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Research Article

FORMULATION AND *IN-VITRO* EVALUATION OF TRIMETAZIDINE DIHYDROCHLORIDE FLOATING BEADS

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ABSTRACT

This study's objective is to develop gastro-retentive floating beads that control the release of trimetazidine dihydrochloride, an effective anti-anginal agent; which is freely soluble in water and suffers from rapid absorption and relatively short plasma half-life (6.0 ± 1.4 h). By emulsion gelation method, trimetazidine calcium alginate floating beads were prepared using sodium alginate solution, hydroxypropyl methyl cellulose and peppermint oil. The effect of sodium alginate concentrations (2, 3, and 4%w/v), peppermint oil percentage (15, 20, and 25% v/v), and hydroxypropyl methyl cellulose type on floating properties besides *in vitro* drug release from the beads were studied. The results indicated that at (2% w/v) sodium alginate solution, bead formulas achieved best floating ability. On the other hand, floating time was longer at intermediate oil percentage (20%v/v), at the same time increased hydroxypropyl methyl cellulose viscosity produced longer floating time and prolonged drug release. The density of the formulated beads was found to be within the range (0.26-0.5g/ml). According to similarity factor, formulas (F9 and F12) which contain (2% w/v sodium alginate, 20% v/v oil and hydroxypropyl methyl cellulose (15 000 centipoises) were the best formulas that showed higher similarity factor (66.19 and 59.08 respectively) of Trimetazidine release in comparison to the reference product, with good floating ability and prolonged duration of more than 8 hours. In conclusion, oil entrapped floating beads give promising results for sustaining the release of highly water soluble drugs.

Keywords: Trimetazidine, Sodium alginate, Gelation, Floating systems, Beads.

INTRODUCTION

Gastro-retentive systems are new drug delivery systems that stay in the gastric region for several hours; hence prolong the gastric residence time (GRT) of drugs. Gastric retention of dosage forms can be achieved by the mechanisms of bioadhesion, floatation, sedimentation and expansion systems [1]. Floating drug delivery systems (FDDS) are low density systems which have sufficient buoyancy to float over the gastric contents and stay in the stomach for a prolonged period of time, where the drug is released slowly at the desired rate, this result in increasing GRT and reducing fluctuation in plasma drug concentration. After drug released, the residual system is emptied from the stomach [2]. Multiple unit floating dosage forms as beads have the advantage of being devoid of "all or nothing" gastric emptying that may occur in single unit dosage forms [3]. Trimetazidine dihydrochloride (TMZ) is an effective anti-anginal agent used in the prophylaxis and management of angina pectoris and in ischemia of neuro-sensory also in Meniere's disease. TMZ is freely soluble in water suffers from rapid absorption and relatively short half-life ($t_{1/2}$ = 6.0 ± 1.4 h) [4]. Emulsion gelation technique used to sustain the release of water-soluble drugs, by preparing emulsion gel beads using sodium alginate. The Oil entrapped gel beads prepared by gently mixing and homogenizing oil and aqueous phase containing sodium alginate which then extruded in to calcium chloride solution [5]. Thus we used this method to sustain the release of TMZ.

MATERIALS AND METHODS

Materials

- TMZ and hydroxypropyl methyl cellulose (HPMC), with two viscosities [K15M (15 000 centipoises) and K100M (100 000 centipoises)], were purchased from Shijiazhuang Aopharm Medical Technology Co., Ltd., China.
- Sodium alginate from British Drug House Ltd, UK.
- Calcium chloride from Merk, Germany.
- Peppermint oil from N.V. FA.J.VanPemb Rock & Co., Holland.
- Vastarel®MR (35mg) tablet from Les Laboratories Servier Industrie, France.
- All other chemicals were of analytical grade.

