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FORMULATION AND CHARACTERIZATION OF MUCOADHESIVE MICROSPHERES USING VERAPAMIL HYDROCHLORIDE AS MODEL DRUG

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Keywords:

Verapamil hydrochloride, Mucoadhesive Microspheres, Emulsification-internal gelation technique, Barium carbonate, *in vitro* wash off test, Non-fickian release

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ABSTRACT

The objective of the current investigation is to reduce dosing frequency and improve patient compliance by designing and systematically evaluating sustained release microspheres of verapamil. Frequent administration and variable low bioavailability (40-60%) after oral administration are problems of conventional dosage forms of verapamil 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. Verapamil-loaded mucoadhesive microspheres were successfully prepared by emulsification-internal gelation technique with a maximum incorporation efficiency of 93.29±0.26%. The scanning electron microscopic study indicated that the microspheres were spherical in shape and the drug remained dispersed in the polymer matrix at amorphous state, which was further confirmed by x-ray diffraction analysis. The *in vitro* wash-off test indicated that the microspheres had good mucoadhesive properties. The wash-off was faster at simulated intestinal fluid (phosphate buffer, pH 7.4) than that at simulated gastric fluid (0.1 M HCl, pH 1.2). The *in vitro* drug release mechanism was non-fickian type controlled by swelling and relaxation of polymer. There was no significant change in drug content and cumulative drug release of drug-loaded microspheres stored at different storage condition after 8 weeks of study.

INTRODUCTION: Verapamil hydrochloride, a calcium channel blocker, is widely used for the treatment of angina pectoris, hypertension and arrhythmias. It is administered orally (tablets, capsules, sustained release tablets/capsules) and parenterally (intravenous). The usual dose of verapamil is 200-280 mg/day.

The conventional tablet and capsule is administered 3 or 4 times a day due to its short biological half-life of about 2-5 hr. The problems of frequent administration and variable low bioavailability (40-60%) after oral administration of conventional tablet or capsules have been attenuated by designing verapamil in the form of sustained release tablet or capsules. The sustained release forms are administered two to four times a day due to its limited residence time in the gastrointestinal tract.

The mucoadhesive microcapsules of verapamil 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 previous studies reported the mucoadhesive drug delivery systems of verapamil in the form of tablets for oral route and transdermal patches; however, there is no report on mucoadhesive microcapsules.

Therefore, the objective of the present study was the development and evaluation of gastroretentive microspheres containing verapamil hydrochloride using various mucoadhesive polymers for prolonged gastrointestinal absorption. An attempt was also made to develop microspheres with high entrapment efficiency.

The method of microencapsulation is based on emulsification-internal gelation technique involving alginate polymers alone and/or in combination with other mucoadhesive polymers. The method was successfully used to encapsulate labile biological materials such as DNA and proteins for immobilization. Encapsulation of bioactive compounds, such as proteins and DNA, by either internal gelation or external gelation revealed that internal gelation provides a better encapsulant protection from hydrolysis but may permit higher losses from microspheres.

Verapamil hydrochloride is a water-soluble cardiovascular drug. The use of external gelation process of microencapsulation involving alginate polymers in the aqueous cross-linking agent would minimize the entrapment efficiency due to the diffusion of verapamil hydrochloride into the aqueous phase during the curing of the gel beads. The other inconveniences include the limitation in reducing microspheres diameter, the teardrop shape of the microparticles produced and difficulty in industrial scale-up. The emulsification-internal gelation techniques of microencapsulation use an external oil phase and thereby may reduce the drug diffusion during encapsulation process and improve the drug entrapment efficiency. The gel bead diameter can be easily controlled and that has scale-up potential.

MATERIALS AND METHODS: The following chemicals and solvents were used: verapamil hydrochloride (a gift sample from Torrent Research Centre, Gandhinagar), sodium alginate, (hydroxypropyl) methyl cellulose (HMPC) and carbopol 934P (Loba Chemie Pvt. Ltd., Mumbai), barium carbonate, chloroform, hydrochloric acid and glacial acetic acid (Ranbaxy Fine Chemicals, Chandigarh), light liquid paraffin, Span 80 (Central Dug House, New Delhi), sodium hydroxide pellets (Qualigens Fine Chemicals, New Delhi), potassium dihydrogen phosphate (Merck Ltd. Mumbai). All the solvents and chemicals used were of analytical grade satisfying pharmacopoeial standards.

Formulation of microspheres: Microspheres containing verapamil hydrochloride were prepared employing sodium alginate alone and in combination with HPMC and carbopol 934 P. The homogeneous polymer(s) solution was prepared in distilled water stirred magnetically with gentle heat. The drug and cross-linking agent were added to the polymer solution and mixed thoroughly by stirring magnetically to form a viscous dispersion which was then extruded through a syringe with a needle of size no. 23 into light liquid paraffin containing 1.5% span 80 and 0.2% glacial acetic acid being kept under magnetic stirring at 100 rpm.

