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Preparation and *in-vitro* evaluation of single and bi-layered beeswax-based microparticles for colon-specific delivery of mesalamine

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Abstract: Beeswax is selected as a natural coating material for the development of new colon specific drug delivery systems charged by mesalamine. In a first step, beeswax microparticles are prepared using hot-melt process of micro-encapsulation where drug:beeswax ratio, stirring speed, emulsifier concentration and pH of external phase are varied for the optimization of the drug entrapment and microparticles' morphology. The effect of the nature of the emulsifier is also discussed by studying the hydrophilic–lipophilic balance (HLB) value. In a second step, to obtain delayed delivery systems, bi-layered microspheres are elaborated by the process of emulsion–solvent evaporation using ethylcellulose or cellulose acetate butyrate as outer enteric coating layer. All formulations are characterized by infrared spectroscopy, X-ray diffraction, scanning electron microscopy and optical microscopy. The drug release is established in simulated gastric, small bowel and colon liquids and the release mechanism is discussed by applying the Korsmeyer–Peppas model.

Keywords: beeswax; mesalamine; colon-specific delivery; double walled microspheres.

INTRODUCTION

Mesalamine or 5-aminosalicylic acid (5-ASA) is an active substance that has been used as a potent non-steroidal anti-inflammatory drug, its efficacy is proved against gastrointestinal tract diseases and especially that of colon part, such as ulcerative colitis and Crohn's desease.[1,2] Obviously, the oral administration of the drug remains a painless and favourite method for patients but it can reduce

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the drug bioavailability and therefore its therapeutic efficacy. In fact, mesalamine is absorbed into the proximal small intestine and metabolized without reaching the therapeutic levels in inflamed sites.[3] So, multiple kinds of colonic drug delivery systems such as gastroretentive dosage forms, pH-dependent dosage forms, or mucoadhesive dosage forms are developed to improve the effectiveness of inflammatory bowel diseases treatments.[3–5] In these systems, the active therapeutic substance is usually coated in a shell that controls the drug release through three parameters often operating in a combined way; the first one is related to the pH values of the gastrointestinal tract, this formula generally showed neglected release in a strongly acidic environment.[5,6] The second parameter is based on the transit time of the gastrointestinal tract;[5,7] for example, as reported by Souza *et al.*,[8] the delayed drug delivery is acquired by the relaxation behaviour of Xylan chains in the first 4 h; in this study, the microspheres were able to reach the large intestine with about 40 % of initial drug load, which could be sufficient for the local treatment of inflammation according to the researchers.[8] Finally, the presence of colonic flora ensures a specific release of the drug, this type of microscopic vector is generally based on polysaccharides since they are indigestible in the small intestine. However, they undergo complete or partial degradation by carbohydrate-active enzymes (CAZymes) in the colon.[9–11] For example, pH––enzyme double-dependent mesalamine colon-specific delivery system were developed by Jin *et al.*;[12] the authors succeeded in developing a dual pH-dependent and enzymatic degradation systems based on chitosan and eudragit S100 for the specific release of mesalamine in the colon.[12]

Thus, in the present investigation, an attempt is made to prepare colon drug delivery microspheres using mesalamine as active ingredient and beeswax as natural coat. Beeswax is a complex mixture composed of hydrocarbons, free fatty acids, esters of fatty acids and fatty alcohol, diesters and exogenous substances.[13] This coat material is chosen, in one hand, for its edible properties and long resistance to external environmental factors (oxygen, humidity, digestive properties).[13,14] In the other hand, it is confirmed by several studies that the drug encapsulation using this lipid material is effective in protecting and improving the release properties of compounds, especially in the medical field, as it allows a prolonged release of the drugs using simple methods with low production costs.[15–19] Beeswax is mostly used as gastro-resistant coat for anti-inflammatory drugs.[15,18,19] and it has been also tested to obtain delayed formulations for an anti-cancer drug.[17] So, the objective of the present work is to develop new reservoir devices by a combination of beeswax, as primary coat and cellulose derivative polymers as second layer, for a colon specific drug delivery. For the research purpose the optimized beeswax microspheres are firstly prepared by emulsion–melt solidification technique and by varying some process parameters, *i.e.*, drug:polymer ratio, stirring speed, emulsifier nature and concentration and the

pH of external phase. Secondly, double walled microspheres are formed using emulsion–solvent evaporation technique where ethylcellulose and cellulose acetate butyrate are chosen and tested as second coat to obtain colon-specific drug delivery systems.

