https://doi.org/ 10.33472/AFJBS.6.10.2024.5085-5100

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

CHARACTERIZATION AND EVALUATION OF ROSIGLITAZONE MALEATE MUCOADHESIVE MICROSPHERES

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Article History

Volume 6,Issue 10, 2024

Received:29 Apr 2024

Accepted : 27 May 2024

doi: 10.33472/AFJBS.6.10.2024.5085-5100

Abstract: Mucoadhesive controlled release dose formulations have grown more popular because of their capacity to adhere and release the loaded medicine over a long period of time. These methods have been used in the past to develop mucoadhesive compositions. For drug delivery devices, mucoadhesion has been a key concern. Characterize, optimize, and assess rosiglitazone maleate microspheres. It is possible to combine mucoadhesion and regulated drug administration with Rosiglitazone Maleate microspheres for the treatment of type 2 diabetes. An emulsion solvent evaporation process was used to manufacture microspheres with mucoadhesive characteristics. Microspheres that were distinct and free-flowing were discovered. They ranged in diameter from 21 to 37 nm. In an in vitro wash-off test, the microspheres displayed good drug trapping and mucoadhesive characteristics. Researchers found that in this investigation, the microspheres released Rosiglitazone Maleate at different rates depending on the polymer used to make them. Up to a 12-hour medication release was seen with F1 and F2 formulations. Most consistently, F1's Carbopol 934 and sodium carboxymethyl cellulose had the greatest mucoadhesive profile and acceptable surface morphology of the many formulations tested. Researchers found that formulation F1 microspheres were the best option for delivering Rosiglitazone Maleate into the gastrointestinal system for an extended period.

Key words: Rosiglitazone maleate, Mucoadhesive microspheres, Evaluation

INTRODUCTION:

Diabetes mellitus (DM) is a gathering of metabolic sicknesses portrayed by hyperglycemia, hypertriglyceridemia and hypercholesterolemia, coming about because of deformities in insulin discharge or activity or both [1]. A few endeavors have been proposed to work on oral

bioavailability, among them microencapsulation addresses a promising concept[2]. Microencapsulation has been utilized to support drug discharge, giving a durable and more dependable delivery with gastrointestinal (GI) bothering decreased or wiped out [3]. Bio adhesive conveyance of medications has acquired unmistakable quality lately for of medication organization. The cozy contact of the mucoadhesive polymer with the mucous surface can bring about an expanded medication maintenance time, expanding bioavailability and expanding contact time among drug and mucosa[4,5,6]. At the point when the mucoadhesive measurements structure is controlled in one or the other tablet or container structure, they could conceivably stick to the mucous surface because of the heaviness of the dose structure and the vivacious development of the GI parcel, bringing about a huge variety. In any case, mucoadhesive microspheres enjoy a few benefits. These incorporate a light weight and a more modest portion variety because of the huge number of microspheres controlled [7]. Rosiglitazone maleate (RSZ) is an antidiabetic drug for type II diabetes that further develops insulin awareness in muscle and fat tissues through actuation of peroxidase proliferator-enacted γ receptor (PPAR γ) that are associated with the record of insulin responsive qualities liable for glucose creation, transport, and utilization[8]. The point of the current work is to portray the rosiglitazone microspheres with natural half-existence of 3-4 h and to assess the convenience and achievability of these microspheres for orally managed drug conveyance framework.

MATERIALS AND METHODOLOGY

MATERIALS

Rosiglitazone (RSZ) was obtained from Indswift Labs Pvt Ltd, Baddi(H.P.). Sodium carboxy methyl cellulose (SCMC) and carbopol-934P (CP), Sodium alginate, Liquid paraffin, Span 20, Tween 80 were procured from Central Drug House, Mumbai. All other reagents used were of analytical grade and purchased from their respective commercial sources.

METHODOLOGY

METHODS

Evaluationofmicrospheres:

- Surface morphology.
- Particle size analysis.
- Drug entrapment efficiency.
- In-vitro mucoadhesivity.
- In-vitro drug release.
- Stability studies.
-

Surface morphology:

The surface morphology and structure will be visualized by scanning electron microscopy (SEM). The samples will be prepared by lightly sprinkling the microspheres powder on a double side adhesive tape which already shucked to on aluminum stubs. The stubs will be then placed into fine coations putter for gold coating. After gold coating samples will be randomly scanned for particle size and surface morphology.

Particle size analysis:

The mucoadhesive microspheres will be examined by optical microscope. The freshly prepared suspension of microspheres will be examined on an optical microscope and size of the microspheres will be measured by using a pre-calibrated ocular micrometer and stage micrometer. Around 300 particlesof each formulation will be observed and counted.

Drug entrapment efficacy:

25 mg of dried microsphere will be weighted accurately and drug will be extracted from microspheres by digesting for 24 hours with 10 ml of 0.1 N HCl (pH-1.2). During this period the suspension will be agitated. After 24 hrs the suspension will be centrifuged at 2000 rpm for about 3 minutes. The supernatant obtained will be assayed spectro photo metrically for drug contents.

Entrapment efficiency will be calculated according to equation.

Entrapment efficiency = (A1 – A2) 100/A1

A1=AmountofRosiglitazonemaleateaddedinitially

A2=AmountofRosiglitazonemaleatedeterminedinsupernatantspectrophotometry.

(A1-A2)=representstheamountofRosiglitazonemaleateentrappedintheformulation.

In-vitro mucoadhesivity:

The Mucoadhesive property of prepared microspheres will be evaluated by in-vitro will be hoff method. A rat stomach mucosa will be tied on the glass slide using a thread. About 100 microspheres will be spread on to wet rinsed tissue specimen and prepared slide will be hung on to one of the grooves of a USP tablet disintegration apparatus. By operating the disintegrating test apparatus, the tissue specimen will be given a slow regular up and down movement in the test fluid at 37±0.5^{0}C. At every 1hrs interval the equipment will be stopped and the number of particles still adhering to tissue will be counted. Percent mucoadhesion will be given by the following formula.

