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PREPARATION AND EVALUATION OF MUCOADHESIVE MICROCAPSULES OF VERAPAMIL

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ABSTRACT

Mucoadhesive microcapsules of verapamil, a peripheral vasodilator, used in the treatment of hypertension, angina pectoris, cardiac arrhythmia, have been prepared from sodium alginate, hydroxypropylmethyl cellulose- K4M & E5LV, Carbopol 934P using 10% w/v calcium chloride solution by ionic gelation method. Drug : polymer ratio was 1:1 in all formulations and polymer mixtures employed were 1:1, 2:1, 3:1, 4:1 of sodium alginate: polymer (hydroxypropyl methyl cellulose- K4M & E5LV, carbopol 934P). Calcium chloride was used for ionic gelatin and cross linking of sodium alginate molecules. Microcapsules were spherical in shape and of sizes between 580 microns to 831 microns. Carbopol 934P was found most effective in controlling drug release from microcapsules followed by hydroxypropyl methyl cellulose- K4M. Drug releases from the best formulations of carbopol 934P and hydroxypropyl methyl cellulose-K4M follow anomalous transport.

INTRODUCTION: Microencapsulation by various polymers and its applications are described in standard text books and journals [1, 2]. Microencapsulation is a suitable technique to achieve controlled release and drug targeting. The main aim of an oral controlled drug delivery should primarily aim at achieving more predictable and increased bioavailability of a drug. The major absorption zone (upper part of the intestine) can not provide complete drug release followed by absorption from the drug delivery system due to rapid transit of the delivery system throughout the zone leading to less bioavailability from the drug delivery system. An attempt has been made for mucoadhesion of the drug delivery system in the upper part of the intestine to make intimate contact and increase duration of contact between the drug delivery system and mucus layer resulting in prolongation of drug release, increased absorption and enhancement of bioavailability of the drugs [3-6].

These considerations have led to the development of oral controlled release microcapsules with mucoadhesive properties. Alginate is easily gelled by the addition of calcium chloride solution to an aqueous solution of sodium alginate, since insoluble calcium alginate will be formed by cationic exchange between Na^+ and Ca^{2+}. The gelation and cross linking are due to stacking of the glucoronic acid (G) blocks of alginate chains with the formation of egg- box- like junction. Alginate microcapsules are non-toxic, have a protective effect on mucous membrane of upper GIT, and have property of re-swelling, so they can act as controlled release systems. Verapamil is a calcium channel blocker generally used in cardiac arrhythmia, angina-pectoris and hypertension. It has an oral bioavailability of 10-35% because of its high first-pass metabolism, and its elimination half life is 5-12 hours [7]. Therefore it is a suitable candidate for the design of mucoadhesive microcapsules.

MATERIALS AND METHOD:

Materials*:* Verapamil hydrochloride was a gift sample from Alkem Lab. (Daman, India). HPMC (E5LV, K4M) were procured from Glenmark Pharmaceutical Ltd (Navi Mumbai, India). Sodium alginate and Magnesium stearate were obtained from SPARC India Ltd. (Vadodara, India).others were of analytical reagent grade.

Preparation of Microcapsules-Orifice Ionic Gelation Method [4]: Coating materials (sodium alginate) and mucoadhesive polymers (HPMC K4M, HPMC E5LV**,** Carbopol 934P) were dissolved in distilled water (40 ml) to form a homogeneous mixture. The core material, Verapamil (1000mg) was added to the polymer solution with the help of magnetic stirrer to form a viscous dispersion. The resulting dispersion was added dropwise with the help of a needle size (20gauze) into the 50 ml calcium chloride solution (10%w/v). The added droplets are retained in the solution for 60 minutes for curing to produce microspheres. Then the microspheres were filtered and washed with distilled water to remove the extra calcium chloride retained and dried at 50^{o}C for 12 hours. The prepared microcapsules were kept in the desiccator for further use. The compositions of the formulations are given in **table 1**.