The microspheres were retained in the light liquid paraffin for 30 min to produce rigid discrete particles. They were collected by decantation and the product thus separated was washed with chloroform to remove the traces of paraffin oil. The microspheres were dried at 40^{o}C under vacuum for 12 h. The compositions of the microspheres formulations are listed in **Table 1**.

TABLE 1: COMPOSITION OF VERAPAMIL HYDROCHLORIDE-LOADED VARIOUS MUCOADHESIVE MICROSPHERES FORMULATIONS

F. N. Codes	Polymer level (% w/v)	Drug level (%w/w)	Cross-linking agent/level (%w/w)
FA	Sodium alginate, 2%	5	BaCO3 / 6%
FB	Sodium alginate, 3%	5	BaCO3 / 6%
FC	Sodium alginate, 4%	5	BaCO3 / 6%
FD	Sodium alginate, 2% + HPMC 1%	5	BaCO3 / 6%
FE	Sodium alginate, 3% + HPMC 1%	5	BaCO3 / 6%
FF	Sodium alginate, 4% + HPMC 1%	5	BaCO3 / 6%
FG	Sodium alginate, 2% + Carbopol 1%	5	BaCO3 / 6%
FH	Sodium alginate, 3% + Carbopol 1%	5	BaCO3 / 6%
FI	Sodium alginate, 4% + Carbopol 1%	5	BaCO3 / 6%

Assay of verapamil hydrochloride Verapamil content in the microcapsules was estimated by a UV spectrophotometric method based on the measurement of absorbance at 235 nm in phosphate buffer of pH 7.4. The method was validated for linearity, accuracy and precision. A linear relationship was obtained between the concentration ranges of 5 & 25 mg/mL. The linear regression equation of the calibration curve was: Absorbance = concentration × 0.0603 ~ 0.0287 (R2 = 0.999). The very low value of intercept indicates its negligible deviation from the origin. The low value of mean standard error (0.4%) and relative standard deviation (0.5%) confirmed the accuracy and precision of the method.

Characterization of Microsphere:

Incorporation efficiency: The amount of verapamil present in the microspheres was determined by extracting the drug into phosphate buffer of pH 7.4 under magnetic stirring for a period of 2 hr. The solution was filtered through Whatman's filter paper no.5, suitably diluted and estimated for drug content spectrophotometrically at 235 nm using Hitachi U-2001 UV-VIS spectrophotometer.

The incorporation efficiency was calculated by the following formula:

Incorporation efficiency (%) = Experimental drug content × 100 / Theoretical drug content.

Size distribution of Microspheres: The size of the microspheres was determined using an optical microscope (Olympus, Japan) fitted with an ocular micrometer. The ocular micrometer was calibrated with a stage micrometer. The mean diameter reported was obtained from a total of more than 100 microspheres.

Scanning Electron Microscopy (SEM): The shape and surface morphology of the drug loaded microspheres were investigated by using JEOL, JSM-6360, scanning electron microscope at 20 kV. Prior to examination samples were gold coated under vacuum (Fine coat, ion sputter, JFC- 1100) to render them electrically conductive. The surface morphology of the microspheres collected after dissolution studies were also investigated.

Mucoadhesive property of Microspheres: The mucoadhesive property of the microspheres was evaluated employing the method described by Lehr et al. with slight modification. The mucoadhesiveness of microspheres was compared with that of a non0bioadhesive material, ethyl cellulose microspheres. The test was performed at both simulated gastric fluid (0.1 M HCl, pH 1.2) and simulated intestinal fluid (phosphate buffer, pH 7.4). The freshly excised pieces of intestinal mucosa (2×3 cm) from goat were mounted onto glass slides (3×1 inch) with cyanoacrylate glue. About 50 microspheres were spread onto each wet rinsed tissue specimen and immediately thereafter the slides with suitable support were hung onto the arm of a USP tablet disintegrating test machine. When the disintegrating test machine was operated, the tissue specimen was given a slow, regular up and down movement in the test fluid at 37^{O}C contained in a 1-liter vessel. At different time intervals up to 8 h the machine was stopped and the number of microspheres still adhering to the tissue was counted.

X-ray diffraction Analysis: The x-ray diffractometry was carried out to investigate the effect of microencapsulation process on crystalinity of the drug. The study was carried out using an x-ray diffractometer (Seifert, Model 3000P, Germany). The pure verapamil hydrochloride powder, blank and drug-loaded microspheres in powder form were scanned from 10^{O} to 60^{O} diffraction angle (2q) range under the following measurement conditions: source, nickel filtered Cu-Ka radiation; voltage, 35 kV; current, 25 mA; scan speed, 0.05 min-1.