Methods

Preparation of TMZ floating beads

TMZ loaded beads were prepared by emulsion gelation method [6-8]. In this method sodium alginate solution (2, 3 and 4%w/v) containing polymer [0.5%w/v HPMC (K15M, K100M)] was prepared. Peppermint oil in the concentration (15, 20, and 25% v/v) was then added with high shear mixing. TMZ was then dispersed in the formed emulsion in fixed drug: alginate ratio (1:8 and 1:5 w/w). This mixture was extruded, using syringe G21 needle into (1% w/v) calcium chloride solution at room temperature.

The gel beads were allowed to stand in solution for 60 min before being separated and washed with distilled water, and then air dried for about 48 h. The composition of different batches of the TMZ loaded alginate beads are given in table1.

Sixteen formulas were prepared by emulsion gelation method to study the effect of oil percentage, sodium alginate concentration, HPMC type and drug to alginate ratio on the beads floating properties, drug loading, entrapment efficiency and release of the drug.

Bulk density of floating beads

In this method floating beads were transferred to a measuring cylinder, tapped manually until constant volume was obtained. This volume is bulk volume (i.e.) it's the true volume of beads and the void space between them. The following equation was used to calculate the bulk density [9]:

(Bulk Density = mass of beads/ bulk volume)

Drug loading (DL) and entrapment efficiency (EE)

An accurately weighed sample of beads (100 mg) was crushed in a mortar; the crushed material dissolved in 75 ml of 0.1N HCl, then completed up to 100 ml. This mixture was filtered and analyzed by UV/visible spectrophotometer at λ max 231 nm against 0.1N HCl as blank. The drug loading and entrapment efficiency percent can be calculated using the following equations [10, 11]

DL% = (Actual drug content/ weight of beads) x 100

EE% = (Actual drug content/ theoretical drug content) x 100

Floating properties

Floating properties of the beads were evaluated in dissolution vessel full with 900 ml of 0.1N HCl. Floating lag time and floating time were measured by visual observation as part of dissolution studies. Floating lag time is the time between the introduction of the beads into the medium and its buoyancy to the upper third of dissolution vessel, while floating time is the time at which the beads remain buoyant over the surface of the medium [12].

Dissolution studies

In vitro dissolution studies were performed for the prepared formulas using USP dissolution apparatus (paddle). An accurately weighed sample of floating alginate beads containing (35mg) of TMZ was dropped into 900 ml of 0.1N HCl maintained at temperature of (37 ± 0.5ºC) and stirred at a speed of 75 rpm.

At predetermined time intervals 5 ml of the sample was withdrawn and the volume was replaced with an equivalent amount of plain dissolution medium [13]. Collected samples were filtered and analyzed at λ max 231 nm.

Release kinetics

The release mechanisms of TMZ from oil-entrapped floating beads were evaluated by using mathematical kinetic model equations that describe the drug dissolution from pharmaceutical dosage form, the commonly used models are: zero-order, first order, Higuchi and korsmeyer-peppas [14].

Selection of the best formula

The selection of the best formulas depends on the comparison of the release profile of TMZ from the prepared alginate beads to the reference release profile of TMZ from marketed modified release tablets (Vastarel® MR) using similarity factor (f_2). The similarity factor (f_2) introduced by Moore and Flanner was calculated using the following equation [15]:

$$f_2 = 50 \times \log \{[1+ 1/n \sum | R_t - T_t |^2]^{-0.5} \times 100\}$$

Where n is the number of dissolution time points, $\mathbf{R_t}$ and $\mathbf{T_t}$ are the reference and test dissolution values at time t.