Analytical Method [8]: Estimations of Verapamil hydrochloride at pH- 1.2, 6.8, 7.4 were done at absorption maxima at 278 nm using UV spectrophotometer (SHIMADZU) in the concentration range 10 to 50 ppm with the help of standard curve.

Evaluations and Characterization:

Determination of Flow properties of Microcapsules: Angle of repose, bulk and tapped density, Carr's index, Hausner's ratio was measured which are given in **Table 2**.

Composition of the formulations:

TABLE 1(A): FORMULATIONS OF VERAPAMIL HYDROCHLORIDE MICROCAPSULES (1-6)

Formulation (F)	Verapamil hydrochloride (mg)	Sodium alginate (By part)	HPMC (E5LV) (By part)	HPMC (K4M) (By part)	Distilled water (ml)	Calcium Chloride solution (10% w/v)
1	1000	1	1	0	Upto 40ml	50 ml
2	1000	2	1	0	Upto 40ml	50 ml
3	1000	3	1	0	Upto 40ml	50 ml
4	1000	4	1	0	Upto 40ml	50 ml
5	1000	2	0	1	Upto 40ml	50 ml
6	1000	3	0	1	Upto 40ml	50 ml

(B) FORMULATIONS OF VERAPAMIL HYDROCHLORIDE MICROCAPSULES (7-9)

Formulation (F)	Verapamil hydrochloride (mg)	Sodium alginate (By part)	Carbopol 934p (By part)	Distilled water (ml)	Calcium Chloride solution (10% w/v)
7	1000	2	1	Upto 40ml	50 ml
8	1000	3	1	Upto 40ml	50 ml
9	1000	4	1	Upto 40ml	50 ml

TABLE 2: FLOW PROPERTIES OF VERAPAMIL MICROCAPSULES]

Formulation (F)	Angle of repose (θ)		Bulk density (g/ml)		Tapped density (g/ml)		Carr's index (%)		Hausner's ratio	
	Mean	S.D(±)	Mean	S.D(±)	Mean	S.D(±)	Mean	S.D(±)	Mean	S.D(±)
1	16.15	0.62	0.43	0.007	0.49	0.005	12.24	1.385	1.14	0.05
2	16.15	0.17	0.43	0.008	0.51	0.003	15.69	1.225	1.19	0.011
3	17.95	0.20	0.42	0.006	0.49	0.007	14.29	0.576	1.17	0.026
4	16.68	0.62	0.42	0.005	0.49	0.006	14.29	1.716	1.17	0.019
5	19.12	0.69	0.43	0.009	0.48	0.009	12.5	1.245	1.14	0.25
6	20.21	0.02	0.43	0.007	0.49	0.005	12.24	1.144	1.14	0.08
7	17.58	0.62	0.52	0.012	0.61	0.008	14.75	1.716	1.17	0.01
8	17.18	0.52	0.51	0.012	0.61	0.005	16.39	1.236	1.20	0.32
9	16.92	0.71	0.52	0.014	0.59	0.006	11.86	0.697	1.13	0.23

*S.D=standard deviation, where n=3

Angle of Repose [9]: Angle of repose has been used as indirect method for quantifying microcapsules' flowability, because of their relationship between interparticular cohesion. It was measured according to fixed funnel standing method.

$$\Theta = \tan^{-1} h/r,$$

Where Θ is the angle of repose, r is the radius, and h is the height.

Bulk density and Tapped density [10]**:** bulk and tapped densities were measured by using 10 ml of graduated cylinder. The sample poured in the cylinder was tapped mechanically for 100 times, then tapped volume was noted down and tapped densities were calculated, each experiment of this was triplicated.

Carr's index [10]**:** compressibility value or Carr's index value of microparticles were computed according to the following equation;

$$\text{Carr's (\%)} = \frac{\text{(tapped density – bulk density)}}{\text{Tapped density}} \times 100$$

Hausner's ratio [10]: was found to be related to interparticular friction and such can be used to predict the flow properties of the microcapsules. It is measured by comparing the tapped density to the bulk density using following equation.

