IJE TRANSACTIONS B: Application Vol. 26, No. 8, (August 2013) 807-814

International Journal of Engineering

Journal Homepage: www.ije.ir

Encapsulation of Peppermint Oil with Arabic Gum-gelatin by Complex Coacervation Method

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PAPER INFO

Paper history:
Received 26 August 2012
Received in revised form 17 December 2012
Accepted 28 February 2013

Keywords:
Encapsulation
Complex Coacervation
Menthol
Arabic Gum
Gelatin
Tannic Acid

ABSTRACT

The gelatin/gum arabic microcapsules encapsulating peppermint oil were prepared by complex coacervation using tannic acid as hardening agent. The effects of various parameters, including concentration of wall material, core material, tannic acid and Tween 80 were investigated on particle size and encapsulation efficiency. For statistical evaluation of the parameters, Taguchi method was concerned. The size of prepared spherical microcapsules was 19-66 micrometers. The results showed that, particle size increased with increasing the core and the wall concentration and decreased with increasing tannic acid and Tween 80 concentration. The efficiency increased by increasing the core and wall concentration and tannic acid and Tween 80 concentration had no effect on efficiency. Maximum efficiency of 82% was achieved under optimal conditions: 4% wall material, 5% core material, 0.75% tannic acid and 0.02% Tween 80. The release of microcapsules was investigated in gastric and intestinal fluid. The microcapsules released most of the core material in simulated gastric fluid (pH 1.2).

doi: *10.5829/idosi.ije.2013.26.08b.01*

1. INTRODUCTION

Primary constituent of peppermint oil is white crystalline substance called menthol ($C_{10}H_{19}OH$). L-menthol is a cyclic terpene alcohol found in high concentrations in peppermint oil and this is the cause of its good scent and wide utilization in pharmaceutical, cosmetic and food products. Typical menthol crystals or granules form with a melting point of 41-43 ° C and among the possible eight isomers of menthol, L-menthol is the most abundant in nature. However, its high volatility restricts its application in processed food. Microencapsulation method is a good solution for this inconvenience [1, 2].

Microencapsulation is a method by which one material or mixture of materials is coated by the other material. This method is designed for protection and isolation and assists in the storage and controlled release [3]. Controlled release of food ingredients at right place and right time is a key functionality that can be provided by microencapsulation [4]. Microcapsules are prepared by different methods such as simple or complex coacervation, phase separation and spry drying [1, 5]. One of the abundant methods studied by authors is complex coacervation [6].

Coacervation, modified emulsification technique, is a relatively simple method. Coacervation consists of the separation of hydrocolloid from primary solution then agglomeration into separate, liquid phase called coacervate. The continuous phase is coacervate and other phase is called equilibrium solution [4, 7]. Coacervation can be divided into two types: with a polymer (simple coacervation) or combining two or more polymer (complex coacervation). When two polymers are used, the two polymers in a specific pH with opposite charges are intertwined and form a coacervate [8, 9]. Microcapsules prepared by this method are insoluble in water and heat resistant and offer controlled release properties [10].

Complex coacervation by gelatin-arabic gum system is recommended due to their abundance, biocompatibility, biodegradability and safe properties suggested by authors. The main reason for using gelatin and arabic gum as wall material is their abundance and biodegradability [11]. These two biopolymers under electrostatic interactions form coacervate layer. Coacervate layer converts to a rigid membrane by

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gelatin cross-linking. Formaldehyde or glutaraldehyde that can react with amino groups of gelatin, typically are used as cross-linker [12]. However, these aldehyde agents are toxic for the human body and in food processing. This is one of the major limitations to produce microcapsules of complex coacervation for food and pharmaceutical industries using this aldehyde [13]. Tannins, plant polyphenols, could be used as a safe alternative cross-linker by ability to link with proteins such as gelatin, through hydrogen bonding and hydrophobic interactions [12].

This study presents complex conservation method in preparing L-menthol microspheres using gelatin-arabic gum and tannin as cross linker. Release performance in simulated gastric fluid is also considered.

