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Development and Optimization of Fixed Dose Antihypertensive Combination Drugs using Double Layer Sustained Release Microsphere Technology

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For the development of double walled microspheres, two polymers *i.e.*, chitosan and eudragit E100 were selected . The inner core which is made up of polymer chitosan will contain drug; propranolol hydrochloride and outer shell which is made up of polymer eudragit E100 contain; frusemide. Since eudragit E100 is dissolving below pH 5, will release the drug (furosemide) and attain therapeutic plasma concentration, which reduces the body fluid thus reduces blood pressure then the inner core chitosan's is a mucoadhesive, contains propranolol hydrochloride, adhesive to mucous layer of stomach or GIT will provide the sustain release of the drug for a longer period (24 h). Therefore, it is the goal of present study to adapt methods of double-walled fabrication with modifications, for the successful encapsulation of water-soluble. Propanolol hydrochloride and water insoluble furosemide, resulting in reduced-initial bursts as well as sustained release profiles suitable for the treatment of hypertension.

Key Words: Double wall microsphere, Chitosan, Furosemide, Propanol hydrochloride.

INTRODUCTION

Microspheres are spherical empty particles with size varying from 50 nm to 2 mm. The microspheres are characteristically free flowing powers consisting of synthetic powder, which are biodegradable in nature ideally having a particle size less than 200 µm. Solid biodegradable microspheres[1] incorporating a drug dispersed or dissolved throughout particle matrix have the potential for the controlled release of drug. Traditional microsphere drug delivery systems using a single polymer have several inherent flaws such as high initial burst, low encapsulation efficiency for highly water soluble drugs, inability to lend themselves to pulsatile or zero order release and lack of sustained release for periods suitable for periodic therapy. Composite double-walled microspheres adapted for the encapsulation of a highly water-soluble, have the ability to circumvent some of these limitations[1]. The limitation of microspheres made of a single polymer encapsulating drugs includes an initial burst caused by the release of the drug trapped on the surface during the encapsulation process and a progressively slower release rate. Therefore, microspheres made with a two-layered structure may have certain advantages over their counterparts made from single polymers. In some applications, where the therapeutic range of the drug is wide or the drug is nontoxic, this burst is not detrimental. However, for molecules with narrow therapeutic ranges or high toxicity, this initial burst of drug can be a problem for the patient. In an attempt to better control the release kinetics, the formation of double-walled microspheres with the drug loaded in the inner core could provide release kinetics with a lower burst effect than polymeric microspheres made from a single polymer. There are several methods of making microspheres with a two-layered structure from polymer blends. One method is to simply encapsulate a therapeutic agent in microspheres using a conventional micro encapsulation technique and then to coat the microspheres with a second polymer. This coating would reduce the burst effect since no protein or drug would be encapsulated on the surface. A second method entails polymer-polymer phase separation of a binary blend of polymer solutions, which results in the formation of microspheres that have a two-layered structure[2]. The solvent evaporation method has been modified to prepare double-walled microspheres. The usual process of micro encapsulation by solvent evaporation entails the formation of an "oil in-water" (o/w) emulsion of a polymer solution in an aqueous nonsolvent. This emulsion creates the spherical droplets, which then harden as the solvent evaporates, creating solid polymer microspheres[3]. To form microspheres from a single polymer, the polymer is dissolved in a volatile organic solvent, such as methylene chloride and mixed with the substance to been capsulated (*i.e.* drug or

