Effect of Formulation Parameters on Encapsulation Efficiency and Release Behavior of *p*-aminobenzoic acid-Loaded Ethylcellulose Microspheres

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Abstract

In the current study, *p*-aminobenzoic acid-loaded ethylcellulose microspheres were prepared under various conditions by solvent evaporation method (o/w). This preparation was carried out with different *p*-aminobenzoic acid:ethylcellulose (PABA:EC) ratios, stirring speed, surfactant nature and concentration in order to investigate their effect on encapsulation efficiency and drug release kinetics. Scanning Electron Microscopy (SEM) studies showed spherical microspheres with a porous surface and different structures. The mean diameter of Sauter (d_{32}) of these microparticles ~~was~~ is in the range from 47 to 165 µm with PVA and from 793 to 870 µm with Tween 80 by adjusting process parameters. However, the encapsulation efficiency varied from 37.52 to 79.05 % suitable for the adjustment of a *p*-aminobenzoic acid with prolonged release. Microspheres were characterized by FTIR, DSC and XRD. The release of cation of *p*-aminobenzoic acid was performed in simulated gastric medium at pH 1.2 and 37 ±0.5 °C by UV-VIS analysis to estimate its content. The release data were best fitted to Higuchi model with high correlation coefficient (r^2) and the obtained values of *n* from Korsmeyer-Peppas showed that the drug release follows the Fickian diffusion mechanism.

Keywords: Microparticles; Solvent Evaporation Method; Diffusion; Drug Release; Kinetic Modeling.

~~EFFECT OF FORMULATION VARIABLES ON MICROSPHERES CHARACTERISTICS~~

INTRODUCTION

It has long been known that *p*-aminobenzoic acid (PABA), supports the production of tetrahydrofolic acid (THFA), enhances the metabolism of amino acids and improves the formation and health of red blood cells[1,2]. Among the recently properties of PABA, ~~we cite~~ we quote~~-~~ its inductor effect on interferon formation in living organisms~~ can be quote~~.[3] It was demonstrated that PABA enhances the treatment efficiency of cornea defaults.[4] PABA also inhibits the melanogenesis *in vitro* and *in vivo*, alone or in combination with chemotherapy

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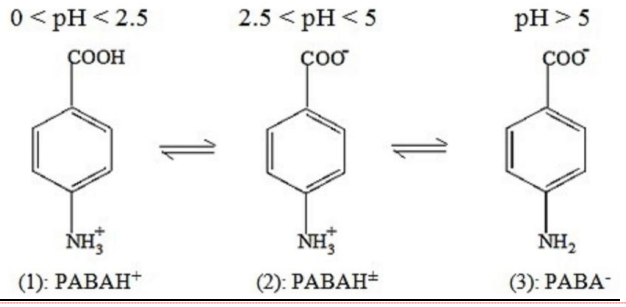
(CT) or radiation therapy[5] (RT). Rather recently, several studies report the biological activities of PABA molecule as an antiviral, antioxidant, antibacterial, antimutagenic and fibrinolytic immuno-modulator agent.[6]

For instance, Akberova has established a new antiviral drug: Actipol® is a 0.007 % PABA solution ~~for the use~~used in ophthalmology.[7] Despite of these new biological properties of PABA ~~cited~~ quoted above, its encapsulation was performed only in the case of solar filter to protect the skin from UVA/B. Microcapsules of PABA with a hardened polymeric shell can be spread through a cream with total elimination of contact between allergenic PABA and the skin.[8] PABA is a water-soluble compound constituting the central part of folic acid and thereby considered as a B-complex factor. It is well tolerated with an easy oral administration.[9] PABA tablet, aminobenzoate potassium capsule, PABA or aminobenzoate potassium are given in food for analytical tests of PABA recovery in 24-hour urine collections.[10] It is required in small amount by the human body but causes liver damage when used ~~with~~ in excess.[11] PABA is an amphoteric compound existing in three forms[12] (Scheme 1) in an aqueous environment at physiological pH with two pK (pK_1 = 2.50 and pK_2 = 4.87). The simulated gastric medium (pH 1.2) promotes the diffusion of the ammonium diacid form (1) with a large majority in this study. The simple UV spectrum of (1), in our case, allows an easy access to %$PABAH^+$ released versus time. Microencapsulation of PABA in microspheres, using ethylcellulose (EC) as a biocompatible polymer matrix, allows its oral administration with sustained release in the stomach at doses below to its toxic level. These microparticle dosage forms can be advantageously used for treating skin cancer. The main objective of this study was to elaborate microspheres of *p*-aminobenzoic acid (PABA) for sustained release using microencapsulation by solvent evaporation method[13-21] and to study the effects of process parameters such as: stirring speed, surfactant nature and concentration, as well as the ratio of PABA:EC on microspheres characteristics, encapsulation efficiency and *in vitro* release behavior. The obtained microspheres were characterized by SEM, FTIR, DSC and XRD. The release from these systems was performed in simulated gastric medium[22] at pH 1.2 and 37±0.5 °C by UV-VIS analysis to estimate the drug content and the influence of different process parameters was also discussed. The obtained release data were analyzed according to Higuchi and Korsmeyer-Peppas models to determine the $PABAH^+$ release mechanism.