EXPERIMENTAL

Materials

Mesalamine is obtained from SALEM laboratory (Algeria), beeswax is obtained from beekeeping farm (west of Algeria), ethyl cellulose (EC, 22 mPa s) and cellulose acetate butyrate (CAB, M = 70000) are purchased from Sigma–Aldrich. Polyvinyl alcohol (PVA, 98––99 % hydrolysed, polymerisation degree:1700–1800, viscosity: 25–32 cP) is from Biochem Chermopharma, Tween 80 origin is Sigma–Aldrich, Span 80 is from Biochem Chermopharma and dichloromethane (DCM) is from Sigma–Aldrich.

Preparation of microspheres and double coated microspheres

Mesalamine microspheres based on beeswax are prepared by using hot-melt technique of micronecapsulation; 2g of beeswax is melted in a water bath at 90 °C, then 0.25 g of mesalamine is added under magnetic stirring. The mixture is then emulsified in 100 mL of hot (85––90 °C) aqueous solution (distilled water with pH 6.1±0.1 or acetate buffer solution at pH 4±0.1), containing 1.5 % of emulsifier and stirred mechanically at 800 rpm using DLS stirrer. After 3 min of emulsion, the system is cooled using an ice bath until 10° C under continuous stirring. After 20 min, solidified microspheres are collected by filtration and washed with distilled water and finally dried at room temperature for 48 h.

To obtain double walled microparticles, solvent evaporation method is used; so selected beeswax microspheres are dispersed in 25mL of DCM coating solution containing 0.4 g of EC or CAB as matrix, the polymer:microspheres ratio is maintained at 1:1. This organic phase is then poured into 100 mL of 1 % of PVA aqueous acetate buffer solution (pH 4) under stirring (800 rpm) for 3 h until the complete solvent evaporation. After filtration, the obtained double walled microspheres are washed with distilled water and dried at room temperature for 48 h.

Microparticles' characterization

For microparticles' characterization, FTIR spectra of drug, beeswax and formulations are registered using a Bruker Alpha FTIR spectrometer in the absorbance range of 4000–400 cm^{-1}. Powder X-Ray diffractogramms of mesalamine, beeswax and D_{11} batch of microspheres are recorded using Rigaku diffractometer type Ultima IV. The samples are scanned in the range of 2θ from 0 to 80° using an incident beam the Kα1 line of copper. Shape and surface morphology of microspheres are examined using Hitachi TM-1000 scanning electron microscope (SEM). The particles are observed using the optical microscopy type Optika stereomicroscope of the series SZM 1 and equipped with a camera and software, in order to identify the size and morphology of microparticles. The mean diameter, d_{10}, is calculated using the following equation and by counting and measuring more than 500 microparticles of each batch:

$$d_{10} = \frac{\sum n_i d_i}{\sum n_i} \tag{1}$$

where d_i is the droplet diameter, n_i is the number of droplets with diameter d_i; d_{10} is the average number diameter.

Determination of the drug content and encapsulation efficiency

5 mg of microspheres are dispersed in 10 mL of a suitable solvent (0.5 M HCl solution) under stirring for 15 min at 90 °C for the complete removal of mesalamine. After cooling at room temperature, the obtained mixture is filtered and analysed by UV–Vis spectrophotometry using Shimadzu 240 1PC spectrophotometer at λ_{max} = 305.8 nm, the drug extraction is carried out in triplicate and the results are expressed by the mean ± *SD*.

The entrapment efficiency and drug content are calculated using the following formulas:

$$\text{Efficiency} = 100\frac{m_{\text{drug extract}}}{m_{\text{initial drug}}} \quad (2)$$

$$\text{Drug loading} = 100\frac{m_{\text{drug extract}}}{m_{\text{microsphers}}} \quad (3)$$

In-vitro *drug release studies*

The drug release is monitored using a Shimadzu 240 1PC dual beam UV–Vis spectrophotometer. 200 mg of microspheres are dispersed in 900 mL of dissolution medium at 37±0.4 °C under magnetic stirring of 100 rpm. At selected time intervals, 3 mL of sample is withdrawn and analysed and quickly reintroduced into the release reactor. The drug release is carried out in simulated gastric medium of pH 1.2 for a period of 2 h, in simulated small bowel liquid (pH 8) for 6 h and in colon simulated liquid at pH 6.5 for 5 days. These mean values of periods are selected based on the available studies on patients with active ulcerative colitis and Crohn disease.[20-23]

Kinetic treatment of mesalamine release data

The release data are mathematically fitted according to Korsmeyer–Peppas kinetic model:

$$\frac{m_t}{m_i} = K_K t^n \quad (4)$$

where m_t is the amount of drug release in time t, m_i is the initial amount of drug in the microsphere and m_t/m_i fractional mass of drug released at time t. K_K is the rate constant of Korsmeyer–Peppas model and n is the diffusion exponent according to Korsmeyer–Peppas, this model is verified for 60 % of the drug released.[24-27] The selected model is applied for each step of drug release in selected time intervals corresponding to the pH medium (from 0–120 min for pH 1.2, from 120–360 min for pH 8 and from 1440–7200 min for pH 6.5).