Table 1: Composition of TMZ oil -entrapped beads

Formula no.	Drug: alginate ratio	Sodium alginate solution %w/v	HPMC K15M %w/v	HPMC K100M %w/v	Peppermint oil % v/v
F1	1:5	4	0.5	-	15
F2	1:5	4	0.5	-	20
F3	1:5	3	0.5	-	15
F4	1:5	3	0.5	-	20
F5	1:5	3	0.5	-	25
F6	1:5	3	-	0.5	15
F7	1:5	3	-	0.5	20
F8	1:5	2	0.5	-	15
F9	1:5	2	0.5	-	20
F10	1:5	2	0.5	-	25
F11	1:8	2	0.5	-	15
F12	1:8	2	0.5	-	20
F13	1:8	2	0.5	-	25
F14	1:5	2	-	0.5	15
F15	1:5	2	-	0.5	20
F16	1:5	2	-	0.5	25

HPMC-K15M: hydroxypropyl methyl cellulose (15 000 centipoises)

HPMC-K100M: hydroxypropyl methyl cellulose (100 000 centipoises)

Morphology and particle size of the floating beads

Morphological examination of the beads surface, particle size and uniformity of the selected formula was studied visually and by scanning electron microscope (SEM) at (magnification 20, 50, and 750x). The sample beads were mounted on a metal grid using a double sided tape coated with gold in a vacuum evaporator.

RESULTS AND DISCUSSION

Bulk density of floating beads

The bulk densities of various TMZ floating bead formulas were found to be within the range (0.26-0.5g/ml) as shown in table 2, (i.e.) the results of all formulations obtained are less than the density of 0.1N HCl (1.004 g/ml).

Drug loading (DL) and entrapment efficiency (EE)

It was found that formulas prepared with 4%w/v sodium alginate solution had much lower EE% than those prepared with 2 and 3%w/v, this is observed by comparing (F1, F3 & F8) and (F2, F4 & F9), which may be due to that the viscosity of the alginate solution increased to the extent that the formation of drops was strongly held up, where inability of calcium ions to penetrate the thick and viscous dispersions of this concentration may occur. This finding is consistent with that reported by Joshi et al and Ghareeb et al [16, 17]. As calcium ions ability to penetrate the dispersion reduced, cross linking decreased hence drug entrapped within the beads decreased. By comparing (F3, F4 &F5), (F8, F9 & F10), (F11, F12 & F13) and (F14, F15 & F16) it was found that formulas affected by using different peppermint oil percentage, as formulas used 25%v/v peppermint oil have lower EE% than those used 15 and 20%v/v.

Table 2: Evaluation parameters of TMZ oil-entrapped beads

Formula No.	Density (g/ml)	EE%	DL%
F1	0.50	41.36	3.31
F2	0.47	65.53	3.39
F3	0.48	80.41	3.45
F4	0.47	86.88	4.08
F5	0.35	71.75	3.86
F6	0.40	84.67	3.20
F7	0.39	83.90	3.15
F8	0.42	97.10	3.29
F9	0.40	98.18	3.64
F10	0.33	84.57	2.95
F11	0.37	96.73	2.96
F12	0.39	99.20	3.76
F13	0.40	86.15	2.24
F14	0.30	86.30	4.46
F15	0.31	84.14	3.13
F16	0.26	69.47	2.82

DL: drug loading, EE: entrapment efficiency

Since TMZ is freely water soluble drug, at intermediate oil percentage the barrier action of entrapped oil droplets protected drug against diffusion to the surrounding medium during gelation process. Further increases in oil percentage to 25%v/v, increased

volume of oil which occupied most of the volume of the bead and prevented the entrapment of sufficient amount of the drug, these results are in line with those reported by Jaiswal D et al [18]. DL% was calculated and ranged from (2.24-4.46), these results with the EE% are shown in table 2.