EXPERIMENTAL

Materials

PABA (*p*-aminobenzoic acid at purity of 99 %, Chemical (China), was chosen as ~~a~~ model drug. Ethylcellulose (EC, ethoxylate at 48 % mass (viscosity 10 mPa s at 5 % mass in toluene / ethanol solution) was purchased from Sigma-Aldrich (USA). Dichloromethane (DCM at purity of > 98 % from Fluka). Polyethylene Glycol sorbitan monooleate (Tween 80) and Polyvinyl alcohol (PVA, 87-89 %, hydrolyzed, Mw = 13000–23000) from Sigma. For kinetic



Scheme 1: Forms of *p*-aminobenzoic acid at different pH values

measurements, the simulated acidic test solution[22] pH 1.2 was prepared by dissolving 2 g of NaCl and 60 mL of HCl solution (1 N) in 1 L of deionized water.

Preparation of Microspheres

PABA-loaded ethylcellulose microspheres were prepared using the oil-in-water solvent evaporation method.[16,21] The effect of various formulation and processing parameters on microspheres characteristics were investigated by varying PABA:EC ratio, stirring speed, nature and concentration of surfactant. Ethylcellulose (EC) was dissolved in 32g of dichloromethane (different amounts of EC 0.25, 0.5, 0.75 and 1g were used, by changing the PABA:EC ratio: 1:1, 1:2, 1:3 and 1:4)~~.~~, ~~t~~Then, the amount of PABA was dispersed in this solution and stirred at 30 °C. This organic phase was slowly poured into 100 mL of PVA or Tween 80 solution (variable concentrations of 0.5, 1 and 2 % (Table I)). The resulting emulsion was continuously agitated in a glass reactor (600 mL, Ø = 80 mm) using a four-blade turbine impeller stirrer (blade length = 50 mm, blade width = 8 mm, IKA, RW20 digital, UK.) with constant stirring speed (speed variation 600, 900 and 1200 rpm) PABA with at room temperature for 4 h. The organic solvent ~~is~~ has been then allowed to evaporate in order to harden the oil droplets. The solidified microspheres were filtered, washed several times with deionized water and dried under vacuum in a desiccator containing $CaCl_2$. The starting composition of the different microspheres prepared along with formulations codes are summarized in Table I.

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Microspheres Characterizations

Determination of the Encapsulation Efficiency

The PABA content in microspheres was determined by dissolving 100 mg of dried microspheres in a sealed bottle containing 100 mL of absolute ethanol under stirring with a rotation speed of 250 rpm at 37 °C for 6 h. The resulting solution was analyzed for PABA content by UV-VIS spectroscopy. The actual drug loading (DL) and encapsulation efficiency (EE) of the microspheres were calculated by the following equations:

TABLE I. Formulations Processing Conditions of Microspheres

Code	PABA:EC ratio	Speed, rpm	Surfactant Concentration, %	
			PVA	Tween 80
F11	1:1	600	1	-
F12	1:2	600	1	-
F13A	1:3	600	1	-
F14	1:4	600	1	-
F13B	1:3	600	0.5	-
F13C	1:3	600	2	-
F13D	1:3	900	1	-
F13E	1:3	1200	1	-
T13A	1:3	600	-	0.5
T13B	1:3	600	-	1

DL / % = (Drug mass in microspheres / Mass of microspheres) × 100 (1)

EE / % = (Actual drug loading / Theoretical drug loading) × 100 (2)

Particle Size

The mean particle size and size distribution (δ) of microspheres were determined by Scanning Electron Microscopy (SEM Quanta 200 FEI) and optical microscopy (OPTIKA 4083. B1). At least 500 microspheres were analyzed for each preparation and the mean diameter was calculated. The particle size distribution was calculated from various equations.[23,24] d_{32}: mean diameter of Sauter, $d_{32} = \sum n_i d_i^{\,3} / \sum n_i d_i^{\,2}$; δ^a: Distribution was calculated as d_{43}/d_{10}; d_{10}: number mean diameter, $d_{10} = \sum n_i d_i / \sum n_i$; d_{43}: weight mean diameter $d_{43} = \sum n_i d_i^{\,4} / \sum n_i d_i^{\,3}$; i: represent an index of population and d_i is the particle diameter population i.

Scanning Electron Microscopy (SEM)

The morphology and surface topography of the prepared microspheres were examined without sputter deposition ~~coating~~ using Scanning Electron Microscopy (SEM Quanta 200 FEI) at 50 Pa s under 12 kV of accelerated ~~tension~~voltage. The microspheres were mounted on double-scotched carbon film fixed on a ~~metal support~~stub.

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Infrared Spectroscopy

The samples were characterized by infrared spectroscopy using a «Bruker Alpha FT-IR Spectrometer», equipped with ALPHA's Platinum ATR with single reflection and diamond ATR module. The infrared spectra of pure PABA, blank ethylcellulose microspheres and the loaded microspheres were analyzed without any prior preparation.