RESULTS AND DISCUSSION

Optimization of beeswax microspheres

In order to obtain the ideal combination of morphology, encapsulation efficiency and yield, some process parameters, *i.e.*, drug:beeswax ratio, stirring speed (*N*, rpm) and external phase pH was studied, and in these experiments PVA is used as surfactant and its concentration was also varied. As shown in Table I, using a drug:beeswax ratio of 1:4 (D_0 formulation), the droplets clung together and formed aggregates. Consequently, to obtain individualized formulations, the drug concentration is reduced to 1:8 (drug:beeswax) and the effects of both the emulsifier concentration and the stirring speed on the drug entrapment

were studied in two external phases of pH 6.1 and 4. The results showed that using pH 6.1 of the external phase, the obtained microparticles are spherical but the drug entrapment efficiency didn't exceed 42 %; it increased when the PVA concentration was risen to 1.5 % and it decreased when the stirring speed of emulsion is increased. For these formulations (D_1–D_5), the microspheres' size (d_{10}) varied from 94 to 133 μm and is largely affected by the stirring speed. In fact, the particle size decreased when the stirring speed of emulsion increased and the results is in agreement with the inertial break-up theory.[28]

TABLE 1. Composition and physicochemical properties of bee wax microspheres

Code	Drug:pol. ratio	N rpm	PVA %	pH of external phase	Particles' shape	Drug loading %±*SD*	Entrapment efficiency %±*SD*	d_{10} μm
D_0	1:4	800	1	6.1±0.1	Aggregate	6.5±0.6	32.5±2.9	122.2
D_1	1:8	800	0.5	6.1±0.1	Spherical	2.3±0.4	20.9±4.1	100.8
D_2	1:8	800	1	6.1±0.1	Spherical	4.1±1.1	37.2±10.3	133.0
D_3	1:8	800	1.5	6.1±0.1	Spherical	4.6±0.5	41.7±4.6	124.0
D_4	1:8	800	2	6.1±0.1	Spherical	3.4±0.4	30.7±4.1	131.9
D_5	1:8	1000	1	6.1±0.1	Spherical	2.4±0.2	21.5±2.2	94.1
D_6	1:8	800	0.5	4.0±0.1	Spherical	2.6±0.2	23.1±2.3	106.2
D_7	1:8	800	1	4.0±0.1	Spherical	5.2±0.1	.471±0.9	139.0
D_8	1:8	800	1.5	4.0±0.1	Spherical	5.2±0.5	47.3±4.7	129.8
D_9	1:8	800	2	4.0±0.1	Spherical	3.6±0.6	32.7±5.7	133.7

In order to improve the drug encapsulation, the beeswax solution was emulsified in an external phase with a lower pH (pH 4) where mesalamine was less soluble.[29] Indeed, the spherical microparticles were obtained and a highest encapsulation efficiency of 47.3 % was obtained for D_8 formulation. On the basis of these experimental results, D_8 experimental conditions were subsequently maintained for the preparation of another formulations where the emulsifier nature was varied as following.

HLB value effect on loaded beeswax microspheres properties

An attention was drawn on the surfactant effect, in fact, it had been proved that the surfactant nature can affect the drug transfer in the external phase and so the drug entrapment.[30] Really, surfactants are agents that reduce the surface tension or interfacial tension, and these excipients can be added in drug formulation especially to improve the drug solubility.[31,32] It was reported that surfactants having HLB (hydrophilic–lipophilic balance) values higher than 15 were best solubilising agents.[33] So, Span 80 was selected and used to reduce the HLB value and consequently to reduce the drug solubility in the aqueous phase.