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

The prepared microparticles have angle of repose varying from (16.15±0.17) to (20.21±0.02) , Carr's index ranging from (11.86±0.697) to (16.39±1.236), similarly Hausner's ratio ranging from (1.13±0.023) to (1.20±0.32)which are given in Table 2.The above data show that these are close approximates to the data which correspond to free- flowing nature of the microparticles .

Particle size analysis of the prepared microcapsules [11]**:** The mean diameter of 10 dried microcapsules was determined by optical microscopy (Metzer, India). The optical microscope was fitted with a stage micrometer by which the sizes of the microcapsules were determined. From the Formulations the highest size was observed in formulation **F1** (0.831±0.058) and lowest size was observed in formulation **F7** (0.58±0.41) which is given in **Table 3**.

TABLE 3: SIZE ANALYSIS OF PREPARED MUCOADHESIVE VERAPAMIL MICROCAPSULES

Formulation	Diameter (mm)
1	0.831±0.058
2	0.819±0.016
3	0.802±0.21
4	0.76±0.16
5	0.75±0.21
6	0.78±0.14
7	0.58±0.41
8	0.58±0.54
9	0.584±0.69

mean±S.D, n=10

Scanning Electron Microscopy of the Formulation [12]: The microcapsules were coated with gold-palladium by using sputter coater (POLARON SC 76430). After fixing the sample in individual stabs, Samples were examined for the surface and internal structure of the microcapsules by using scanning electron microscope which is given in **fig. 1**.

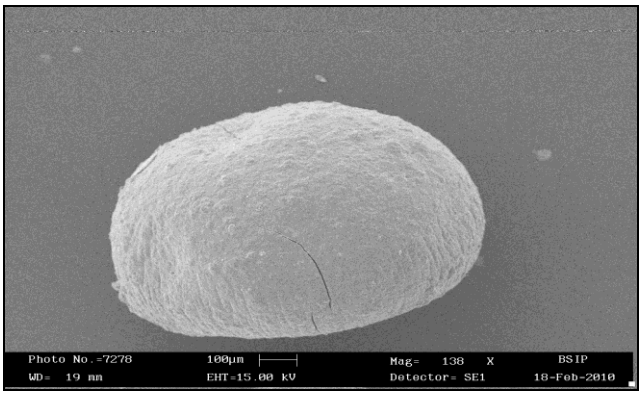

FIG. 1: SEM IMAGE SHOWING SURFACE VIEW OF VERAPAMIL MICROCAPSULE CONTAINING SODIUM ALGINATE AND HPMC (K4M)

Percent drug loading and encapsulation efficiency: Formulation F7 showed lowest percentage of drug loading (29.09%) and lowest percentage of encapsulation efficiency (58.18%), similarly formulation F8 showed highest percentage of drug loading (34.89%) and highest encapsulation efficiency (69.78).

***In vitro* wash-off test for mucoadhesion** [4]**:** The mucoadhesive property of the microcapsules was evaluated by an *in vitro* adhesion testing method known as the wash-off method. Freshly excised pieces of intestinal mucosa (4x5cm) from sheep were mounted onto glass slides (3x1 inch) with cyanoacrylate glue. Two glass slides were connected with a suitable support. About 50 microcapsules were spread onto each wet rinsed tissue specimen, and immediately thereafter the support was 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 (400 ml) at 37^{o}C contained in a 1000ml vessel of the machine. At the end of 1 hr, and at hourly intervals up to 10 hr, the machine was stopped and the number of microcapsules still adhering to the tissue was counted. The test was performed both in simulated gastric fluid (pH 1.2) and simulated intestinal fluid (pH 7.4 phosphate buffer). The data of test are shown in **Table 4**.