***In vitro* Release Study:** The United States Pharmacopoeia basket-type dissolution rate test apparatus was used for all the in vitro release studies. An accurately weighed quantity of the microspheres (100 mg) was suspended in 900 ml of phosphate buffer of pH 7.4. The dissolution medium was stirred at 50 rpm and maintained at constant temperature (37±0.5^{o}C). At preset time intervals, 5 mL aliquots were withdrawn and replaced by an equal volume of fresh pre-warmed dissolution medium maintaining sink condition throughout the experiment. After suitable dilution, the samples were analyzed for drug content at 235 nm using Hitachi U-2001 UV-VIS spectro-photometer.

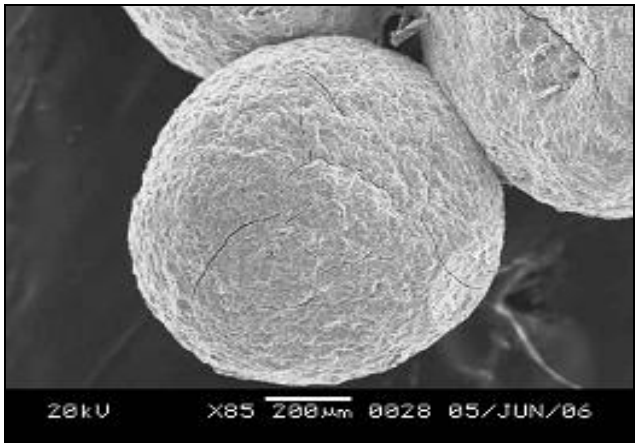
The concentration of verapamil in samples were calculated using regression equation of the calibration curve of verapamil in phosphate buffer of pH 7.4 and corrected to compensate the loss due to sample withdrawal, using the equation proposed by Hayton and Chen.

Accelerated Stability study: The drug-loaded microspheres were stored at various storage conditions (room temperature, 37^{o}C and 45^{o}C/75% RH) in airtight sealed vials. The drug content of the microspheres was determined at regular time intervals and the drug release profiles were studied at 0 and 60 days.

RESULTS AND DISCUSSION: Verapamil-loaded mucoadhesive microspheres were prepared by emulsification-internal gelation technique. Verapamil hydrochloride, a hydrophilic drug, can partition out into the aqueous processing phase during the preparation of microspheres by external gelation method. Depending on the processing conditions as much as 80 to 90% of the drug can partition out into the external aqueous processing medium. In this study attempt was made to encapsulate verapamil hydrochloride with sufficiently high encapsulation efficiency. An external oil phase (liquid paraffin) was used as the harvesting medium with the expectation that for verapamil hydrochloride it would be non0favorable to diffuse out of the microspheres before they form as rigid and discrete particles. The emulsification-internal gelation technique use an oil soluble acid (0.2% glacial acetic acid) in the external oil phase, which diffuse through the oil-water interface into the polymeric dispersed globules containing barium carbonate, resulting in the release of free Ba^{2+}.

The sodium ion (Na^{+}) of alginate is exchanged with Ba^{2+} initiating gelation reaction to form barium alginate gel beads. Verapamil-loaded mucoadhesive microspheres composed of alginate alone and in combination with HPMC and carbopol were prepared by the emulsification- internal gelation technique. The microspheres were found to be discrete, spherical, free flowing and of the monolithic matrix type. The microspheres were uniform in size with a mean size range of 807.95±6.5 to 1007.86±11.6 mm which fall in the arbitrary particle size range of 5 ~ 5000 mm. The particle size ranges are shown in **Table 2**. The size of the microspheres was in increasing trend with increasing the alginate concentration. This may be due to the increase in viscosity, which in turn increases in droplet size during addition of the polymer dispersion to the harvesting medium. The use of oil soluble surfactant (Span 80) permits the remarkable reduction in size of alginate gel beads as the result of decreasing the interfacial tension and preventing the droplets coalescence.

The SEM photomicrographs (**Figure 1**) indicated that the microspheres were spherical in shape having particle size of 200 mm and the drug remained dispersed in the polymer matrix at amorphous state. **Table 2** and SEM photomicrographs in Figure 1 reveal that the mean microspheres size as observed by optical microscope is significantly higher than that observed under scanning electron microscope. It might be explained by the fact that the incompletely dried microspheres (remaining at swollen state) were observed under optical microscope, whereas the microsphere particles were fully dried when SEM study was performed.