protein), before adding to an aqueous non-solvent bath. The solvent evaporation method has been used extensively to prepare microspheres from PLA and PLGA. In the modified solvent evaporation process used to form double-walled microspheres, two polymer solutions are briefly mixed before adding to the aqueous non-solvent bath. As the solvent is slowly lost, the droplets of the polymer-polymer solution become more concentrated and the polymers begin to phase-separate. A homogeneous polymer solution undergoes phase separation into one phase rich in one polymer and a second phase rich in the second polymer. For the treatment of hypertension, combination therapy is used. In practice large majority of hypertensives require two or more drugs. Near about 70 % patients who achieve target BP (blood pressure) were being treated with two drugs. Even initial treatment of mild to moderate hypertension with low dose combination is being advocated as an alternative strategy *i.e.* combination of β-blocker and diuretics. In present formulation, there are two drugs; propranolol hydrochloride and furosemide were selected as the combination of β-blocker and diuretics. In spite of above reason, we are using diuretic furosemide and β-blocker propranolol hydrochloride, in present formulation. Since both these drugs don't have any type of chemical and pharmacological interaction so that we have selected the combination of these drugs. But, when propranolol hydrochloride is co-administered with furosemide, the plasma concentration of propranolol hydrochloride is increased. Due to above reason in this double walled microspheres formulation, inner core which is made up of chitosan polymer contains propranolol hydrochloride will maintain sustain release of drug (24 h) and outer shell which is made up of eudragit E100 (dissolve below pH 5) contains furosemide, will release the drug in the stomach and reduces the blood pressure.

EXPERIMENTAL

Preparation of chitosan core microspheres: Chitosan was obtained as gift sample from Central Institute of Fisheries Technology, Kochi. The microsphere system was prepared by ionic precipitation and chemical cross linking method[4]. A specific amount of chitosan was dissolved in 100 mL of 0.1 M acetic acid solution. To the above solution 1 % (w/v) Tween-80 was added with constants stirring. Then sodium sulphate (20 % w/v) solution was added during the stirring process, drop wise, until uniform turbidity was observed. To this, 1 % w/v cross linking agent, glutaraldehyde was added and solution was stirred for additional 1 h to stabilize the microspheres. The stirring was made by mechanical stirrer. Now the microsphere suspension was centrifuged at 3000 rpm for 0.5 h and microspheres were collected. The microspheres were washed twice with distilled water and freeze-dried.

Preparation of double walled microspheres: Double walled micro spheres were prepared by emulsion evaporation method. Eudragit E 100 (Alembic Ltd. Vadodara, India) (2 % w/v) solutions in dichloromethane were prepared and the drug (furosemide) was dispersed. The aqueous phase was added with Span-80 solution (2 % v/v). The organic phase was added drop-wise to aqueous phase to form w/o emulsion and homogenized for 15 min at 2000 rpm. The resulting emulsion was added to the aqueous solution of poly(vinyl alcohol) (2 % w/v) with stirring at 1500 rpm for 2 h until the organic phase was evaporated. The microspheres were prepared in aqueous poly(vinyl alcohol) solution was filtered, washed and dried.

Process variables: There are various process variables which could affect the preparation and properties of the microspheres. The preparation procedure was accordingly optimized and validated.

Optimization of process variables: The preparation of chitosan microspheres involves various process variables *viz.,* (i) effect of polymer concentration; (ii) effect of sodium sulphate (20 % w/v); (iii) effect of surfactant and (iv) effect of stirring rate.

The effects of variables were observed on the final particle size, drug loading and percentage yield of microspheres. During the preparation of a particular system, the other variables were kept constant.

Drug contents: The drug content was calculated as per method[5] 100 mg of dried microspheres were weighted accurately and drug was extracted from microspheres by digesting for 36 h with 10 mL of phosphate buffer saline (PBS pH 7.4) containing 60 % methanol. During this period the suspension was agitated. After 36 h, the suspension was centrifuged at 3000 rpm for *ca.* 0.5 h. The supernatant obtained was assayed spectrophotometrically for drug contents.

Yield of microspheres: After drying of microspheres in the round bottom flask, the microspheres were collected and weighted accurately.

$$\text{Yield of micropheres} = \frac{\text{Total weight of microparticles}}{\text{Total weight of drug} + \text{Total weight of polymer}}$$

***In vitro* drug release studies:** The different formulations were prepared by changing the drug-polymer ratio and subjected to *in vitro* drug release study in SGF (pH 1.2) and PBS (pH 7.4) solutions respectively.