Floating properties

The beads floating properties data shown in table 3 indicated that floating ability affected by sodium alginate concentration, peppermint oil percentage and HPMC type. At a given sodium alginate solution concentration, the formulas used 15%v/v peppermint oil had short floating time and long floating lag time, as peppermint oil percentage increased to 20%v/v the floating ability of the beads improved and they had immediate floating lag time and floating time more than 8h, further increase in oil percentage to 25%v/v did not affect the floating lag time but this change resulted in decrease in the floating time. Emulsification of peppermint oil in the alginate solution and fast gelation with the entrapment of the oil within alginate gel matrices resulted in a large number of tiny oil pockets, which may be the reason for the beads buoyancy. On the other hand, oil leakage was observed in formulas prepared using 25% v/v of oil; this is in agreement with Prasad SK et al [19], which may be the reason for their reduced floating time. While at a given peppermint oil percentage, the use of 4% w/v sodium alginate solution in formulas produced poor floating ability, and by decreasing the concentration to 3 and 2%w/v better results was obtained, which can be observed by comparing formulas (F1, F3 & F8) and (F2, F4 & F9). Increasing viscosity of formulas, with 4%w/v sodium alginate concentration, resulted in the formation of beads with irregular shape, which may affect their floating properties.

By comparing formulas (F4 & F7), (F9 & F15) and (F10 & F16) it was found that changing HPMC type from K15M to K100M with the higher viscosity resulted in floating time improvement. This may be explained that when HPMC viscosity increased the beads had fewer pores distributed on their surface, this might produce higher oil entrapment and also reduced oil leakage thus enhanced floating properties.

Table 3: Evaluations of TMZ oil-entrapped beads floating properties

Formula No.	Floating lag time (min)	Floating time (h)
F1	N/A*	N/A*
F2	10	4
F3	5	3
F4	Immediate	6
F5	Immediate	5
F6	N/A*	N/A*
F7	Immediate	12
F8	Immediate	6
F9	Immediate	9
F10	Immediate	5
F11	10	3
F12	Immediate	10
F13	Immediate	6
F14	Immediate	6
F15	Immediate	>12
F16	Immediate	10

* N/A: means some of the beads floated and others settled down

Because of the poor floating properties of formulas prepared with 4% sodium alginate (F1&F2) and formulas used 15% peppermint oil (F3, F6, F8, F11and F14); they did not subjected to further drug release study evaluation.

Dissolution studies

It was found that formulas were retarded as we increased the oil percentage from 20 to 25% v/v while other factors are constant, this is observed by comparing (F9 &F10), (F12 &F13) and (F15 & F16) as shown in fig.1. This may be attributed to an additional diffusion layer to the release of the drug. These results are in line with that reported by More et al [20].

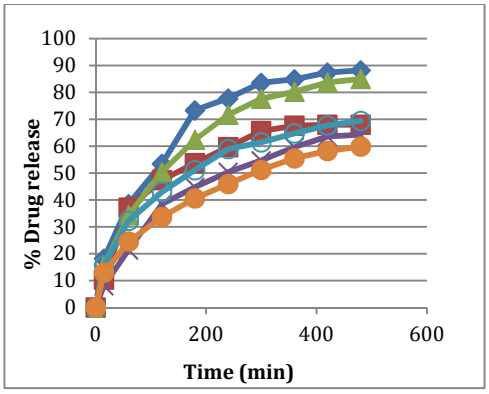

Fig.1: The Effect of Oil Percentage on TMZ Release in 0.1N HCL at 37°c

♦: F9-20% oil, ■: F10-25% oil, ▲: F12-20% oil, ×: F13-25% oil, ○: F15-20% oil, ●: F16-25% oil

On the other hand, it was found that when sodium alginate concentration decreased from 3 to 2% w/v while other factors are constant the TMZ release from the beads enhanced as seen by comparing (F4 & F9) in fig.2. These results may explained by that lower alginate amount result in less cross-linking between sodium alginate and the divalent cat-ion thus less drug entrapped and release enhanced this is in agreement with results that stated by Morshed et al [21].

