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Preparation of a Delayed Releasing Device of Hydroxypropyl Methylcellulose Phthalate Macrocapsules Containing Peppermint Oil

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A B S T R A C T

In this study, pH-sensitive macrocapsules containing peppermint oil (PO) were prepared using a new emulsification/polymer precipitation technique. An aqueous alkaline solution of hydroxypropyl methylcellulose phthalate (HPMCP) was used to emulsify PO in water (O/W). This emulsion was then added dropwise to a solution of citric acid to result in a solid dispersion of macrocapsules. Screening studies showed that three different factors including the polymer and the acid concentrations, as well as the essential oil to water (O/W) ratio have considerable effects on the encapsulation process. Different formulations of macrocapsules were prepared and characterized in terms of PO loading, encapsulation efficiency, and release using GC-FID analysis. A 2-level factorial design with 4-centre points was then used to optimize PO loading and encapsulation efficiency against the above mentioned parameters. Optical microscopy showed a polydispersed O/W emulsion, whereas the macrocapsules were uniformly dispersed with mean diameter of 1 mm. The prepared macrocapsules could protect PO from acidic condition of stomach and release it in a simulated intestinal fluid.

Key Words:

delayed release;
encapsulation;
HPMCP;
macrocapsules;
peppermint oil;
pH-sensitive polymer.

INTRODUCTION

Natural and synthetic polymers have been extensively used for encapsulation of different chemicals including; paints, perfumes, pesticides, probiotics, flavours, and drugs [1-3]. Encapsulation of drugs has attracted a number of research groups due to the potential advantages of this technology for drug targeting, designing the release profile of active compounds and extending the shelf life of the encapsulated medicine [4-5]. Moreover, in oral drug delivery as the preferred route of administration, many advantages can be considered for polymeric particulate systems compared to the single unit dosage forms including: (a) uniform distribution of drug-containing particles and less local damage to the gastrointestinal (GI) mucosa,

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(b) reproducibility of transport in GI tract, (c) adding controlled release properties to the particulate system, and (d) good content uniformity and drug loading [6]. Particulate delivery systems can be highly desirable for treatment of a common site specific disease of GI tract, which is irritable bowel syndrome (IBS) [7]. Population-based studies in the United States estimate the prevalence of IBS at 10% to 20% and the incidence at 1% to 2% per year [8]. Similar reports have been published about the IBS distribution among the Iranian populations in the recent years [9-10]. Peppermint oil (PO) is an effective therapy for the symptoms of IBS through its relaxation of smooth muscles, carminative, and antibacterial actions. Enteric delivery of PO is crucial for drug therapy and also for avoiding contact with the gastric mucosa and gastric pH which decomposes the essential oil [11]. Therefore, providing simultaneous enteric delivery of PO with the particulate delivery systems would be highly desirable.

Different techniques have been used to encapsulate solids and liquids, among which coacervation [12], emulsification/solvent evaporation [13], and spray drying have been more frequently reported [14,15]. Polymeric particulate carriers were recently investigated for enteric delivery of PO in IBS treatment due to the potential advantages of this systems as described above [6,11,16]. However, complicated techniques and a requirement to coat the resulting particles have made these techniques inconvenient [11,16]. The objective of this study was to develop a simple technique for fabrication of spherical macrocapsules for site-specific delivery of PO. Moreover, as the traditional formulation of drug delivery systems involved a good deal of effort and time, experimental design was used to investigate the effect of the key parameters on the final characteristics of the macrocapsules. In addition to the advantages provided by the experimental design, this technique could indicate the relative significance of the key variables and their interactions.

EXPERIMENTAL

Materials and Methods

Peppermint oil (PO, grade BP) was purchased from Zardband Co. (Tehran, Iran). HPMCP (HP-55) manufactured by Shin-Etsu Chemical Co., Ltd. (Tokyo, Japan). Sodium hydrogen carbonate and citric acid were purchased from Sigma Chemical Co., (Poole, UK). Also, menthol standard, disodium hydrogen phosphate and hydrochloric acid were supplied from Merck (Darmstadt, Germany).