For the purpose, new formulations (D_{10} and D_{11}) were prepared using Tween 80 (HLB_A = 15) as hydrophilic surfactant and a mixture of Tween 80

(HLB_A = 15) and Span 80 (HLB_B = 4.3) as lipophilic surfactant (Table II). The *HLB* value of the surfactant mixture was set to 9; this value of *HLB* is required for the lipid phase such as beeswax to form stable emulsions.[34] The surfactant ratio is determined according to the following theoretical calculation:[35]

$$A = \frac{100(9 - HLB_B)}{HLB_A - HLB_B} \quad (5)$$

The results as shown in Table II are promising, in other words the drug entrapment efficiency was successfully increased using the surfactant mixture; indeed, the drug entrapment efficiency in formulation D_{11} reached 86 %, in this case the HLB value is lower and the lipophilic character of emulsifier mixture may prevent the drug loss. However, the use of Tween 80 alone has led to the decrease of drug entrapment.

TABLE II. Effect of surfactant on bee wax microspheres properties

Code	Emulsifier nature	*HLB* value	Drug loading %±*SD*	Entrapment efficiency %±*SD*	d_{10} / μm
D_8	PVA	18	5.2±0.5	47.3±4.7	129.8
D_{10}	Tween 80	15	4.7±0.8	42.3±7.5	70.2
D_{11}	(Span 80 +Tween 80)	9	9.6±0.9	86.2±8.1	91.6

Regarding the particles' size, using Tween 80, the microsphere size clearly decreased compared to PVA. In fact, the *HLB* value has a significant effect on the droplet size,[36] the same results are obtained by Kim and Cho[36] where small droplets are obtained at *HLB* value between 13.5 and 14.5. While the microparticles' size increased when the mixture Tween 80:Span 80 is used as remarked by Kim and Cho,[36] the size droplet increased when *HLB* value was lower than 13.

Double-walled microspheres' characteristics

The experimental conditions of batch D_{11} gave the most desired results with the highest encapsulation efficiency of 86.2 % and the suitable spherical particles, so they are chosen for the second coating process. Ethylcellulose (EC) and cellulose acetate butyrate (CAB) are selected for covering the beeswax microspheres of the D_{11} batch, as described in the Experimental. The results are displayed in Table III.

TABLE III. Double walled microspheres' characteristics

Code	Polymer matrix	Particles morphology	Drug loading %±*SD*	Entrapment efficiency % ±*SD*	d_{10} / μm
F1	EC	Spherical	8.6±0.4	77.9±3.5	144.7
F2	CAB	Spherical aggregates	7.8±0.1	70.6±0.1	131.6

Through this study, actually, the particles' size increased due to the presence of the second wall but a difference is noticed between the two polymeric coats. The microspheres coated by EC are more spherical and larger than those obtained by CAB. The double coating process induced a loss of drug entrapment of 1 and 1.8 % for EC and CAB, respectively. The lower loss can be related to the higher viscosity of EC organic phase which can limit the drug migration to the external phase.[37,38] We concluded that the EC–beeswax formulations called F1 gave the best results, *i.e.*, the spherical microparticles with the uppermost drug entrapment.

Microparticles' characterization

Infrared spectroscopy analysis is performed to identify the interaction between mesalamine and coating polymers. So, FT-IR spectra of the individual constituents and microparticles are acquired as shown in Fig. 1. The infrared spectrum of pure mesalamine displayed characteristic bands[39,40] like C–N stretching vibration at 1262 cm^{-1}, the bending vibration of N–H at 1616 cm^{-1}, C=O of acrylic acid stretching vibration at 1645 cm^{-1} and a large band between 2500–3300 cm^{-1} which corresponds to the stretching vibration of O–H of acid bond and NH_2 group, and C–H bond of the aromatic group at 807–833 cm^{-1}.

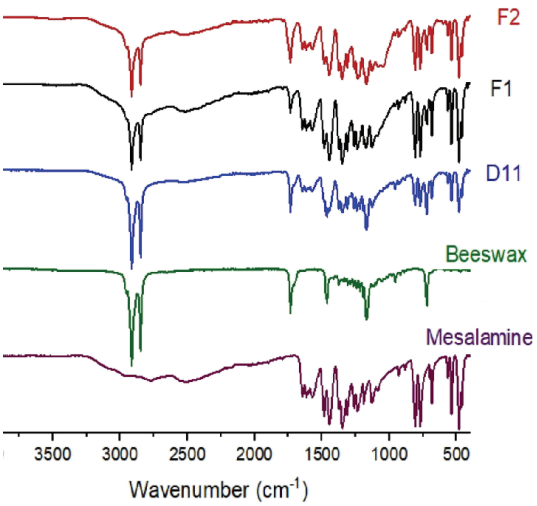

Fig. 1. Infrared spectra of mesalamine, beeswax and formulations (F1, F2 and D_{11}).