2. MATERIALS AND METHODS

2. 1. Materials L-menthol and gelatin used were purchased from Merck (Darmstadt, Germany). Gum arabic used in this study was obtained from PANREAC QUIMICA SAU. All other chemicals were analytical grade and from Merck (Darmstadt, Germany).

2. 2. Design of Experiments In order to avoid the traditional methods of testing and error guessing, Taguchi method was used in this study. Wall material concentration (%), concentration of core material (%), tannic acid concentration (%) and Tween 80 concentration (%) were considered to check. Factor levels according to tests carried out and studies are shown in Table 1 [13]. Designed experiments corresponding to the levels reported in Table 1 are summarized in Table 2.

2. 3. Preparation of Microcapsules Arabic gum and gelatin solution were prepared with known concentrations. To prepare gelatin solution, 50 °C water bath was used to facilitate dissolution of the gelatin. After preparing the solution, menthol, which was melted at 50 °C was added to gelatin solution. After that gum arabic and Tween 80 soloution, with a 1:1 mass ratio of gelatin to gum arabic was added to mixture. Then, in a 40 °C water bath, the pH of mixture was adjusted to 4 by adding acetic acid 10%. Then the mixture was putted in ice bath until the temperature reached 4 °C. Tannic acid was added to the mixture and put on stirrer to reach ambient temperature. Coacervate liquid deposited at the bottom of the container was washed with distilled water and dried using a freeze dryer.

2. 4. Morphology of Microcapsules The morphology of microcapsules was observed by scanning electron microscope (SEM: CamScan MV2300). The samples were covered with a fine layer of gold using an SEM sputter coater.

TABLE 1. Factors and levels selected for tests

Factors	Symbol	Levels			
		1	2	3	4
Wall material concentration (%)	A	1	2	3	4
Menthol concentration (%)	B	2	3	4	5
Tannic acid concentration (%)	C	0.5	0.75	1.25	1.5
Tween 80 concentration (%)	D	0.02	0.04	0.08	0.1

TABLE 2. Proportions of gum arabic and gelatin as per mixture design

Experimental run no.	A	B	C	D
1	1	1	1	1
2	1	2	2	2
3	1	3	3	3
4	1	4	4	4
5	2	1	2	3
6	2	2	1	4
7	2	3	4	1
8	2	4	3	2
9	3	1	3	4
10	3	2	4	3
11	3	3	1	2
12	3	4	2	1
13	4	1	4	2
14	4	2	3	1
15	4	3	2	4
16	4	4	1	3

2. 5. Determination of Microcapsules Efficiency In order to determine microcapsules efficiency, 0.1g of powder was dissolved in 1 mL of NaOH 2N which 2 mL of acetone containing benzyl alcohol was located as internal standard (1 µL / mL of aceton) in the upper phase, followed by mixing with a rota mixer for 1 min. Then mixture was placed at 90 ºC water bath for 10 min. One microliter of acetone solution containing

released menthol was analyzed by a PR2100 Perichrom GC (Saulx-les-Chartreux, France), equipped with a Flame Ionisation Detector and a HP-PONA column (50 m, 0.201 mm i.d. and 0.5 μm film thickness). Injector and detector temperatures were maintained at 200 and 250 °C, respectively. The oven temperature was maintained at 50 °C for 1 min and increased to 170 °C at the rate of 10 °C/min, then maintained at that temperature for 10 min. The internal standard method was used to calculate the amount of menthol. Encapsulation efficiency was calculated from Equation (1):

$$\text{Encapsulation efficiency} = \frac{\text{Amount of encapsulated menthol}}{\text{Total amount of added menthol}} \times 100 \quad (1)$$