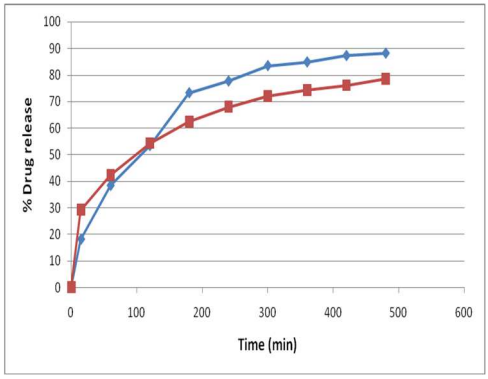

Fig.2: The Effect of Sodium Alginate Concentration on TMZ Release in 0.1N HCL at 37° C

■: F4 - 3% sodium alginate, ▯: F9 - 2% sodium alginate

It was found that TMZ release profile from all bead formulas delayed when HPMC changed from K15M to K100M as shown in (fig.3), (i.e.) as the viscosity of HPMC polymer increases the drug release rate decreases.

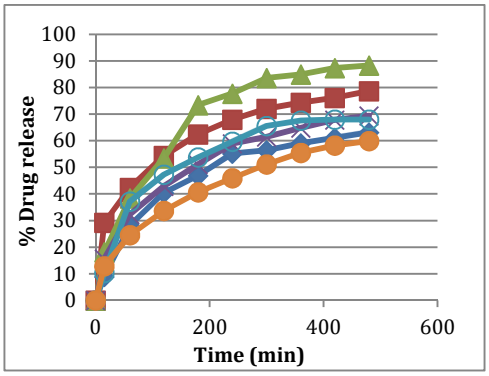

Fig.3: The Effect of HPMC Type on TMZ Release in 0.1N HCL at 37°C

■: F4– K15M, ▯: F7– K100M, ▲: F9– K15M, ×: F15– K100M, ○: F10–K15M ●: F16–K100M

Release kinetics

The drug transport inside pharmaceutical systems and its release sometimes involves multiple steps provoked by different physical or chemical phenomena make it difficult to get a mathematical model describing it in the correct way [14]. The results are illustrated in Table 4. The release pattern of TMZ from oil-entrapped beads in 0.1N HCl showed higher correlation when fitted to Korsmeyer-peppas kinetic model, R2 (correlation coefficient) value range (0.916-0.999).

This indicates that combination of diffusion and erosion processes are responsible for the release of the drug. The exponent (n) value, as calculated from Korsmeyer-Peppas model, for TMZ oil-entrapped bead formulas almost all ranged 0.43<n<0.85 (spherical geometry) which indicate that these formulas had Anomalous non-fickian drug release behavior from the beads.

Table 4: Release mechanisms of TMZ from oil-entrapped beads

Formula No.	R^2 value				
	Zero-order	First-order	Higuchi	Korsmeyer-peppas	n
F4	0.873	0.785	0.971	0.994	0.294
F5	0.906	0.849	0.975	0.982	0.225
F7	0.824	0.627	0.944	0.950	0.591
F9	0.820	0.692	0.938	0.974	0.522
F10	0.772	0.572	0.949	0.916	0.599
F12	0.870	0.720	0.907	0.985	0.568
F13	0.889	0.698	0.940	0.978	0.678
F15	0.875	0.729	0.972	0.988	0.474
F16	0.931	0.798	0.994	0.999	0.450

R^2: correlation coefficient, n: Korsmeyer-peppas exponent

Selection of the best formula

From the comparison of the release profile of TMZ from the prepared alginate beads to the reference release profile of TMZ from marketed modified release tablets (Vastarel® MR), as shown in fig.4, by using similarity factor (*f2*), it was found that dissolution profile of formulas (F9 and F12) similar to that of the marketed modified release tablet (f2= 66.19 and 59.08 respectively); these results are within the public standard range stated in references [15].

