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Original Article

Preparation and Evaluation of Mucoadhesive Microspheres of Simvastatin by Ionic Gelation Technique

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ABSTRACT

A primary object of using mucoadhesive formulations orally would achieve a substantial increase in the length of stay of the drug in GI tract stability problem in the intestinal fluid can be improved. Mucoadhesive microsphere carrier systems were made from the biodegradable polymers in sustained drug delivery. The objective of the present study is related to the preparation and evaluation of mucoadhesive microspheres of simvastatin by using different polymers like Sodium alginate, HPMC K100M, Sodium CMC, Ethyl cellulose, Methyl cellulose, Guar gum, Xanthan gum and Carbopol 940 in different ratios by the Ionic deletion method. The prepared batches of mucoadhesive microspheres of simvastatin were evaluated for the flow properties; drug content, entrapment efficiency, and Percent mucoadhesive property, *in vitro* dissolution studies of all 12 formulations were performed. From the all batches F10 (Drug: Sod. Alginate: Methyl cellulose 1:2:1) batch is considered to be the most promising formulation batch because among all the batches it shows better extent of drug release 97.11% (8hrs), good entrapment efficiency (78%), and *in vitro* wash-off test shows the good mucoadhesive property. Simvastatin release from alginate – Methyl cellulose (F10) was slow and extended over a period of 8 hrs and these microspheres were found suitable for the oral controlled release formulation.

Keywords: Mucoadhesive drug delivery system, Hydroxyl propyl methyl cellulose, Sodium alginate, Carboxy methyl cellulose.

INTRODUCTION

Microspheres are discrete spherical particles ranging in average particle size from 1 to 1000µm. Mucoadhesive microsphere carrier systems are made from

the biodegradable polymers in sustained drug delivery. Recently, dosage forms that can precisely control the release rates and target drugs to a specific body site have made an enormous impact in the formulation and development of novel drug delivery systems. Good[1] defined mucoadhesion as the state in which two materials, at least one biological in nature, are held together for an extended period of time by interfacial forces. It is also defined as the ability of a material (synthetic or biological) to adhere to a biological tissue for an extended period of time[2].

Mucoadhesive polymers are water-soluble and water insoluble polymers, which are swellable networks, jointed by cross-linking agents. Mucoadhesive polymers that adhere to the mucin-epithelial surface can be conveniently divided into three broad classes:

- Polymers that become sticky when placed in water and owe their mucoadhesion to stickiness.
- Polymers that adhere through nonspecific, noncovalent interactions those are primarily electrostatic in nature (although hydrogen and hydrophobic bonding may be significant).
- Polymers that bind to specific receptor sites on tile self surface.

Characteristics of an ideal mucoadhesive polymer[3]

- The polymer and its degradation products should be nontoxic and should be non absorbable from the gastrointestinal tract.
- It should be nonirritant to the mucous membrane.
- It should preferably form a strong noncovalent bond with the mucin-epithelial cell surfaces.
- It should adhere quickly to most tissues and should possess some site-specificity.
- It should allow daily incorporation to the drug and offer no hindrance to its release.
- The polymer must not decompose on storage or during the shelf life of the dosage form.

Advantages of mucoadhesive drug delivery system[4]

- Prolonged residence time at the site of action or absorption;
- Localization of the drug delivery system at a given target site;
- An increase in the drug concentration gradient due to the intestine contact of the particles with the mucosal surface;
- Direct contact with intestinal cells, which is the step earlier to particle absorption.

MATERIALS AND METHODS

Simvastatin was obtained as a gift sample from Hetero Pharma, Hyderabad. Carbopol 940P, Xanthan gum, Ethyl cellulose, Guar gum, Methyl cellulose, Sodium.CMC, Sodium alginate, Calcium chloride from SD Fine Chemicals Ltd., Mumbai.

Experimental techniques in mucoadhesive drug delivery[5,6]

- **Wilhelmy plate method:** A glass plate is coated with a bioadhesive polymer and immersed in beaker of mucin solution. A microbalance is connected to a plate to measure the dynamic force and plate as the beaker is lowered away from the mucin solution. The force measured is then related to the wettability of the mucin on the polymer surface and correspond to the adhesive force between the bioadhesive and the mucin. This technique has the advantage of being expensive and rapid, although disadvantage includes possible errors

resulting from capillary forces, hysteresis and polymer dissolution in the mucin solution.