2. 6. Determination of Release Profile of Coacervate Microcapsules in Simulated Digestive Fluids The microcapsules release profiles were investigated with two simulated digestive fluids prepared according to US Pharmacopoeia: simulated gastric fluid (SGF, pH 1.2) prepared by dissolving 2 g of NaCl and 7 mL of HCl in distilled water with or without 3.2 g of pepsin and diluting to 1 L, and simulated intestinal fluid (SIF, pH 7.5) by dissolving 6.8 g of K_2HPO_4 and 190 mL of 0.2 N NaOH in distilled water with or without 10 g of pancreatin and diluting to 1 L [14]. Accurately weighed amounts (about 0.3 g) of microspheres were immersed in 40 mL of SGF, incubated for 2 h at 37 °C in a shaking water bath at 100 rpm, transferred to 40 mL of SIF, and incubated under the same condition for 6 h.

The remaining amount of menthol was measured according to the method that was described in the previous section. Amount of released menthol was obtained from Equation (2):

$$\text{Released menthol} = \frac{\text{Amount of encapsulated menthol} - \text{Amount of remained menthol}}{\text{Amount of encapsulated menthol}} \times 100 \quad (2)$$

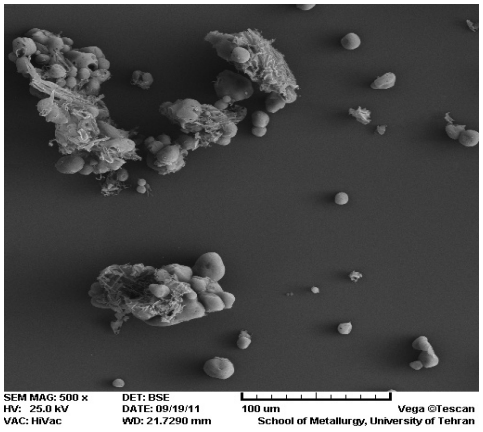
3. RESULTS AND DISCUSSION

3. 1. Size and Morphology of Microcapsules Scanning electron microscope was used to study morphology and size of microcapsules. Representative examples are shown in Figures 1 and 2 that show the fabricated spherical microspheres (Run No. 5, 8, 9, 10 in Table 2) had typical smooth surface.

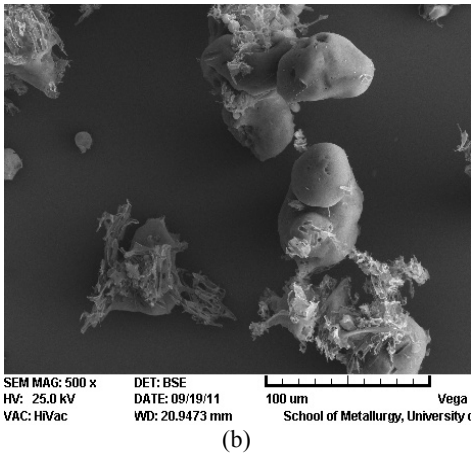
Table 3 shows the efficiency and particle size of microcapsule samples shown in Table 2. All particle sizes of prepared microcapsule samples were less than 100 micrometer.

TABLE 3. Efficiency and particle size of microcapsule samples

Experimental run no.	Efficiency (%)	Particle size (μm)
1	53	30
2	62	27
3	58	24
4	65	18
5	57	22
6	62	30
7	67	38
8	68	44
9	59	19
10	65	26
11	68	51
12	79	62
13	64	32
14	71	50
15	73	48
16	80	69



(a)



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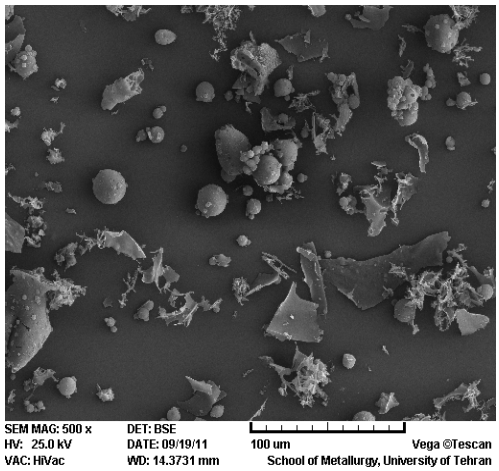