%mucoadhesion=(no.ofparticles/no.of applied microspheres) ×10

In-vitro drug release:

The release rate of Rosiglitazone maleate from microspheres will be determined using United States Pharmacopeia (USP) dissolution testing apparatus2 (paddletype). The dissolution test will be performed using 900mL of 0.1NHCl, at37±0.5°C and 50rpm. As ample(10mL) of the solution will be withdrawn from the dissolution apparatus hourly for 12hrs, and the samples will be replaced with fresh dissolution medium to maintain the sink condition. The samples will be filtered through a membrane filter and diluted to a suitable concentration with 0.1NHCl. Absorbance of these solutions will be measured at 242nm using a model 1700-EShimadzu, double-beam spectrophotometer. Cumulative percentage drug release will be calculated using an equation obtained from a standard curve.

Stability studies:

Selected formulation of microspheres stored in amber colored glass bottle at 25±1^{0}C, 40±1^{0}C and 50±1^{0}C for a period of 40 days and observed for any change in percentage residual drug content. Sample will be analysed for residual drug content at the time interval of 10days for 40days.

RESULTS AND DISCUSSION

EVALUATION OF MUCOADHESIVE MICROSPHERES:

The 6 formulations of mucoadhesive microspheres of Rosiglitazone maleate will be prepared by water in oil (w/o) emulsification solvent evaporation techniques using various ratios of sodium CMC, carbopol-934 and sodium alginate. All these formulations will be evaluated for surface morphology, particle size analysis, drug entrapment efficiency, in-vitro mucoadhesivity and in-vitro drug release study.

SURFACE MORPHOLOGY:

Surface morphology of the mucoadhesive microspheres will be examined by scanning electron microscopy. The SEM showed that the blend of sodium CMC and carbopol-934 produced spherical with smooth surface microspheres due to their high solubility in water. Microspheres of sodium CMC alone produced smooth surface spherical shape microspheres.

While sodium alginate microspheres will be of irregular shape with a rough morphology due to less water solubility and non-uniform evaporation of water from the surface of microspheres. This indicates that in the blending of sodium CMCwithcarbopol-934 at1:1 ratio, the uniformly shaped microspheres with smooth surface could be obtained.The SEM of microspheres of formulation F1, F2, F3, F4, F5 and F6.

PARTICLE SIZE ANALYSIS:

Particle size analysis of different formulations will be done by optical microscopy. The average particle size will be found to be in the range of 29.20± 1.88 to 37.54±3.24 μm. Data for the particle size of microspheres of various formulations are shown in table no. 24 and expressed in figure no. 26. For the purpose of accessing effect of stirring speed and polymer concentrations on particle size of microspheres, 6 batches will be prepared attwo different stirring speeds and two different polymer concentrations.

The mean particle size will be significantly increases with increasing polymer concentration this maybe due to high viscosityof polymer solution. High viscosityof polymer concentration requires high energy for breaking of droplets. Particle size decreased with increasing stirring speed due to the fact that increased in stirring speed, produce high energy, which leads to further decrease in droplets size.

DRUGENTRAPMENT EFFICIENCY:

Drug content in different formulations will be estimated by U Vspectrophotometric method. Drug entrapment efficiency of microspheres will be optimized by preparing 6 formulations of microspheres using various ratios of sodiumCMC, carbopol-934 and sodium alginate.

Effect of polymer ratios or type of polymer used in the formulation, on the drug entrapment efficiency is listed in table no.25 and expressed in figure no.27. It may, therefore, be reasoned that the entrapment of the drug, which is dependent on the successful molecular association of the drug with the polymer, is dictated by the moieties and functional group make up the constitutional repeat unit of the polymer molecules.

Percent drug loading efficiency of microspheres will be found in the range of 53.00±3.68 to 80.00± 3.23% (table-25). Formulation F2 containing carbopol-934 showed maximum % drug loading about 80% whereas formulation F6 containing carbopol-940/ sodium alginate showed minimum % drug loading about 53% as compared to other formulations. Rank order of % drug loading of various formulations will be found to be as follows:

F2 > F4 > F1 > F5 > F3 > F6

IN-VITROMUCOADHESIVITYTEST:

To assess the mucoadhesive property of microspheres, In-vitro will be h-off test will be performed for all the formulations. A dhesion of the polymer with the mucus membrane mediated by hydration in the case of hydrophilic polymers. Upon hydration, these polymers become sticky and adhere to mucus membrane. In the case of sodium CMC anionic nature of polymer responsible for mucoadhesion.

Carbopol possess various carboxyl groups. When mobile at the wet mucosal surface, they orientate these mucoadhesivesites towards mucosa and make interactions through hydrogen bonding. At the end of 4 hrs % mucoadhesion will be found to be 61.33±1.78, 45.22±2.12, 39.65±2.69, 36.43±1.78, 58.37±2.33, 35.08±2.68 for formulation F1, F2, F3, F4, F5 and F6 respectivelyand at the end of end of 8 hrs % mucoadhesion will be found tobe24.54±1.54, 15.11±1.44, 11.32±1.56, 07.08±1.45,06.08±0.85,04.17±1.10.

Formulation F1 containing sodium CMC showed the highest mucoadhesivity. The greater mucoadhesivity of sodium CMC microspheres will be due to an ionic nature of the polymer. Formulation F6 containing sodium alginate/carbopol showed the lowest mucoadhesivity due to their regular surface of microspheres.