- ***Ex vivo* fluorescence method** of measuring bioadhesion in which human epithelial cells are labeled with the fluorescent probes pyrene are fluorescein isothiocyanate. These cells are then combined with bio adhesive polymer. When a photo excited moiety combines with an unexcited moiety, an eximer is formed the ratio of eximer of monomers is monitored as a function of time in order to assess the affinity of the cells for the mucin. There are some minor problems associated with it, i.e., a migration of pyrene from the cells may act to reduce eximer formation, showing an underestimated value for the affinity of the cells for the mucin.
- **Flow channel technique:** In this method bio adhesive spherical polymer particle was placed on the mucus surface inside a Plexiglas channel. A laminar flow of air or a viscoelastic solution was directed over the particle while photographs were taken to determine the static and bio adhesive behavior of the particle.
- **Falling film:** In this method measuring the ability of a polymer in a flowing fluid to adhere to mucus. Using this method, small spherical latex particles are coated with a bio adhesive polymer and combined with a buffer solution to create a suspension of particles with a known concentration. The solution with the contained microspheres is then pumped over a rat small intestine that has been cut lengthwise and placed in semi cylindrical trough. The eluted solution and the particles are collected in the beaker and the collected particles are counted using an electronic particle counter. The fraction particles that adhered to the mucus during the flow experiment are then related to the bio adhesion of the polymer.
- **Tensiometric technique:** In these techniques, the tensile strength is needed to separate a bio adhesive from tissues is measured. One such technique is that in which an animal tissue is placed in a clamp on a tissue device and brought into contact with a bio adhesive polymer tablet. Swelling of that tablet occurs at the interface over time while it is in contact with the mucus. A vertical force is applied until the tablet and mucus separate and this force is used to calculate the work of addition. If a good bio adhesive material is used, the addition of the mucus to the polymer is stronger than the cohesion of the mucous gel, causing mucin molecule to part from mucous gel, upon separation.
- ***In vivo* technique[7]:** This is developed based on γ-scintinography. Using this method, a bio adhesive device is labeled with Tc or in, administered to an animal while the residence time of the device in the body is monitored by a gamma camera. The length of the time the device spends in the gastric area is related to the mucoadhesive ability of the device. This technique is advantageous because it is noninvasive.

Preparation of microspheres[8]

All the formulations were prepared by orifice ionic gelation method. The compositions of different formulations are given in Table No: 1 the microspheres were prepared as per the procedure given below and the aim is to prolong the release of Simvastatin drug.

Procedure

1. Simvastatin and all other polymers were individually passed through sieve no 60.

2. The required quantities of Sodium alginate and the mucoadhesive polymer were dissolved in purified water to form a homogenous polymer solution.

3. The Drug, Simvastatin was added to the polymer solution and mixed thoroughly with a stirrer to form a viscous dispersion.
4. The resulting dispersion was then added manually drop wise into calcium chloride (10 % w/v) solution through a syringe with a needle of size no. 18.
5. The added droplets were retained in the calcium chloride solution for 15 minutes to complete the curing reaction and to produce the spherical rigid microspheres.
6. The microspheres were collected by decantation, and the product, thus separated was washed repeatedly with water and dried at 45^0C for12 hours.

Evaluation[8]

Estimation of simvastatin

A spectrophotometric method based on the measurement of absorbance at λ max 239 nm in pH 7.0 phosphate with 0.5% SLS (official in USP) buffer was used in the present study for the estimation of Simvastatin. Finally the calibration curve was plotted between concentration (x-axis) and absorbance (y-axis).

Evaluation of microspheres[8]

Drug content

Powder equivalent to 10 mg of Simvastatin was dissolved in 20 ml methanol and volume made up to 100 ml with p^H 7.0 phosphate buffer with 0.5% SLS. The Solution was filtered through Whatman filter paper no. 41 to obtain the stock Solution A. The Stock Solution A (1 ml) was diluted to 10 ml to obtain the stock Solution B.The Absorbance of the resulting solution is observed at λ max 239nm using the U.V. Spectrophotometer.

Entrapment efficiency

Entrapment efficiency was calculated using the following formula:

Entrapment efficiency = Estimated percentage drug content / Theoretical percentage drug content x 100.

In vitro wash-off test for microspheres[8]

The mucoadhesive properties of the microspheres were evaluated by the *In vitro* wash-off test.

A 4-cm by 4-cm piece of goat intestine mucosa was tied onto a glass slide using thread. Microspheres were spread (∽100) onto the wet, rinsed, tissue specimen and the prepared slide was hung on to one of the groves of a USP tablet disintegrating test apparatus. The disintegrating test apparatus was operated such that the tissue specimen was given regular up and down movements in the beakers containing the simulated gastric fluid USP (pH 1.2), and the pH 7.0 Phosphate buffer. At the end of 30 minutes, 1 hour, and at hourly intervals up to 8 hours, the number of microspheres still adhering on to the tissue was counted. The results of the *In vitro* wash-off test of batches F1 to F12.

Mucoadhesion property=No. of microspheres adhered / No. of microspheres applied x 100.

In vitro dissolution studies of microspheres[10]

Dissolution parameters

Apparatus -- USP-II, Paddle
Dissolution Medium -- 900 ml of pH 7.0 phosphate buffer with 0.5% SLS.
RPM -- 50
Sampling intervals (hrs) -- 0.5, 1, 2, 3, 4, 6 and 8.
Temperature -- 37°C ± 0.5°C

Procedure

900ml of pH 7.0 phosphate buffer was placed in the dissolution vessel and the USP dissolution apparatus –II (Paddle Method) was assembled. The medium was allowed to equilibrate to temperature of 37°C ± 0.5°C. Microspheres were placed in the dissolution vessel and the vessel was covered, the

apparatus was operated for 8hrs at 50 rpm. At definite time intervals the 5 ml of the dissolution fluid was withdrawn, filtered and again 5ml blank sample was replaced. Suitable dilutions were done with the dissolution fluid and the samples were analyzed spectrophotometrically at λ max 239 nm using a UV-spectrophotometer (Lab India).