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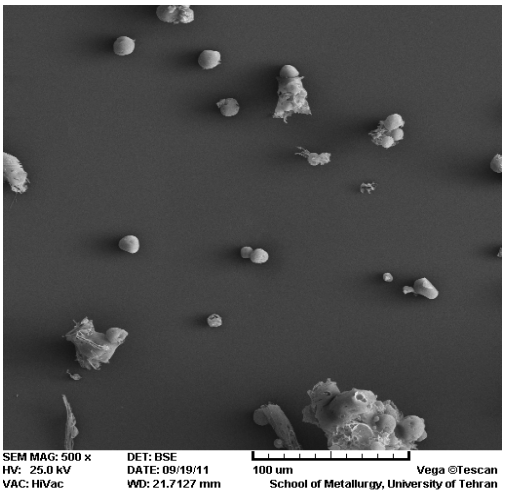
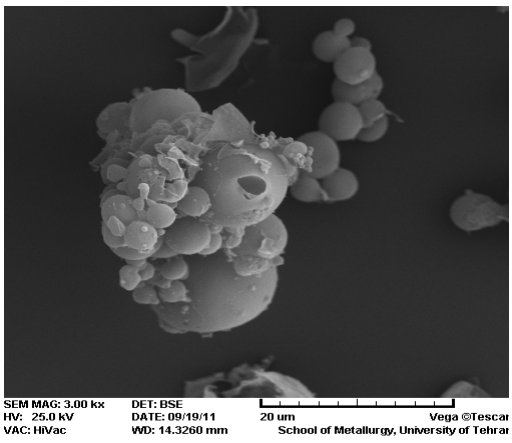
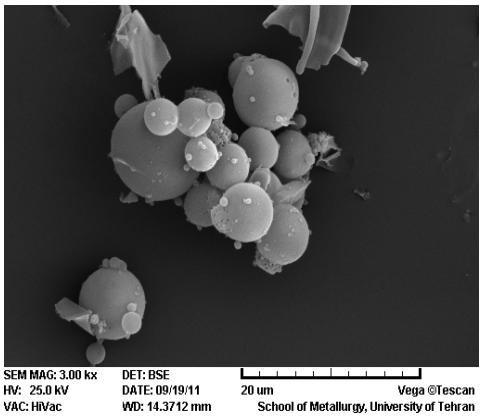


(d)

Figure 1. SEM image of microcapsules with magnification 500 X : (a) sample 5, (b) sample 8, (c) sample 10 and (d) sample 4.



(a)



(b)

Figure 2. SEM image of microcapsules with magnification of 3000 X: (a) sample 9 and (b) sample 10

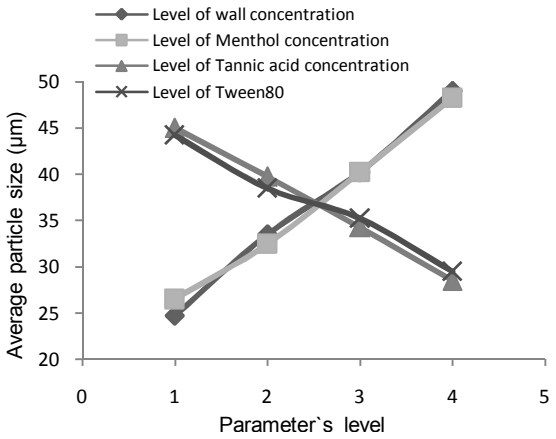


Figure 3. Effects of wall concentration, menthol, tannic acid and Tween 80 on average particle size