TABLE 4: *IN VITRO* WASH-OFF TEST FOR MUCOADHESION

Formulation (F)	pH used	% of microcapsules adhering to the tissue at various time interval* in hours				
		1	2	4	6	8
1	7.4	85.3±1.4	64.96±2.10	42.56±2.4	24.22±1.6	5.32±1.4
2	7.4	81.20±1.6	57.56±2.3	27.12±2.6	16.22±2.2	4.22±1.8
3	7.4	79.22±2.4	55.65±2.2	24.32±2.3	12.96±2.1	-
4	7.4	82.28±1.3	62.98±1.4	26.11±2.1	22.56±2.3	5.78±1.8
5	7.4	81.23±2.2	63.22±1.7	35.19±1.2	15.23±1.7	
6	7.4	82.33±1.7	56.99±2.3	36.22±1.8	16.33±1.7	-
7	7.4	72.9±2.2	53.85±1.2	24.9±2.5	14.77±1.2	-
8	7.4	86.8±1.2	54.45±1.8	26.22±2.2	18.36±2.1	
9	7.4	77.22±1.7	51.22±1.2	28.11±1.8	12.83±1.7	-

***In Vitro* drug release study** [13]: *In vitro* dissolution studies were carried out in Microcapsules at 37^{o}C at 50 rpm with USP dissolution apparatus II; 200mg verapamil microcapsules were placed into the dissolution apparatus. The in vitro studies were performed at two different pH values; (i) 1.2 (simulated gastric fluid), (ii) 7.4 (simulated intestinal fluid). An accurately weighed sample responded in dissolution media consisting 900 ml of 0.1 N (pH- 1.2) HCl and the dissolution was done for two hours. At the end of two hours, 28.062gm of disodium hydrogen phosphate and 10.305gm of potassium dihydrogen phosphate with 0.171gm of sodium chloride were added to change the pH upto 7.4 and after that the study was performed for 12 hrs. The sample (5ml) was withdrawn at each hour interval and replaced with same volume of medium and the withdrawn samples were diluted if required and then estimated for verapamil concentration at 278nm spectrophotometrically (by using UV- VISIBLE double beam spectro photometer Shimadzu). Finally the drug content in all fluids was determined from the calibration curve of Verapamil hydrochloride. Drug release in cumulative percentage from different formulations versus time were compared which are given in **fig. 2-4**.

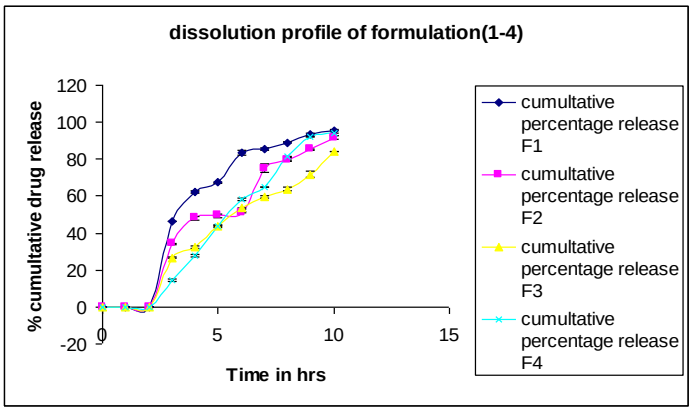

FIG. 2: DISSOLUTION PROFILE COMPARISION OF FORMULATION (1-4)

*Where dissolution is done first two hours in pH 1.2 then in pH 7.4 for rest of the time

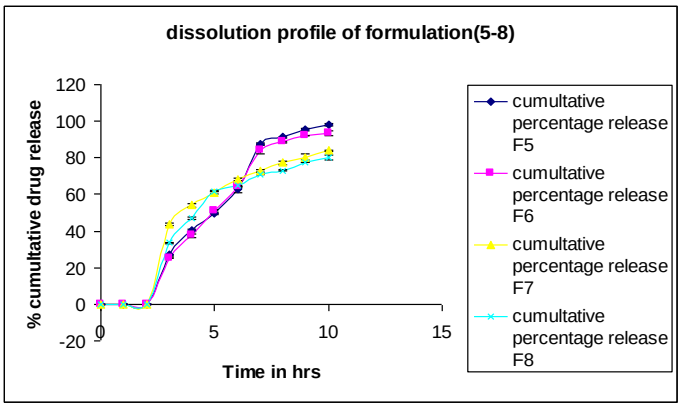

FIG. 3: DISSOLUTION PROFILE COMPARISION OF FORMULATION (5-8)

