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Research Article

FORMULATION, DEVELOPMENT AND *IN-VITRO* EVALUATION OF VALSARTAN MUCOADHESIVE MICROCAPSULES

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ABSTRACT:

Sustained release alginate microcapsules of valsartan were prepared by orifice-ionic gelation method using Hydroxy Propyl Methyl Cellulose, (*viz*, 50 cps, K4M) as mucoadhesive polymer. Microcapsules were discrete spherical and free flowing. Encapsulation efficiency varied from 68.42% to 85.46%. Microcapsules were evaluated for drug excipients interactions (DSC & IR spectroscopy), % yield, drug content uniformity, particle size distribution, surface morphology(scanning electron microscopy), percentage moisture loss, *in vitro* drug release profile, and mucoadhesion study by *in vitro* wash off test, short term stability. The formulation prepared by using alginate – Hydroxy propyl methyl cellulose (K4M) in the ratio of 5:1 along with magnesium stearate, emerged as the overall best formulation based upon their drug release characteristics (in phosphate buffer 6.8). This formulation showed slow release up to 14 hrs. *In vitro* drug release followed first order kinetics, fickian diffusion mechanism ($n<0.5$) and the results had proven the release of the best formulation had extended up to 13.5 hrs according to $t_{50\%}$, $t_{70\%}$ and $t_{90\%}$ values. All the microcapsules exhibited good mucoadhesive property in the *in –vitro* wash off test. Accelerated stability studies were carried out for short term studies of formulations according to ICH and Q 1 A (R2) guidelines. These mucoadhesive microcapsules are thus suitable for oral controlled release of valsartan.

Key words: Valsartan, mucoadhesive microcapsules, orifice ionic gelation method, Hydroxy Propyl Methyl Cellulose (50 cps, K4M), Magnesium stearate.

INTRODUCTION:

Micro-encapsulation is a process in which tiny particles or droplets are surrounded by a coating to give small capsules many useful properties. In a relatively simplistic form, a microcapsule is a small sphere with a uniform wall around it. The novel design of an oral controlled drug delivery system should primarily be aimed at a achieving more predictable and increased bioavailability of drugs. But there are several difficulties, which include restraining/ localizing the drug delivery system within the regions of the gastro intestinal tract and the highly variable nature of gastro emptying process (few minutes to 12 hrs and above)[1].

The major absorption zone (stomach or upper part of the intestine), can result in incomplete drug release from the drug delivery system leading to diminished efficacy of the administered dose. Therefore, restraining a drug delivery system in a specific region of the gastro intestinal tract due to its mucoadhesiveness increases the intimacy and duration of contact between a drug containing polymer and mucus surface. Such a drug delivery system offers numerous advantages, especially for drugs exhibiting an absorption window or for drugs with a stability problem. Alginate is easily gelled by the addition of Ca^{2+} to an aqueous solution of sodium alginate, since insoluble calcium alginate is formed by cations exchange between Na^{+} and Ca^{2+}.Then an aqueous solution of sodium alginate was added drop wise to an aqueous solution of calcium chloride, a spherical gel is termed as "Alginate Bead"[1].

Valsartan (VAL) is a potent and specific competitive antagonist –II $–AT_1$ receptor. It is used orally for the treatment of hypertension and has a low bioavailability of 23%, because of its poor absorption in lower gastro intestinal tract. It undergoes little or no hepatic metabolism and its elimination half life is 6 hrs. Therefore, it is selected as a suitable drug for the design of mucoadhesive microcapsules with a view to improve its oral bioavailability and increase its drug release in a sustained manner.

MATERIALS AND METHODS:

Valsartan (VAL) is a gift sample from Aurobindo pharmaceutical (Hyderabad, India), Hydroxy propyl methyl cellulose (K4M) obtained from ParasChem Suppliers, Pune, Hydroxy propyl methyl cellulose (50 cps), sodium alginate, calcium chloride and magnesium stearate was provided by Qualigens, Mumbai. The concentration of

valsartan was measured with UV – visible spectrophotometer, Shimazdu. All the reagents used were in analytical grade.