The beeswax spectrum showed alkanes adsorption bands assigned to C–H stretching at 2954 and 2848 cm^{-1}, C–H bend or scissoring at 1472 cm^{-1}; and CH_2 rocking seen in long-chain alkanes (718 cm^{-1}). The sharp band observed at 1736 cm^{-1} is attributed to the carbonyls in the ester linkages between the fatty acids and glycerol backbone.[41]

All absorption bands of mesalamine and beeswax are observed in the infrared spectrum of microparticles as shown for D_{11} in Fig. 1, which confirms

the actual mesalamine loading and also the absence of chemical interactions between the components during the formulation process.

In addition to the bands already distinguished in D_{11} spectrum, the FT-IR spectra of double walled formulation, *i.e.*, F1 and F2 showed a small absorption at 1056 cm^{-1}, which can correspond to the glycosidic C–O–C vibration band of cellulose.

Beeswax X-ray diffractogram (Fig. 2) displayed sharp and intense peaks at 2θ 22.1 and 24.51° in addition to weaker peaks at 2θ 19.98 and 36.7°. The mesalamine pattern showed a range of intense peaks at 2θ 8, 15.6 and 17.1° and other weak peaks between 23.1 and 48.9° confirming crystalline forms of beeswax and mesalamine. An important diminution of the mesalamine peak intensity is observed in the XRD pattern of microparticles, indicating a reduced drug crystallinity after the process formulation. Beeswax XRD peaks remained virtually unchanged. It has been also seen that mesalamine loses its crystallinity when it is loaded in PLGA nanoparticles.[42]

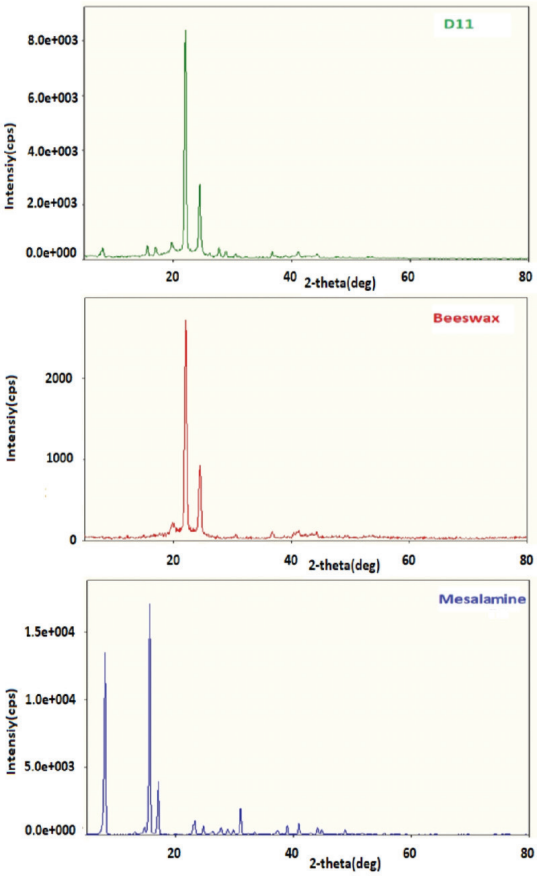

Fig 2. X-ray diffractograms of beeswax, mesalamine and microparticles of D_{11}.

Microscopic observation of the lots D_8, D_{10}, D_{11} and F1, as shown in Fig. 3, revealed spherical shape of microparticles. The surface of beeswax microspheres and the double walled microspheres appeared in SEM micrographs (Fig. 4) with rough and spotted state. The surface of the double walled microparticles is different from that simple beeswax microspheres; EC gave rise to an uniform external layer, as remarked in the case of F_1batch.

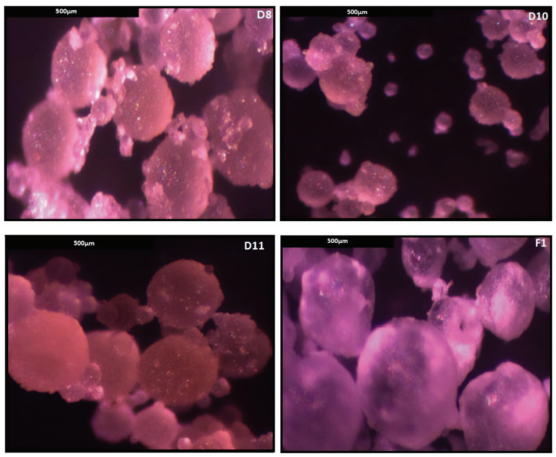

Fig. 3. Optical microscopy images of microparticles of lots D_8, D_{10}, D_{11} and F1.

