

$R = K_H t^{0.5}$ -- (3)

Where
R = cumulative percent drug release
K_H = higuchi model rate constant

iv) korsermeyer peppas model:

$M t / M \alpha = K_k t^n$

$\log M t / M \alpha = \log K_k + n \log t$ -- (4)

Where K_k = Korsermeyer Peppas rate constant 'Mt/Mα' is the fractional drug release, n = diffusional exponent, which characterizes the mechanism of drug release (Simon Benita, 2007).

Diffusional exponent (n)		Drug release mechanism
0.43	--	Fickian diffusion
0.43- 0.85	--	Anamolous (non- fickian) transport
0.85- 1	--	Case II transport
> 1	--	Supercase II transport

The obtained regression co-efficient (which neared 0.999) was used to understand the release pattern of the drug from the sustained release microspheres.

Stability studies [13]

Stability of a drug product is the ability of a particular formulation, in a specific container, to remain within its physico-chemical, therapeutic and toxicological specifications. Stability testing provides evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity and light and enables recommended storage conditions, retest periods and shelf lives to be established. Mucoadhesive microspheres were filled in HDPE containers at 40±2°C/75±5% RH for 90days as per ICH specifies.

3. RESULTS AND DISCUSSION

Drug and Excipient compatibility studies (FT-IR)

The compatibility between the drug and the selected lipid and other excipient was evaluated using FTIR peak matching method.

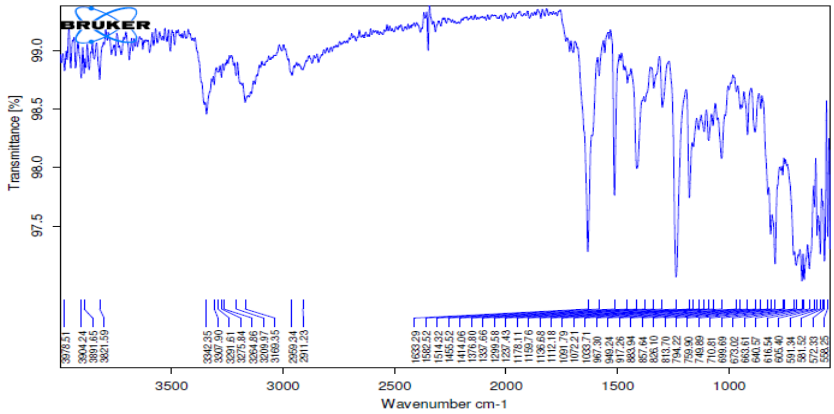

Fig-1: FTIR spectra data for Diltiazem

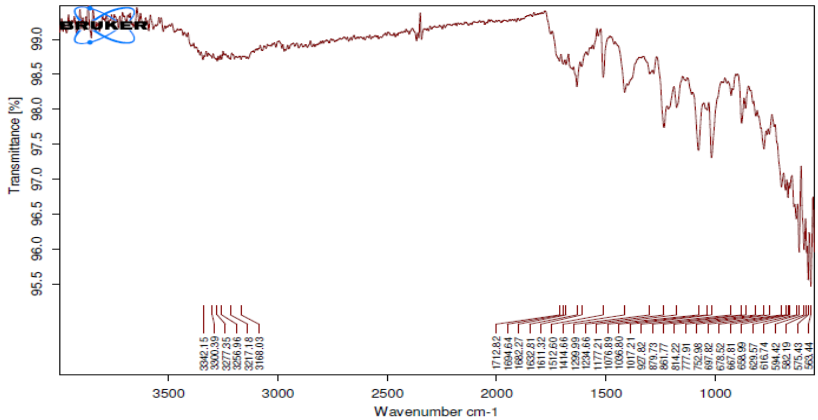

Fig-2: FTIR spectra data for optimized formulation

Formulation and Evaluation of sustained release Microspheres of Diltiazem

Characterization of Diltiazem microspheres

The formulated Diltiazem microparticles were examined for different parameters. And effect of preparation and process variables such as drug polymer ratio, speed, type of polymer and combination of polymers on particle size, yield, Mucoadhesion study, swelling index, entrapment efficiency, and *in-vitro* release of Diltiazem from sustained microspheres were also studied.