Differential Scanning Calorimetry (DSC)

Thermal analysis (DSC) was performed on the microspheres using a Differential Scanning Calorimeter (NETZSCH DSC 200 PC). All the samples were prepared by weighing (≈ 14 mg of pure PABA, ≈ 32 mg of blank ethylcellulose microspheres and the loaded microspheres) into aluminum pans hermetically sealed. The analysis was performed at a rate of 5 °C min^{-1} with a heating range from 20 to 300 °C.

XRD Diffraction Spectroscopy

The XRD spectrums of the pure PABA, blank ethylcellulose microspheres and the loaded microspheres formulations were recorded with D8 Advance BRUKER diffractometer~~. The XRD was performed with~~at a ~~the~~ diffraction angle 2Θ from 5° to 70°.

In vitro $PABAH^+$ *Release*

The *in vitro* release studies of the PABA-loaded ethylcellulose microspheres were carried out using an appropriate glass dissolution reactor[15,21] plunged in a bath regulated at 37 ±0.5 °C. This reactor allows us to withdraw solution without microspheres. The $PABAH^+$ release kinetics from microspheres was followed by using UV-VIS spectrometer (Shimadzu UV-VIS 2401 PC, Japan) with a cell compartment thermostat at 37 °C. At the desired time, 100 mg of microspheres was soaked in 500 mL of buffer solution[22] pH 1.2. The dispersion medium was stirred at a rotation speed of 250 rpm; at predetermined time intervals, 3 ml of solution was withdrawn, analyzed by UV spectroscopy, and replaced in the reactor. The samples were analyzed at 220 nm for $PABAH^+$ concentrations using UV-VIS spectroscopy.

RESULTS AND DISCUSSION

Microspheres Characterizations

The microspheres surface and morphology were studied using SEM (Fig. 1 and 2). The formulation parameters of the various PABA-loaded ethylcellulose microspheres prepared along are summarized in Table I.

Fig. 1 shows the effect of surfactants PVA and Tween 80 on the microspheres prepared under the same operative conditions (1:3 of PABA:EC ratio and 600 rpm stirring speed). ~~We have remarked that~~ The PVA microspheres (Fig. 1a) are spherical and smaller than Tween 80 microspheres with a rough and porous surface. It is obvious that the surface of these particles

obtained with Tween 80 is much rougher with bigger pores in the surface (Fig. 1d). Indeed we have obtained microspheres with a mean diameter of Sauter (d_{32}) in the range of 121 to 143 µm with PVA and in the range of 793 to 870 µm with Tween 80 (Table II). Comparative effects between PVA and Tween 80 described in Table II show their direct action on the actual drug loading (DL), encapsulation efficiency, the mean diameter of Sauter (d_{32}) and the distribution (δ) of these microparticulate systems. We have obtained small microspheres sizes with PVA. This observation is in agreement with literature.[16] Furthermore, PVA acts more strongly than Tween 80 on the reduction of the interfacial tension decreasing the coalescence

TABLE II The effects of Various Formulations Processing Conditions on Microspheres Characteristics and Encapsulation Results; DL – Drug Loading; EE – Encapsulation Efficiency

Code	Theoretical DL,%	Actual DL,%	EE / %	d_{10} / µm	d_{32} / µm	d_{43} / µm	δ [a]
F11	50.00	25.10	50.20	80.95±2.00	90.00±0.67	148.45±2.61	1.83±0.01
F12	33.33	19.45	58.35	73.15±0.52	106.04±0.50	127.73±0.61	1.74±0.02
F13A	25.00	16.85	67.40	87.45±1.98	121.11±2.97	147.21±0.50	1.69±0.04
F14	20.00	15.81	79.05	114.73±1.12	164.61±0.72	182.17±0.79	1.59±0.01
F13B	25.00	15.26	61.04	83.83±1.64	142.91±3.34	159.54±3.84	1.90±0.08
F13C	25.00	17.55	70.20	69.02±2.79	77.54±1.26	91.13±2.93	1.31±0.01
F13D	25.00	14.06	56.24	64.05±0.78	62.74±0.08	94.46±0.84	1.47±0.00
F13E	25.00	13.00	52.00	40.05±0.09	47.04±0.11	50.12±0.68	1.25±0.02
T13A	25.00	09.38	37.52	441.30±0.69	869.28±1.06	987.45±3.87	2.23±0.01
T13B	25.00	12.52	50.08	397.64±3.36	793.21±2.55	965.58±0.99	2.42±0.02

of the emulsion droplets. Increasing PVA concentration promotes both of the actual drug loading and encapsulation efficiency, decreases the mean diameter d_{32} and the distribution (δ). In our research PVA gives a high EE than Tween 80. This can be explained by the structure and porosity in the surface of these microparticles. For Tween 80, the presence of big pores in the surface of particles led to an important loss of PABA resulting in a decrease of DL and EE. The comparison of the SEM images of Fig. 1 a-d (F13A and T13B) performed at the same surfactant concentration (1%) PVA and Tween 80 respectively, reinforces these results.

