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Research Article

Influence of Some Formulation Variables on the Optimization of pH-dependent, Colon-targeted, Sustained-release Mesalamine Microspheres

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Abstract. The aim of this work was to understand the influence of different formulation variables on the optimization of pH-dependent, colon-targeted, sustained-release mesalamine microspheres prepared by O/O emulsion solvent evaporation method, employing pH-dependent Eudragit S and hydrophobic pH-independent ethylcellulose polymers. Formulation variables studied included concentration of Eudragit S in the internal phase and the ratios between; internal to external phase, drug to Eudragit S and Eudragit S to ethylcellulose to mesalamine. Prepared microspheres were evaluated by carrying out *in vitro* release studies and determination of particle size, production yield, and encapsulation efficiency. In addition, morphology of microspheres was examined using optical and scanning electron microscopy. Emulsion solvent evaporation method was found to be sensitive to the studied formulation variables. Particle size and encapsulation efficiency increased by increasing Eudragit S concentration in the internal phase, ratio of internal to external phase, and ratio of Eudragit S to the drug. Employing Eudragit S alone in preparation of the microspheres is only successful in forming acid-resistant microspheres with pulsatile release pattern at high pH. Eudragit S and ethylcellulose blend microspheres were able to control release under acidic condition and to extend drug release at high pH. The stability studies carried out at 40°C/75% RH for 6 months proved the stability of the optimized formulation. From the results of this investigation, microencapsulation of mesalamine in microspheres using blend of Eudragit S and ethylcellulose could constitute a promising approach for site-specific and controlled delivery of drug in colon.

KEY WORDS: colon; ethylcellulose; eudragit; mesalamine; sustained release.

INTRODUCTION

Mesalamine (5-aminosalicylic acid, 5-ASA) is widely used in long-term treatment of ulcerative colitis by its topical mode of action on the inflammation in colonic mucosa (1). In order to achieve effective oral 5-ASA treatment with minimal side effect and acceptable patient compliance, the delivery system has to overcome many obstacles. Upon oral administration, 5-ASA exhibits rapid and nearly complete absorption from the upper intestine, resulting not only in systemic side effects but also in lowering the dose reaching the colon with the subsequent decreased probability of therapeutic success (2). Furthermore, in the case of ulcerative colitis, inflammation is observed in all regions of the colon. Therefore, if 5-ASA is released in a pulsatile manner in the ascending colon, 5-ASA would be diluted during its passage in the colon, consequently insufficient concentration of 5-ASA could be delivered to the transverse and descending colon resulting in reduced clinical effectiveness (3).

The effective use of most of the current 5-ASA formulation requires multiple daily dosing with up to 12 tablets or capsules. Reduced patient compliance and disease control are the results of this inconvenience of frequent daily dosing and the number of tablets or capsules required per day (4).

Accordingly, in order to overcome these problems, in formulating 5-ASA in a successful delivery system, it is tremendously important to minimize 5-ASA release in the upper gastrointestinal (GI) tract and to localize 5-ASA release in the colon in a sustained-release manner.

Single-unit dosage forms (tablets and capsules) for modified release colonic delivery suffer from problems such as unpredictable gastric emptying, GI transit variations resulting from intersubject variability in transit patterns, and incomplete drug delivery in GI tract due to the risk of not dissolving the polymer coat on the large, low surface area-coated tablets. On the other hand, multiparticulate drug delivery system for colonic delivery shows several advantages over single-unit dosage forms. Being of smaller size, it is expected to provide less inter- and intra-individual variability, more rapid and uniform gastric emptying, more uniform dispersion and reproducible transit through GI tract (5,6).

However, in microparticulate delivery systems, it is challenging to develop a colon-targeted sustained-release dosage form. It suffers from the risk of early dissolution and release of the drug before reaching the colon due to its large surface area (7). This is more difficult in the case of 5-ASA due to its physiochemical properties. 5-ASA exhibits

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amphoteric property, its solubility is increased at acidic pH values (pH<2) in the stomach and at more alkaline values (pH>5.5) in the lower part of the small intestine (8).

The aim of this study is to investigate the feasibility of development of pH-dependent, colon-targeted, sustained-release mesalamine microspheres prepared by O/O emulsion solvent evaporation method employing pH-dependent Eudragit S and hydrophobic pH-independent ethylcellulose polymers. The influence of some formulation variables such as concentration of Eudragit S in the internal phase and the ratios between internal to external phase, drug to Eudragit S and Eudragit S to ethylcellulose blends on the particle size, morphology, production yield, and encapsulation efficiency as well as *in vitro* drug release will be studied.

MATERIALS AND METHODS

Materials

5-ASA was kindly donated by Minapharm Pharmaceuticals, (Cairo, Egypt), ethylcellulose (EC) 300 cps and Span 80 were purchased from Sigma-Aldrich (Germany), magnesium stearate (Adwic, El-Nasr Pharmaceutical Chemicals Co., Egypt), Eudragit®S 100 polymer (ES100), generously donated by Röhm Pharma, GmbH, Germany. All other chemicals and solvents were of analytical grade.

Methods

Preparation of Microspheres

The composition of 5-ASA microspheres formulations with varying proportion of ES100 alone and in combination with EC is presented in Table I. 5-ASA microspheres were prepared by oil/oil solvent evaporation method. At room temperature, 5-ASA and the specified amount of magnesium stearate were dispersed in acetone (internal phase) to which ES100 either alone or with EC was previously dissolved. The resultant suspension was added at once to a specified amount of liquid paraffin (external phase) containing 2% (*v*/*v*) Span 80 under stirring using a mechanical stirrer at a speed of (2,000 rpm) for the initial 5 min and at 1,000 rpm for the following 4–6 h under ambient conditions until the organic solvent completely evaporated and precipitating out the microspheres. The microspheres were filtered, washed with 100 ml *n*-hexane, overnight air dried and then stored in a desiccator until further use.