Using Taguchi software, the main effects plot for the size of microcapsules was provided. Figure 3 shows that particle size increased with increasing the core and the wall concentration and decreased with increasing tannic acid and Tween 80 concentration. When the wall material concentration increased, the spherical multinuclear microcapsules became bigger. This was consistent with the conclusion obtained by Andrianov, who observed that the mean size of microcapsules increased with the increase of the wall material concentration [15]. Increasing particle size with increase of wall concentration could be due to the interconnection with tannic acid. There are more gelatin molecules in the system as a result, the molecules are more concentrated. Increasing particle size with increase of core concentration could be due to the formation of more and larger particles in the oil-water emulsion, in the presence of more oil. According to the

researchers' views, effect of core/wall ratio on the loading and particle size of coacervate microcapsules was very significant [10]. Increasing particle size with increase of tannic acid was expected but as shown in the diagram, it decreased. When head of all connections of tannic acid are free, best crosslinking between tannic acid and gelatin occurs. By increasing ratio of tannic acid to gelatin, all free heads of gelatin can be occupied by tannic acid, therefore there is less chance for tannic acid to find out free gelatin in its neighborhood, as a result, crosslinking chance is reduced and smaller microcapsules are formed. Cross linking between gelatin molecules and tannic acid occurs mainly through hydrogen bonding and hydrophobic interactions, as adding non-ionic surfactant Tween 80 prevents cross linking.

Analysis of variance with Taguchi software showed the influence of each parameter on the particle size of microcapsul samples. The impact of each constituent concentration (%) was as follows: wall material, menthol, tannic acid, Tween 80 and others; 35.6, 29.9, 16.4, 12.0, and 5.9, respectively. That shows all parameters affect the size of the microcapsules.

3. 2. Determination of Microcapsules Efficiency

Main effects plot for means of efficiency was prepared by using Taguchi software. Figure 4 shows that the efficiency increased by increasing the concentration of wall materials and menthol, also tannic acid concentration and Tween 80 concentration had no effect on efficiency. The positive impact of two parameters of wall materials and menthol concentration could be due to the impact on the formation of emulsions and complex coacervation. In the presence of higher amounts of natural biopolymer, more stable emulsion particles are formed and a thick coacervate layer is formed around the emulsion particles. An important parameter is the core/wall ratio. Efficiency increases with increasing core material, if the wall material, is high. Results obtained by Dong shows that as the core/wall ratio was increased, the efficiency of microcapsules remained constant [10].

Analysis of variance showed that the impact of parameters percent including: wall material, menthol, tannic acid, Tween 80 and others were 39.8, 50.5, 2.1, 0.8, and 6.6, respectively. That shows the parameters affecting the efficiency were wall material concentration and menthol concentration.

Aldehyde compounds such as formaldehyde and glutaraldehyde are known as cross-linkers for complex coacervation but, they are toxic and are not allowable in food processing. In this study, tannic acid a natural polyphenols, was used instead of aldehyde compounds. The efficiency obtained in the present study was lower than the efficiency of complex coacervation gum arabic - gelatin with glutaraldehyde and formaldehyde.

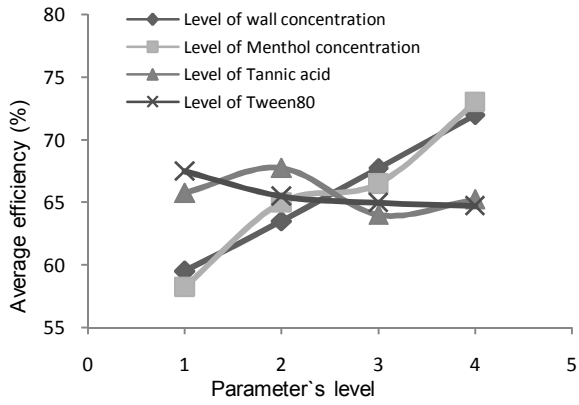


Figure 4. Effects of wall concentration, menthol, tannic acid and Tween 80 on average efficiency