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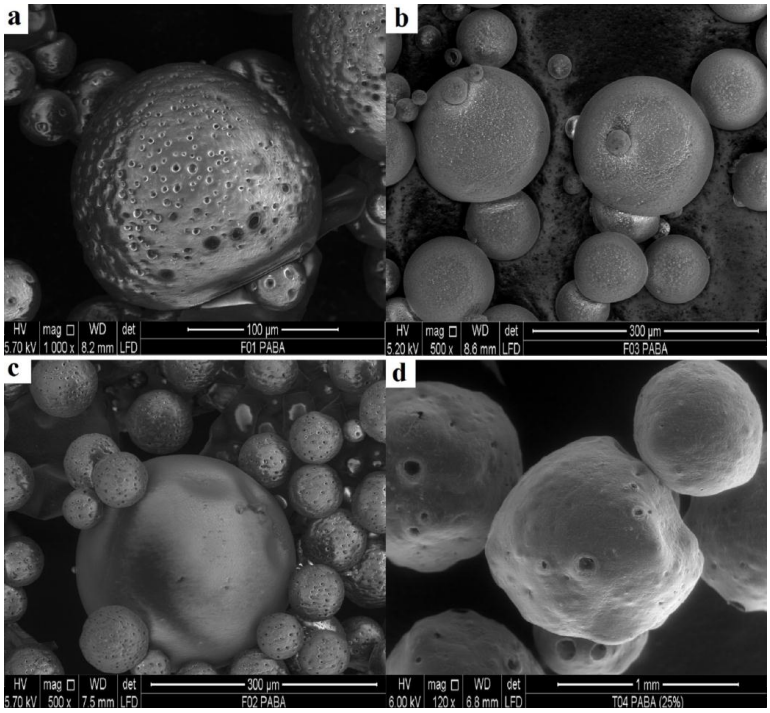

Fig. 1. SEM micrographs of the loaded microspheres. Preparation conditions: a: F13A, 1% PVA, b: F13B, 0.5% PVA, c: F13C, 2 % PVA and d: T13B, 1% Tween 80. Microspheres were prepared with the ratio 1:3 of PABA:EC and stirring speed at 600 rpm

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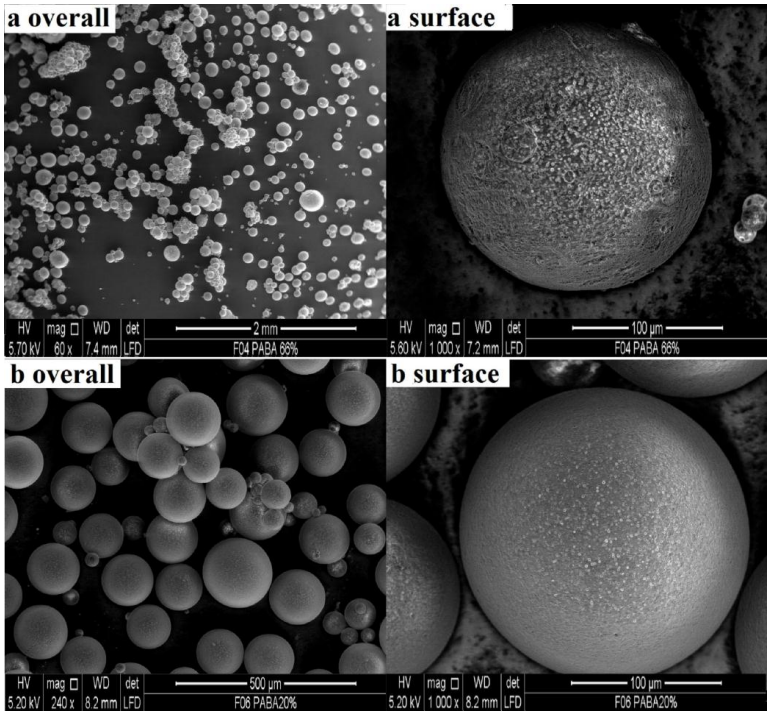

Fig. 2. SEM micrographs of the surface and morphology of the loaded microspheres prepared with different PABA:EC ratios. Preparation conditions: a- F12, 1:2 PABA:EC ratio and b- F14, 1:4 PABA:EC ratio. Microspheres were prepared with 1% PVA and stirring speed at 600 rpm

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Fig. 2 showed spherical microspheres prepared under the same operative conditions with different PABA:EC ratio 1:2 and 1:4. The microspheres formulated by higher EC content 1:4 (F14) shows a smooth and homogenous surface without any visible pores (Fig. 2b). ~~We have remarked~~ Some microspheres had ~~with~~ a rough and porous surface when we used ~~the~~ 1:2 PABA:EC ratio (Fig. 2a). An increase in the microspheres sizes (d_{32}) was observed with increasing the amount of EC (Table II). It was reported that increasing in organic phase viscosity due to increasing in polymer concentration yields larger microspheres[25,26]. By increasing the amount of EC from 1:1 to 1:4, the encapsulation efficiency increases from 50.20 % to 79.05 % with 1 % PVA and stirring speed 600 rpm constants. This can be explained by the fact that increasing the dispersed droplets viscosity hinders the loss of PABA[20,27].

The mean particle size and size distribution can be determined by the operative conditions of microencapsulation process[26-29]. From the results of microspheres sizes (Table II), we have observed that under the same operative conditions, increasing stirring speed from 600 rpm to 900 rpm and to 1200 rpm has affected the microspheres characteristics: the mean diameter of Sauter (d_{32}) was decreased from 121.11 µm to 62.74 µm and to 47.04 µm, the distribution (δ) is improved from 1.69 to 1.47 and to 1.25 and the encapsulation efficiency EE decreased from 67.40 % to 52.00 %. The obtained result about particle size is also reported by André ~~and~~ *et al* [26].