Physical Characterization of the Microspheres

Morphological and Particle Size Analyses. The prepared microspheres from different formulations were studied for appearance and size using optical microscopy. Images of microspheres were acquired using an optical microscope (Leica Imaging Systems, Cambridge, UK) equipped with digital camera (JVC, Victor Co, Yokohama, Japan). The particle size was measured with optical microscope using a calibrated eye piece micrometer. A small amount of dry microspheres was suspended in purified water. Small drops from the obtained suspension were placed on a clean glass slide. The slide containing the microspheres suspension was mounted on the microscope and 100 particles were measured using the calibrated ocular micrometer (9).

Production Yield. Production yield of the microspheres was determined by measuring the last weight of the microspheres (WM) produced and calculating the theoretical weight of microspheres based on the initial weight of the raw materials used (WR). The ratio of WM to WR was then calculated and multiplied by 100 and expressed as a percentage.

Table I. Composition of Microspheres Formulations Using ES100 Alone and in Combination with Varying Proportion of EC

Formulation	5-ASA (mg)	Drug: ES100 ratio	ES 100 (%)[a]	Drug/EC ratio	Mg stearate (%)[b]	Internal/external phase ratio	Surfactant (%)[c]
Microspheres prepared with varying ES100 concentration in the internal phase							
MF1	500	1:1	2	–	5	1:2	2
MF2	500	1:1	1	–	5	1:2	2
MF3	500	1:1	0.667	–	5	1:2	2
Microspheres prepared with different internal-to-external-phase ratio							
MF2	500	1:1	1	–	5	1:2	2
MF4	500	1:1	1	–	5	1:4	2
MF5	500	1:1	1	–	5	1:6	2
MF6	500	1:1	1	–	5	1:8	2
Microspheres prepared with varying drug to ES 100 ratio							
MF7	500	1:0.25	1	–	5	1:2	2
MF8	500	1:0.5	1	–	5	1:2	2
MF9	500	1:0.7	1	–	5	1:2	2
MF2	500	1:1	1	–	5	1:2	2
Microspheres with varying drug to polymeric blends ratio							
MES70EC10	500	1:0.7	1	1:0.1	5	1:2	2
MES85EC10	500	1:0.85	1	1:0.1	5	1:2	2
MES100EC10	500	1:1	1	1:0.1	5	1:2	2

[a] Percent *w*/*v* of internal phase
[b] Percent *w*/*w* of sum of drug and polymer(s) content
[c] Percent *v*/*v* of external phase.

Content of 5-ASA in the Microspheres and Encapsulation Efficiency. For estimating drug content, accurately weighed quantity of each formulation containing 50 mg of drug was dissolved in 50 ml of freshly prepared phosphate buffer (pH 7.4). The resulting mixture was kept shaking on a mechanical shaker for 48 h, filtered and suitably diluted, then analyzed spectrophotometrically (Jenway UV/Vis Spectrophotometer, Barloworld Scientific Limited, Essex, UK) at predetermined λ_{max} of 5-ASA (330 nm). The encapsulation efficiency of microspheres was determined by taking the ratio of the actual drug content to the theoretical drug content expressed in percentage.

In Vitro Release Studies. The release characteristics of 5-ASA from the prepared formulations were determined according to the USP dissolution I basket method using a dissolution tester (Vision® Classic 6^{TM} Dissolution Tester, Hanson Research Corporation, CA, USA) at 37±0.5°C with a rotation speed of 100 rpm. The release profile was studied in a medium of changing pH. The initial condition was 350 ml of 0.1 N HCl (pH 1.2) for 0–2 h. At the end of the second hour, the pH of the media was raised to 4.5 and the total dissolution media volume to 600 ml by the addition of 250 ml solution composed of 3.75 g of KH_2PO_4 and 1.2 g of NaOH. At the end of the fourth hour, pH was raised to 7.4 by adding 300 ml of phosphate buffer concentrate (2.18 g of KH_2PO_4 and 1.46 g of NaOH in distilled water; 10–13). At predetermined time intervals, a 5-ml sample was withdrawn and replaced with fresh dissolution media. Collected samples were filtered through 0.45-μm Millipore filters. After appropriate dilutions, the concentration of 5-ASA in samples was spectrophotometrically measured at predetermined $\lambda_{max(s)}$. Cumulative percentages of drug released from the microspheres were calculated and plotted as a function of time.

Kinetic Analysis of Release Data. The data obtained from the *in vitro* release studies were kinetically analyzed using Excel 2007 (Microsoft, USA) software to determine the mechanism and the order of drug release from different formulations. Generally, the kinetics of 5-ASA release from the prepared formulations was determined by finding the best fit of the dissolution data (fraction of drug release *versus* time) to distinct models: zero-order (14), Higuchi (15), and first-order (16) kinetics. Furthermore, in order to better characterize the drug release mechanism, the Korsmeyer–Peppas (17) and Hixson–Crowell (18) models were applied.

Scanning Electron Microscopy Analysis. The surface and cross-sectional characteristics of microspheres resulted from selected formulation were studied by scanning electron microscopy using the Jeol, JXA-840A (Tokyo, Japan) electron microscope. Samples were prepared by dropping the microspheres onto metallic slides that had a double-sided adhesive tape adhered to their surfaces. The microspheres were then made electrically conductive by coating, in vacuum, with a thin layer of gold.