For determination of drug release behaviour of chitosan microspheres, 50 mg of chitosan microspheres where suspended in small amount of water. This suspension was placed in an open ended test tube; one end of test tube was tide with cellophane membrane and the test tube was placed in the beaker containing 100 mL of release media (SGF/PBS). This solution was stirred at 100 rpm with magnetic stirrer at 37 ± 10 ºC. Sink conditions were maintained during the drug dissolution study. Sampling was done at specific interval (1 h). At each sampling, 1 mL of the solution withdrawn and was replaced with fresh media. The drug concentration was measured at respective lmax in respective medium using "Shimadzu-1700 pharmaspec UV/visible spctophotometer" after proper dilution.

The above drug release procedure was applied on the different formulations (PC_1, PC_2, PC_3 and PC_4), which were prepared by changing the drug polymer ratio, in different pH (SGF and PBS) media. The study was done continuous for 10 h and the total release of the drug after 24 h was also observed by using "Shimadzu-1700 pharmaspec UV/visible spectrophotometer" after proper dilution. The cumulative percentage drug release profile at interval of 1 h was calculated.

RESULTS AND DISCUSSION

Size and surface morphology of chitosan microspheres: The freshly prepared suspensions of microspheres were examined on an optical microscope and size of the microspheres was measured by using a pre-calibrated ocular micrometer and stage micrometer (Fig. 1). The least count of ocular microscope was calculated as 8.1 µm. Around 100 particles of each formulation were counted and observed. The observations are shown in Tables 1-4 after using different variables.

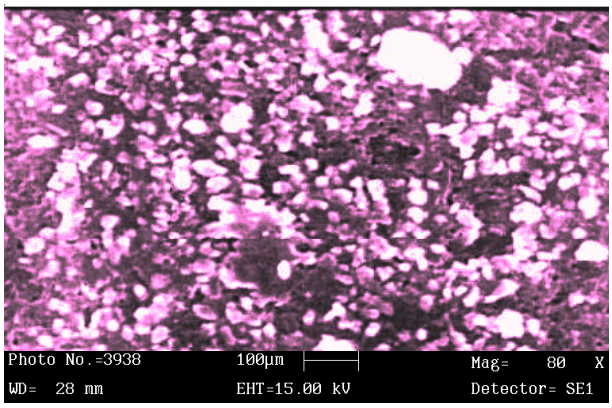

Fig. 1. SEM, photo micrograph of chitosan microspheres

TABLE-1
EFFECT OF POLYMER CONCENTRATION

Formulation code	Drug: Polymer ratio	Size of microspheres (µm)	Drug entrapped (%)	Yield of microspheres (%)
PC_1	1:1.0	16.53±0.14	8.07±1.33	60.50±2.67
PC_2	1:1.5	15.29±0.31	17.13±0.85	83.12±1.55
PC_3	1:2.0	15.66±0.26	12.05±0.97	77.24±1.48
PC_4	1:2.5	17.09±0.19	8.91±1.31	67.48±0.91

Size and surface morphology of double walled microspheres: The double walled microspheres were examined by optical microscope and electron microscope (Fig. 2). The least count of ocular microscope was calculated as 8.1 µm. Around 100 particles of each formulation were counted and observed. The observations are shown in Tables 5-7 after using different variables.