FTIR studies

The FTIR spectra of the drug (alone), polymers (alone) and the drug-polymer mixture were recorded by the potassium bromide pellet method.

SEM studies

The External surface morphology was evaluated by using the SEM (Horizon 230, CIPRA Labs, Hyderabad).The microspheres were mounted directly on the SEM sample stub using the double sided sticking tape and coated with gold film (thickness 200nm) under the reduced pressure (0.001 mm of Hg). The voltage was used is 5KV.

RESULTS

See table 2-10 and figure 2-8.

FTIR studies

See figure 9.

DISCUSSION

Microspheres of Simvastatin with a coat consisting of sodium alginate and different mucoadhesive polymers - Sodium CMC, Methylcellulose, Carbopol 940P, HPMC K100M, Ethyl cellulose, in 1:1, with HPMC K100M, Carbopol 940P, Guar gum, Xanthan gum, Methyl cellulose in 1:2, with Guar gum, and Xanthan gum 1:3 could be prepared by the orifice-ionic chelation process.

Microspheres with a coat consisting of sodium alginate and a mucoadhesive polymer exhibited good mucoadhesive properties in the *in vitro* wash-off test. The microencapsulation efficiency was in the range of 57% to 96% being highest for F4 and lowest for F5. The result of *in vitro* wash-off test studies indicate that the formulation F10, having considerable mucoadhesive property[9].

From the all batches F10 (Drug: Sod. Alginate: Methyl cellulose = 1:2:1) batch is considered to be the most promising formulation batch because among all the batches it shows better extent of drug release 97.11% (8hrs), good entrapment efficiency (78%), and *in vitro* wash-off test shows the good mucoadhesive property. Simvastatin release from alginate – Methyl cellulose (F10) was slow and extended over a period of 8 hrs and these microspheres were found suitable for the oral controlled release formulation. The FTIR studies indicated the lack of drug – polymer interactions in the Optimized formulation (F10).

SEM studies

It was observed that the optimized formulation (F10) of the mucoadhesive microspheres was spherical and completely covered with the coat polymer. At higher magnification, pores were observed. The pores can influence the rate of release of the drug from the microspheres. (See figure 10).

SUMMARY AND CONCLUSION

The objective of the present study was to prepare and evaluate mucoadhesive microspheres of Simvastatin. The microspheres were prepared by the orifice-inotropic gelation method using polymers such as HPMC (K 100 M), Carbopol 940P, Sodium CMC, Guar gum, Sodium Alginate, Ethyl Cellulose, Methyl Cellulose, Xanthan gum and 10% Calcium Chloride solution[11].

The prepared batches of microsphere were evaluated for Micromeritic study such as tapped density, bulk density, Carr's index, Hausner ratio and angle of repose.

Microspheres with a coat consisting of sodium alginate and a mucoadhesive polymer exhibited good mucoadhesive properties in the *in vitro* wash-off test. The result of *in vitro* wash-off test studies indicate that the formulation F10, having considerable mucoadhesive property.

The FTIR studies indicated the lack of drug – polymer interactions in the Optimized formulation (F10). The SEM results indicated that the shape of Mucoadhesive microspheres was spherical and completely covered with the coat polymer.

Simvastatin release from the microspheres was studied in phosphate buffer (pH 7.0) for 8 hours. Drug release from the microspheres was slow and depended on the composition of the coat. From the all batches F10 (Drug: Sod. Alginate: Methyl cellulose = 1:2:1) batch is considered to be the most promising formulation batch because among all the batches it shows better extent of drug release 97.11% (8hrs), good entrapment efficiency (78%), and *in vitro* wash-off test shows the good mucoadhesive property. Simvastatin release from alginate – Methyl cellulose (F10) was slow and extended over a period of 8 hrs and these microspheres were found suitable for the oral controlled release formulation.

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Table 1. Composition of different formulations

Batch code	Coat composition	Ratio
F1	Drug: Sod. Alginate	1:1
F2	Drug: Sod. Alginate : Carbopol (940)	1:0.9:0.1
F3	Drug: Sod. Alginate : HPMC (K100M)	1:0.9:0.1
F4	Drug: Sod. Alginate : Sod.CMC	1:0.9:0.1
F5	Drug: Sod. Alginate : Ethyl cellulose	1:0.9:0.1
F6	Drug: Sod. Alginate : methyl cellulose	1:0.9:0.1
F7	Drug: Sod. Alginate	1:2
F8	Drug: Sod. Alginate : Carbopol (940)	1:2:1
F9	Drug: Sod. Alginate : HPMC (K100M)	1:2:1
F10	Drug: Sod. Alginate : Methyl cellulose	1:2:1
F11	Drug: Sod. Alginate : Xanthan gum	1:2:1
F12	Drug: Sod. Alginate : Guar gum	1:2:1

Table 2. Calibration curve of simvastatin at λ max 239nm

Concentration (µg/ml)	Absorbance
2	0.138
4	0.265
6	0.367
8	0.490
10	0.602
12	0.729
14	0.818
16	0.945