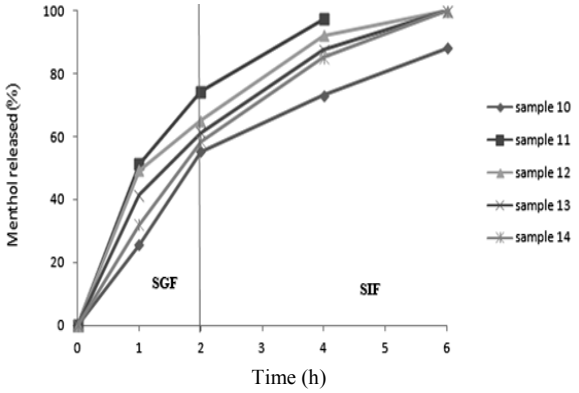


Figure 5. Effect of time on released menthol for different samples 10, 11, 12, 13, 14 as shown in Table 2 in SGF and SIF

3. 3. Determination of the Optimal Mode

Since the desired particle size is less than 100 micrometers and the size of the prepared microcapsules were less than 100 micrometers, so the efficiency factor is important to determine the optimal mode. Optimal mode for efficiency using the Taguchi software was determined as follows:

The concentration of wall materials: Level 4
The concentration of menthol: Level 4
The concentration of tannic acid: Level 2
The concentration of combination of Tween 80: Level 1

and the efficiency of microcapsules prepared in this condition was 82%.

3. 4. Oil Release from Microcapsules

Oil release under the two environments, simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) were studied. Due to investigations, peppermint oil is effective prescribed for colon syndrome, so menthol release in the intestine is desirable. As Figure 5 shows a considerable amount of menthol in simulated gastric

fluid is released and if the goal is to be used as a drug delivery application, this result is not suitable and considerable. The effectiveness of food additives are improved by a timely and targeted release of the range of food ingredients with optimal dosage of additives; thereby, improvement of cost-effectiveness for the food manufacturer [4].

4. CONCLUSION

The gum arabic-gelatin microcapsules, encapsulating peppermint oil were prepared by coacervation using tannic acid as cross-linking agent. In complex coacervation, cross-linking agent factor is important. In this study, tannic acid a natural polyphenols, was utilized instead of aldehyde compounds. In appearance, spherical microspheres with smooth surface were formed. The results showed that, increasing concentration of core and wall materials, increases efficiency. Evaluation of microcapsules release in the SGF and SIF indicated that large amount of menthol is released in the SGF environment that is not suitable solution for medicinal cases.

5. ACKNOWLEDGMENT

This research has been supported by Iran National Science Foundation (INSF) through the Grant Number 90004980.