PREPARATION OF MICROCAPSULES:

Orifice ionic gelation method :(syringe method)

Orifice ionic gelation method is also been successfully used to prepare large sized alginate beads. In this method microcapsules are prepared by employing sodium alginate in combination with different mucoadhesive polymers like Hydroxy propyl methyl cellulose (K4M), Hydroxy propyl methyl cellulose(50 cps) in the different polymer ratios like 1:1, 3:1, 5:1and 9:1are dissolved in purified water to form a homogenous polymer solution.[1]

A homogenous polymer solution was prepared by coating material (sodium alginate) and mucoadhesive polymer was dissolved in 32 ml to form a homogenous polymer solution. The core material valsartan (1 g) was added to the polymer solution, it was properly stirred in homogenous solution at a 500 rpm. The resulting dispersion was dropped drop wise to a calcium chloride (10% w/v) [40 ml] through a syringe fitted with a needle of 18 gauge. The added droplets were retained in the calcium chloride solution for 30 min to complete the curing reaction and to produce spherical rigid microcapsules. The microcapsules were collected by decantation and the product was thus separated and washed repeatedly with water and dried at 45° for 12 hrs. Since the microcapsules prepared by the above method sustain the release up to 8 hrs to 10 hrs only, further modifications were made in this method. These modifications include (i) increasing the cross-linking time for further 3 to 6 hrs (Formulations MC 5 to MC 8). (ii) Addition of magnesium stearate (#200 mesh) in 2 to 4%w/w concentration (Formulation MC 7 to MC 8)after thoroughly mixing with the drug valsartan by spatulaion (on a butter paper).The prepared microcapsules were stored in a desiccators for further use [2] (Table 1).

Table 1: Formulation Table of Microcapsules:

Formulation code	Sodium alginate:HPMC ratio	Valsartan (mg)	Sodium alginate (mg)	Magnesium Stearate (mg)	HPMC(50cps) (mg)	HPMC(K4M) (mg)
MC 1	1:1	1000	500	-	500	-
MC2	3:1	1000	750	-	250	-
MC3	5:1	1000	800	-	200	-
MC4	9:1	1000	900	-	100	-
MC5	5:1	1000	800	-	-	200
MC6	9:1	1000	900	-	-	100
MC7	5:1	1000	800	2%	-	200
MC8	5:1	1000	800	4%	-	200

EVALUATION PARAMETERS FOR MICROCAPSULES:

i) Percentage yield: [3, 4]

The total amount of microcapsules obtained were weighed and evaluated for percentage yield and the yield was calculated as per the equation

$$\text{Percentage yield} = \frac{\text{Practical yield}}{\text{Theoretical yield}} \times 100$$

ii) Size analysis:

For size distribution analysis, different sieves in a batch were separated by sieving, using a set of standard sieves (IP). The amount retained on different sieves was weighed.

iii) Drug content estimation: [5] Valsartan microcapsules (25mg) from each batch initially stirred in 3ml of sodium citrate solution (1%w/v) until complete dissolution. Methanol (7ml) was added to above solution to gel the

solubilised calcium alginate and further solubilize valsartan. This solution was filtered through what man filter no. 1 filter paper. The filtrate was assayed for drug content by measuring the absorbance at 250nm after suitable dilution.

iv) Encapsulation efficiency: [6]

Encapsulation efficiency was calculated using the equation:

$$\text{Encapsulation efficiency} = \frac{\text{Estimated practical \% drug content in microcapsules}}{\text{Estimated theoretical \%drug content in microcapsules}}$$

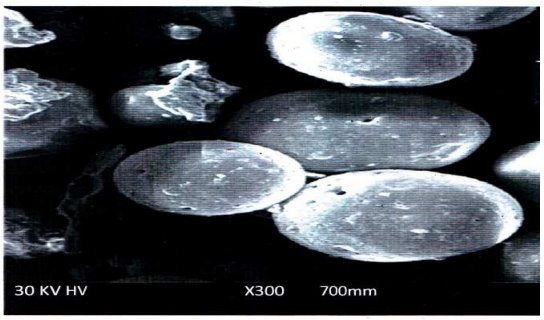
v) Percentage moisture loss: [7]