Differential Scanning Calorimetry. Thermal analysis by differential scanning calorimetry (DSC) was carried out on the plain drug and the optimized formulation using Shimadzu thermal analyzer (Shimadzu DSC 60, TA-60 WS, Japan) equipped with a computerized data station. Samples were placed in an aluminum pan and heated at a rate of 10°C/min in the temperature range of 30–350°C. The thermal analysis was performed under dynamic nitrogen atmosphere.

Stability Study. According to the International Conference on Harmonisation guidelines, the microspheres from selected formulation were exposed to 6 months stability study at 40°C/75% RH (19–21). At the end of 3 and 6 months, the formulations were evaluated by visual observation of any physical changes that occurred, determination of drug content, and carrying out *in vitro* release studies for 16 h.

Statistical Analysis. A one-way analysis of variance test was performed to analyze the effect of formulation factors on

Table II. Physical Characterization of 5-ASA-Loaded Microspheres Formulations

Batches	Physical appearance	Particle size[a] (μm)	Yield (%) (mean±SD, n=2)	Encapsulation efficiency (%) (mean±SD, n=2)
Microspheres prepared with varying ES100 concentration in the internal phase				
MF1	Irregular, discrete	276.53±22.74	84.45±2.45	90.15±2.54
MF2	Spherical, discrete	264.04±21.10	94.42±1.88	90.55±3.45
MF3	Irregular, discrete	105.38±26.95	83.52±2.17	87.94±1.48
Microspheres prepared with different internal-to-external-phase ratio				
MF2	Spherical, discrete	264.04±21.10	94.42±1.88	90.55±3.45
MF4	irregular, discrete	190.54±29.21	89.25±0.47	81.79±0.98
MF5	Irregular, discrete	151.68±26.61	91.54±2.96	79.25±1.22
MF6	Irregular, aggregated	139.76±19.40	96.24±2.54	74.68±2.17
Microspheres prepared with varying drug to ES 100 ratio				
MF7	No spheres	–	–	–
MF8	No spheres	–	–	–
MF9	Irregular aggregated	180.25±26.48	94.99±1.24	85.85±2.88
MF2	Spherical, discrete	264.04±21.10	94.42±1.88	90.55±3.45
Microspheres with varying drug to polymeric blends ratio				
MES70EC10	Spherical, discrete	151.29±24.54	91.00±3.45	84.15±2.93
MES85EC10	Spherical, discrete	347.89±22.83	94.99±1.99	90.44±1.26
MES100EC10	Spherical, discrete	595.27±46.40	98.75±1.55	92.89±2.35

[a] Average of 100 particles±SD

characteristics of 5-ASA-loaded microspheres. A p value less than 0.05 was considered as representing a significant difference

RESULTS AND DISCUSSION

Preparation of Microspheres

To prepare 5-ASA-loaded microspheres, the O/O (oil in oil) emulsion solvent evaporation technique was used. The method is correctly referred as O/O instead of W/O (water in oil) as a polymeric solution in organic solvent is considered as oil in microencapsulation terminology (22). This method was proven to be effective, robust, and universal in encapsulation of drugs with different physiochemical properties (5). Liquid paraffin was used as the dispersion medium or external phase because of the insolubility of 5-ASA, ES100, and EC in liquid paraffin. To prevent droplets coalescence during emulsification and solvent evaporation, an emulsifying agent, Span 80, was used to reduce the interfacial tension between the droplets and the external phase (23) and magnesium stearate was added as droplet stabilizer (24). Employing higher stirring speed during the preparation process was aimed at decreasing microspheres