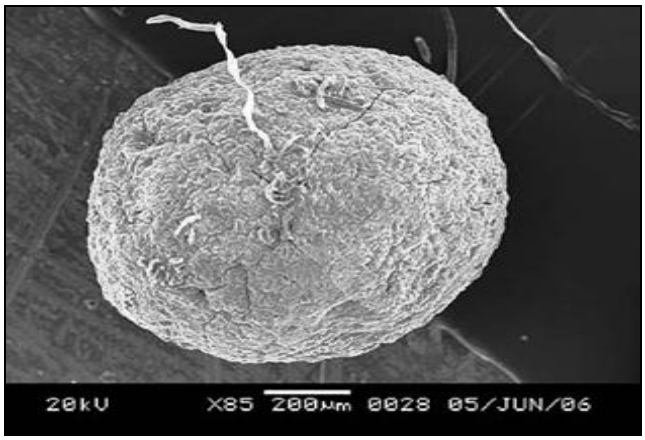


FIG. 1: SEM PHOTOMICROGRAPH OF DRUG-LOADED
(a) sodium alginate microsphere, (b) sodium alginate-HPMC microsphere, (c) sodium alginate-carbopol 934 microsphere

TABLE 2: INCORPORATION EFFICIENCY AND PARTICLE SIZE OF VERAPAMIL HYDROCHLORIDE-LOADED MICROSPHERES

F. N. Codes	Incorporation efficiency (mean ± SD) mm	Particle size (mean ± SD) mm
FA	63.16 ± 0.40	807.95 ± 6.5
FB	79.31 ± 0.57	876.30 ± 9.0
FC	83.79 ±0.46	1007.86 ± 11.6
FD	81.46 ± 0.30	851.00 ± 9.3
FE	82.57 ± 0.29	882.30 ± 4.6
FF	93.29 ± 0.26	913.00 ± 4.66
FG	70.80 ± 0.40	884.59 ± 7.0
FH	68.00 ± 0.35	940.60 ± 3.7
FI	87.70 ± 0.10	908.47 ± 4.2

The effects of alginate concentrations and polymer compositions on the drug incorporation efficiency of microspheres are shown in **Table 2**. The highest incorporation efficiency (93.29±0.26%) was achieved with 4% w/v sodium alginate in combination with 1% HPMC, which is followed by 4% w/v sodium alginate in combination with 1% w/v carbopol (loading efficiency 87.70±0.10%). Three different concentrations of sodium alginate (2%, 3% and 4%) were used.

The higher incorporation efficiency was observed as the concentration of alginate increased. This may be attributed to the greater availability of active barium binding sites in the polymeric chains and consequently the greater degree of cross linking as the quantity of sodium alginate increased, resulting in the formation of nonporous microspheres. The drug loading efficiency greatly improved when alginate was blended with carbopol at 1% level.

Mucoadhesive property of Microspheres: The microspheres consisting of sodium alginate alone and in combination with HPMC and carbopol exhibited good mucoadhesive properties as observed in *in vitro* wash-off test when compared to a non-mucoadhesive polymer, ethyl cellulose microspheres. The wash off was slow in the case of microspheres consisting of alginate-mucoadhesive polymers when compared to that of ethyl cellulose microspheres (**Table 3**). The wash-off was faster at simulated intestinal pH (7.4) than that at simulated gastric pH (1.2). Robinson *et al*., reported that the solubility, hydration and mucoadhesivity of the polymers depend on the pH of the medium. The rapid wash-off observed at simulated intestinal pH may be due to the ionization of carboxyl acid group and other functional groups in the polymers, which increase their solubility and reduce adhesive strength.

TABLE 3: RESULTS OF *IN-VITRO* WASH OFF OF VERAPAMIL HYDROCHLORIDE LOADED MICROSPHERE

F. Code	Percentage of microsphere adhere to mucosa of goat intestine ±SD (N=3)															
	Time (in Hrs)															
	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8
	In 0.1 M HCl (pH 1.2)								In Phosphate buffer (pH 7.4)							
FA	100	100	100	98±0.23	93±0.26	90±0.13	84±0.18	76±0.52	100	98±0.18	96±0.29	92±0.49	84±0.54	82±0.83	78±0.21	72±0.76
FB	100	92±0.27	84±0.51	76±0.86	70±1.0	64±0.72	62±0.69	56±0.74	100	100	94±0.67	88±0.45	80±0.77	74±0.85	64±0.38	66±0.73
FC	100	100	100	96±0.31	94±0.83	86±0.27	86±0.52	85±0.26	100	100	100	100	100	100	91±0.83	73±0.35
FD	100	88±0.37	96±0.91	96±0.86	90±0.71	84±0.09	84±82	82±.59	100	98±0.28	96±0.99	86±0.31	80±0.83	81±0.74	78±0.98	74±0.61
FE	94	90±0.29	90±0.25	90±0.19	86±0.72	88±0.84	80±0.29	72±0.57	100	95±0.38	94±0.53	94±0.82	94±0.89	94±0.29	92±0.51	86±0.46
FF	100	96±0.38	90±0.97	91±0.89	81±0.86	82±0.48	78±0.37	74±0.98	100	100	100	100	100	100	97±0.59	88±0.47
FG	98	98±0.91	98±0.87	96±0.28	92v0.37	83v0.78	81±0.39	80±0.33	100	98±0.32	98±0.18	95±1.06	94±0.88	92±0.41	88±0.39	84±0.31
FH	100	100	100	98±0.39	93±0.73	91±0.81	90±0.89	83±0.25	100	100	96±0.59	92±0.62	90±0.89	88±0.26	84±0.96	83±0.82
H	100	100	98	96	94	92	86	83	98	92	92	90	92	92	90	78
EC	85	54	11	2	0	0	0.2	0	-	-	-	-	-	-	-	-