The valsartan loaded microcapsules was evaluated for percentage moisture loss, which sharing an idea about its hydrophilic nature. The microcapsules weighed initially kept in a desicator containing $Cacl_2$ at 37ºC for 24 hours. The final weight was noted when no further change in weight of sample was observed.

$$\text{Moisture loss} = \frac{\text{Initial weight} - \text{final weight}}{\text{Final weight}} \times 100$$

vii) Scanning electron microscopy (SEM):

The microcapsules were observed under a scanning electron microscopy (SEM-3400 N, SEM HITACHI). They were mounted directly onto SEM sample stub using double-sided sticking tape and coated with gold film with ion spillter with gold target with resolution 3 nm(30 KV HV Mode),10 nm (30 KV HV Mode),40 nm (30 LV Mode) and a vacuum system is fitted to it.(Figure. 2)



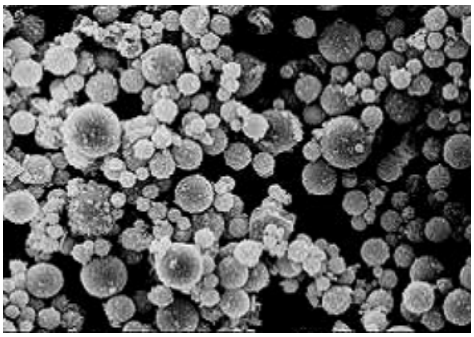


Figure 2: Scanning electron photomicrography of formulation MC 8, magnification 5To300.000v. Resolution 30 KV HV Mode, 10nm (30 KV HV Mode, 40nm (30 KV LV Mode), Detector –standard detector SE, BSC, Vacuum system based –TMP/RP based Model S-3400 N SEM –HITACHI.

viii) *In vitro* wash off test for mucoadhesion: [8, 9]

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 (4×5 cm) from sheep were mounted onto glass slides (3×1inch) with cyanoacrylate glue. Two glass slides were connected with a suitable each wet rinsed tissue specimen, and immediately thereafter the 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 (400ml) at 37ºC contained in a 1000 ml vessel of the machine. At the end of 1 hr and at hourly interval up to 8 hr, the machine was stopped and the number of microcapsules still adhering to the tissue was counted. The test was performed in both in pH 1.2 and simulated intestinal fluid (pH6.8 phosphate buffer).The data of *in vitro* wash off test for pH 6.8 are shown in (Table 3).

ix) *In vitro* drug release studies: [10, 11]

Drug release study was carried out in USP basket type dissolution test apparatus (Veego – VDR-8DR, USP standards).A quantity of microcapsules equivalent to 80 mg of valsartan was used for the test. Dissolution medium was phosphate buffer having a pH 6.8. Volume of dissolution medium was 900 ml, and bath temperature was maintained at 37±0.5ºC throughout the study. At specified time intervals, 2ml samples were withdrawn by means of a syringe fitted with prefilter and replaced immediately with 2ml of fresh medium. The absorbance of sample was measured at 250nm after suitable dilution with the medium. All the studies are conducted in triplicate (n=3) (Table 4, Figure .1).

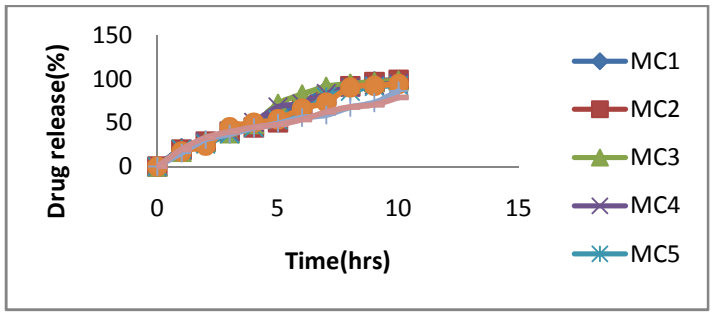


Figure 1: Percentage *in vitro* drug release of microcapsule formulations.

xi) Drug release mechanism and release: [12, 13]

The *in vitro* drug release data was fitted into four popular models of data treatment for the matrix formulations as follows:

(1)Zero order kinetics (2) first order kinetics (3) Higuchi's square root model, (4)Peppas model the data obtained from the stability studies was subjected to the statistical (student "t" in order to find out any significant in the drug content and dissolution parameters of the promising formulation(MC8)after the storage for 3 months at a temperature of 40°±1° ambient humidity conditions (Table 5).