Table-2: Effect of drug polymer ratio on Yield of microspheres, Particle size, Drug entrapment efficiency

F.code	%yield	Particle size	Drug Entrapment Efficiency	Mucoadhesive strength
F1	75.23	189.90	76.16	56.98
F2	78.21	184.21	79.23	53.16
F3	71.67	186.75	82.78	49.56
F4	74.15	187.25	78.93	53.89
F5	76.45	187.28	83.99	55.96
F6	77.50	183.15	79.74	59.63
F7	79.82	179.03	88.15	65.23
F8	81.23	182.10	71.45	63.16
F9	75.98	183.64	84.56	58.69

The entrapment efficiency ranged between 71.45 to 88.15 and was dependent on polymer concentration. Entrapment efficiency increased with increasing concentration of polymer. Formulation F7 showed highest entrapment efficiency 88.15 which may be attributed to the presence of high concentration of polymer that led to high viscosity, which prevented diffusion of drug from polymeric droplets.

Table-3: Swelling index of all formulation

Time	F1	F2	F3	F4	F5	F6	F7	F8	F9
1	0.36	0.35	0.39	0.36	0.41	0.43	0.37	0.31	0.32
2	0.41	0.31	0.34	0.38	0.36	0.40	0.32	0.38	0.39
3	0.32	0.43	0.37	0.40	0.42	0.39	0.36	0.29	0.42
4	0.38	0.41	0.40	0.36	0.39	0.43	0.46	0.35	0.40

Table-4: Drug release studies of all formulations

TIME (hours)	F1	F 2	F3	F4	F5	F6	F7	F8	F9
0	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
1	28.45	25.89	27.44	24.22	24.12	22.99	27.85	25.12	23.09
2	36.18	33.78	33.85	35.10	35.10	35.10	36.85	33.45	31.23
3	48.56	49.58	45.85	41.55	41.85	45.89	49.88	48.91	45.92
4	53.12	52.99	53.78	53.55	52.32	52.90	58.12	53.124	52.09
5	65.12	68.88	65.64	61.14	60.75	63.75	66.64	65.12	63.52
6	70.36	78.75	75.71	74.61	71.54	76.90	71.63	70.97	72.60
7	81.86	86.71	82.99	84.81	80.12	85.06	88.96	87.14	85.23
8	90.56	91.58	93.65	95.61	93.61	92.85	98.65	97.77	95.60

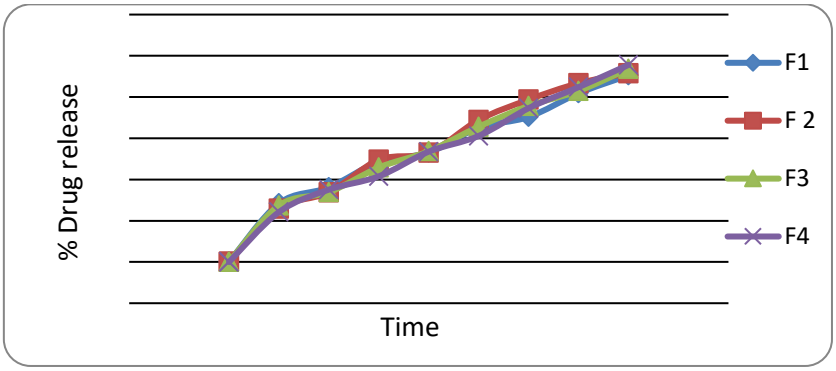

Fig-3: Comparative Dissolution profile of F1-F4

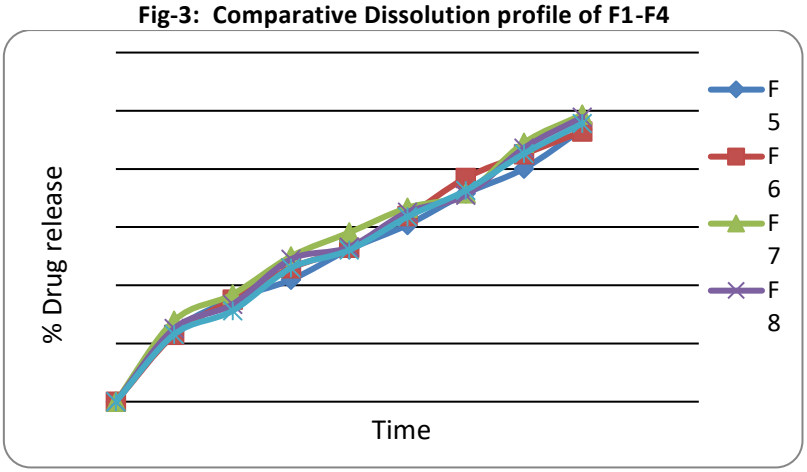

Fig-4: Comparative Dissolution profile of F5-F9

The *in vitro* release of diltiazem was influenced by altering the drug to polymer ratio and the mechanism of drug release was by diffusion controlled. The percentage drug release for each of the formulation was found to be 92.85 to 98.65. The formulation 7 shows 98.65% of drug release at the end of 8 hrs. So it is the best formulation releasing the drug at a sustained rate for a period of 8 hr increase drug absorption further increasing the bioavailability.