The rank order of % mucoadhesivity of all the formulations will be found to be as follows (after8 hours):

F1 > F2 > F3 > F5> F4 > F6

IN-VITRODRUG RELEASESTUDY:

% drug release from microspheres will be optimized by preparing 6 formulations of microspheres using various ratios of polymers, for this in-vitro drug release study of all the formulations containing drug will be performed in 0.1NHCl at 37^0C ±1. It will be found that the release profile of Rosiglitazone maleate will be different for the different formulations. Rosiglitazone maleate release form these microspheres will be slow, extended and dependent on the type of polymer used.

After the end of 5hrs, the drug release will be found to be 58.22±1.90, 49.00±2.45, 32.00±2.34, 42.22±2.94, 38.44±1.33, and 40.11±1.39 percent for formulations F1, F2, F3, F4, F5 and F6 respectively. At the end of 5hrs less than 50% drug release from all formulations except formulation F1. After the end of 10hrs, the drug release will be found to be 91.45±2.55, 83.43±2.55, 56.23±2.10, 84.22±3.33, 67.54±3.44, and 78.76±2.33 percent for formulations F1, F2, F3, F4, F5 and F6 respectively. Data for the release of the drug from microspheres of various formulations are shown in table no. 28 and expressed infigure no. 30.

Formulation F1 containing sodium CMC showed the maximum release 91.45±2.55 % after 10hrs, due to rapid swelling property and high dissolution of sodium CMC in dissolution environment(0.1NHCl). Microspheres of sodium CMC may hydrated to form gel and dissolution medium permeation into the microspheres is facilated due to high swelling action of the sodium CMC which leads to more medium for the transport of the drug is available.

While sodium alginate microspheres (F3) showed the least drug release 56.23±2.10 % after 10hrs due to less swelling action and irregular surface of microspheres as compared to sodium CMC microspheres. The slowing of drug release from sodium alginate microspheres is probably due to the less swelling action of the polymer leads to reducing in access of the solvent to the microspheres.

STABILITY STUDY:

The stability studies will be carried out by storing drug loaded microspheres of formulations F1, F2, F3 and F4 at different temperaturei.e.25^{0}C, 40^{0}C, 50^{0}C for 40days and their % drug retained will be calculated forevery 10days intervals. The data tells that after 40days at 50^{0}C temperature the maximum drug degrades from microspheres will be 4.38, 4.42, 4.31, and 4.35 percent for the formulations F1, F2, F3 and F4 respectively. After 40days at 25^{0}C the minimum drug loss from microspheres will be 1.12, 1.08, 1.22 and 1.18 percent for the formulations F1, F2, F3 and F4 respectively. Data for the stability studies are shown in table no.29-30 and expressed in figure. The stability profile of Rosiglitazone maleate loaded microspheres suggests that the storage of microspheres at elevated temperature resulting greater loss of drug as comparedto low temperature.

Table 1:Interference of polymers in estimation of drug

Description	**λmax**
Drug	242.00
Drug+SCMC	242.35
Drug+CP-934	242.56
Drug+SA	242.48

SCMC- sodium carboxy methyl cellulose
CP- carbopol
SA- sodium alginate

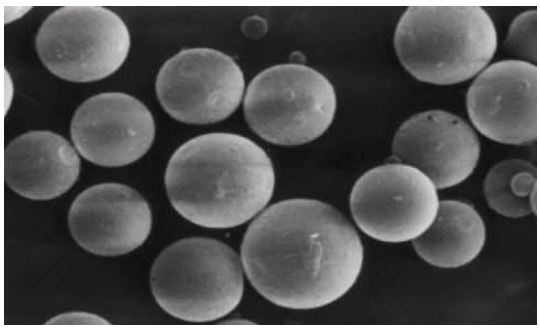

Figure1: Scanning electron photomicrograph of formulation F1 showing population of microspheres.

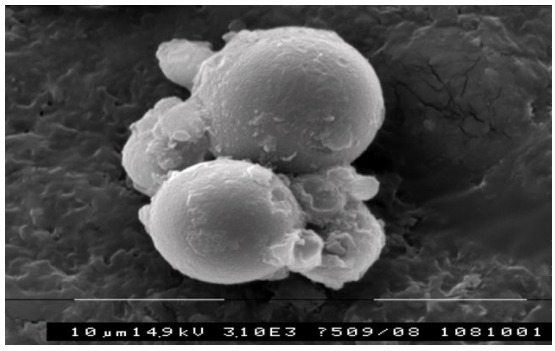

Figure2: Scanning electron photomicrograph of formulation F2 showing population of microspheres.

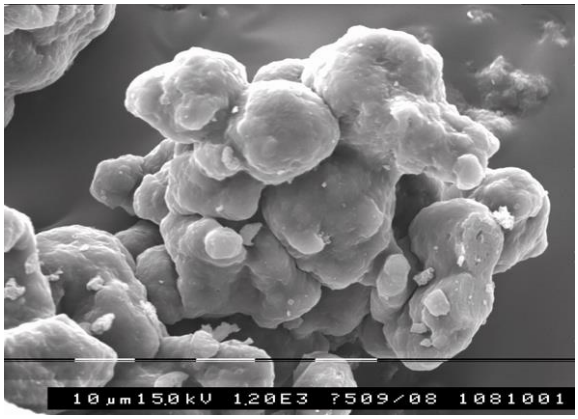

Figure3: Scanning electron photomicrograph of formulation F3 showing population of microspheres.

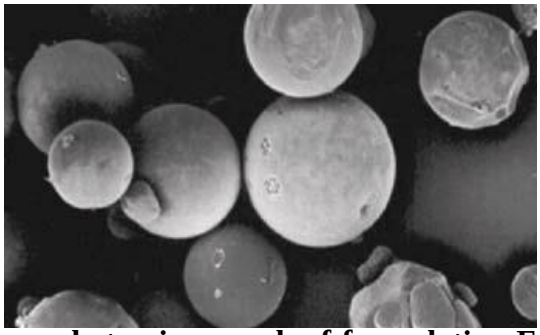

Figure4: Scanning electron photomicrograph of formulation F4 showing population of microspheres.