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The infrared spectra of microspheres were compared with PABA and polymer matrix ethyl cellulose spectrums (Fig. 3). The analysis showed the effective presence of similar characteristics bands of PABA in the microspheres spectrum at the same wave number: (νC-OH) at 1659.60 cm^{-1} and at 1285.15 cm^{-1},the characteristic bands of COOH group[30], (C-N) at 1343cm^{-1}, (νC=O), the aromatic vibration band (C=C) at 1622 cm^{-1} and the amino group (N-H) at 3359-3457 cm^{-1}. The microspheres spectrum appears as the sum of pure PABA and EC.

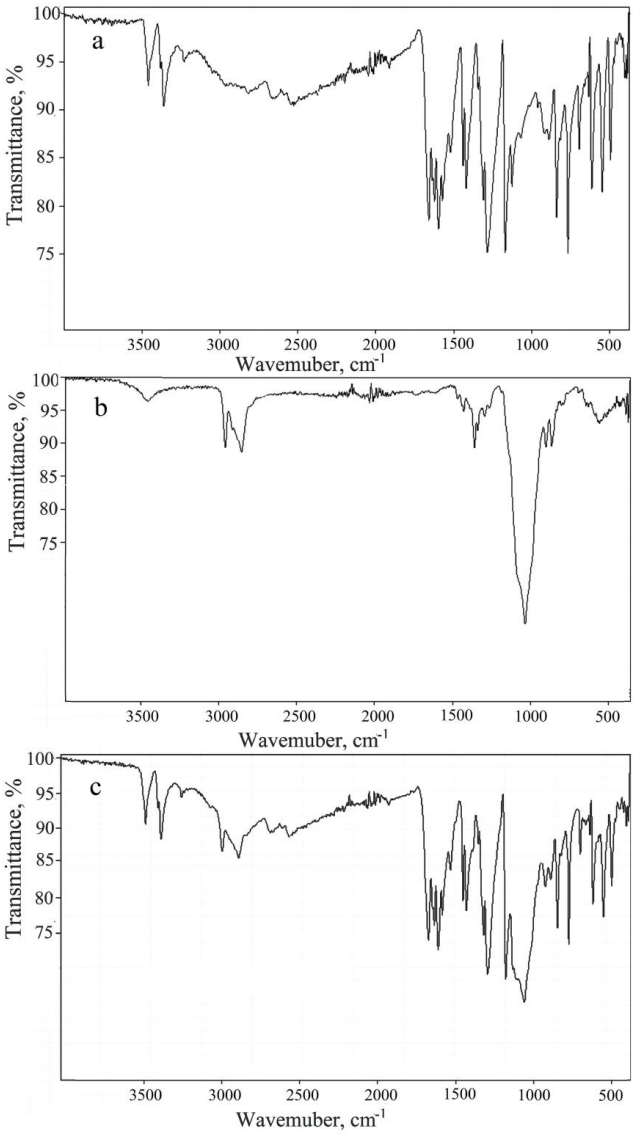

Fig. 3. Infrared spectra of (a)- Pure PABA, (b)- Blank ethylcellulose microspheres and (c)- The loaded microspheres (F14)

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The compatibility of PABA in the microspheres formulations was studied by DSC (Fig.4). By comparing the DSC (a), (b) and (c) of Figure 4, ~~we have noted~~ a sharp endothermic peak (191°C, lit. 190°C) corresponding to the melting point of PABA (Fig. 4a) accompanied by an endothermic peak positioned at 250 °C have been noted. The loaded microspheres (Fig. 4 (c)) showed a wide endothermic peak at 170 °C, indicating the melting point of PABA in the presence of EC. This change in the peak position can be a consequence of some interaction between polymer matrix EC and the drug. The thermal transition of the polymer EC in the

ethylcellulose microspheres (blank test) was observed at ~85 °C which corresponding to the glass transition temperature of EC. The Tg was difficult to detect, it is generally depending on the molecular