The results of the wash-off test indicated that the microspheres had fairly good mucoadhesive properties. Our result is supported by the report of Chowdary and Rao who used the microcapsules of glipizide with a coat consisting of alginate and a mucoadhesive polymer ñ sodium carboxymethyl cellulose, methyl cellulose, carbopol and (hydroxypropyl) methyl cellulose. The mucoadhesive behaviours of various microsphere formulations are shown in **Figures 2 & 3**. The developed mucoadhesive microspheres would adhere to the GI walls, thus resisting gastric emptying.

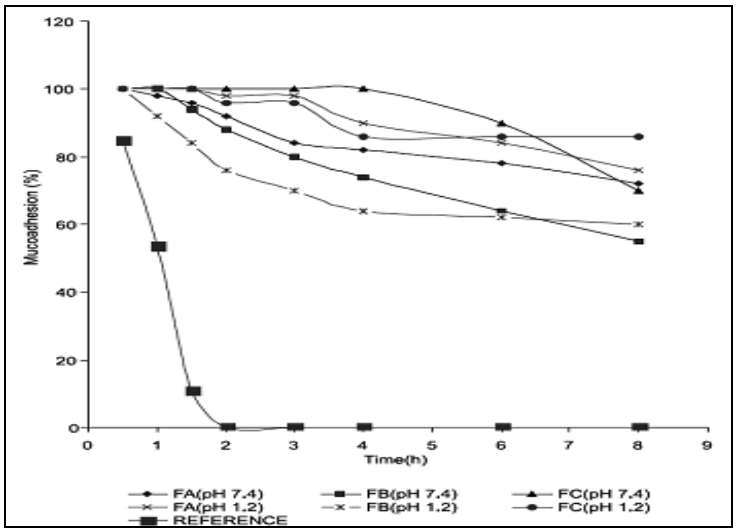


FIG. 2: MUCOADHESION BEHAVIOR OF MICROSPHERE FORMULATIONS (FA n FC) IN 0.1 M HCl, (pH 1.2) AND PHOSPHATE BUFFER, (pH 7.4) MEDIUM

It would ensure the prolong residence time at the absorption site to facilitate intimate contact with the absorption surface and thereby improve and enhance the bioavailability.

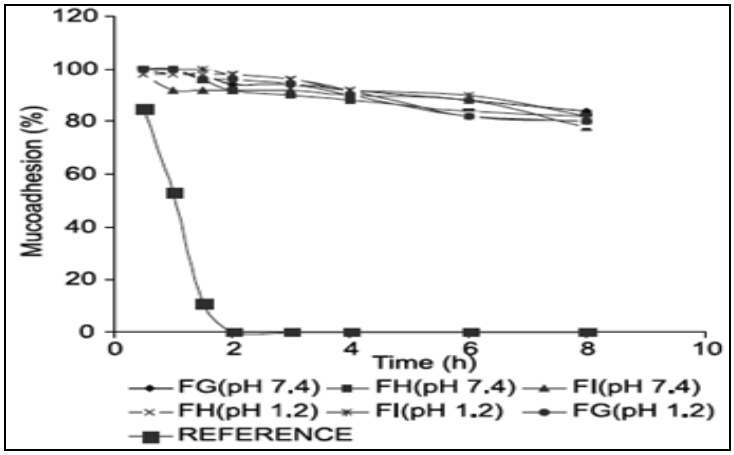


FIG. 3: MUCOADHESION BEHAVIOR OF MICROSPHERE FORMULATIONS (FG-FI) IN 0.1 M HCl, (pH 1.2) AND PHOSPHATE BUFFER, (pH 7.4) MEDIUM

***In vitro* drug release:** The *in vitro* drug release studies were carried out in the simulated gastric fluid (0.1 M HCl, pH 1.2) and simulated intestinal fluid (phosphate buffer, pH 7.4). The microspheres were prepared by ionic internal gelation technique using $BaCO_3$ as cross linking agent. The microspheres cross-linked with Ba^{2+} showed delay in disintegration and consequently a slow release of drug was obtained. It can be explained with the fact that the large size of barium ions (1.74) produced hard and nonporous microspheres.

Therefore, the exchange of Ba^{2+} ions in the microspheres with Na^{+} ions of the phosphate buffer and their removal as insoluble barium phosphate was obstacle and attributed as delayed swelling of the microspheres and slow release. Our results are in good agreement with the report of Das and Senapati who used the alginate microspheres containing furosemide prepared by the ionic external gelation technique using $BaCl_2$. Sodium alginate at three different concentrations (2%, 3% and 4% w/v) alone and in combination with 1% w/v of HPMC and/or carbopol 934 P was utilized for the preparation of microspheres. The drug release behaviors are shown in the **Figures 4 & 5**.