Table 2: Effect of stirring speed on particle size of microspheres of batches of MS1, MS2, MS3, MS4, MS5 and MS6.

Formulation code	**Particlesize(µm) At 500rpm**	**Particlesize(µm) At 1000rpm**
MS1	31.54±2.43	26.87±1.43
MS2	29.20±1.88	25.36±1.79
MS3	37.54±3.24	29.22±2.45
MS4	32.62±2.78	26.85±1.95
MS5	35.12±2.84	28.33±2.32
MS6	33.85±3.15	27.85±1.85

All batches will be prepared at 2% polymer concentration and every batch will be prepared at two different stirring speeds (500rpm and1000rpm).

All values are represented as mean ± standard deviation(n=3).

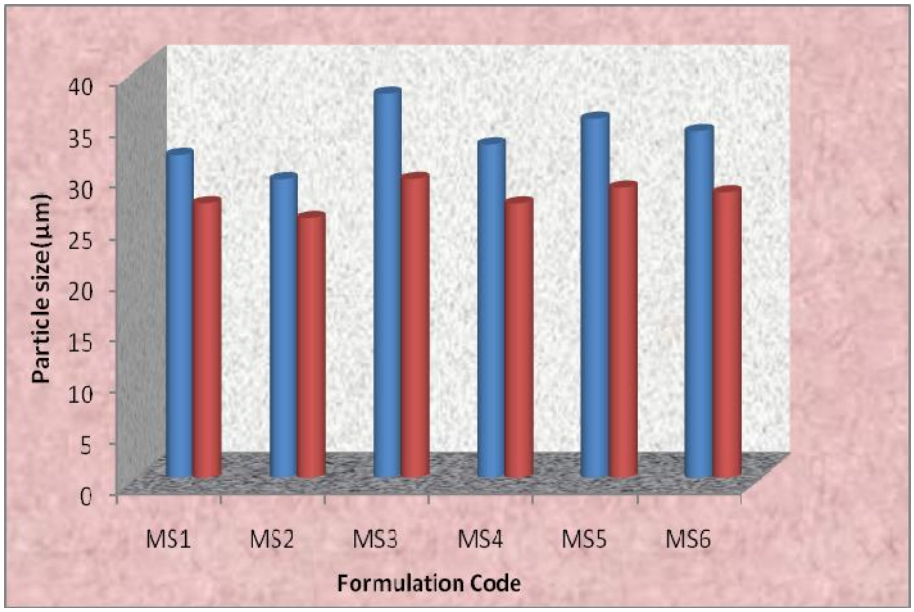

Figure5: Effect of stirring speed (■500rpm, ■1000rpm) on particle size of microspheres of batches of MS1, MS2, MS3, MS4, MS5 and MS6.

Table 3: Batches of MC1, MC2, MC3, MC4, MC5 and MC6.

Formulation code	Particle size(µm) at 1% polymer concentration	Particle size(µm) at 2% Polymer concentration
MC1	27.23±1.89	31.54±2.43
MC2	25.56±1.75	29.20±1.88
MC3	30.42±2.12	37.54±3.24
MC4	28.32±2.25	32.62±2.78
MC5	30.56±2.58	35.12±2.84
MC6	28.85±1.96	33.85±3.15

Values are represented as mean ±s tandard deviation (n=3).

All batches are prepared at 500rpm stirring speed and every batch are prepared at two different polymer concentrations (2%,1%).

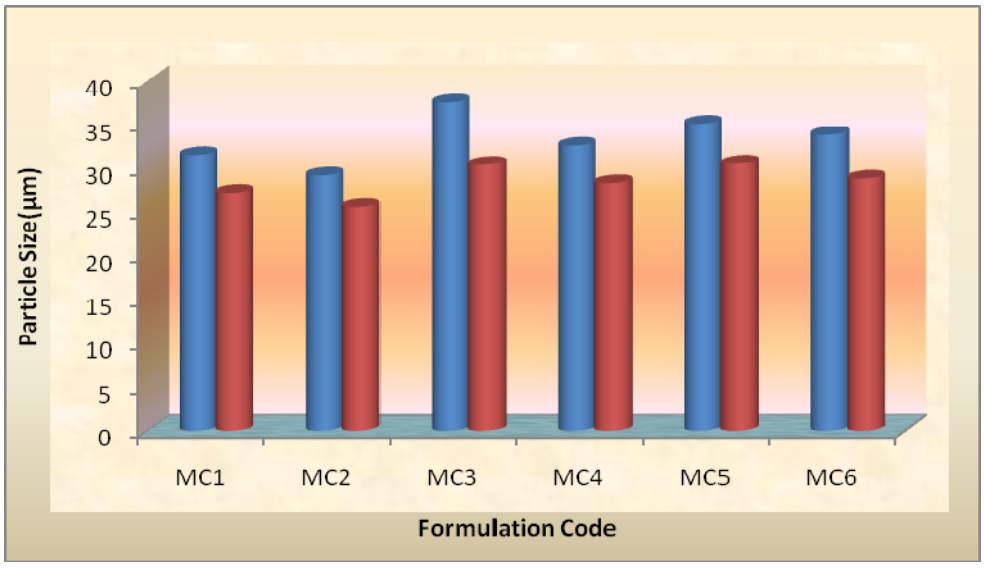

Figure6: Effect of polymer concentration (■2%, ■1%) on particle size of batches of MC1, MC2, MC3, MC4, MC5 and MC.

Table 4: Particle size of microsphere of formulations F1, F2, F3, F4, F5 and F6.