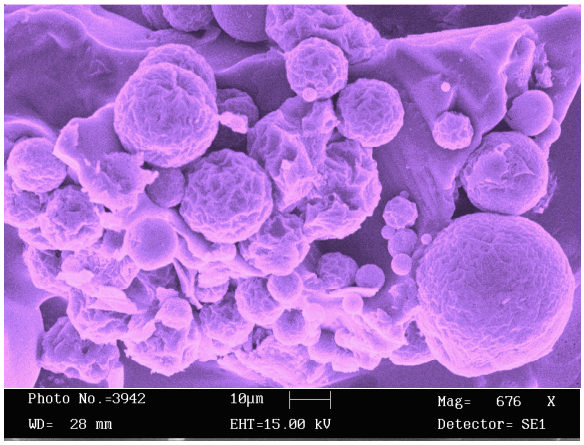

Fig. 2. SEM of double walled microspheres

***In vitro* drug release studies:** *In vitro* drug release from the various microspheres was performed in different mediums: (i) SGF (pH-1.2); (ii) PBS (pH-7.4).

During the release study of double walled microspheres, it may be possible that both drugs are present in the same release study medium. So that, for the measurement of release profile of both drugs, the analytical method must be necessary to develop. The outer layer is made up of eudragit E 100 which dissolves in stomach (below pH 5) is expected to release furosemide and the inner layer which consists of polymer chitosan dissolves throughout the GIT. Since, outer core, which is made

TABLE-2
EFFECT OF AMOUNT OF SODIUM SULPHATE (20 % w/v)

Formulation code	Sodium sulphate (20 % w/v) (mL) used	Size of microspheres (µm)	Drug entrapped (%)	Yield of microspheres (%)
S_1	5.0	18.26 ± 0.18	16.11 ± 0.11	45.01 ± 1.25
S_2	7.5	14.09 ± 0.21	15.98 ± 1.02	79.26 ± 1.35
S_3	10.0	13.82 ± 0.86	14.57 ± 0.95	75.85 ± 0.73
S_4	12.5	9.97 ± 0.61	11.23 ± 0.45	62.83 ± 0.17

TABLE-3
EFFECT OF SURFACTANT (TWEEN-80) CONCENTRATION

Formulation code	Drug:Polymer ratio	Concentration of Tween-80 (%)	Size of microspheres (µm)	Drug entrapped (%	Yield of microspheres (%)
TW_1	1:1.5	0.5	16.18 ± 0.25	15.16 ± 0.85	74.12 ± 1.07
TW_2	1:1.5	1.0	15.59 ± 0.74	18.78 ± 0.31	82.26 ± 1.12
TW_3	1:1.5	1.5	15.86 ± 0.86	16.04 ± 0.87	78.56 ± 1.42
TW_4	1:1.5	2.0	17.11 ± 0.18	15.68 ± 0.49	73.11 ± 0.84

TABLE-4
EFFECT OF STIRRING RATE

Formulation code	Stirring rate (rpm)	Size of microspheres (µm)	Drug entrapped (%)	Yield of microspheres (%)
SR_1	500	21.59 ± 0.61	11.65 ± 0.49	77.15 ± 1.23
SR_2	1000	18.27 ± 0.12	14.23 ± 0.58	79.13 ± 0.96
SR_3	1500	15.06 ± 0.25	17.95 ± 0.54	81.62 ± 1.44
SR_4	2000	11.84 ± 0.14	15.85 ± 0.19	74.55 ± 1.12

TABLE-5
EFFECT OF CORE MICROSPHERES CONCENTRATION AND POLYMER CONCENTRATION

Formulation code	Core microspheres: Polymer ratio	Size of microspheres (μm)	Drug furosemide (mg)	Drug entrapped (%)	Yield of microspheres (%)
EC_1	1:1	28.35 ± 2.26	200	42.15 ± 1.16	56.61 ± 1.23
EC_2	1:2	33.53 ± 2.42	200	58.07 ± 1.33	78.50 ± 2.67
EC_3	1:3	34.29 ± 1.81	200	67.13 ± 0.85	83.12 ± 1.55
EC_4	1:4	35.66 ± 2.26	200	72.35 ± 1.15	85.24 ± 1.48
EC_5	1:5	51.09 ± 1.19	200	45.21 ± 1.56	82.48 ± 0.91