Surface topography by scanning electron microscopy (SEM)

SEM photograph of optimized microspheres at 100× magnification, at 1000× magnification. SEM photographs showed discrete, spherical microspheres.

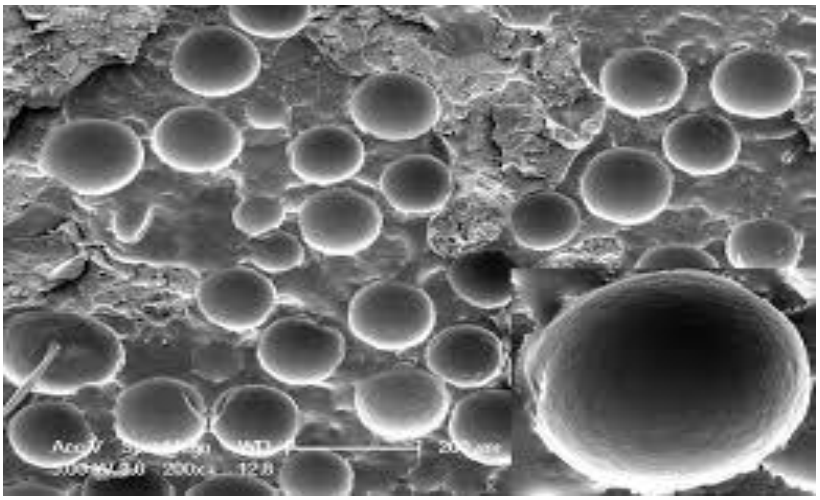

Fig-5: SEM analysis of Microsphere

The prepared microspheres were discrete, free flowing and uniform with a size range of 300 to 500 µm. The SEM photomicrographs showed that microspheres were nearly spherical completely covered by the coat polymer with occasional pits on the surface. The particle size of mucoadhesive microspheres was found between 179.03µm to 189.90 µm. The practical yield of microspheres prepared using emulsification technique.

Mucoadhesive Study

Diltiazem mucoadhesive microspheres varied between 49.56-65.23 % and was dependent on polymer concentration.

Release kinetics

The mechanism of Diltiazem microspheres was studied by the data obtained from *in-vitro* release studies into zero-order, first-order, Higuchi's, korsermeyer peppas kinetic models. On application of different release kinetic models mentioned earlier, it was found that optimized formulations showed better fitting with the zero-order release and korsermeyer peppas model.