Formulation code	Particle size (µm)
F1	31.54±2.43
F2	29.20±1.88
F3	37.54±3.24
F4	32.62±2.78
F5	35.12±2.84
F6	33.85±3.15

Value are represented as mean ± standard deviation (n=3).

All formulation are prepared at 2% polymer concentration and 500rpm stirring speed. Span20(0.5%) used as emulsifying agent.

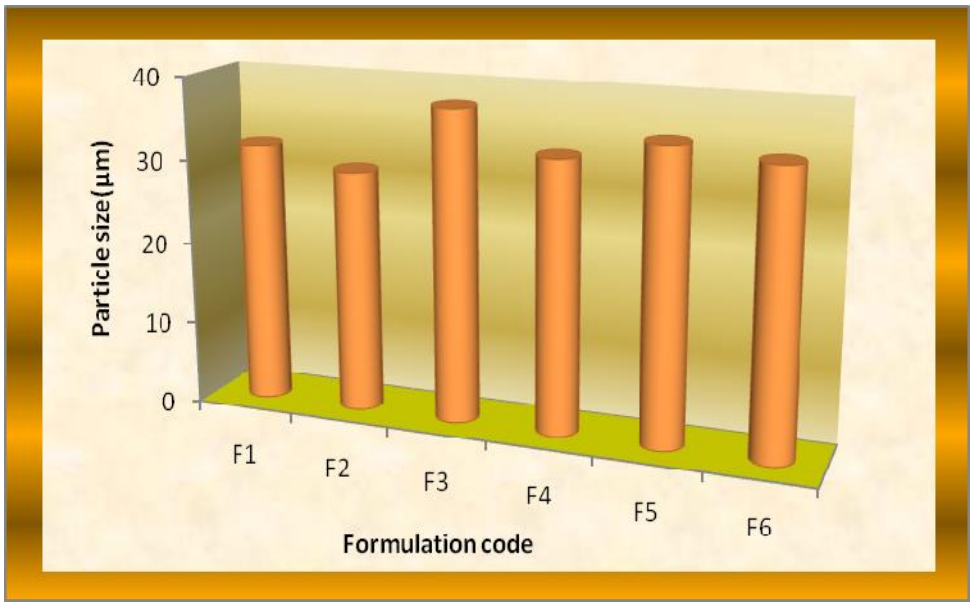

Figure 7: Particle size of microsphere of formulations F1, F2, F3, F4, F5 and F6.

Table5: Drug Entrapment efficiency of mucoadhesive microspheres of formulations F1, F2, F3, F4, F5 and F6.

Formulation code	Theoretical loading (mg)	Practical loading(mg)	%Drug entrapment
F1	100	68	68±2.45
F2	100	80	80±3.23
F3	100	56	56±2.86
F4	100	78	78±2.75
F5	100	62	62±2.84
F6	100	53	53±3.68

Value are represented as mean ± standard deviation (n=3).

All formulations are prepared at 2% polymer concentration and 500rpm stirring speed. Span20 (0.5%) used as emulsifying agent.

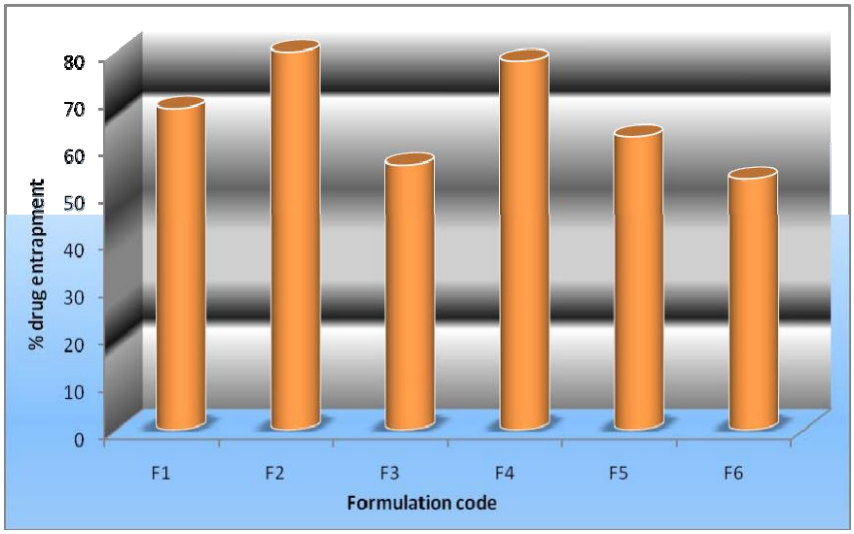

Figure8: Drug Entrapment efficiency of mucoadhesive microspheres of formulations F1, F2, F3, F4, F5 and F6.

Table6: Comparative %mucoadhesion of microspheres of formulations F1, F2, F3, F4, F5 and F6.

Time(hrs)	F1	F2	F3	F4	F5	F6
1	89.33±2.33	87.22±2.43	84.23±2.83	69.20±1.90	93.33±2.95	73.44±2.32
2	76.66±2.54	68.66±2.43	64.54±3.22	60.08±2/23	84.44±3.24	51.09±3.10
3	68.00±1.98	58.33±2.76	54.34±2.09	49.18±1.80	71.33±2.55	43.33±2.90
4	61.33±1.78	45.22±2.12	39.65±2.69	36.43±1.78	58.37±2.33	35.08±2.68
5	52.64±1.90	34.43±1.98	32.15±2.10	28.43±1.85	35.24±2.76	24.20±2.83
6	42.31±185	27.54±1.86	25.17±1.98	21.52±1.47	20.22±1.95	15.22±2.32
7	33.76±1.70	19.32±1.57	18.50±1.35	09.26±1.29	10.18±1.33	08.30±1.65
8	24.54±1.54	15.11±1.44	11.32±1.56	06.08±1.45	07.08±0.85	04.17±1.10
9	18.34±1.65	11.65±1.08	05.21±1.10	03.04±0.80	03.45±0.90	00.00±0.00
10	13.12±1.12	07.26±1.25	03.08±0.78	00.00±0.00	00.00±0.00	00.00±0.00

Values are represented as mean ± standard deviation (n=3).