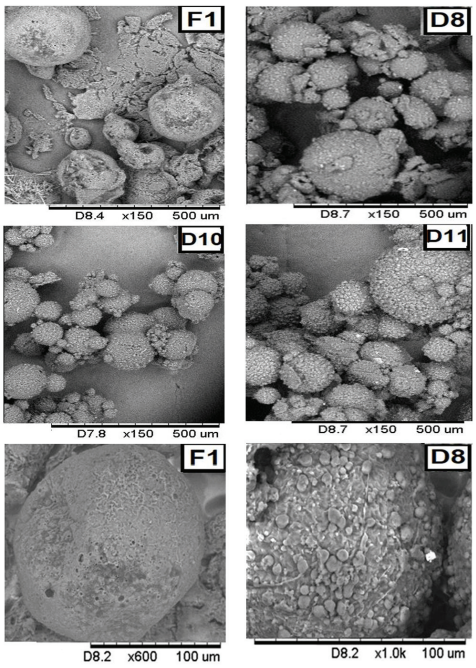

Fig. 4. SEM micrographs of microparticles of lots D_8, D_{10}, D_{11} and F1.

In-vitro *drug release studies*

The drug release is carried out in three selected simulated digestive liquids with pH 1.2, 6.5 and 8. The mesalamine release profiles of D_8, D_{10}, D_{11} beeswax lots and F1 and F2 double walled beeswax microspheres are given in Fig. 5. It is well distinguished that in the acidic medium (pH 1.2), the drug is discharged from simple beeswax formulations (D_8, D_{10}, D_{11}) and is not discharged by double walled microspheres F1and F2. In pH 8, the drug release began slightly in F1 and F2 formulations and increased in pH 6.5.

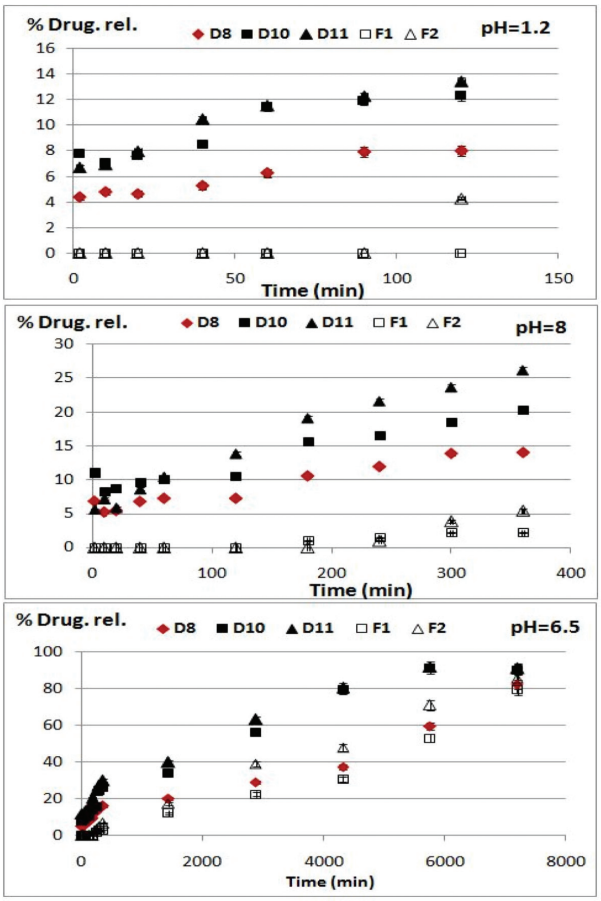

Fig. 5. Drug release profiles of mesalamine from single beeswax and double walled beeswax microparticles in simulated liquids (pH 1.2, 8 and 6.5).

The release data are summarized in Table IV. The percentage of mesalamine released from the formulations D_8, D_{10} and D_{11} and detected in the acidic medium (pH 1.2) after 120 min of release time varied from 8 to 13.5 %. How-

ever, for the double walled microparticles of F1, the drug release didn't occur during 120 min of contact time. In the simulated small bowel liquid (pH 8) and after 6 h of contact time, the drug release varied from 14 to 26 % and from 2.1 to 5.4 % for simple beeswax formulations (D_8, D_{10}, D_{11}) and double coated formulations (F1 and F2), respectively. Finally, in the colon simulated liquid where pH 6.5 and after 96 h (4 days) of contact time, the % of drug release for D_8 and F1 didn't exceed 60 % and for D_{10} and D_{11} reached 91 % and remained at 70 % for F2 microspheres.