Table 3. Flow properties of different formulations

Formulation	Angle of repose	Bulk density(g/ml)	Tapped density (g/ml)	Hausner ratio	Compressibility index
F1	12	0.816	0.816	1	0
F2	14	0.672	0.717	1.06	6.2
F3	11	0.556	0.602	1.08	7.6
F4	12	0.692	0.721	1.04	4.02
F5	15	0.297	0.371	1.24	9.2
F6	13	0.656	0.772	1.17	7.8
F7	16	0.454	0.552	1.21	17.75
F8	19	0.772	0.821	1.06	5.96
F9	14	0.659	0.721	1.09	8.59
*F10	19	0.604	0.679	1.12	11.04
F11	18	0.721	0.869	1.20	17.03
F12	16	0.526	0.619	1.17	15.02

Table 4. Dissolution profile of mucoadhesive microspheres of simvastatin (F1, F2, F3)

Time (hrs)	Cumulative percent drug release (n = 3±SD)		
	F1	F2	F3
0.5	12.6 ± 2.0	21.42 ±1.00	10.46 ±2.48
1	35.42 ±3.2	34.68 ±1.25	21.27 ±1.2
2	50.55 ±1.21	64.73 ±1.34	36.3 ±7.34
3	80.04 ±1.65	75.91 ±1.9	69.26 ±8.7
4	88.68 ±3.47	91.67 ±1.30	101.8 ±2.8
6	108.4 ±2.02	102.18 ±0.93	---

Table 5. Dissolution profile of mucoadhesive microspheres of simvastatin (F4, F5, F6)

Time (hrs)	Cumulative percent drug release (n = 3±SD)		
	F4	F5	F6
0.5	12±1.8	13.7±2.2	22.5±0.9
1	22.86±5.52	16.87±0.67	49.28±5.8
2	55.6±5.3	28.37±7.17	83.86±3.06
3	71.46±1.22	42.22±7.65	89.74±1.92
4	97.89±1.48	48.39±4.19	107.82±1.35
6	106.67±1.88	54.78±4.84	----
8	----	58.21±3.84	----

Table 6. Dissolution profile of mucoadhesive microspheres of simvastatin (F7, F8, F9)

Time (hrs)	Cumulative percent drug release (n = 3±SD)		
	F7	F8	F9
0.5	13.65±4.56	32.79±2.51	12.45±1.58
1	40.27±3.03	42.42±1.59	31.69±4.34
2	56.16±3.67	65.94±1.73	58.89±2.52
3	63.54±5.75	91.39±0.99	72.41±1.87
4	84.24±4.2	102.59±1.56	88.58±5.8
6	105.75±6.76	----	108±1.73

Table 7. Dissolution profile of mucoadhesive microspheres of simvastatin (F10, F11, F12)

Time (hrs)	Cumulative Percent Drug Release (n = 3±SD)		
	F10	F11	F12
0.5	7.05±0.18	11.49±2.52	14.4±0.61
1	14.26±0.63	19.54±4.51	29.34±0.62
2	24.11±1.25	30.46±7.02	38.26±2.22
3	26.95±0.15	37.66±7.59	54.9±3.83
4	32.5±4.13	39.39±7.81	54.9±0.67
6	58.07±3.16	53.93±1.89	73.65±3.21
8	97.11±2.98	65.52±3.44	---

Table 8. Percent mucoadhesive property of the microspheres of simvastatin in ph 1.2 HCL buffer

Time (hr)	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
0.5	33	41	22	40	54	40	41	50	78	76	54	61
1	21	36	8	35	46	28	32	38	69	68	40	46
2	---	21	---	24	35	10	24	21	45	52	21	38
3	---	12	---	13	26	---	16	---	38	43	10	28
4	---	---	---	---	14	---	4	---	24	37	---	20
5	---	---	---	---	---	---	---	---	12	28	---	12
6	---	---	---	---	---	---	---	---	5	14	---	----
7	---	---	---	---	---	---	---	---	---	2	---	--
8	---	---	---	---	---	---	---	---	---	---	---	---

Table 9. Percent mucoadhesive property of the microspheres of simvastatin in ph 7.0 phosphate buffer

Time (hr)	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
0.5	44	51	48	30	56	52	28	54	70	78	56	64
1	20	36	31	29	38	44	18	42	54	69	42	54
2	---	14	27	---	29	13	---	34	40	60	32	38
3	---	---	---	---	13	---	---	12	28	55	26	38
4	---	---	---	---	---	---	---	---	18	43	15	24
5	---	---	---	---	---	---	---	---	10	39	8	---
6	---	---	---	---	---	---	---	---	---	26	---	----
7	---	---	---	---	---	---	---	---	---	8	---	---
8	---	---	---	---	---	---	---	---	---	--	---	---

Table 10. Quality control parameters of mucoadhesive microspheres of simvastatin

S. No	Batch code	Drug content		Encapsulation efficiency
		Theoretical (percentage)	Practical (percentage)	
1	F1	50	39.70	79.40±0.025
2	F2	50	42.02	84.05±0.027
3	F3	50	39.03	78.07±0.027
4	F4	50	48.33	96.67±0.02
5	F5	50	28.73	57.47±0.012
6	F6	33.33	26.24	78.73±0.013
7	F7	25	19.14	76.57±0.032
8	F8	25	17.47	69.91±0.013
9	F9	25	18.60	74.40±0.017
10	F10	25	19.37	77.51±0.025
11	F11	25	18.10	69.64±0.019
12	F12	20	14	70.0±0.014