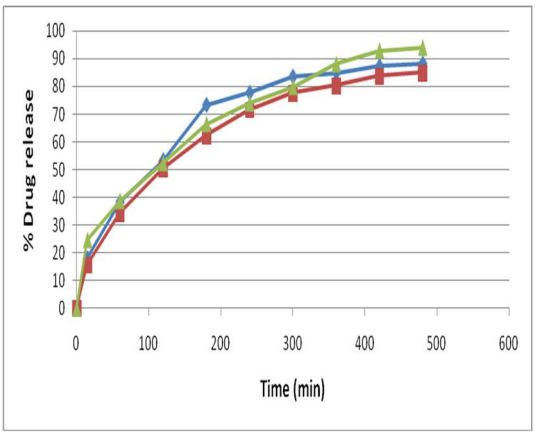

Fig. 4: Release Profile of Candidate Formulas (F9 & F12) in Comparison to Reference Vastarel®MR Tablet in 0.1N HCl at 37°C

♦: F9, ■: F12, ▲: Vastarel®MR

Morphology and particle size of the floating beads

By SEM; three formulas (F7, F9 and F12) were studied for their size range, shape and morphology where alginate beads showed average size of the dried beads was found to be 1.48mm, 1.54mm and 1.53mm for the formulas F7, F9 and F12 respectively fig.5, spherical geometry of the beads shown in fig.6. Oil entrapped beads had an "orange peel" surface with corrugations where pores or channels distributed throughout the surface, which was clearer with F9 & F12 while the bead surface of F7 looks smoother as shown in fig.7.

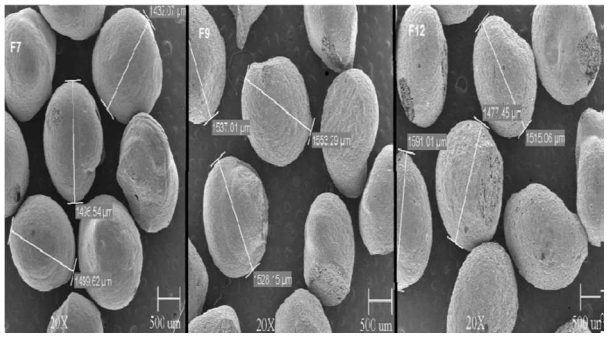

Fig. 5: SEM Micrographs of Formulas (F7, F9, and F12) at Magnification 20X

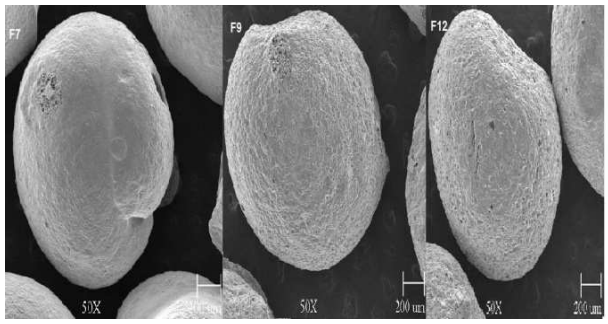

Fig. 6: SEM Micrographs of Formulas (F7, F9, F12) at Magnification 50X

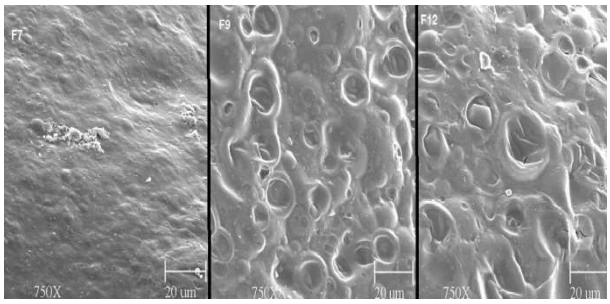

Fig. 7: SEM Micrographs of Formulas (F7, F9, F12) at Magnification 750X

CONCLUSION

In this study, the emulsion gelation method used to prepare oil entrapped floating beads, gives promising results for sustaining the release of highly water soluble drugs that have fast absorption and short plasma half life as the drug used in this study TMZ.

Two formulas (F9 and F12) were selected as promising formulas required further stability and *in vivo* studies suggested for future.

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