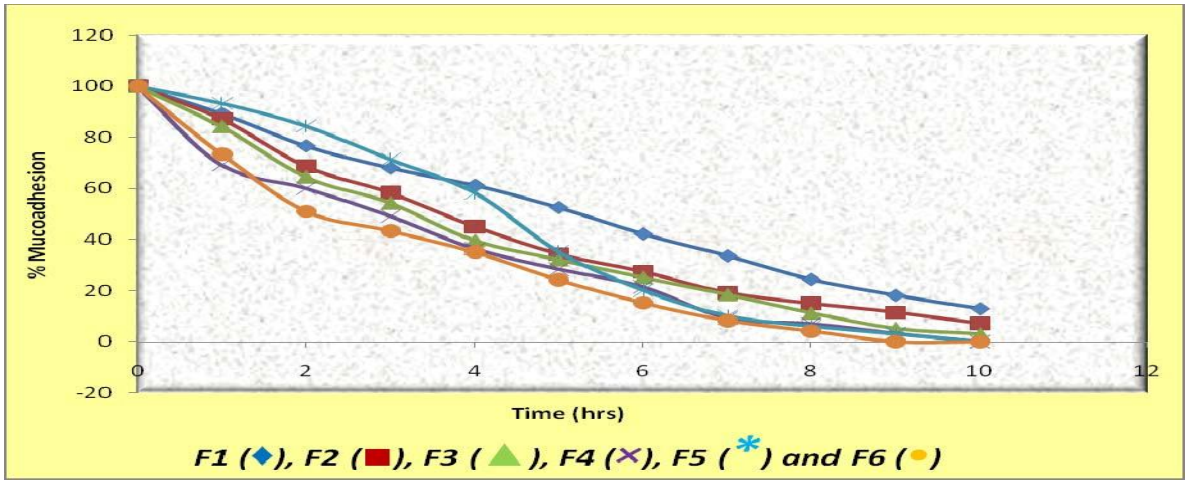

Figure9: Comparative %mucoadhesion of microspheres of formulations F1, F2, F3, F4, F5 and F6.

Table7: Comparative %mucoadhesion of drug entrapment and %yield of microspheres of formulations F1, F2, F3, F4, F5 and F6.

Formulation code	%Mucoadhesion after 1hrs	%Drug entrapment	Particle size (µm)	%yield
F1	89.33±2.33	68±2.45	31.54±2.43	78.45±3.20
F2	87.22±2.43	80±3.23	29.20±1.88	74.56±2.80
F3	84.23±2.83	56±2.86	37.54±3.24	81.10±2.95
F4	69.20±1.90	78±2.75	32.62±2.78	79.52±2.84
F5	93.33±2.95	62±2.84	35.12±2.84	76.12±3.10
F6	73.44±2.32	53±3.68	33.85±3.15	77.44±2.60

Values are represented as mean ± standard deviation (n=3).
All formulation are prepared at 2% polymer concentration and 500rpm stirring speed.

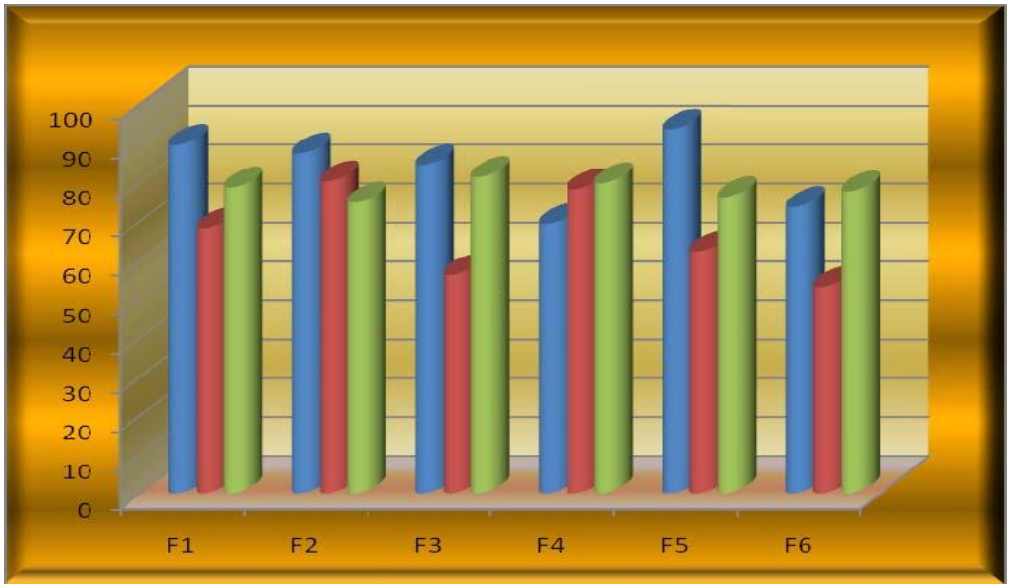

Figure10: Comparative% mucoadhesion, drug entrapment and %yield of microspheres of formulations F1, F2, F3, F4, F5 and F6 (■%mucoadhesion, ■%drugentrapment, ■%yield).

Table8: Comparative cumulative %drug release from microsphere of formulation F1, F2, F3, F4, F5 and F6.