TABLE IV. Mesalamine release data in simulated gastric (pH 1.2), small bowel (pH 8) and colon (pH 6.5) liquids

Formulation	pH	Drug rel., %	Korsmeyer–Pepas model			
			Time interval, min	K	n	R^2
D8	1.2	7.9	0–120	0.024	0. 23	0.843
	8	14.0	120–360	0.004	0.61	0.950
	6.5	59.3	1440–7200	0.001	0.73	0.932
D10	1.2	12.3	0–120	0.037	0.25	0.918
	8	20.2	120–360	0.008	0.55	0.932
	6.5	90.6	1440–7200	0.003	0.64	0.964
D11	1.2	13.4	0–120	0.052	0.18	0,879
	8	26.1	120–360	0.010	0.55	0.977
	6.5	91.6	1440–7200	0.008	0.54	0.963
F1	1.2	0	0–120	–	–	–
	8	2.1	120–360	–	–	–
	6.5	52.9	1440–7200	$3\ 10^{-5}$	1.12	0.959
F2	1.2	4.2	0–120	–	–	–
	8	5.4	120–360	–	–	–
	6.5	70.7	1440–7200	1510^{-5}	0.97	0.987

In summary, the mesalamine is slowly discharged from D_8 microparticles which are prepared using PVA as emulsifier, in contrary to D_{10} and D_{11} beeswax microspheres, that are prepared using Tween 80 and a mixture of Tween 80 and Span 80. So, D_8 beeswax microparticles can be used for sustained and prolonged drug release. The results can be related to the microparticles' size, since it is known that the smaller particle size results in an increase of dissolution because the contact surface area is increased;[43] in fact, the D8 microparticles' size is higher than those of D_{10} and D_{11} and then it causes a slowly drug dissolving and release.

Regarding the bi-layered beeswax microparticles F1 and F2, these devices exhibited a delayed release effect in simulated gastric media due to the acid resistance of the outer enteric coating layer,[5,44] so they can be considered as colon-specific delivery systems. In fact, the coating using pH-sensitive polymers or

biodegradables matrices, such as polysaccharides, provides delayed release and protects the active drug from gastric fluid.[45].

In addition, the results showed that the percentages of drug released in the first eight hours, *i.e.*, before reaching the simulated colon region are 22, 33 and 50 % for D_8, D_{10} and D_{11} formulations, respectively. It didn't exceed 10 % for the F2 double coated microparticles and is lower than 3% for F1 microparticles.

Release data analysis

In order to identify the drug dissolution mechanism through beeswax and double coated beeswax microparticles in each release medium, the Korsmeyers––Peppas model was tested. This model is developed to analyse both Fickian and non-Fickian release of drug from swelling or non-swelling polymeric delivery systems. As reported in Table IV, the release data are fitted according to the selected model in the appropriate time intervals which are specified in Table IV. In the semi-empirical model of Korsmeyer–Peppas (Eq. (4)), the exponent of time (n) is related to the drug release mechanism. So, n-values are indicative for diffusion or relaxation/erosion-controlled drug release. In one hand, the limit values of n depend on the dosage form geometry; it is equal to 0.50, 0.45 and 0.43 for slabs, cylinders and spheres, respectively. In the other hand, this limit is also dependent on the width of spherical polymeric particles' size distribution.[46] If we consider the spherical geometry, when $n = 0.43$, the release mechanism is controlled by Fick's diffusion. When $0.43 < n < 0.85$, the drug release mechanism is related to non-Fick's diffusion or anomalous transport. When $n = 0.85$, the drug release mechanism is governed by Case-II transport.[47] It is also reported that when $n > 0.85$, the release mechanism belongs in matrix erosion or relaxation and generally when the exponent $n < 0.5$, the drug release mechanism is related to the quasi-Fickian diffusion model, and the value of $n = 1$ indicates zero order release.[48–50]

So, regarding the mesalamine release from D_8, D_{10} and D_{11} formulations, and for the first two hours corresponding to the retention time in the stomach, the coefficient of regression R^2 of Korsmeyer–Peppas model varied between 0.843––0.918 (Table IV). On the basis of n-values of Korsmeyer–Pepas equation which are lower than 0.43, the release mechanism can be assumed to be the quasi-Fickian diffusion model, it assumed to non-swellable matrix diffusion.[49] In this step of drug release (pH 1.2), the formulations F1 and F2 didn't discharge the drug.