Preparation of Macrocapsules

An emulsion system of oil in water/precipitation technique was developed for encapsulation of PO in a pH sensitive polymer (i.e., HPMCP). This new technique was adopted from the work reported by Cerdeira et al. [17] for encapsulation of a model micronized powder. Screening studies using different concentrations of reagents and working conditions were carried out to fabricate the macrocapsules. Briefly, an appropriate amount of PO (Table 1) was emulsified in an alkaline solution (2% (w/v) $NaHCO_3$) of HPMCP (HP-55, 8-16% (w/v)) using a magnetic stirrer (500 rpm) for 1 h. The resulting emulsion was then added dropwise through a needle (i.d. of 1.8 mm) to 100 mL of citric acid solution (10% (w/v)) under mechanical stirring. The resulting macrocapsules were then filtered and dried at room temperature. In order to control the emulsification of PO in the polymer solution, optical microscopy using an Olympus optical microscope (BX51TF, Japan) equipped with a digital camera along with MpegTV software was used. Briefly, a droplet of the emulsion was put on a glass slide and the distribution of the PO droplets was observed. The external appearance and the internal structure of the resulting macrocapsules (using a surgical blade for cross-sectioning the particles) were recorded using a CAMAG Reprostar 3 camera (Muttenz, Switzerland).

Determination of PO Loading and Encapsulation Efficiency

The amount of encapsulated PO was determined by dissolving a sample of 20 mg macrocapsules in 5 mL acetone, which already contained 100 ppm linalool as the internal standard. GC-FID analyses of the oil were conducted using a Thermoquest-Finnigan instrument equipped with a DB-1 fused silica column (30×0.25 mm i.d., film thickness 0.25 μm). Nitrogen was used as the carrier gas at the constant flow of 1.1 mL/min. The oven temperature was raised from 60ºC to 110ºC at a rate of 5ºC/min and then to 250ºC at a rate of

Table 1. 2-level factorial design with 4 replications of centre point for preparation of PO-loaded macrocapsules.

Run	Macrocapsule Formulation	Ratio O/W (%v/v) (A)	Polymer (%w/v) (B)	Acid (%w/v) (C)	Loading (%w/w)	Efficiency (%)	Drug release test
1	F8	30	16	15	65.4	66.0	P*
2	F3	10	16	5	37.0	74.9	P
3	F6	30	8	15	96.8	65.2	P
4	F7	10	16	15	25.5	56.7	P
5	F10	20	12	10	52.5	64.0	P
6	F12	20	12	10	55.7	61.8	P
7	F2	30	8	5	78.4	34.3	P
8	F5	10	8	15	40.2	56.8	P
9	F9	20	12	10	68.2	75.8	P
10	F4	30	16	5	72.7	68.6	P
11	F1	10	8	5	28.4	34.4	P
12	F11	20	12	10	56.8	63.1	P

(*) Passed the test

10ºC/min. The injector and detector (FID) temperatures were kept at 250ºC and 300ºC, respectively. 1 µL of the test solutions and the standards were injected into the GC-FID for analysis. Statistical analysis was carried out to validate the analytical method.

The menthol content was determined by plotting a standard curve relating the ratio of AUC for menthol/linalool with the menthol concentartion. An equation was generated by fitting a linear regression model to the data generated for standard concentrations. The PO loading (eqn 1) and encapsulation efficiency (eqn 2) were then determined using the following equations:

$$\text{Loading (\%)} = \frac{\text{weight of loaded PO}}{\text{weight of PO loaded sample}} \times 100 \quad (1)$$

$$\text{Encapsulation efficiency (\%)} = \frac{\text{loading} \times \text{TMW (mg)} \times 100}{\text{added PO (mg)}} \quad (2)$$

(TMW = total macroencapsule weight)

Drug Release Test

Drug release test was performed according to an adapted procedure [11] from the United States Pharmacopoeia 25, using paddle apparatus. An amount of 200 mg macrocapsules was placed in a dissolution vessel containing 100 mL of 0.1 N HCl solution in the lower phase and 50 mL *n*-hexane containing internal standard in the upper phase. After 2 h, samples were collected from the dissolution vessels and the amount of the released PO in organic phase was determined using the above mentioned GC-FID technique. The samples were then transferred into 100 mL of sodium phosphate buffer solution with pH 6.8 (50 mM) in the lower phase and 50 mL of *n*-hexane containing the internal standard in the upper phase and the amount of essential oil released in the organic phase was measured after 90 min. The collected samples were concentrated at the stream of nitrogen gas up to 1 mL and kept at low temperature for analysis by GC method, as previously described. Each experiment was carried out in triplicate.