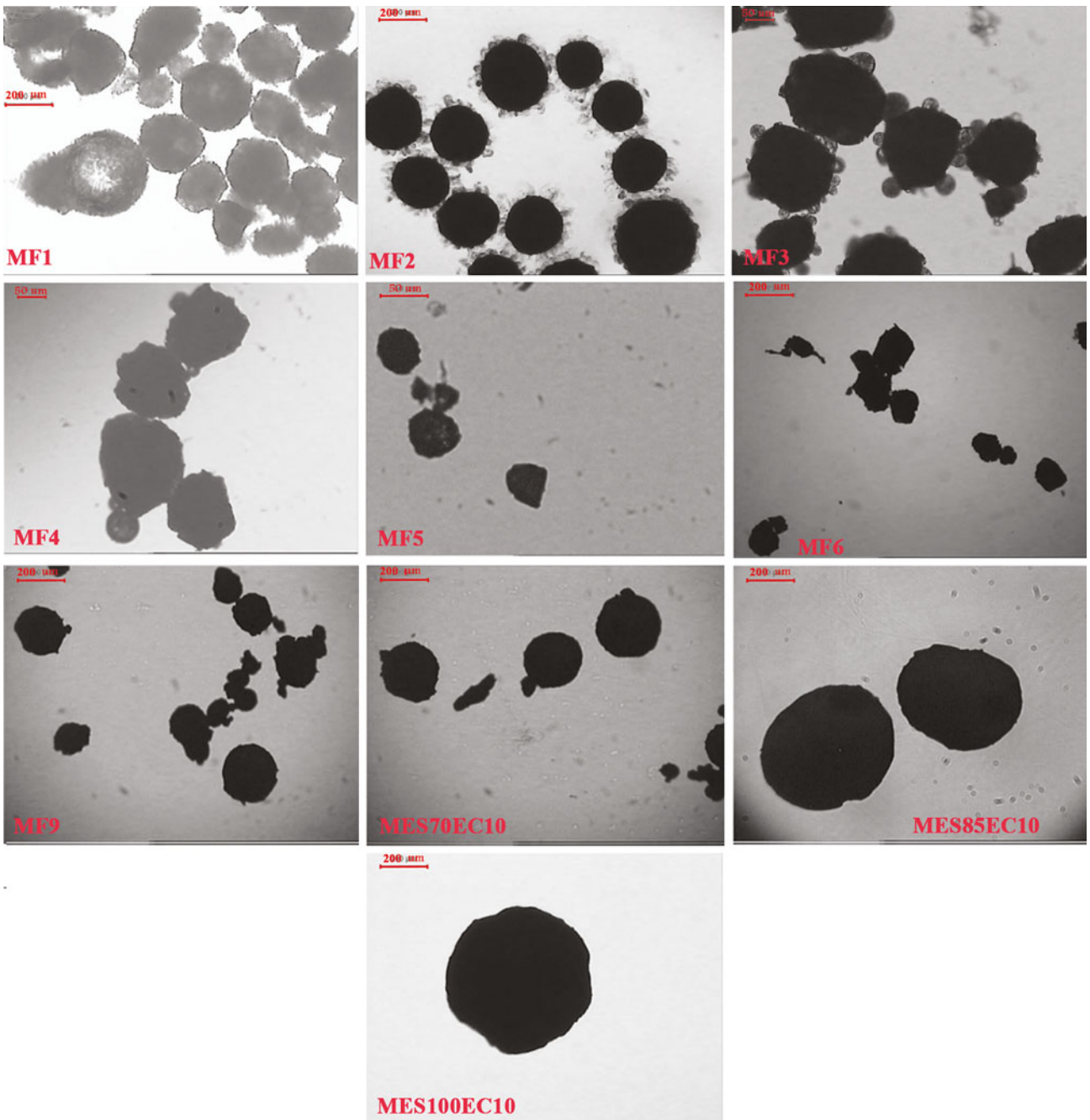

Fig. 1. Photomicrograpgs of different 5-ASA microspheres formulations. *Bar* 200 μm except for MF3, MF4 and MF5 (*bar* 50 μm)

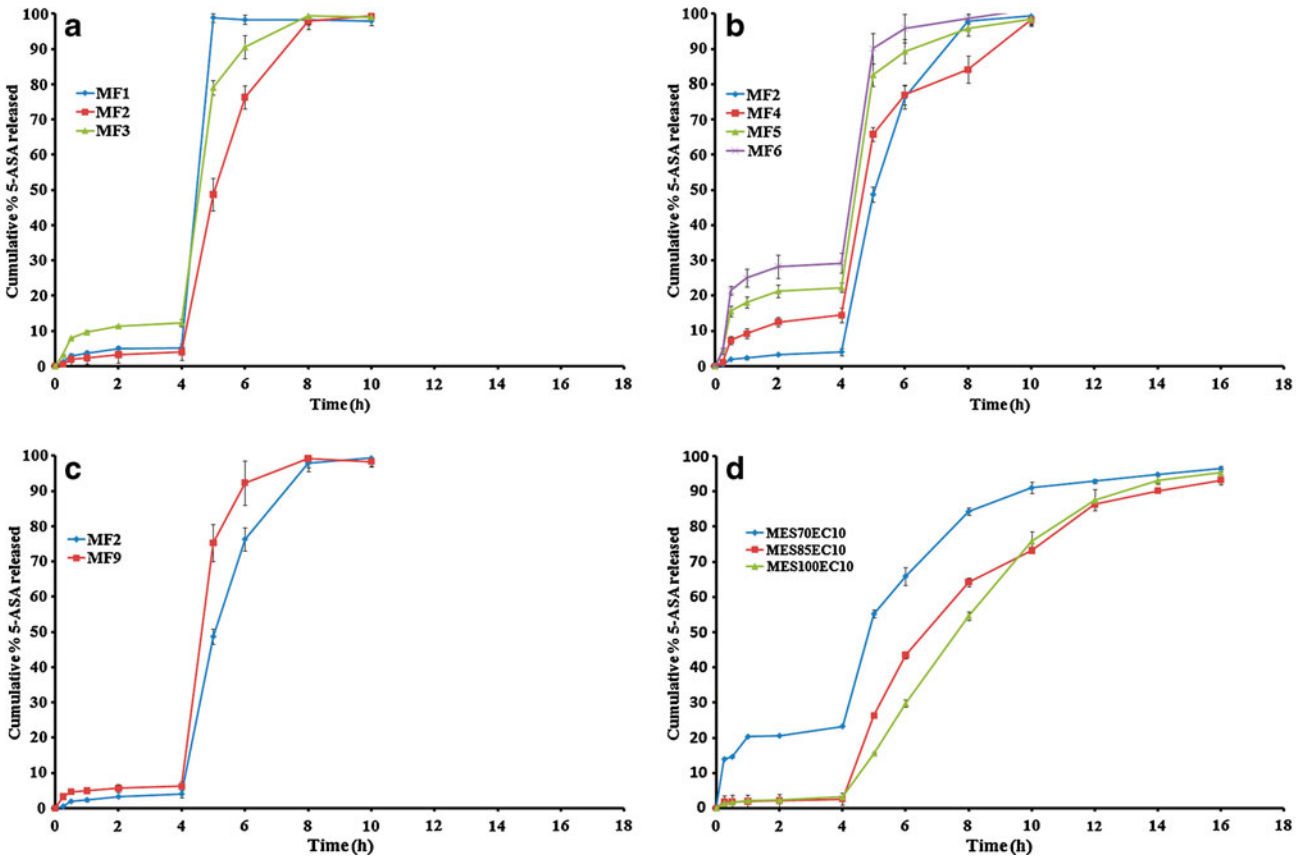

Fig. 2. *In vitro* release of 5-ASA from different microspheres formulations

mean size. *n*-Hexane was used to clean the surface of microspheres because it removes liquid paraffin without disturbing the integrity of the microspheres (25).