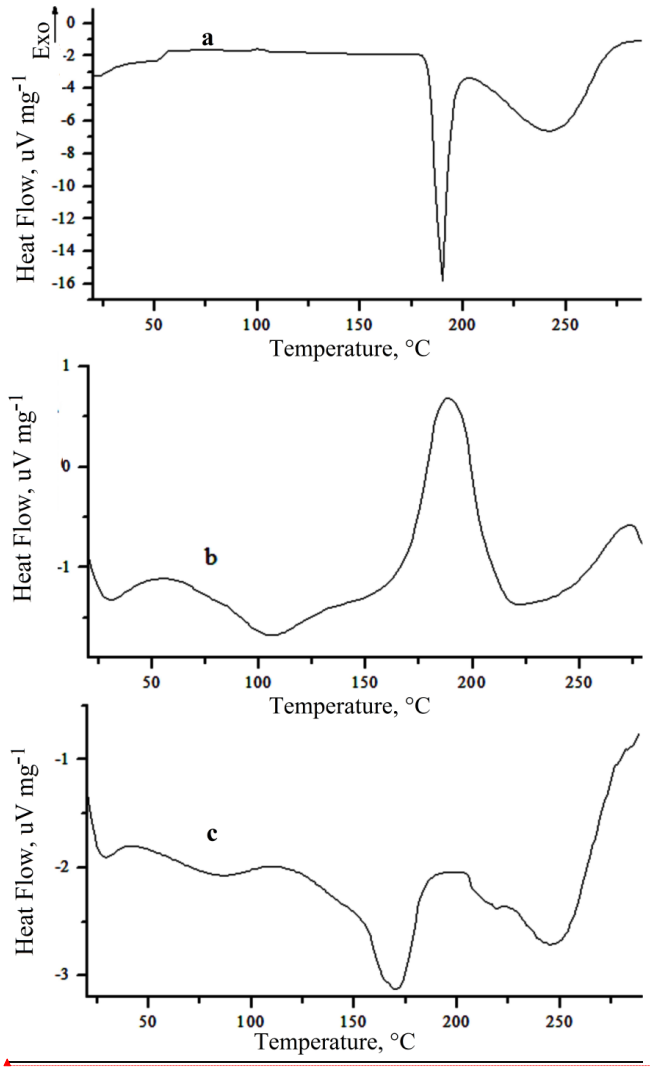

Fig. 4. DSC thermograms of (a)- Pure PABA, (b)- Blank ethylcellulose microspheres and (c)- The loaded microspheres (F14)

weight and the degree of substitution of the used ethylcellulose. It has been noticed by other authors[31-33] that the variability of the thermal transition temperature Tg is thoroughly depending on the structure of EC and the addition of some compounds like plasticizer in order to increase the porosity of the microspheres[32]. ~~It was has been noticed by other authors[31] that~~ the elongation, flexibility and Tg decreased significantly with the increase of EC ethoxyls groups[31] ~~EC~~. At higher temperatures, two peaks "endo/exothermic" at 106-190 ° C appeared

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in our case (b), generally observed for the pure EC ~~ethylcellulose~~. The exothermic peak at around 190 °C is associated with oxidative degradation of ethylcellulose[31]. Finally, the endothermic peak positioned at 250 °C on the Fig. 4 (a) and (c) accompanying PABA and appears to be a compound derived from eventual degradation of PABA at a temperature higher than its melting point at 191 °C. In conclusion, we can ensure that the microspheres are well loaded with PABA, this is also attested by its release in the simulated ~~test~~ solution pH 1.2.

Fig. 5 shows XRD technique has been used with DSC to study the physical state of the drug in the microspheres. The crystalline nature of PABA was clearly demonstrated by its characteristics XRD pattern containing well define peaks with the diffraction angle 2 Θ from 5° to 70° (Fig. 5). However, PABA-loaded ethylcellulose microspheres exhibited characteristic diffraction pattern, which was less intense as compared to pure PABA.

Data kinetics of in vitro PABAH$^+$ release

To analyze the drug release kinetics, the cumulative release data versus time were fitted to Higuchi[34] and Korsmeyer-Peppas equations[35,36]. Higuchi ~~describes~~ described drug release by a simple equation of the type:

$$Q_t = k_H t^{1/2} \quad (3)$$

Where, Q_t is the cumulative percent of drug released at time t and k_H is ~~a~~ the dissolution constant characteristic of the equation. The cumulative %PABAH$^+$ release was proportional to square root time suggesting that the drug release from microspheres was diffusion controlled (Fig. 6). The calculated values of rate constants k_H were influenced by varying the process parameters: stirring speed, surfactant nature and concentration as well as the amount of polymer matrix EC. The best fit with the highest correlation coefficient r^2 was observed in the Higuchi model (Table III). The obtained values of rate constants (k_H) were decreased by increasing the amount of polymer, decreasing surfactant concentration and stirring speed (Table III).

Further, to find out the mechanism of drug release, the obtained release data versus time were fitted to Korsmeyer and Peppas semi-empirical equation as follows:

$$M_t / M_{t\infty} = k_{KP}.t^n \quad (4)$$

Here $M_t / M_{t\infty}$ is the fractional drug release at time t, M_t and $M_{t\infty}$ represent the masses of drug released at time t and $t\infty$ respectively, k_{KP} is the rate constant and n is an empirical parameter