Figure 1. The diagrammatic representation of *In vitro* wash off test

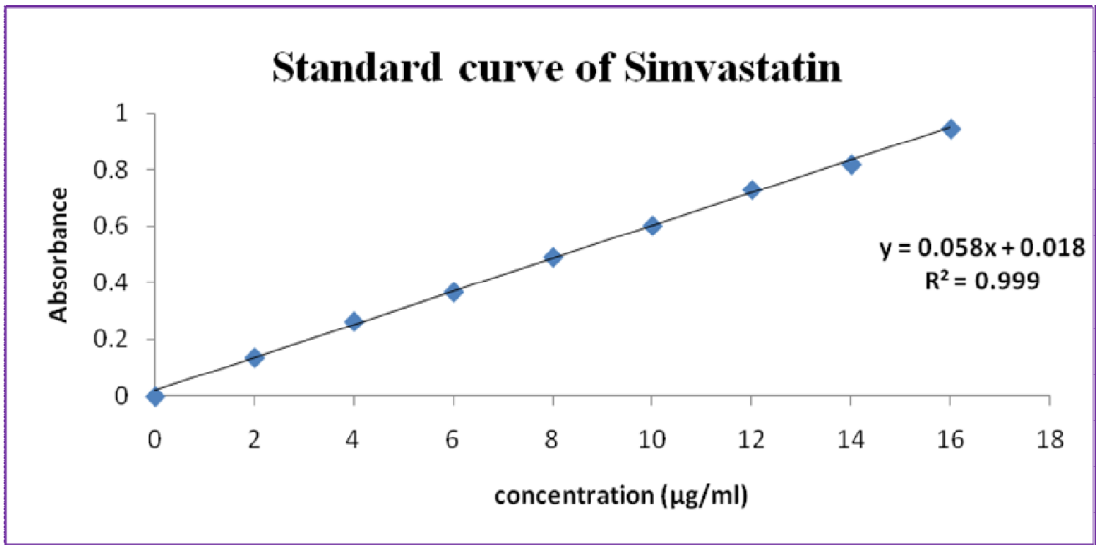

Figure 2. Calibration curve of Simvastatin in pH 7.0 buffer with 0.5% SLS

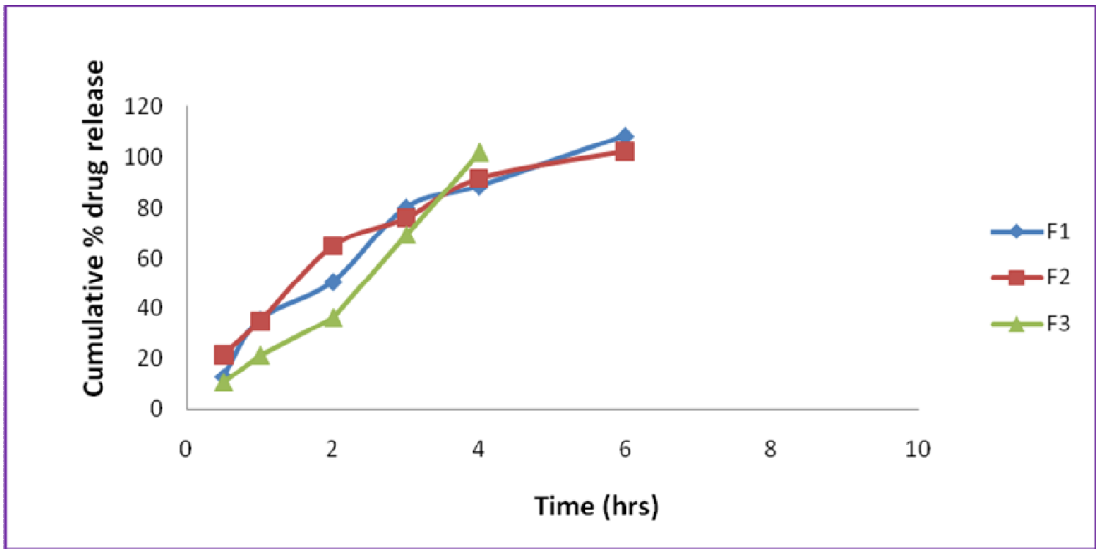

Figure 3. Dissolution profile of mucoadhesive microspheres of simvastatin (F1, F2, F3)