Physical Characterization of the Microspheres

The physical appearance, mean particle size, yield, and encapsulation efficiency of the prepared formulations are presented in Table II. It is clear that the production yield remained high (ranged from 83.52% to 98.75%) under the entire studied formulation variables. This could suggest the success and the efficiency of the proposed method.

Microspheres Prepared with Varying ES100 Concentration in the Internal Phase

The microspheres prepared with different ES100 concentrations in the internal phase were characterized by different characters. Only microspheres of Formula MF2 exhibited acceptable free-flowing spherical shape with no agglomeration as shown in Fig. 1. Decreasing ES100 concentration in the internal phase from 2% (formula MF1) to 1% (formula MF2) resulted in insignificant decrease in particle sizes from 276.53 to 264.04 μm but further decrease in ES100 concentration to 0.667% (formula MF3) led to a significant decrease in particle size ($p<0.05$) to 105.38 μm (Table II).

Table III. Determination Coefficients of Different Mathematical Models for 5-ASA Release from ES100-EC Microspheres

Formulation	Zero order[a]	First order[b]	Diffusion[c]	Peppas[c] (*n*)	Hixson–Crowell[d]	Correlation time span (*h*)
MES70EC10	0.881	*0.986*	0.983	0.997 (0.349)	0.964	4–8
MES85EC10	0.952	0.985	*0.989*	0.986 (0.559)	0.988	5–12
MES100EC10	0.980	0.989	*0.997*	0.995 (0.845)	0.999	5–12

Better results of R^2 are in italics
M_t/M_∞, the fraction of drug released up to time; t, k the kinetic constant; C constant
[a] Zero order, $M_t/M_\infty = kt + C$
[b] First order, $Mt/M\infty = 1 - e^{-kt}$
[c] Higuchi, $M_t/M_\infty = k(t)^{0.5} + C$
[d] Korsmeyer–Peppas, $M_t/M_\infty = kt^n + C$
[d] Hixon–Crowell $M_t/M_\infty = 1 - (1 - kt)^3$

This could be explained on the basis that higher polymer concentration produced a more viscous dispersion which reduces the shearing action, resulting in larger droplets during the emulsification and consequently larger microspheres were produced after evaporation (24). It also could be suggested that the higher ES100 concentration led to higher frequency of collision, resulting in fusion of the droplets producing larger microspheres (26).