TABLE5: DRUG RELEASE KINETIC STUDIES OF MICROCAPSULE FORMULATIONS

Formulation	Zero order Kinetics	First order kinetics	Higuchi square root equation	Korsemeyer-Peppas model		Best fit model
	R	r	r	r	n	
MC 1	0.9873	0.9199	0.9916	0.9927	0.4539	Peppas
MC 2	0.9853	0.9165	0.9803	0.9877	0.4437	Peppas
MC 3	0.9902	0.9388	0.9903	0.9925	0.5002	Peppas
MC 4	0.9877	0.9612	0.9628	0.9934	0.4771	Peppas
MC 5	0.9947	0.9447	0.9531	0.9989	0.4226	Peppas
MC 6	0.9835	0.9223	0.9562	0.9855	0.4583	Peppas
MC 7	0.9755	0.9691	0.9697	0.9906	0.3744	Peppas
MC 8	0.9408	0.9949	0.9935	0.9971	0.3481	Peppas

(n < 0.5 ,indicates it follows diffusion controlled manner)

The criteria for selecting more appropriate model was based on the goodness of the test, Koresmeyer and peppas equation; $Mt/M\acute{\alpha}=kt^n$; whereas Mt/ Mά is the fraction of drug released at time "t" k=constant. Incorporating of structural and geometric characteristics of controlled release device n=diffusion release exponent indicative of

release mechanism. The best fit model was determined statically employing comparison of correlation coefficient. 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.

RESULT AND DISCUSSION:

All the prepared microcapsules were found to be spherical, discrete and free flowing with yellowish white color. The SEM studies of microcapsules (MC-8) reveals that the microcapsules were perfectly spherical in shape and show a rough porous surface with free drug crystals (Figure.2). Percent yield was in the range of 65.6% to 88.65%(Table.2). The mean diameter of microcapsules was found to be in the range of 750.631µm to 823.28 µm (Table.2). The mean percent drug content of the microcapsules ranged from 34.21% - 42.73 % (Table 2). The low values of standard deviation indicate that uniform distribution of the drug within the various batches of microcapsules prepared. Encapsulation efficiency ranges approximately from 68.42% - 85.46%. Results reveal that the encapsulation efficiency of microcapsules increases with an increase in alginate concentration (Table2).

Table 2: Evaluation of Microcapsules.

Sl. No:	Formulation code	Yield (%)	Microcapsules with mean diameter in microns (%)		Mean % drug content (X±S.D)* Average of three determinations	Encapsulation efficiency (%)	Percentage moisture loss (%) (x±s.d).
			1204(22/25) Mesh	710(14/16) mesh			
1	MC1	77.4	23.20	76.80	34.21±0.03	68.42	7.273±0.155
2	MC2	73.2	15.20	84.80	38.12±0.05	76.25	4.837±0.078
3	MC3	82.2	20.05	79.95	38.47±0.04	75.95	2.876±0.090
4	MC4	82	20.26	79.94	39.41±0.01	78.83	3.258±0.141

5	MC5	71.6	14.93	85.06	36.99±0.01	73.90	2.529±0.050
6	MC6	65.6	13.94	86.06	40.77±0.01	81.54	1.838±0.063
7	MC7	87.95	11.19	88.81	41.61±0.06	83.22	2.516±0.040
8	MC8	88.65	23.20	76.80	42.73±0.04	85.46	1.244±0.130

Microcapsules with a coat consisting of alginate and a mucoadhesive polymer exhibited good mucoadhesive properties in the *in vitro* wash off test .The wash off test was faster at a intestinal pH than at a gastric pH. It was observed that the pH of the medium was critical for the degree of hydration, solubility and mucoadhesion of the polymers. The rapid wash off test observed at intestinal pH is due to ionization of carboxyl and other functional groups in the polymers at this pH, increases their solubility and reduces adhesive strength. The results of the wash off test indicated that the microcapsules had very good mucoadhesive properties with more than 64.9% retention for 8 hrs in phosphate buffer rather and the intestinal adhesive property and values has tabulated in (Table 3).