It was observed that the amount of drug release decrease with an increase in the concentration of sodium alginate. It can be attributed to an increase in the densities of the polymer matrix resulting in larger microspheres and this in turn increase the diffusion path length, which the drug molecules have to be traverse. It was observed that alginate microspheres had swollen more in phosphate buffer of pH 7.4 than in 0.1 M HCl (pH 1.2).

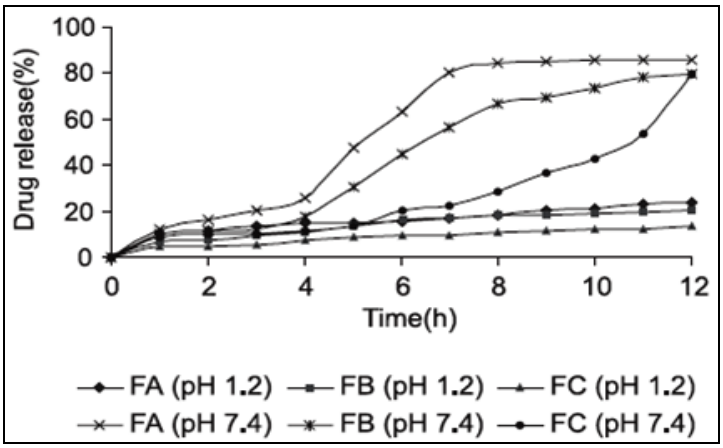


FIG. 4: EFFECT OF CONCENTRATION OF POLYMER ON DRUG RELEASE PATTERN OF MICROSPHERES IN 0.1 M HCl, (pH 1.2) AND IN PHOSPHATE BUFFER, (pH 7.4) FOR THE FORMULATIONS FA, FB AND FC

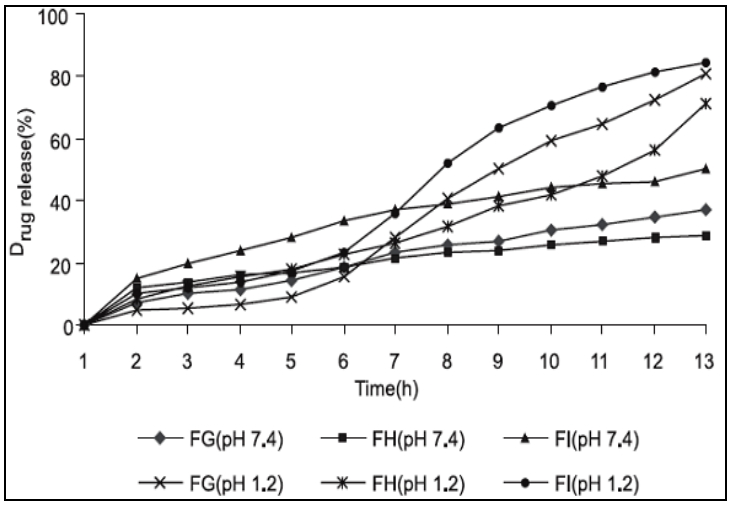


FIG. 5: EFFECT OF CONCENTRATION OF CROSS-LINKING AGENT ON DRUG RELEASE PATTERN OF MICROSPHERES IN 0.1 M HCl, (pH 1.2) AND IN PHOSPHATE BUFFER, (pH 7.4) FOR THE FORMULATIONS FG, FH AND FI

Therefore, the release would depend on diffusion of verapamil through the insoluble matrix of alginate polymer in 0.1 M HCl and a sustained drug release behavior was observed. In contrast, swelling and erosion of the microspheres prepared from alginate polymer was observed in phosphate buffer of pH 7.4. Slow erosion of barium cross-linked alginate microspheres could occur through slight degradation of alginate backbone into smaller fragments. In addition, the exchange of Ba^{2+} ions in the microspheres with Na+ ions of the phosphate buffer causes the sustained erosion of the microspheres, which greatly increase the drug release rate in phosphate buffer of pH 7.4.

To retard or sustain the drug release from the microspheres, (hydroxypropyl) methyl cellulose and carbopol 934P were blended with the alginate matrix. The microspheres retained their spherical shape after dissolution experiment in 0.1 M HCl. The microspheres composed of only sodium alginate converted to gel form after dissolution experiment in phosphate buffer of pH 7.4, while the microspheres composed of sodium alginate along with HPMC and/or carbopol lose their spherical shape after dissolution experiment.

The microspheres were collected from the dissolution medium and dried at 40^{O}C under vacuum for 12 h. The microspheres samples were then completely dried under vacuum and gold coated before performing the SEM study. The surface of the microspheres after dissolution experiment showed pores (SEM photographs, **Figure 6A, 6B**) suggesting that the drug was released through these pores and the mechanism of drug release was diffusion-controlled.