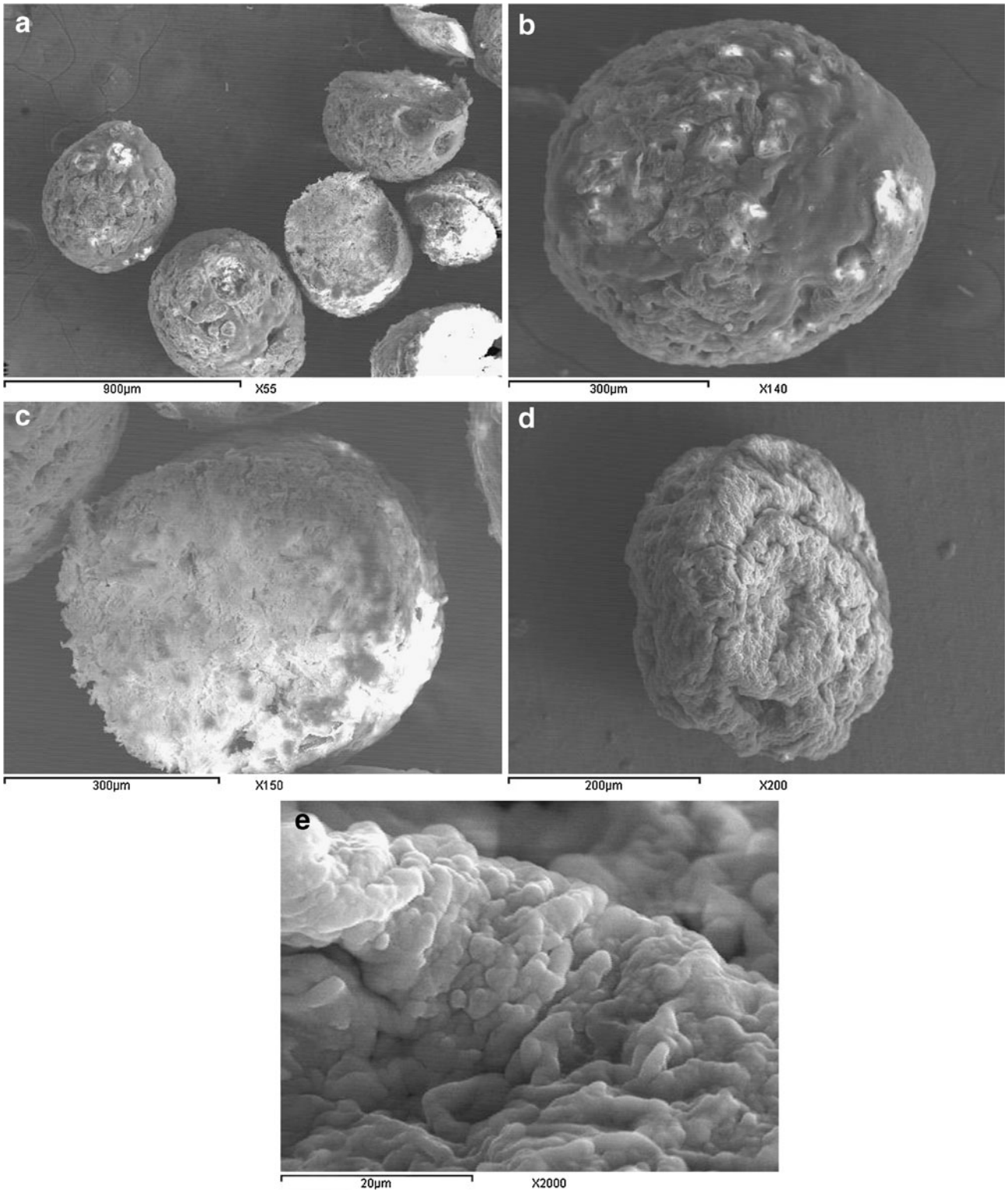

Fig. 3. Scanning electron micrographs of formula MES100EC10; **a** at 55× magnification before release studies (some microspheres were transversely cut), **b** at 140× magnification before release, **c** cross section at 150× magnification before release, **d** at 200× magnification after release, **e** at 2,000× magnification after release

As shown in Table II, encapsulation efficiency was slightly decreased by decreasing ES100 concentration in the internal phase. Sachin *et al.* (27) reported that a more viscous internal phase is formed by higher polymer concentration, which results in solidification at a faster rate. This reduces the leaching of drug to the external phase; consequently the encapsulation efficiency is increased.

Microspheres Prepared with Different Internal-to-External Phase Ratio

Three additional formulations (MF4–MF6) were prepared and evaluated to establish an optimum internal-to-external-phase ratio. Formulations (MF4–MF6) were prepared with varying internal-to-external-phase ratios at fixed ES100 concentration at 1% *w/v* of the internal phase and drug to ES100 ratio at 1:1, and compared to MF2. These formulations showed less spherical characters compared to MF2, especially, with decreasing internal to external phase ratio (Fig. 1.). Also, as shown in Table II, the particle size was significantly decreased ($p<0.05$) from 264.04 (MF2) to 190.54 (MF4) to151.68 (MF5) to 139.76 (MF6)μm on decreasing internal phase to external phase ratio from 1:2 to 1:4 to 1:6 to 1:8, respectively. These results could be explained by the fact that when the internal phase ratio increase, the shearing action of the mixer probably decreases resulting in formation of larger emulsion droplets and thereby creating relatively larger microspheres. Additionally, the mean distances between the droplets could be decreased with increasing the internal phase ratio. Consequently, the chances of coalescence between the droplets are increased, leading to aggregation of the microspheres prepared (28).

As shown in Table II, the encapsulation efficiency was significantly reduced ($P<0.05$) by increasing the volume of the external phase to that of the internal phase. The encapsulation efficiencies were reduced from 90.55–81.79% to 79.25–74.68% for formulations MF2, MF4, MF5, and MF6, respectively, on increasing the volume of the external phase relative to the volume of the internal phase from 1:2 to 1:4 to 1:6 to 1:8 respectively. Kendall *et al.* (29) suggested that encapsulation efficiency is related to particle size. The larger the droplet size, the smaller the interfacial surface area and thus, the less drug transfer to liquid paraffin phase. Consequently, higher encapsulation efficiency is achieved.

Microspheres Prepared with Varying Drug to ES100 Ratio

To determine the drug to ES100 ratio sufficient to prevent premature drug release in the precolonic stages of the *in vitro* release studies, four formulations (MF7–MF9 and MF2) with different drug to ES100 ratios were prepared and characterized. Formulations MF7 and MF8 did not give microspheres at all. Images of formulations MF9 and MF2 are presented in Fig. 1 and it is clear that the microspheres of formula MF9 is irregular and aggregated in shape.

As revealed from Table II, decreasing drug: ES100 ratio from 1:0.7 (MF9) to 1:1 (MF2) resulted in a significant increase in both particle size from 180.25 μm (MF9) to 264.04 μm (MF2) and encapsulation efficiency from 85.85% (MF9) to 90.55% (MF2). Comoglu *et al.* (30) reported that the encapsulation efficiency of microspheres is affected by drug/polymer ratio. When the amount of the polymer is decreased, there is no sufficient polymer in the media to produce microspheres for the entire drug. Consequently, greater amount of the drug is lost, resulting in the formation of the microspheres with lower drug content.

Microspheres with Varying Drug-to-Polymeric-Blends Ratio

In order to investigate the effect of incorporation of the pH-independent hydrophobic polymer EC and pH-dependent ES100 polymer blend on the physical characters of the microspheres, three formulations with fixed drug: EC ratio at 1:0.1 and different drug: ES100 ratios at 1:0.7, 1:0.85 and 1:1 (MES70EC10, MES85EC10, and MES100EC10, respectively) were prepared. It is clear from Fig. 1 that the three formulations exhibited spherical free-flowing shape. As depicted in Table II, similar trends as those shown and discussed in the previous formulations were observed. Decreasing drug: ES100 ratio from 1:0.7 (MES70EC10) to 1:0.85 (MES85EC10) to 1:1 (MES100EC10) resulted in significant increase in particle size from 151.29 to 347.89 to 595.27 μm, and encapsulation efficiency was significantly increased from 84.15–90.44% to 92.88%, respectively.