Zero order kinetics

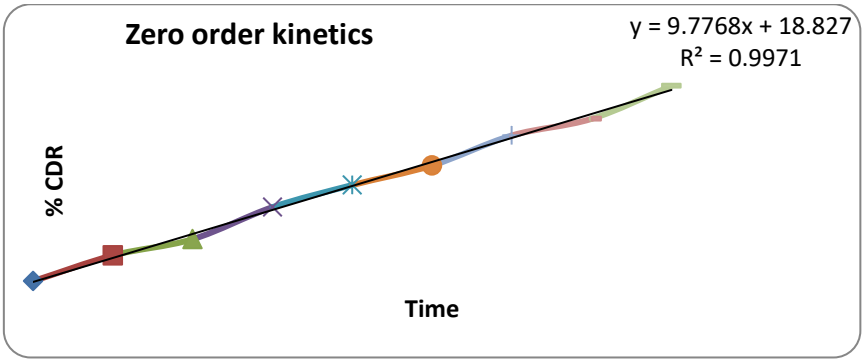

Fig-6: zero order plot for optimized formula

First order kinetics

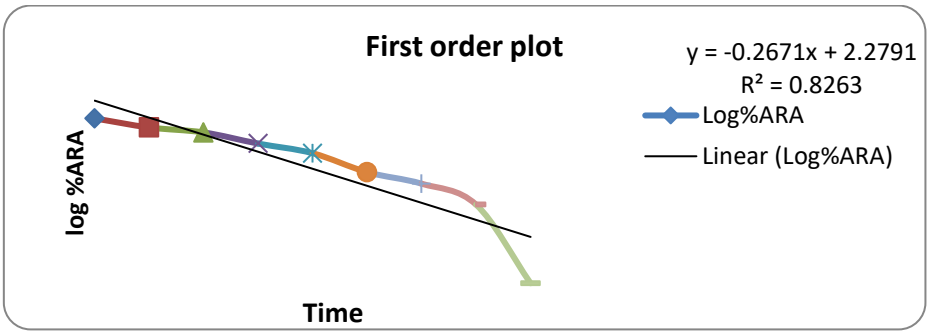

Fig-7: First order for optimized formula

Higuchi model

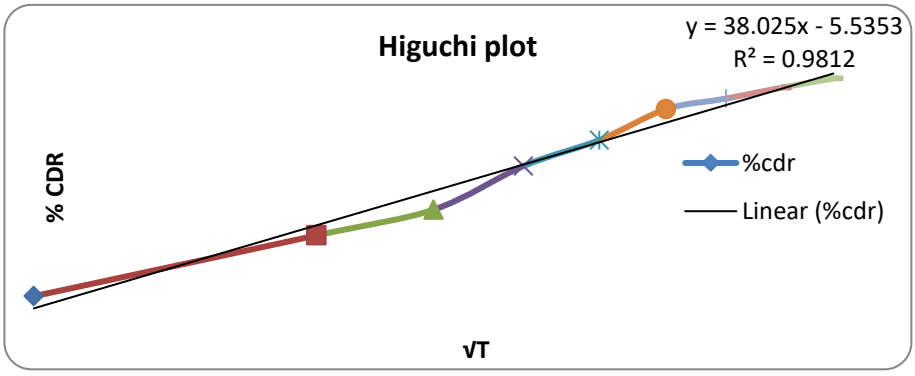

Fig-8: Higuchi plot for optimized formula

Korsmayer peppas

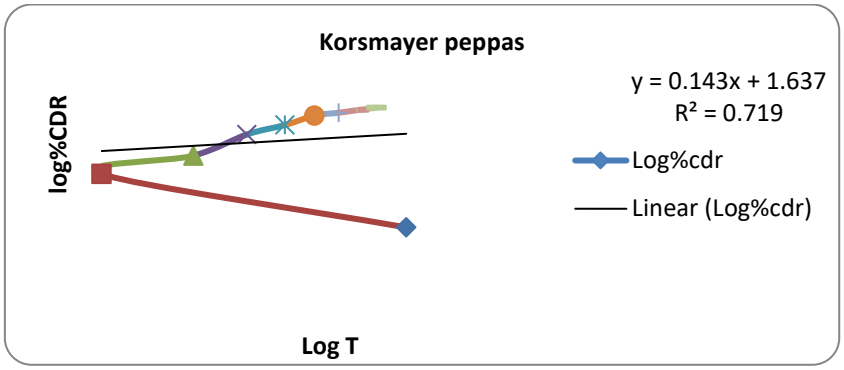

Fig-9: Korsmayer peppas plot for optimized formula

The drug release from the diltiazem microspheres was found to follow Zero order release based on the "r" value obtained for Zero order (0.997) and first order (0.826) for F7 formulation. Also, the drug release mechanism was found to be "Diffusion" based on the "r" value of 0.981 obtained for Higuchi's plot. Similarly, the drug release mechanism was found to be of Anomalous diffusion mechanism based on the "r ^{2}" value of 0.719 obtained for Peppa's equation

Table-6: Drug release kinetics

S.no	Kinetic model	R^2 value
1	Zero order kinetics	0.997
2	First order kinetics	0.826
3	Higuchi model	0.981
4	Krossmayer peppas	0.719

Stability studies

The stability studies were carried out for the formulation 7 at 40˚C ± 2˚C/75% ± 5% RH for 3 months as per ICH guidelines and the results are summarized. The results indicated that the microspheres did not show any significant physical changes during the study period. The results of stability studies show that there is about 98.65 percentage of the drug is present in the formulation and 98.52 percentage of in vitro drug release after storage at 40˚C for 90 days, it indicates the good stability of the diltiazem microspheres.

Table-7: Stability studies of optimized formulations at 40 ± 2 ^{0}C and 75 ± 5% RH for 3 months

F.Code	Parameters	Initial	1st Month	2nd Month	3rd Month	Limits as per specifications
F7	**25^{0}C/60%RH % Release**	98.65	98.63	98.62	98.60	Not less than 85%
F7	**30^{0}C/75% RH % Release**	98.65	98.61	98.59	98.56	Not less than 85%
F7	**40^{0}C/75% RH % Release**	98.65	98.60	98.57	98.52	Not less than 85%

4. CONCLUSION

Diltiazem mucoadhesive microspheres were successfully prepared by emulsification technique with a maximum incorporation efficiency of 88.15. The microspheres were spherical in shape and the drug remained dispersed in the polymer matrix at amorphous state. The prepared microspheres exhibited good mucoadhesive properties as observed in in vitro wash-off test when compared to a non-mucoadhesive polymer, ethyl cellulose microspheres. The drug release mechanism was non-fickian type controlled by swelling and relaxation of polymer chain. There was no significant change in drug content of drug-loaded microspheres, stored at different storage conditions after 3 months of study.

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