6. REFERENCES

1. Soottitantawat, A., Takayama, K., Okamura, K., Muranaka, D., Yoshii, H., Furuta, T., Ohkawara, M., and Linko, P., "Microencapsulation of l-menthol by spray drying and its release characteristics", ***Innovative Food Science & Emerging Technologies***, Vol. 6, No. 2, (2005), 163-170.
2. Mortenson, M. A. and Reineccius, G. A., "Encapsulation and release of menthol. Part 1: The influence of osan modification of carriers on the encapsulation of l-menthol by spray drying", ***Flavour and Fragrance Journal***, Vol. 23, No. 6, (2008), 392-397.
3. Madene, A., Jacquot, M., Scher, J. and Desobry, S., "Flavour encapsulation and controlled release–a review", ***International Journal of Food Science & Technology***, Vol. 41, No. 1, (2006), 1-21.
4. Gouin, S., "Microencapsulation: Industrial appraisal of existing technologies and trends", ***Trends in Food Science & Technology***, Vol. 15, No. 7, (2004), 330-347.
5. Sarkar, S., Gupta, S., Variyar, P. S., Sharma, A. and Singhal, R. S., "Irradiation depolymerised guar gum as partial replacement of gum arabic for microencapsulation of mint oil", ***Carbohydrate Polymers***, (2012).
6. Mayya, K. S., Bhattacharyya, A. and Argillier, J. F., "Micro-encapsulation by complex coacervation: Influence of surfactant", ***Polymer International***, Vol. 52, No. 4, (2003), 644-647.
7. KORUS, J., "Microencapsulation of flavours in starch matrix by coacervation method", ***Polish Journal of Food and Nutrition Sciences***, Vol. 10, No. 1, (2001), 17-23.
8. Dong, Z., Toure, A., Jia, C., Zhang, X. and Xu, S., "Effect of processing parameters on the formation of spherical multinuclear microcapsules encapsulating peppermint oil by coacervation", ***Journal of Microencapsulation***, Vol. 24, No. 7, (2007), 634-646.
9. de Kruif, C. G., Weinbreck, F. and de Vries, R., "Complex coacervation of proteins and anionic polysaccharides", ***Current Opinion in Colloid & Interface Science***, Vol. 9, No. 5, (2004), 340-349.
10. Dong, Z., Ma, Y., Hayat, K., Jia, C., Xia, S., and Zhang, X., "Morphology and release profile of microcapsules encapsulating peppermint oil by complex coacervation", ***Journal of Food Engineering***, Vol. 104, No. 3, (2011), 455-460.
11. Najafi, A., "Microencapsulation of orange oil by complexcoacervation and its release behavior", ***International Journal of Engineering***, Vol. 17, No. 4, (2004), 333-342.
12. Xing, F., Cheng, G., Yang, B. and Ma, L., "Microencapsulation of capsaicin by the complex coacervation of gelatin, acacia and tannins", ***Journal of Applied Polymer Science***, Vol. 91, No. 4, (2004), 2669-2675.
13. Zhang, Z.-Q., Pan, C.-H. and Chung, D., "Tannic acid cross-linked gelatin–gum arabic coacervate microspheres for sustained release of allyl isothiocyanate: Characterization and in vitro release study", ***Food Research International***, Vol. 44, No. 4, (2011), 1000-1007.
14. Andrianov, A. K., Chen, J. and Payne, L. G., "Preparation of hydrogel microspheres by coacervation of aqueous polyphosphazene solutions", ***Biomaterials***, Vol. 19, No. 1-3, (1998), 109-115.
15. Anal, A. K., Stevens, W. F. and Remuñán-López, C., "Ionotropic cross-linked chitosan microspheres for controlled release of ampicillin", ***International Journal of Pharmaceutics***, Vol. 312, No. 1, (2006), 166-173.

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P A P E R I N F O

Paper history:
Received 26 August 2012
Received in revised form 17 December 2012
Accepted 28 February 2013

Keywords:
Encapsulation
Complex Coacervation
Menthol
Arabic Gum
Gelatin
Tannic Acid

چکیده

میکروکپسول L–منتول با روش کواسرویشن مرکب، با استفاده از صمغ عربی و ژلاتین به عنوان مواد دیواره و تانیک اسید به عنوان عامل اتصال عرضی تهیه شد. در این مطالعه، تأثیر پارامترهای مختلفی، شامل درصد غلظت مواد دیواره، مواد هسته، تانیک اسید و توئین۸۰ روی اندازه ذرات و بازده بررسی شد. در این مطالعه برای صرفه‌جویی در زمان و هزینه از روش تحلیل آماری تاگوچی استفاده گردید. نتایج بدست آمده از این مطالعه نشان می‌دهد، با افزایش درصد غلظت مواد دیواره و هسته و کاهش درصد غلظت تانیک اسید و توئین۸۰، بازده افزایش می‌یابد. میکروکپسول‌های تهیه شده از نظر شکل کروی بودند و اندازه آن‌ها ٦٦–١٩ میکرومتر بوده‌است. بازده ماکزیمم ۸۲٪ در شرایط بهینه: ٤٪ مواد دیواره، ۵٪ مواد هسته، ۰/۷۵٪ تانیک اسید و ۰/۰۲٪ توئین۸۰ به دست آمد. مطالعه در مورد رهایش نشان می‌دهد که بیشترین مواد هسته در محیط شبیه‌سازی شده معده (pH 1.2) آزاد می‌شود.

doi: *10.5829/idosi.ije.2013.26.08b.01*