In Vitro Release Studies

In the *in vitro* release studies, pH condition was chosen in an attempt to approximately simulate GI conditions without enzymes. The pH condition used was pH 1.2 for a period of 2 h (stomach), pH 4.5 for 2 h (duodenum) followed by pH 7.4 (distal ileum and colon) for the remaining period of the study (13).

The successful formulation of a colon-targeted delivery system requires minimum release of the drug during its transit in the stomach and the upper small intestine to ensure maximum dose reaches the colon. Accordingly, the amount of drug released after 4 h, representing the passage of the formulation in the upper GI tract, must be reduced and considered as a parameter for the evaluation of the prepared formulation.

Eudragit S100 (ES100) is an anionic copolymer of methacrylic acid and methyl methacrylate, the ratio of the free carboxyl groups to the ester groups is approximately 1:2. It is practically insoluble in water but it is soluble in intestinal fluid from pH 7.0 upwards (31). Due to the pH-dependent solubility of this polymer, it was selected to avoid the rapid dissolution of 5-ASA during the initial transit of the

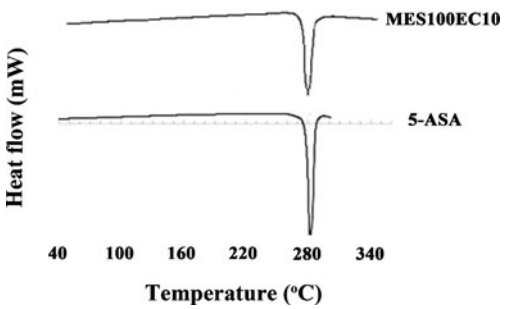

Fig. 4. DSC thermogram of 5-ASA and formula MES100EC10

Table IV. Effect of Storage at 45°C/75%RH on the Microspheres of Formulation MES100EC10

Time	Color	Drug Content (mean±SD)	f_2[a]
Zero time	Brownish yellow	99.14±0.16	100
3 Months	Brownish yellow	98.04±0.27	85.25
6 Months	Brownish yellow	98.66±0.94	78.52

[a] Similarity factors of the *in vitro* release profiles in relation to that at zero time

microspheres through the gastric cavity and the upper small intestine.

Microspheres Prepared with Varying ES100 Concentration in the Internal Phase

As shown in Fig. 2a, formula MF3 prepared with ES100 concentration of 0.667% of the internal phase released the largest amount of 5-ASA after 4 h (12.22%). As expected, increasing ES100 concentration to 1% or 2% (formulations MF2 and MF3) resulted in a decrease of the amount of 5-ASA released after 4 h (4%). This could be due to the increased amount of ES100 incorporated in the microspheres with increasing the concentration of ES100 in the internal phase. When the pH of the dissolution medium was increased to 7.4 (above the ES100 dissolving pH, pH 7), rapid 5-ASA release was observed and a complete release of 5-ASA was achieved in less than 4 h.

Microspheres Prepared with Different Internal-to-External-Phase Ratio

Figure 2b depicts that decreasing internal-to-external-phase ratio from 1:2 to 1:4 to 1:6 to 1:8 resulted in significant increase in amount of 5-ASA released after 4 h from 4% (MF2) to 14% (MF4) to 22% (MF5) to 29% (MF6), respectively. This result could be explained by taking in consideration the particles size of the microspheres previously discussed. A faster release of 5-ASA is expected from smaller microspheres due larger surface area exposed to the dissolution medium (32).

Microspheres Prepared with Varying Drug to ES100 Ratio

The *in vitro* drug release profiles showing the effect of varying 5-ASA to ES100 ratio is shown in Fig. 2c. 5-ASA release in the first 4 h from formulations MF9 and MF2 was restricted to be less than 6.5% and their release profiles showed 5-ASA pulsatile release behavior after the first 4 h of the *in vitro* release studies. It could be inferred from the previous results that the use of ES100 alone in preparation of 5-ASA microspheres is only successful in preventing the premature release of 5-ASA in the precolonic stages of the *in vitro* release studies but it is not suitable for sustained-release formulation.

Microspheres with Varying Drug-to-Polymeric-Blends Ratio

Since our target was to develop sustained-release of 5-ASA during its transit through the colon, a strategy of using mixture of time-dependent polymer (EC) combined with pH-dependent polymer (ES100) was attempted to prolong the release of the drug after reaching ES100 dissolving pH (i.e., pH 7) in the colon. EC is a water-insoluble polymer; it has been widely used in microencapsulation in order to retard the release of many drugs (33–36). Being hydrophobic in nature (37), EC is expected to prolong the release of 5-ASA from ES100-based microspheres at pHs higher than the threshold of ES100 ionization (pH 7).

As shown in Fig. 2d, formulation containing 1:0.7 5-ASA to ES100 ratio (MES70EC10) released the highest percent of the 5-ASA in the first 4 h (23.28%). Decreasing 5-ASA: ES100 ratio to 1:0.85 (MES80EC10) or 1:1 (MES100EC10) resulted in a decrease in the amount of 5-ASA released at 4 h