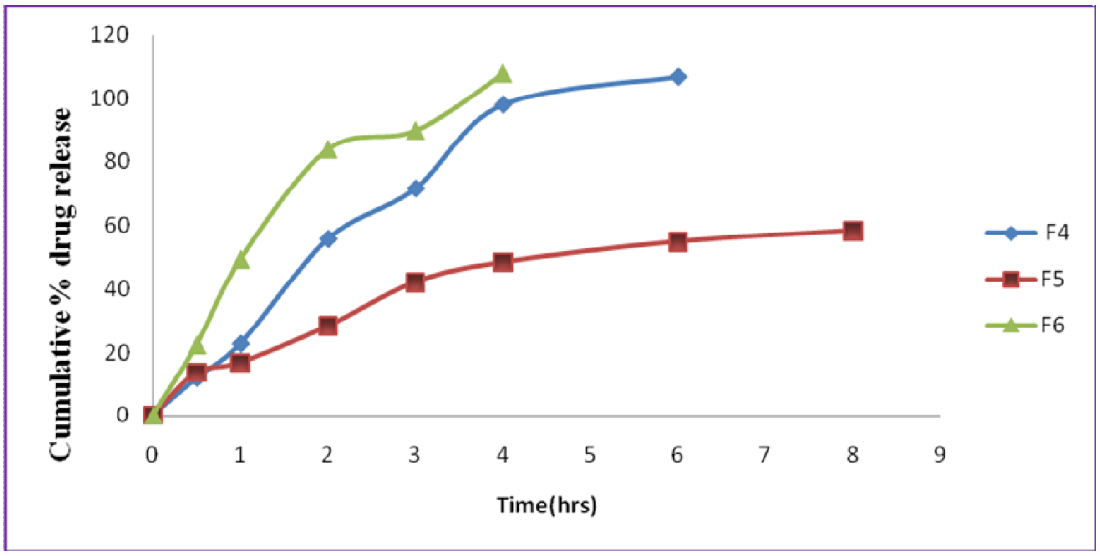

Figure 4. Dissolution profile of mucoadhesive microspheres of simvastatin (F4, F5, F6)

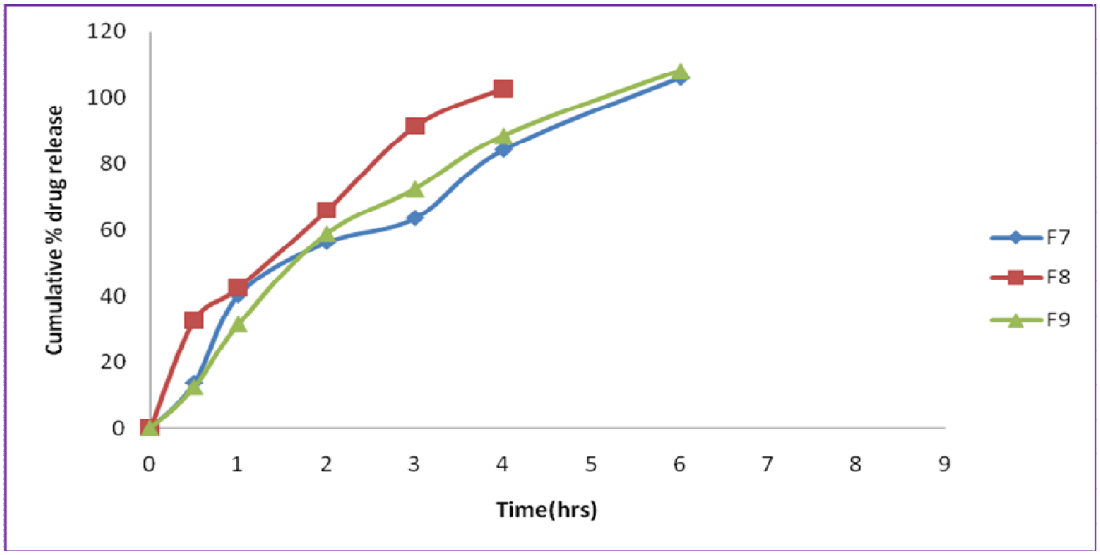

Figure 5. Dissolution profile of mucoadhesive microspheres of simvastatin (F7, F8, F9)

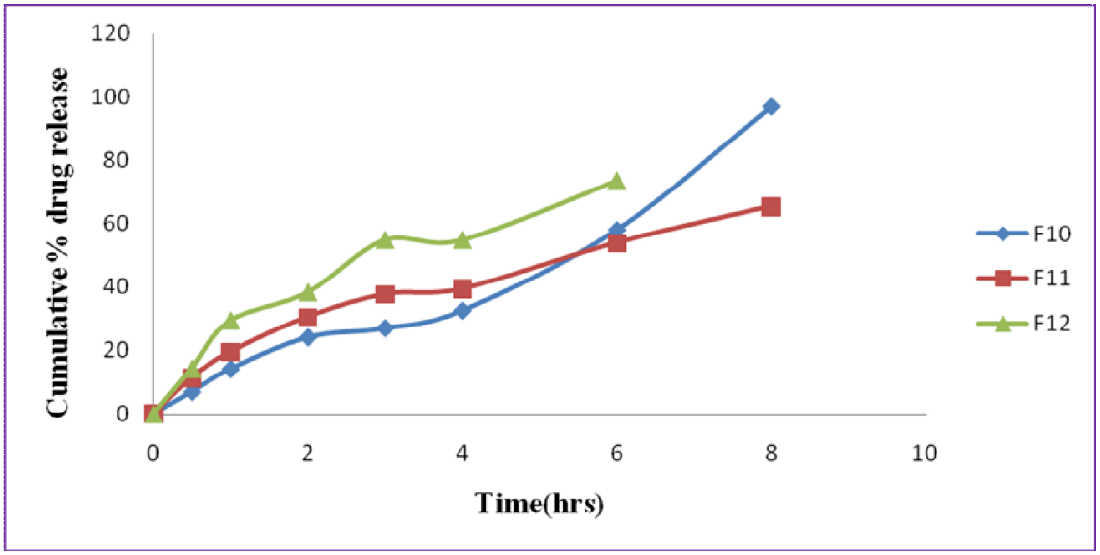

Figure 6. Dissolution profile of mucoadhesive microspheres of simvastatin (F10, F11, F12)