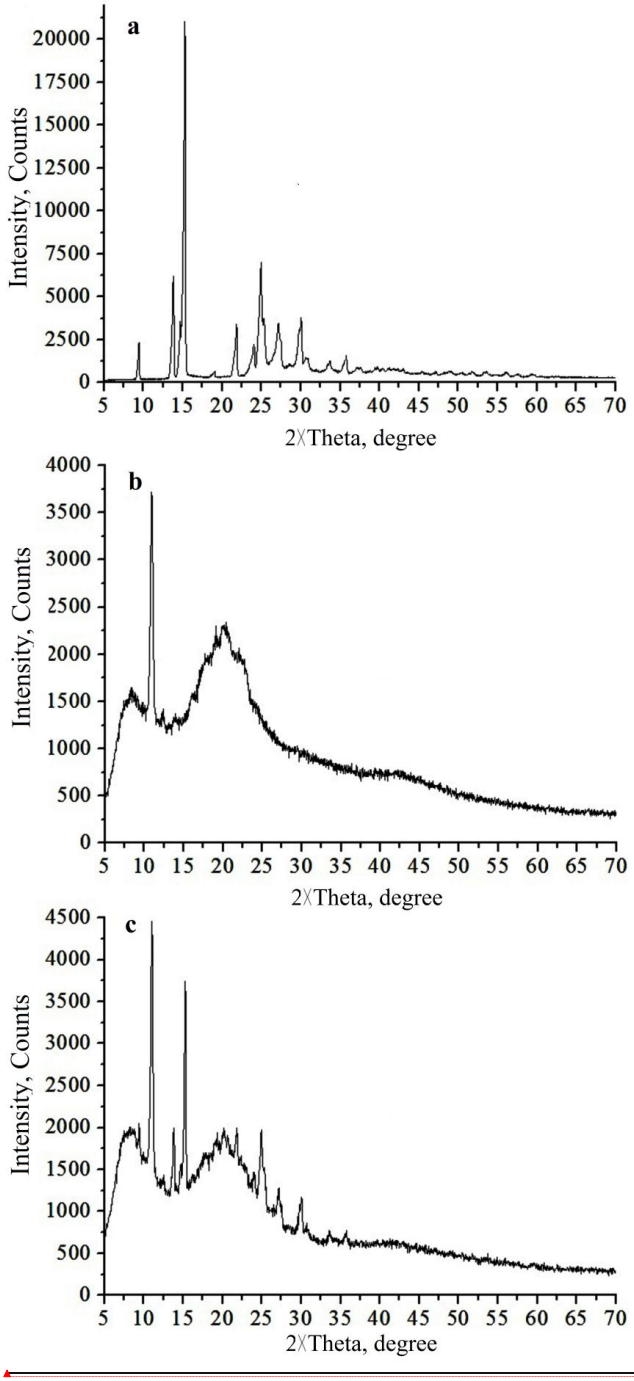

Fig. 5. X-RD spectrums of (a)- Pure PABA, (b)- Blank ethylcellulose microspheres and (c)- The loaded microspheres (F14)

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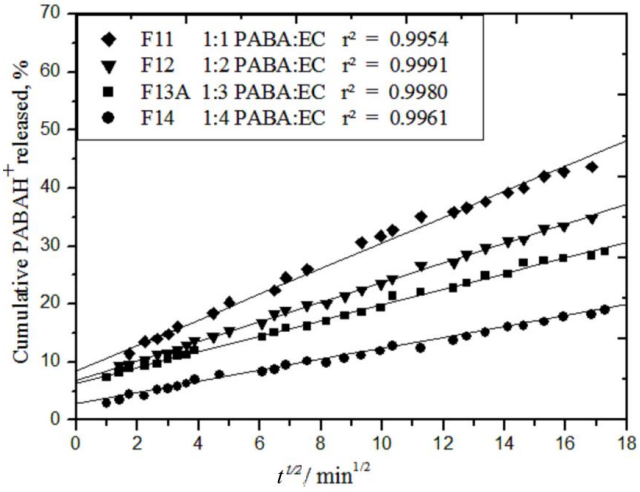

Fig. 6. Higuchi plots of $PABAH^+$ release % from microspheres in pH 1.2 at 37 °C having different PABA:EC ratios (1:1, 1:2,1:3 and 1:4) with 1 % PVA and 600 rpm constants.

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characterizing the release mechanism. We have estimated the values of n, k_H (Higuchi) and k_{KP} (Korsmeyer-Peppas) for all formulations by applying these equations and the obtained values are given in Table III. Divers values of n for cylinders and spheres are described by Ritger and Peppas.[37,38] For spheres geometry, a value of n = 0.43 corresponds to Fickian diffusion (Case I), n = 0.85 to Case II transport (zero order release) and n > 0.89 to Super Case II drug release. For the intermediary values ranging between 0.43 and 0.85 are attributed to anomalous or non-Fickian transport (erosion and diffusion) occurs.[37] The values of n for the microspheres prepared by varying the process parameters are ranging from 0.29 to 0.39 (Table III), indicating the shift of Fickian transport. For the Korsmeyer-Peppas model, the values of rate constants ~~were~~ are depended on the obtained n values.

TABLE III. Release Kinetic Parameters of the Model Equations Applied to the *In vitro* Release of $PABAH^+$ from Microspheres; k_H and $k_{K\text{-}P}$ - The release rate constants for Higuchi and Korsmeyer-Peppas models , respectively; n - The release exponent of Korsmeyer-Peppas model; r^2 - The correlation coefficient

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Kinetic models					
	Higuchi model		Korsmeyer-Peppas model		
Code	k_H / $min^{-1/2}$	r^2	n	$k_{K\text{-}P}$ / min^{-n}	r^2
F11	2.209	0.9954	0.29	0.00066	0.9971
F12	1.689	0.9991	0.31	0.00039	0.9971
F13A	1.349	0.9980	0.30	0.00029	0.9932
F14	0.936	0.9967	0.39	0.00033	0.9881
F13B	1.182	0.9950	0.37	0.00040	0.9937
F13C	2.061	0.9970	0.34	0.00055	0.9963
F13D	2.155	0.9982	0.33	0.00051	0.9952
F13E	2.468	0.9969	0.32	0.00075	0.9962
T13A	1.415	0.9956	0.29	0.00032	0.9955