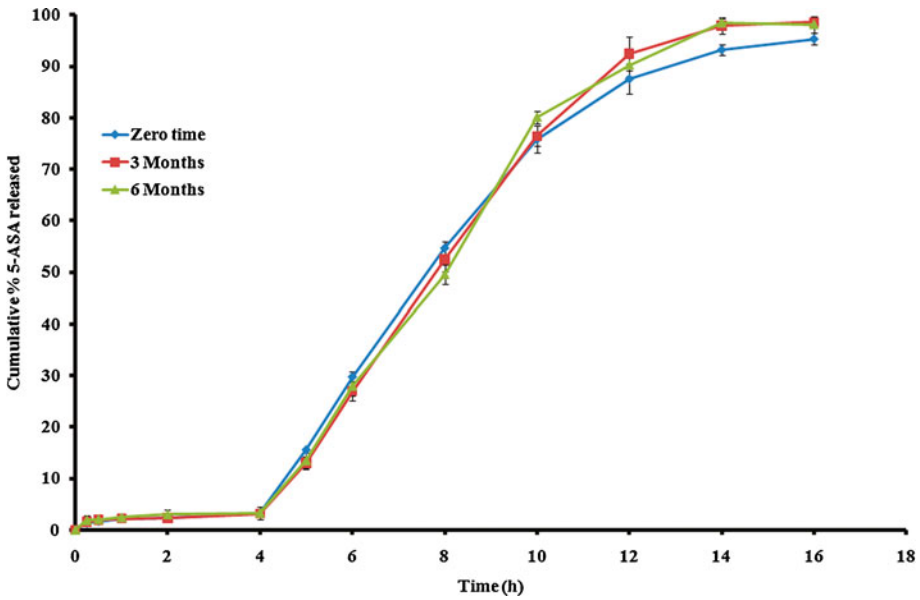

Fig. 5. Effect of storage at 40°/75%RH on the release of 5-ASA from formula MES100EC10

to be less than 3.5%. Also, it is clear that the addition of EC resulted in prolongation of 5-ASA release from ES100-based microspheres. Such effect could be attributed to the hydrophobic nature of the polymer.

It could be concluded that formula MES100EC10 showed the most suitable characters for a sustained-release colonic 5-ASA delivery system. It exhibits high production yield (98.75%) and encapsulation efficiency (92.89%). Also, it minimizes 5-ASA release in the first 4 h (2.5%) and extends drug release over the next 12 h in the *in vitro* release study. Therefore, formula MES100EC10 was selected for scanning electron microscopy analysis and stability studies.

Kinetic Analysis of Release Data

As revealed from Table III, the release of drug from formula MES70EC10 followed first-order kinetics. In first-order system, drug release is dependent on the remaining concentration of drug in the microspheres. The other two formulations followed Higuchi diffusion model. The release profiles were also fitted to Korsmeyer–Peppas equation and the n values were determined. Formulation containing 5-ASA: ES100 ratio in 1:0.7 (MES70EC10) exhibited $n<0.43$, indicating that the drug release is following Fickian diffusion mechanism. However, formulations containing higher amounts of ES100 (MES85EC10 and MES100EC10) showed n value that lie between 0.43 and 0.85, the range being indicative of anomalous behavior of drug release, where swelling, diffusion, and erosion play an important role (38).

It could be suggested that in the first 4 h of the *in vitro* release studies, 5-ASA release was minimized by the polymeric matrix composed of the two polymers, whereas on raising the pH to 7.4, ES100 is dissolved and 5-ASA release is retarded by diffusion through hydrophobic EC polymer. This suggestion will be further investigated by the results of the scanning electron microscopy study.

The release data from the three formulations were also well fitted according to Hixson–Crowell model especially with increasing amount of ES100. This indicates that there is a change in surface area and the diameter of the microspheres with progressive dissolution of the matrix as a function of time (18).

Scanning Electron Microscopy Analysis

Scanning electron microscopy was utilized to observe the surface and inner part of the microspheres from formula MES100EC10 before and after the *in vitro* release studies. It can be observed that the surface of the microspheres was uneven and there are some grooves scattered on the surface (Fig. 3a, b). Figure 3c shows the inner part of microspheres after making transverse section. Figure 3d shows the microsphere after finishing the *in vitro* release study. While Fig. 3e depicts the surface of the microspheres after the *in vitro* release study at higher magnification (2,000×). The release study showed that ES100 and 5-ASA were completely dissolved and only EC matrix was left. This changed the surface characteristics of these microspheres as shown in Fig. 3d, e.

Differential Scanning Calorimetry

Figure 4 shows the DSC thermograms of 5-ASA and 5-ASA-loaded microspheres (formula MES100EC10). The thermograms showed the characteristic melting endothermic peak of 5-ASA at 280°C (39). These results suggested that no polymorphic changes of the drug occurred during preparation of the microspheres and the dispersion of the drug in the polymeric matrices preserves its crystalline form. This could be attributed to the insolubility of 5-ASA in acetone used in the preparation of the microspheres.

Stability Study

Table IV show the effect of storage at 45°C/75%RH on the characters of the selected optimized formulation (MES100EC10). Neither physical changes nor significant changes in drug content of optimized formula had been detected on storage. The *in vitro* release profiles of the stored samples are presented in Fig. 5. The values of the similarity factor (f_2) indicated statistically insignificant difference in the *in vitro* drug release profile from stored samples compared to fresh ones (Table IV). These results could suggest the stability of the optimized formulation (MES100EC10).

CONCLUSION

The proposed oil/oil solvent evaporation method is suitable for preparation of two types of microspheres suitable for 5-ASA colon targeting. Pulsatile-type colon targeting microspheres could be successfully achieved with formulation using Eudragit S alone, but such formulation is not suitable for sustained-release purposes. On the other hand, microspheres fabricated from the pH-dependent Eudragit S and pH-independent hydrophobic ethylcellulose polymers blend presents a promising approach to optimize the microspheres that is able to minimize release under acidic condition and to extend drug release at high pH. However, the formulator must keep in mind that the O/O solvent evaporation method is sensitive to different formulation variables such as the concentration of Eudragit S in the internal phase and the ratios between internal to external phases, 5-ASA to Eudragit S, and ethylcellulose to Eudragit S to drug.

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