TABLE-6
EFFECT OF STIRRING RATE

Formulation code	Stirring rate (rpm)	Size of microspheres (μm)	Drug entrapped (%)	Yield of microspheres (%)
EC_4R_1	500	45.46 ± 1.35	71.62 ± 0.52	82.52 ± 1.64
EC_4R_2	1000	38.29 ± 1.25	75.83 ± 0.64	82.78 ± 1.87
EC_4R_3	1500	34.47 ± 1.65	78.61 ± 0.53	83.18 ± 0.98
EC_4R_4	2000	32.66 ± 0.89	81.45 ± 0.35	84.96 ± 1.24
EC_4R_5	2500	31.32 ± 0.54	79.15 ± 0.58	83.21 ± 2.15

TABLE-7
EFFECT OF SURFACTANT (SPAN-80) CONCENTRATION

Formulation code	Stirring rate (rpm)	Size of microspheres (μm)	Drug entrapped (%)	Yield of microspheres (%)
$EC_4R_4S_1$	0.5	38.35 ± 2.26	72.32 ± 0.43	75.82 ± 1.65
$EC_4R_4S_2$	1.0	33.53 ± 2.42	79.23 ± 0.68	82.43 ± 1.74
$EC_4R_4S_3$	1.5	31.29 ± 1.81	82.71 ± 0.35	83.38 ± 0.98
$EC_4R_4S_4$	2.0	32.66 ± 2.26	80.15 ± 1.02	79.96 ± 1.24
$EC_4R_4S_5$	2.5	32.89 ± 2.53	78.57 ± 1.24	83.13 ± 2.14
$EC_4R_4S_1$	0.5	38.35 ± 2.26	72.32 ± 0.43	75.82 ± 1.65

TABLE-8
In vitro CUMULATIVE % DRUG RELEASE PROFILE IN PBS (pH 1.2)

Time (h)	Drug release (%)			
	Formulation code			
	PC_1	PC_2	PC_3	PC_4
0	0.00	0.00	0.00	0.00
1	7.42	3.63	3.24	3.61
2	14.61	8.82	7.21	6.92
3	21.16	20.14	18.32	12.24
4	33.16	25.44	22.21	20.43
5	41.83	32.64	28.54	24.67
6	46.65	40.58	36.21	32..22
7	55.47	49.11	45.25	40.88
8	62.24	58.97	55.20	53.24
9	68.24	64.55	63.27	60.73
10	72.81	70.70	68.23	63.12
24	98.73	98.24	97.16	96.38

TABLE-9
In vitro CUMULATIVE % DRUG RELEASE PROFILE IN PBS (pH 7.4)

Time (h)	Drug release (%)			
	Formulation code			
	PC_1	PC_2	PC_3	PC_4
0	0.00	0.00	0.00	0.00
1	2.85	2.50	2.41	2.11
2	3.71	4.17	3.28	3.14
3	6.73	8.11	5.80	5.23
4	14.28	12.24	10.24	9.12
5	18.62	19.24	17.24	15.42
6	26.54	25.24	24.21	22.31
7	33.25	33.67	32.00	30.21
8	44.57	42.35	42.15	40.45
9	50.68	49.76	48.64	45.76
10	64.21	63.27	62.07	60.98
24	91.24	90.09	89.56	87.89

up eudragit E 100 is soluble in 0.1N HCl, the microspheres was dissolved in simulated gastric fluid (pH-1.2) and release the outer shell's drug which will give quick action. But, after dissolution of outer shell, inner core (microspheres of chitosan) will be free and give sustain release of the drug (propranolol hydrochloride). Since, chitosan microsphers are mucoadhesive in nature, so, some chitosan microsphers will remain in stomach and rest will be passed to the stomach. The cumulative percentage drug release profile at interval of 1 h was calculated and given in Tables 8 and 9 for simulated gastric fluid (pH 1.2) and PBS (pH 7.4), respectively.

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