Time(hrs)	F1	F2	F3	F4	F5	F6
1	10.22±0.95	07.11±1.23	05.22±1.90	08.44±1.23	06.22±0.90	07.22±0.94

2	24.33±1.40	22.33±2.32	10.33±2.44	16.10±1.35	12.21±1.10	14.55±1.42
3	36.43±1.50	31.22±1.95	17.43±1.22	27.22±1.56	20.33±1.22	24.42±1.56
4	48.45±1.45	38.22±2.21	22.11±2.10	33.22±2.11	30.44±1.3	32.22±1.65
5	58.22±1.90	49.00±2.45	32.00±2.34	42.22±2.94	38.44±1.33	40.11±1.39
6	67.55±1.98	56.21±2.34	36.12±3.45	51.44±2.55	44.55±1.87	48.43±2.26
7	72.12±2.43	64.22±2.45	40.11±2.67	66.00±3.94	52.22±1.94	58.08±2.89
8	80.32±3.20	70.22±3.18	46.75±2.95	73.22±2.45	58.56±2.22	65.78±3.24
9	86.32±2.80	76.05±2.38	50.24±2.33	79.90±3.19	62.09±2.34	72.45±3.33
10	91.45±2.55	83.43±2.55	56.23±2.10	84.22±3.33	67.54±3.44	78.76±2.33
11	95.10±2.10	90.32±1.85	61.44±2.80	90.22±3.65	72.45±3.21	84.55±2.12
12	98.55±1.15	95.65±1.70	64.32±2.35	93.43±2.10	76.33±3.90	92.33±1.95

Values are represented as mean ± standard deviation (n=3).

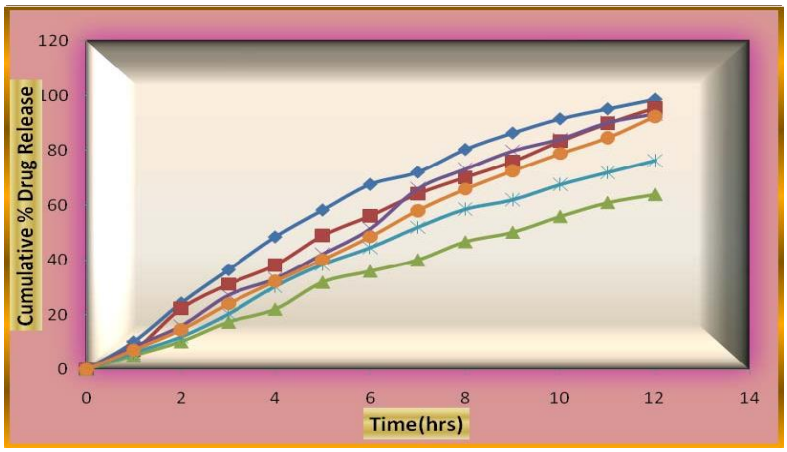

Figure11: Comparative cumulative %drug releases from microsphere of formulation F1(♦), F2(■), F3(▲), F4(×), F5 (*) and F6(•).

Table 9: Stability study of formulations F1 and F2

S No.	**Time(days)**	**%Drug retained**					
		At 25^{0}C		**At 40^{0}C**		**At 50^{0}C**	
		F1	**F2**	**F1**	**F2**	**F1**	**F2**
1	0	100	100	100	100	100	100
2	10	99.70	99.68	99.32	99.23	99.12	99.09

3	20	99.42	99.38	98.61	98.54	98.09	97.95
4	30	99.15	99.12	97.89	97.95	96.79	96.70
5	40	98.88	98.92	97.20	97.15	95.65	95.58

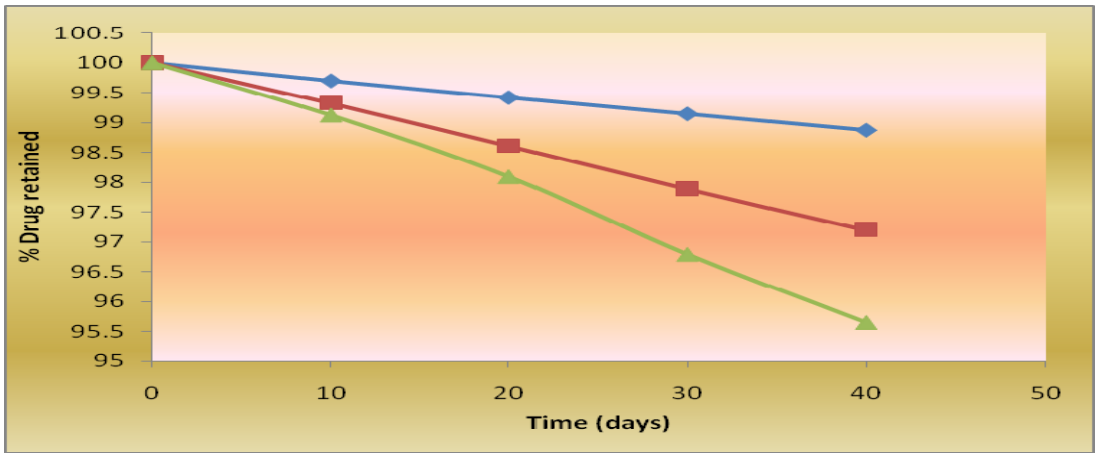

Figure 12: Stability study of formulation F1 (♦at 25^{0}C, ■at 40^{0}C, ▲ at 50^{0}C).