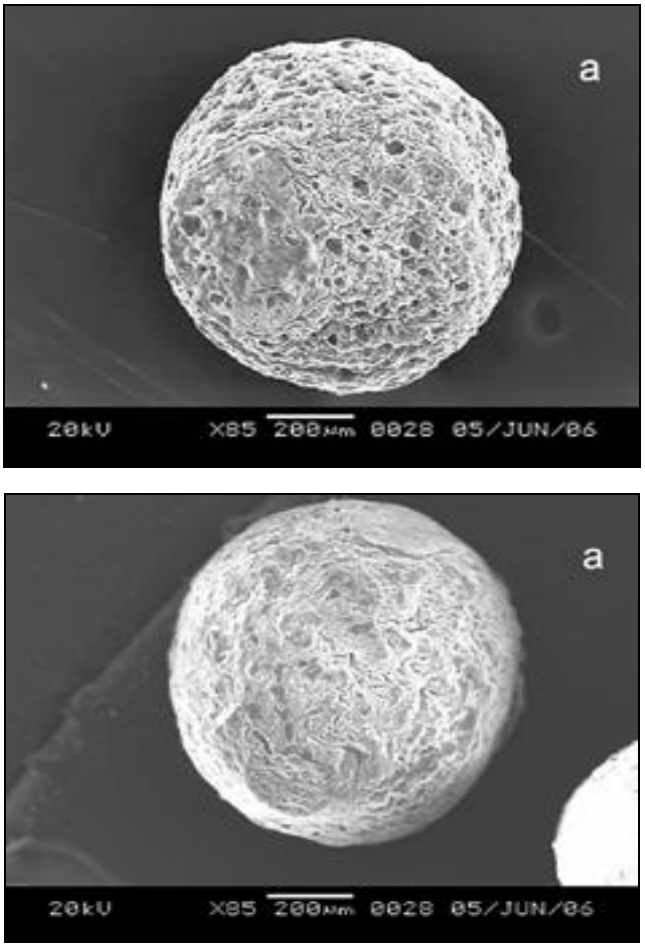


FIG. 6A: SEM PHOTOMICROGRAPHS OF DRUG-LOADED SODIUM

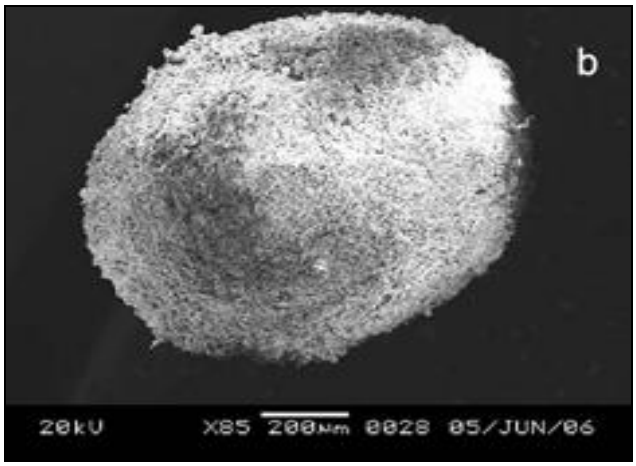


FIG. 6B: SEM PHOTOMICROGRAPHS OF DRUG-LOADED SODIUM ALGINATE CARBOPOL 934 P MICROSPHERES

(a) after dissolution alginate-HPMC microspheres; (a) after dissolution in 0.1 M HCl of pH 1.2 and; (b) after dissolution in HCl of pH 1.2 and; (b) after dissolution in phosphate buffer phosphate buffer of pH 7.4

The size range of the microsphere samples was smaller than the size range of the microspheres listed in Table 2. The reason has been discussed during physical characterization of the microspheres. In order to understand the mode of release of drug from swellable matrices, the release data were fitted to the following power law equation:

$$Mt / M\mu = Ktn$$

Where Mt and Mμ are the amounts of drug released at time t and the overall amount released, respectively, K is the release constant and *n* is the release exponent indicative of the release mechanism. The value for *n* is ≤ 0.45 for fickian release, > 0.45 and < 0.89 for non-fickian release, 0.89 for case II release and > 0.89 for super case II type release. The values of *n* and the coefficient of determination (r2) obtained are listed in **Table 4**.

TABLE 4: VALUES FOR RELEASE EXPONENT (*N*) AND COEFFICIENT FOR DETERMINATION (r^2) DERIVED FROM Mt / Mμ = Ktn kinetic model

F. N. Codes	n	r^2
FA	0.528	0.929
FB	0.896	0.960
FC	0.670	0.998
FD	0.780	0.885
FE	0.782	0.910
FF	0.552	0.964
FG	0.641	0.917
FH	0.880	0.897

The values of *n* fell within the range of 0.528 to 0.896, indicating non-fickian type release. This kind of release is the characteristics of swelling-controlled system in which the rate of solvent uptake into a polymer is largely determined by the rate of swelling and relaxation of the polymer chains. It is assumed that the drug molecules diffuse out through a dissolving gellike layer formed around the drug during the dissolving process. Kulkarni *et al*., observed the same type of release behavior of neem seed oil from alginate beads cross-linked with glutaraldehyde. Das and Senapati also observed the non-fickian type release behavior of furosemide from alginate microspheres cross-linked with Ca^{2+}.