*Where dissolution is done first two hours in pH 1.2 then in pH 7.4 for rest of the time

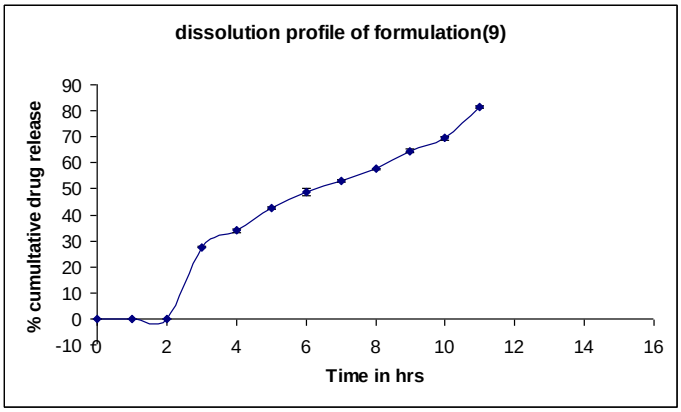

FIG. 4: DISSOLUTION PROFILE COMPARISION OF FORMULATION (9)

*Where dissolution is done first two hours in pH 1.2 then in pH 7.4 for rest of the time

Drug Release Mechanism [14, 15, 16]**:** To describe kinetics of drug release from the controlled release microcapsules, mathematical models, such as zero order, first order and Higuchi square root of time model, Korsmeyer and Peppas equation were used, when the release mechanism is not well known or when more than one type of release phenomena could be involved. The criterion for selecting most appropriate model was based on goodness of fit test.

Korsmeyer and Peppas equation: $Mt/M\alpha = Kt^n$,

Where, $Mt/M\alpha$ is the fraction of drug released at time t, K=constant incorporating of structural and geometric characteristic of controlled release device, n=diffusional release exponent indicative of release mechanisms.

The best fit model was determined statistically employing comparison of correlation coefficients. The drug release rate from the formulations and the respective half lives were calculated. The preparation of graphs and statistical calculations were carried out with the help of computer. The formulations F9 andF6 containing carbopol 934P and HPMC (K4M) respectively showed better and prolonged drug release making the two formulations most effective. Highest percentage of swelling was observed in F2 (165.31±3.3) and highest percentage of erosion was found in F6 (59.8±1.5)**.** Similarly Lowest percentage of swelling was observed in F8 (128.43±3.7) and lowest percentage of erosion was found in F1 (36.5±1.6) in pH media 7.4.

Infrared Spectroscopy (I.R.): Infrared spectrum was taken in the Perkin Elmer (spectrum RX -1) by scanning the sample in potassium bromide (KBr) discs. Before taking the spectrum of the sample, a blank spectrum of air back ground was taken. The sample of pure drug, pure polymer and the

formulations containing both the drug and polymer were scanned and plotted with the help of Bruker software. No interaction between the drug and polymer was found as evident from analysis of characteristics peaks.

Statistical Analysis: Anova was applied to F1, F2, F3, F6, F8, F9 to see whether significant differences are there in release characteristics at $p \leq 0.05$ level due to variation in polymer concentrations and variation in polymer. Results showed all formulations were significantly different in release characteristics.

Stability Study: Microcapsules of formulations F6, F9 were put on short term stability study at 30^{o}C and 40^{o}C/75 RH for a period of three months. Microcapsules showed no significant changes in drug content and dissolution profile at 30^{o}C but significant changes were observed at 40^{o}C.So microcapsules needs storing in a dry place at a temperature not exceeding30^{o}C.

CONCLUSION: Microcapsules were spherical in shape and of good flow properties with mucoadhesion upto 8 hours. Carbopol was most effective to control the release of the drug. Drug release mostly followed Higuchi equation and anomalous transport. There was no interaction between drug and excipients. Microcapsules were stable at 30^{o}C in dry atmosphere.

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