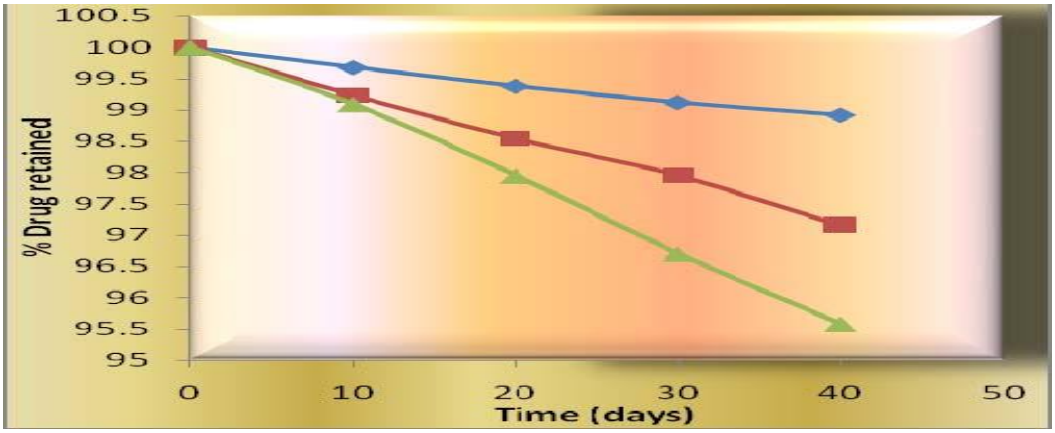

Figure 13: Stability study of formulation F2 (♦at 25^{0}C,■ at 40^{0}C, ▲ at 50^{0}C).

Table 4.23: Stability study of formulations F3 and F4

S No.	**Time(days)**	**%Drug retained**					
		At 25^{0}C		**At 40^{0}C**		**At 50^{0}C**	
		F3	**F4**	**F3**	**F4**	**F3**	**F4**
1	0	100	100	100	100	100	100
2	10	99.60	99.71	99.22	99.29	99.18	99.09
3	20	99.32	99.42	98.51	98.59	98.13	97.99
4	30	99.05	99.15	97.84	97.98	96.89	96.82
5	40	98.78	98.82	97.15	97.25	95.69	95.62

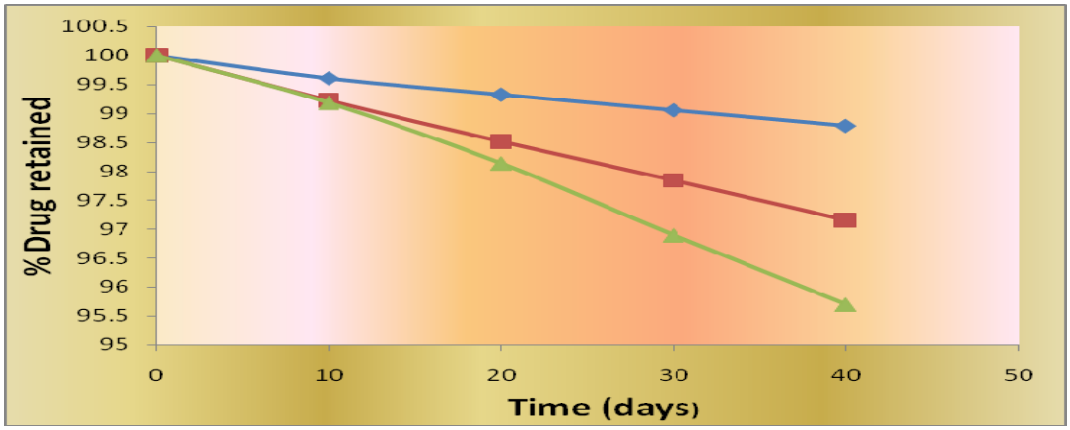

Figure 14: Stability study of formulation F3 (♦ at 25^{0}C, ■at 40^{0}C, ▲ at 50^{0}C).

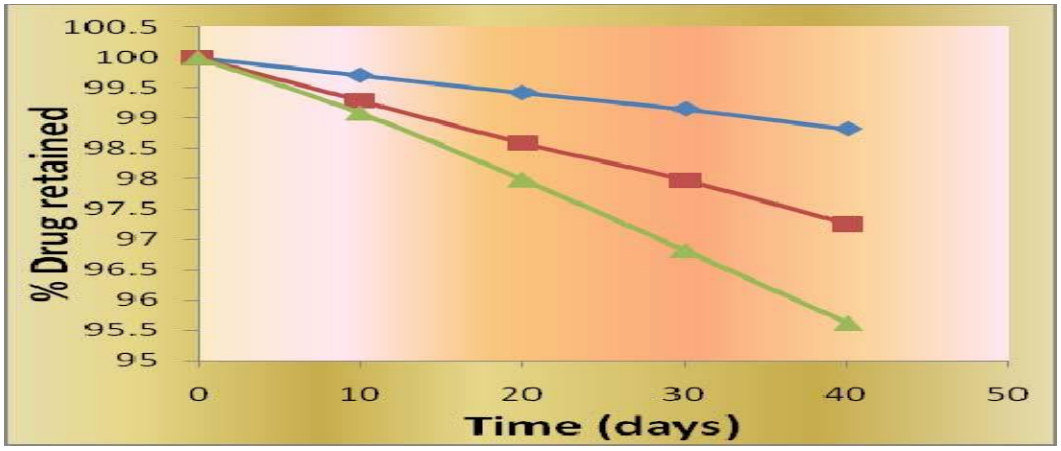

Figure 15:Stability study of formulation F4 (♦at 25^{0}C, ■at 40^{0}C, ▲ at 50^{0}C).

CONCLUSION

Among the mucoadhesive microspheres of Rosiglitazone maleate prepared using sodium carboxy methyl cellulose, carbopol-934 and sodium alginate polymers, the formulation F1 (containing sodium carboxymethyl cellulose) and F2 (containing carbopol-934) showed reproducible results and the best mucoadhesive profile with good surface morphology. Among all the formulations of microspheres, formulation F1 containing sodium carboxy methyl cellulose showed best sustained release effect.

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