X-ray Diffraction Analysis: The x-ray powder diffraction pattern of pure verapamil hydrochloride, blank microspheres and drug loaded microspheres are shown in **Figure 7**. The diffractograms of the drug-loaded microspheres revealed the presence of amorphous verapamil since there was a dramatic decrease of the intensity of the signal as compared to the diffractograms of the pure verapamil. The SEM photomicrograms of the drug loaded microspheres also indicated that the drug was dispersed at amorphous state in the polymer matrices.

Stability Studies: As described in **Table 5**, there was no significant change in drug content of drug-loaded microspheres, stored at room temperature, 37°C and 45°C/75% RH, after 8 weeks of study. The cumulative release of verapamil from microspheres stored at different storage conditions during weeks 0 and 8 showed that there was no significant effect of temperature of storage on the drug release.

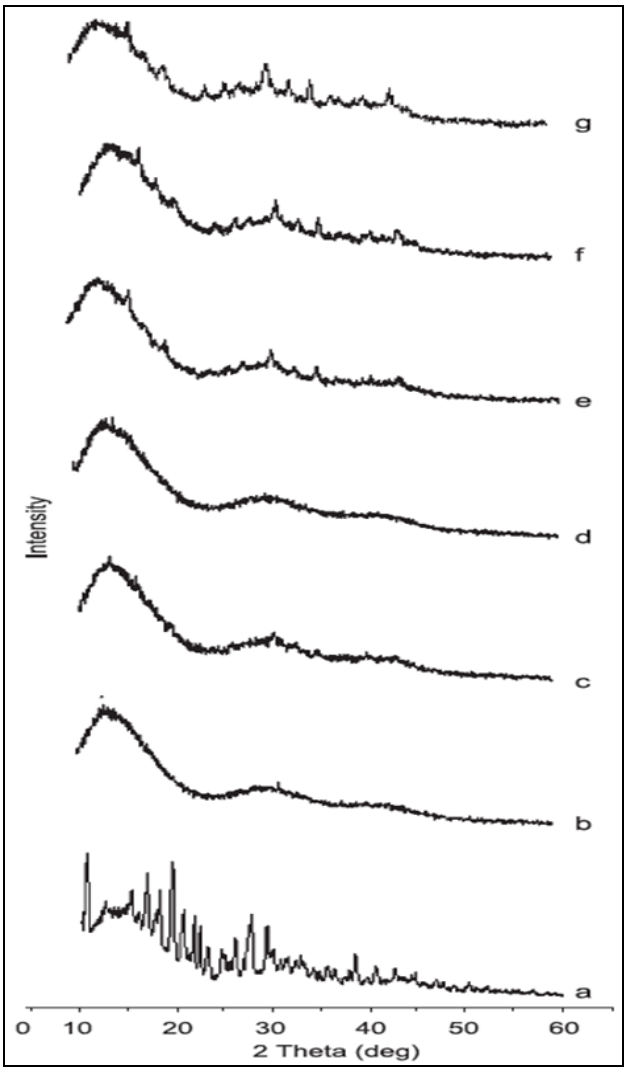


FIG. 7: X-RAY POWDER DIFFRACTION PATTERN OF
(a) pure verapamil, (b) blank alginate microspheres, (c) blank alginate-HPMC microsphere, (d) blank alginate-carbopol 934 P microsphere, (e) drug loaded alginate microsphere, (f) drug loaded alginate-HPMC microsphere, (g) drug loaded alginate-carbopol 934 P microsphere

TABLE 5: CHANGE IN DRUG CONTENT OF DRUG-LOADED MICROSPHERES, STORED AT ROOM TEMPERATURE, 37°C AND 45°C/75% RH, AFTER 8 WEEKS OF STUDY

Storage Conditions	Time (weeks)	Formulation Codes		
		FB	FE	FH
Room temperature	0	90.26	87.24	75.00
	4	89.52	85.10	75.50
	8	88.12	82.01	71.01
37°C	0	90.26	87.24	75.00
	4	87.89	84.12	70.89
	8	87.02	80.79	65.08
45°C/75%RH	0	90.26	87.24	75.00
	4	84.71	81.01	68.12
	8	78.05	78.05	63.17

CONCLUSION: Verapamil-loaded mucoadhesive microspheres were successfully prepared by emulsification-internal gelation technique with a maximum incorporation efficiency of 93.29 ± 0.26%. 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 8 weeks of study.

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