The influence of different process parameters and the content of matrix polymer on the active agent release rate were studied. The plots of cumulative $PABAH^+$ % released versus time from different microspheres formulations are shown in Fig. 7 and 8. After the end of 24 h of dissolution, percentages of $PABAH^+$ released from microspheres F11, F12, F13A and F14 ~~were~~ are 67.62 %, 56.65 %, 51.53 % and 43.47 % respectively (Fig. 7). This study indicated that the release rate decreases with higher polymer (EC) concentration: increasing the amount of polymer from 1:1 to 1:4 decreases the $PABAH^+$ release rate due to the less porosity in the surface of microspheres (Fig. 2a-b). By increasing the amount of EC, the drug loading (DL) was increased. Nevertheless, the release rate was faster. However, we have noted the significant effect of stirring speed on microspheres sizes and distribution (δ). Increasing stirring speed by decreasing the size of microspheres (d_{32}) uniformly dispersed (δ = 1.25) led to a faster release rate (Fig. 8). The release of the active agent is inversely proportional to the size of microspheres.[39,40] Figure 8 shows that the cumulative % release is more rapid from the smaller microspheres prepared at a high PVA concentration. It was reported that for the smaller microspheres, a larger effective area produces a greater number of drug molecules in the surface of the microspheres, which leading to a faster drug release.[21] The release rate from microspheres T13B prepared by Tween 80 in the aqueous phase is more rapid than that observed with PVA, this is due to the porosity and the surface morphology of these microspheres (Fig. 1d). Thus, the results showed that the $PABAH^+$ release rate from these systems can be modulated by adjusting the formulation processing parameters.

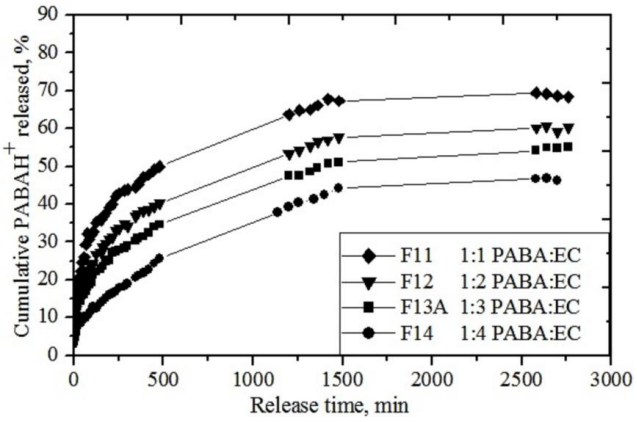

Fig. 7. Cumulative release of $PABAH^+$ % from the microspheres in pH 1.2 at 37 °C having different PABA:EC ratios (1:1, 1:2,1:3 and 1:4 with 1% PVA and 600 rpm constants**)**

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CONCLUSION

The present study demonstrated the effects of formulation process parameters on PABA-

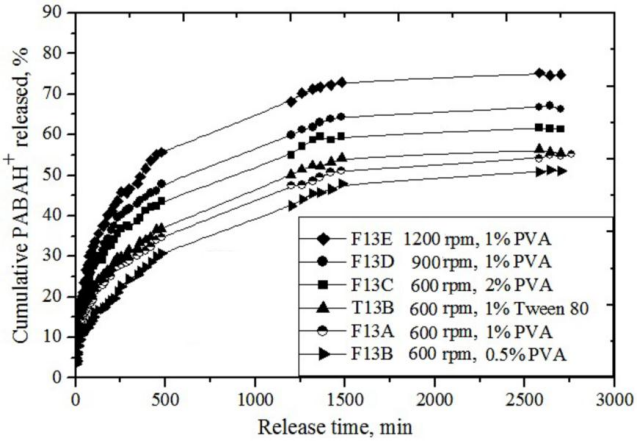

Fig. 8. Cumulative release of $PABAH^+$ % from the different microspheres in pH 1.2 at 37 °C (with 1:3 PABA:EC ratio constant)

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loaded ethylcellulose microspheres characteristics and *in vitro* release behavior using the microencapsulation by solvent evaporation technique. It was shown that the surfactant nature could minimize the loss of PABA, affects microspheres sizes and structure morphology. PVA gives perfectly spherical microspheres with a smooth and porous surface while Tween 80 led to slightly spherical shapes with rough surface and large pores. We have obtained systems with large ranges of size (the mean diameter of Sauter d_{32} in the range of 47 to165 µm with PVA and 793 to 870 µm with Tween 80) by modifying the process conditions. The particle size (d_{32}) and distribution (δ) can be successfully controlled by surfactant nature and concentration, stirring speed as well as the amount of polymer. The encapsulation efficiency (EE) can be improved especially by increasing PVA concentration and the amount of EC. The release kinetics using Higuchi and Korsmeyer-Peppas equations showed a Fickian diffusion mechanism and the release rate can be controlled by adjusting the microencapsulation processing parameters that have significant effects on particle sizes.

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