Factorial Design

A 2-level factorial design was used to avoid the traditional pharmaceutical formulation approach of one variable at a time. Using this method, joint effects of independent variables can also be evaluated. The number of the required experiments in the statistical design depends on a number of independent variables. The responses were measured for each trial and then simple linear, interactive, or other suitable models fit-

ted by a regression analysis and the F-value was used to identify the statistically significant terms. The model was also used for drawing response surface plots to visualize the impact of the changing variables at a glance. Our primary studies showed that the O/W ratio in the PO emulsion and the polymer concentration were of the most important and independent variables, which highly affected the emulsion stability and finally the encapsulation process. The citric acid concentration was also considered as the third parameter, which could potentially affect the responses. The design and the statistical analysis of the experiments were performed using Design Expert software (version 6.0).

RESULTS AND DISCUSSION

Typical chromatograms of a standard and a sample solution of menthol, containing linalool as the internal standard are shown in Figures 1a and 1b. The results of the method validation (Table 2) showed a very good linearity ($r^2 = 0.995$) within the specified menthol concentration range (i.e., 200-2000 ppm).

The results of the emulsification of PO in water, using less than 0.3 volume fraction of PO and different concentrations of HPMCP in the alkaline solution, led to a homogeneous and stable emulsion. The prepared emulsions were stable in the refrigerator conditions (i.e., 4ºC) for investigated period of one week. The use of the alkaline solution of HPMCP as the suspending agent for etodolac particles (an anti-inflammatory drug model) was previously reported by Cerderia et al. [17]. However, a new adaptation of the mentioned technique for emulsifying PO in water using solubilized HPMCP is presented in this work. The emulsification capability of the ionized polymer may be attributed to a thin protective layer of the polymer around the PO droplets which prevent the coalescence of the primary emulsion droplets [18].

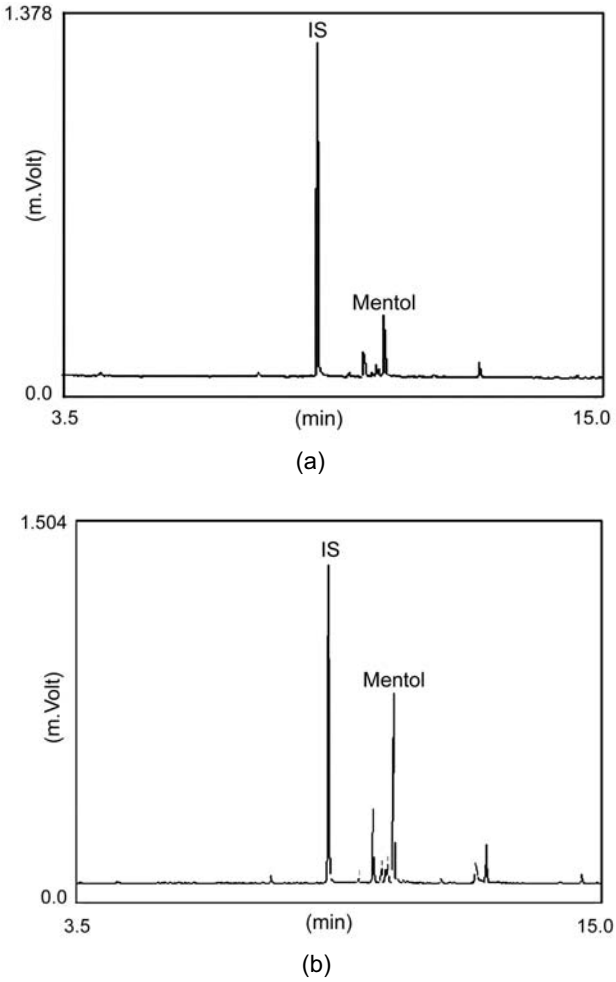


Figure 1. GC-FID Chromatograms of: (a) standard solution and (b) the sample extracted from macrocapsule.