Table 3: *in vitro* wash off test to assess mucoadhesive properties of microcapsules.

%of microcapsules adhering to the tissue at various time interval* in hours						
Phosphate buffer (6.8)						
Formulations	pH used	1	2	4	6	8
MC1	6.8	97.3 ±0.9	90.3±0.9	87.6±2.0	60.5±1.2	44.5±2.0
MC2	6.8	96±1.07	91±0	87±2.0	72.3±1.2	52.3±1.8
MC3	6.8	98±2.0	95.6±1.2	85.3±3.3	79±2.0	53.6±2.2
MC4	6.8	98±2.1	95.2±1.2	90.5±2.0	78.7±1.9	51.2±1.5
MC5	6.8	98.6±2.0	96.6±1.2	92.6±1.2	72.9±1.2	59.1±1.5

MC6	6.8	98.4±2.0	97±1.2	90.3±2.0	62.9±1.2	49.5±3.5
MC7	6.8	99± 1.3	97±0	91.6±1.2	79.9±1.9	61.3±1.2
MC8	6.8	100±0	97.3±0.9	92.6±2.0	84.2±3.1	64.9±1.2

*Average of three determinations (S.D)

Microcapsules of alginate-HPMC (K4M) were observed that drug release from the microcapsules produces a sustained release, it was evident that followed a diffusion controlled from the best fit model data obtained (n<0.5). The formulation prepared by alginate-HPMC (K4M) in the ratio of 5:1 produced a sustained release of nearly 92% approximately and along with 4% magnesium stearate (it has the property of retarding the dissolution time)it further more prolonged the release, and so it emerged as the best formulation ($t_{50\%}$=4.8h,$t_{70\%}$=8.7h,$t_{90\%}$=13.6ah), based upon drug release characteristics(in pH 6.8 phosphate buffer). This formulation shows slow and extended release up to 13.6 hrs according to $t_{90\%}$ values (Table 4).

Table 4*: in – vitro* drug release data for microcapsules.

SL.No.	Formulation code	$t_{50\%(hrs)}$	$t_{70\%(hrs)}$	$t_{90\%(hrs)}$	Average percent drug release(10 hrs)*
1	MC 1	3.7	5.8	8.3	98.485±0.20
2	MC 2	4.4	6.1	8.9	98.667±0.32
3	MC 3	3.7	5.4	7.7	98.226±0.97
4	MC 4	3.7	6.1	8.5	94.991±0.19
5	MC 5	4.5	6.6	9.3	92.412±0.07
6	MC 6	4.1	6.3	8.9	94.165±0.24
7	MC 7	5.0	8.1	11.8	86.152±0.53
8	MC 8	4.8	8.7	13.6	78.983±0.26

*Average of three determinations

Accelerated stability studies were carried out using the ICH and Q1 A (R2) guidelines meant for storing the product for 90 days. Products have been kept in the stability chamber. Products were kept in stability chamber with the temperature of 25±2ºC (60% ±5%), 40ºC ±2ºC (75%RH±5%). The sample time points were 30, 60 and 90 days. The formulations will be monitored for changes in particle size, physical changes, morphology, drug content, entrapment efficiency and drug release profile.

CONCLUSION:

The formulation MC8 containing drug: polymer in the ratio of 1:1 and polymer ratio 5:1 with the combination of sodium alginate, HPMC K4M and magnesium stearate (4%) was concluded to be the best formulation(released 78.983% drug in 10 hrs, $t_{90\%}$ was found to be 13.6 hrs), regarding all the studies evaluated in order to achieve objective of this study. The novel formulation design facilitated the successful development of valsartan microcapsules formulations. Our data concluded that combination of sodium alginate along with HPMC(K4M) produced a sustained release and on further addition of magnesium stearate may be an effective strategy for the designing and development of valsartan mucoadhesive microcapsules for easy, reproducible and cost effective method to prove its potential for safe and effective controlled for oral drug delivery.

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