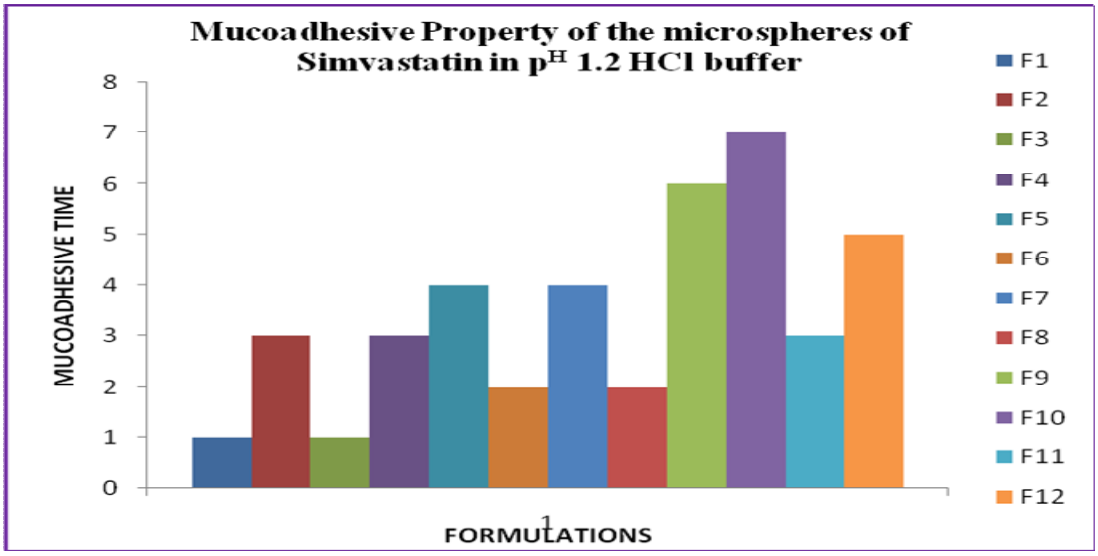

Figure 7. Mucoadhesive Property of different formulations in p^H 1.2 HCl buffer

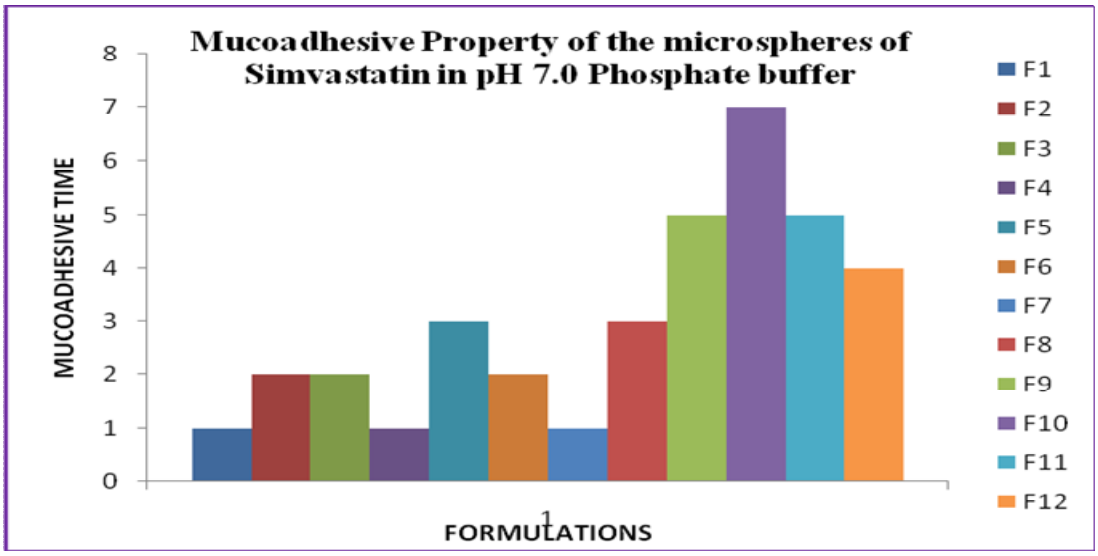

Figure 8. Mucoadhesive Property of different formulations in pH 7.0 phosphate buffer