Different formulations were prepared using a statistical design. The characteristics of the resulting macrocapsules are summarized in Table 1. The results of the optical microscopy (Figure 2) for the PO emulsion (Formulation 2) showed that the droplets of PO were polydispersed in respect with the size diameters range from below 5 to over 50 µm. Emulsification involves a complex interaction between the applied mechanical forces and the viscosity of the emulsion medium. These factors

Table 2. Analytical characteristics of the GC-FID method for menthol assay.

Compound	Range (ppm)	Equation	r^2	LOD (ppm)	LOQ (ppm)	RSD (%)	Recovery (%)
Menthol	200-2000	Y=0.0015X-0.05	0.995	9.8	32.0	1.8	80

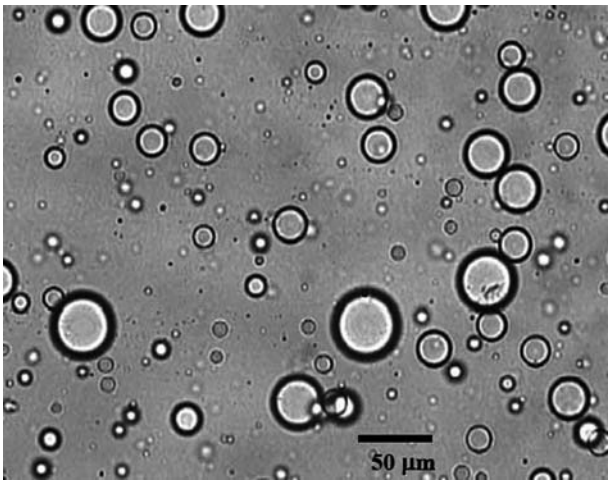


Figure 2. Optical microscopy of O/W emulsion.

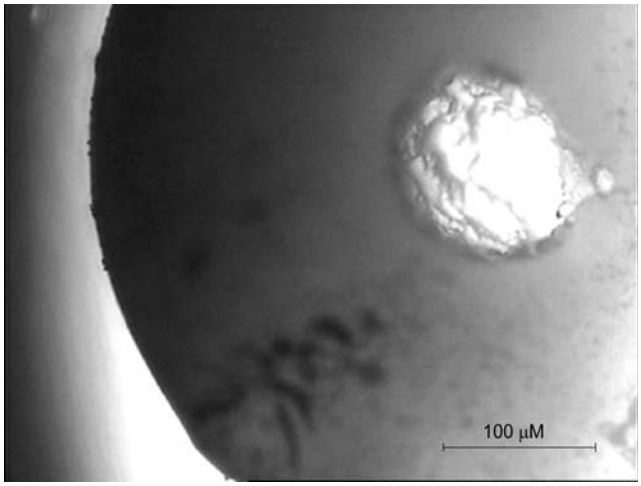


Figure 4. Photograph of the cross-sectioned macrocapsule.

contribute to the overall rheological behaviour of the emulsion and the shear stresses experienced by PO droplets. Therefore, a polydispersed emulsion may be the results of weak mechanical force provided by magnetic stirrer, while may be the results of a powerful emulsifier (ionized HPMCP) to produce a stable emulsion.

Preparation of macrocapsules by adding the primary emulsion to the acidic medium led to solid macrocapsules with mean size 1 mm (Figure 3). The resulting macrocapsules were spherical and monodispersed and had no relationship with the PO droplets in the emulsion with an extensive particle size range (Figure2). Therefore, the size of the resulting macrocapsules may be attributed to the parameters like size and hight of needle and also polymer concentration.