In the simulated small bowel medium (pH 8), the model is fitted from 120 to 360 min so during the next four hours, and the results showed that R^2 varied from 0.932–0.977 for D_8, D_{10} and D_{11} formulations. However, the value of the Korsmeyer–Pepas exponent, n, is close to 0.55, which belongs to $0.43 < n < 0.85$ demonstrating that the release mechanism corresponds to a non-Fickian or anomalous transport, so that the release mechanism is governed by simultaneously

various phenomena such as diffusion and relaxation.[46,49] Concerning the double coated microparticles of F1 and F2, the experimental data are not sufficient to apply the mathematical model.

Finally, in the colon simulated medium (pH 6.5), the model is fitted from the first to fifth day of drug release. The results showed that the mesalamine is discharged in this medium from all formulations. For the beeswax microparticles, the coefficient R^2 of Korsmeyer–Pepas model varied from 0.932–0.964 and the n exponent varied from 0.54 to 0.73, indicating that the drug release is also anomalous and is governed by a non-Fick's transport. About the double walled formulations (F1 and F2), the release data are well fitted according to the Korsmeyer–Pepas model (R^2 0.959–0.987) and the values of the exponent n are 1.12 and 0.97 for F1 and F2, respectively, so they can be considered as close to unit, in this case, the release mechanism is controlled by Case II transport or zero order release. The obtained release profiles obviously indicate that the drug release is actually delayed using these new formulations.

CONCLUSION

The objective of this work is to develop colon specific drug delivery systems that use natural materials and should be capable to deliver the most drug quantity in colon region. Really, beeswax is chosen as matrix for mesalamine drug formulation and successful results are being recorded. The optimization of the microencapsulation process by varying some parameters such as drug:beeswax ratio, emulsifier concentration and nature, and the stirring speed of emulsion permitted to allow spherical microparticles, with a drug entrapment efficiency varying from 42 to 86 %. The D_8 bath of microparticles offered a slow and prolonged drug release which became faster in simulated colon liquid. In addition, bi-layered microparticles which are prepared using cellulose derivatives, *i.e.*, ethylcellulose and cellulose acetate butyrate are promising, in fact, the drug release didn't occur in acidic medium from these new devices. The drug release mechanism is related to the quasi-Fickian diffusion model in acidic medium (pH 1.2), to a non-Fick's transport in the simulated small bowel medium (pH 8) and in the colon simulated medium (pH 6.5), it is assumed to a non-Fick's transport for beeswax formulations and controlled by Case II transport or zero order release for the double coated microparticles. In conclusion, this bi-layer coating methodology can successfully be applied to produce delayed and especially colon specific drug release formulations.

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ИЗВОД

ПРИПРЕМА И *IN VITRO* ЕВАЛУАЦИЈА ЈЕДНОСЛОЈНИХ И ДВОСЛОЈНИХ МИКРОЧЕСТИЦА НА БАЗИ ПЧЕЛИЊЕГ ВОСКА ЗА КОЛОН-СПЕЦИФИЧНУ ДОСТАВУ МЕСАЛАМИНА

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Пчелињи восак је одабран као природни материјал за облагање ради у циљу нових система за доставу и контролисано отпуштање лекова специфичних за колон, уз додатак месаламина. У првом кораку, припремљене су микрочестице пчелињег воска коришћењем поступка микроинкапсулације топљењем, где је вариран однос лек:пчелињи восак, брзина мешања, концентрација емулгатора и pH спољне фазе ради оптимизације задржавања лека и морфологије микрочестица. Такође је разматран утицај природе емулгатора праћењем вредности хидрофилно–липофилног баланса (*HLB*). У другом кораку, како би се добили системи за одложено отпуштање лека, израђене су двослојне микросфере поступком испаравања растварача уз употребу етилцелулозе и ацетилбутирата целулозе као спољног слоја за облагање. Све формулације су окарактерисане инфрацрвеном спектроскопијом, рендгенском дифракционом анализом, скенирајућом електронском микроскопијом и оптичком микроскопијом. Ослобађање лека је испитивано у симулираним течностима желуца, танког црева и дебелог црева, а механизам ослобађања је разматран применом Korsmeyer–Peppas модела.

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