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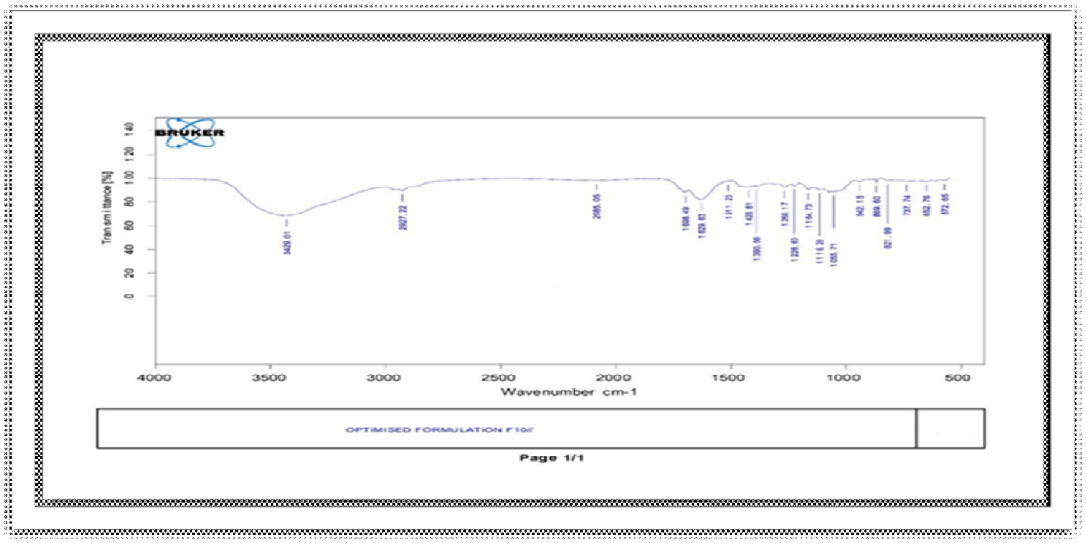

Figure 9. FTIR spectrum of optimized formulation

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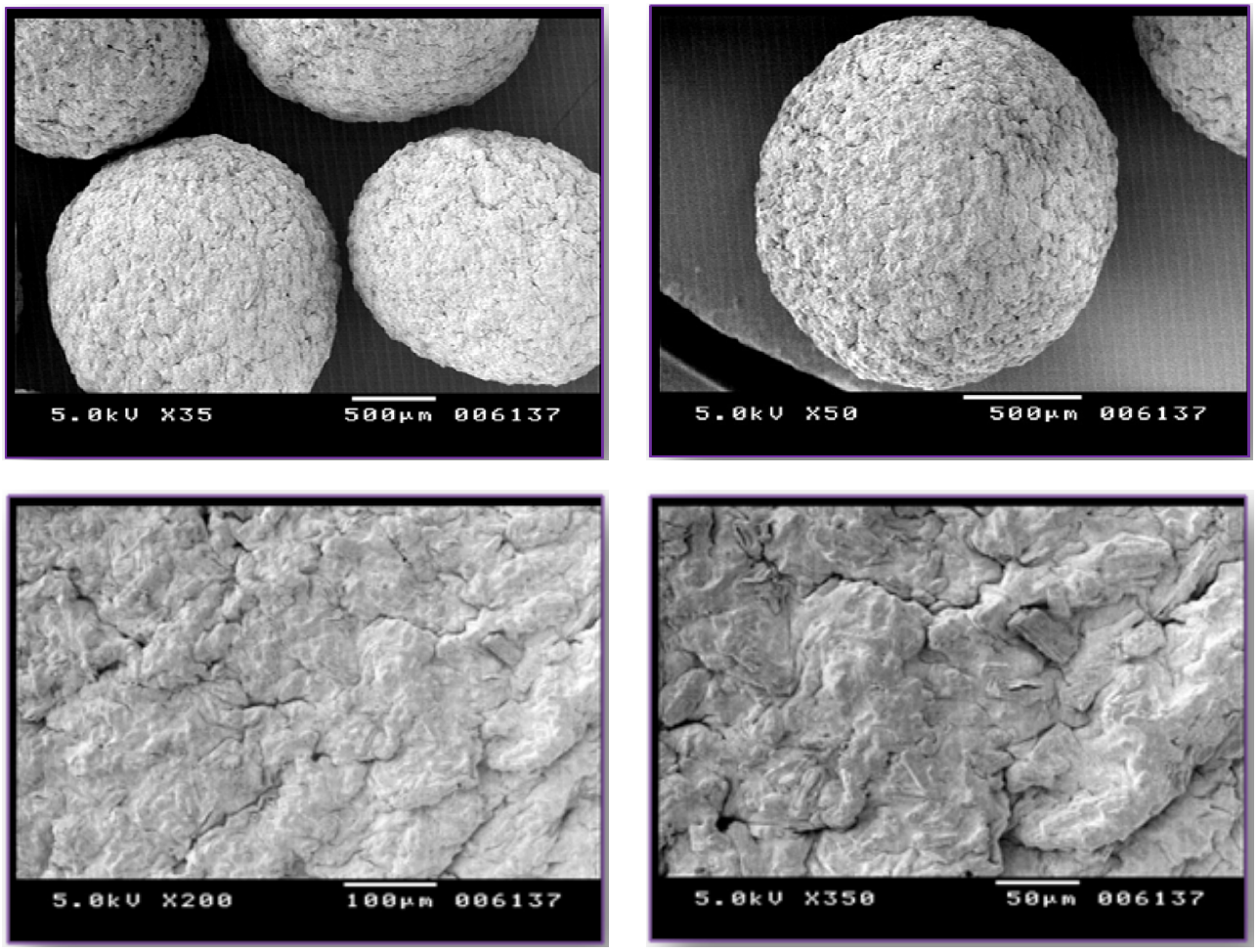

Figure 10. SEM images of optimized formulation (F10)