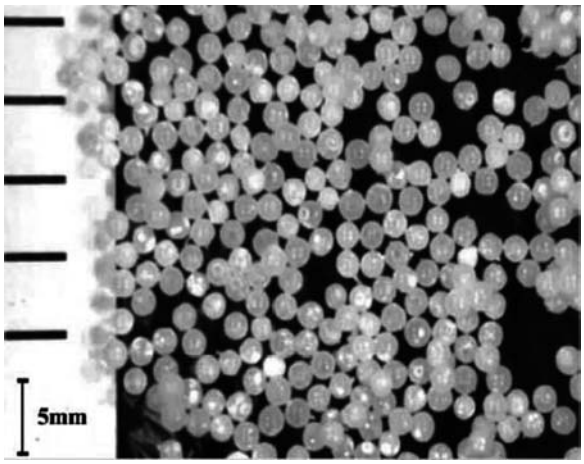


Figure 3. Photograph of the PO loaded macrocapsules.

Since every droplet of the primary emulsion formed macrocapsule upon contact with the acid medium, it was assumed that either the macrocapsules have many small PO droplets entrapped within the polymeric matrix or PO droplets coalesced to form a larger capsule due to the mechanical forces exerted on PO droplets. However, the internal structure of the macrocapsules (Figure 4) suggests that the PO droplets coalesce to produce a large reservoir (capsule) inside the polymeric shell.

This observation in line with that of Cerderia et al. [17] may highlight the importance of polymer precipitation on the outer surface of the droplets to form the macrocapsules shell. Therefore, HPMCP plays dual actions of emulsion stabilization on the first step followed by addition of the primary emulsion to the acid medium, and shell formation or encapsulation on the second step.

The present method of PO loaded macrocapsules preparation has many advantages compared to the cross linking procedure, as reported by Sibanda and his colleagues [11], including simple, fast, and reliable procedure. Moreover, in the above mentioned study the prepared oilispheres could not protect the PO in acid medium, unless a complementary coating was applied over the surface of the particles [11]. However, all the formulations prepared using the present emulsification/polymer precipitation technique have passed the drug release test as described before. The results of the dissolution test showed that less than 10% of the loaded PO was released into the

Table 3. Analysis of variance for 2-level factorial design and loading response.

Source	Sum of squares	DF	Mean square	F Value	Prob > F	
Model	4149.61	1	4149.61	45.23	< 0.0001	significant
A*	4149.60	1	4149.60	45.23	< 0.0001	-
Residual	825.73	9	91.75	-	-	-
Lack of fit	685.07	6	114.18	2.44	0.2485	not significant
Pure error	140.66	3	46.89	-	-	-
Cor total	4995.51	11	-	-	-	-

(*) (O/W ratio)

organic phase in acid medium, or in another word, the polymeric shell (HPMCP) could successfully protect PO from the acidic environment. However, upon exposure to the alkaline solution the macrocapsules released more than 80% of the loaded PO.

Experimental Design and Optimization of the PO Loading

The results of the statistical analysis of PO loadings are summarized in Table 3 and Figure 5a.

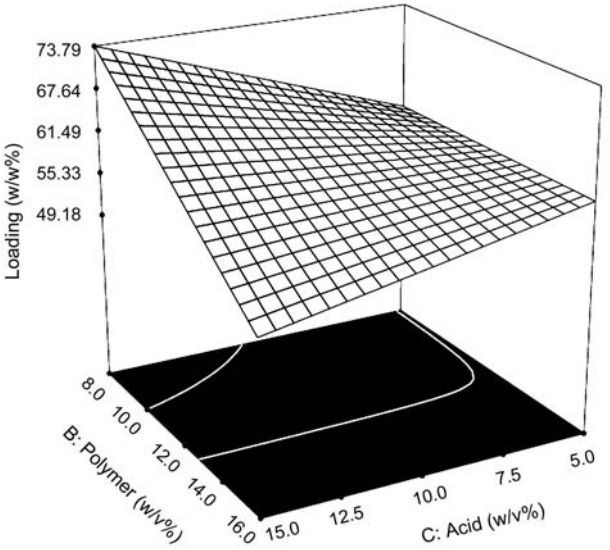
The PO loading in the macrocapsules is an important parameter which determines the conditions where the maximum loading with desired release characteristics can be achieved. The results show that the model F-values 45.23 proposes a valid relationship between the selected variables and PO loading within the specified variable ranges. In another word, there is only a 0.01% chance that a model with such large F-value could occur by experimental errors. In this model, the O/W ratio (F<0.0001) is a significant factor, wherein, as the volume fraction of PO increases, the PO loading also increases. This linear relationship can be summarized in the following equation:

$$\text{Loading} = 10.0000 + 2.2775\ (\text{O/W}) \qquad (3)$$

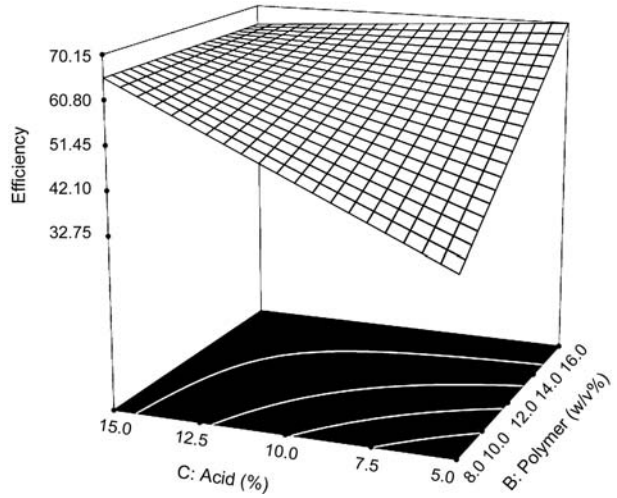
therefore, within the specified range of O/W ratio (i.e., 10-30%) as the O/W ratio increases, the loading of PO also increases. However, acid and polymer concentrations do not show a direct impact on the PO loading.

Experimental Design and Optimization of the Encapsulation Efficiency

The results of statistical analysis for encapsulation



(a)



(b)

Figure 5. Surface response curves for: (a) loading and (b) efficiency.

Table 4. Analysis of variance for 2-level factorial design and encapsulation efficiency response.

Source	Sum of squares	DF	Mean square	F Value	Prob > F	
Model	1619.48	5	323.90	11.93	0.0083	significant
B	712.53	1	712.53	26.24	0.0037	-
C	132.03	1	132.03	4.86	0.0786	-
BC	686.35	1	686.35	25.27	0.0040	-
Residual	135.78	5	27.16	-	-	-
Lack of fit	9.81	2	4.91	0.12	0.8936	not significant
Pure error	125.97	3	41.99	-		-
Cor total	1974.27	11		-		-

B: Polymer conc; C: Acid Conc; BC: Interaction between Polymer and Acid Conc.

efficiency are summarized in Table 4 and can be graphically observed in Figure 5b.

Encapsulation efficiency is even a more important parameter than loading, due to the high value of the essential oil in the encapsulation process. Therefore, the optimization of the encapsulation conditions where the loss of the essential oil is minimized would be highly desirable. The results show that the model F-value of 11.93 is proposing a significant relationship between the selected variables and the encapsulation efficiency. Values of prob.>F less than 0.050 indicate that the factors are significant. In this case, polymer concentration (B), acid concentration (C) and their interaction with the citric acid concentration (BC) are the significant model factors. Therefore, the final equation in terms of the actual factors can be summarized as follows:

$$\text{Efficiency} = -25.67 + 6.99\,B + 5.16\,C - 0.46\,BC \quad (4)$$

therefore, despite the loading response, polymer and acid concentrations have a direct impact on the increasing of the efficiency of encapsulation. In another word, increasing the concentrations of these latest parameters directly results in lesser PO loss which may be related to the more efficient transformation of O/W droplets to the macrocapsules. Interestingly, the interaction of polymer and acid concentrations negatively affect the encapsulation efficiency, which highlights the importance of the balance between these factors on the responses.

CONCLUSION

PO loaded HPMCP macrocapsules were successfully formulated and evaluated for the delayed-release action. Key parameters, including the O/W ratios, polymer and citric acid concentrations were studied by factorial design for their influences on loading and system's efficiency. The O/W ratio had the major effect on the loading of PO in the macrocapsules whereas, polymer and acid concentrations increased the encapsulation efficiency response. The negative impact of the interaction of these two last parameters on the efficiency of encapsulation may highlight the importance of phase transformation on the encapsulation process. Further investigations are necessary to study the stability profile of the encapsulated PO.

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