INDOMETHACIN-LOADED MICROSPHERES: PREPARATION, CHARACTERIZATION AND IN-VITRO EVALUATION REGARDING ETHYLCELLULOSE MATRIX MATERIAL

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Abstract

Indomethacin-loaded microspheres of ethylcellulose were prepared by the emulsion solvent evaporation technique. The aim of this work was to investigate the influence of process variation in polymer type via viscosity grades of ethylcellulose N10 and N100, drug to polymer ratio, stirring rate of the propeller and surfactant type on the micromeritic properties of microspheres such as particle size distribution, bulk and tapped density, surface topography, tangent of angle of repose, compressibility index, Hausner ratio and flow rates. All microspheres presented a narrow particle size distribution and good flow characters according to USP 28-NF 23 criteria, besides microspheres were more spherical in shape in their manufacture with ethylcellulose N100 and higher ratio of both polymers. Thus, in the case of ethylcellulose, the viscosity and ratio of the polymer in dispersion medium were found to be the controlling factors of drug release. Ethylcellulose N10 and N100 membrane materials indicated difference in release patterns of microspheres. Microspheres exhibited lower burst effect with decreased drug release rate, when the drug was incorporated with ethylcellulose N100 and higher ratio of each polymer. Therefore, indomethacin release from ethylcellulose microspheres could not be evaluated by any of the kinetic models.

Key words: *Emulsion-solvent evaporation technique, Ethylcellulose, Indomethacin, Microspheres, Modified release*

İndometazin Yüklenmiş Mikroküreler: Hazırlanmaları, Özelliklerinin Belirlenmesi ve Etilselüloz Matris Materyaline Yönelik İn-Vitro Değerlendirilmeleri

İndometazin yüklenmiş etilselüloz mikroküreleri, emülsiyon çözücü buharlaştırma yöntemi ile hazırlanmıştır. Bu çalışmada, farklı viskozitelere sahip etilselüloz N10 ve N100 polimer tipleri, etkin madde/polimer oranı, pervane karıştırma hızı ve sürfaktan tipi olan hazırlama yöntemi değişkenlerinin; mikrokürelerin partikül büyüklüğü dağılımı, küme ve sıkıştırılmış dansite, yüzey özellikleri, yığın açısının tanjantı, sıkıştırılabilirlik indeksi, Hausner oranı ve akış hızları gibi mikromeritik özelliklerine etkilerini araştırmak amaçlanmıştır. Tüm mikroküreler USP 28-NF 23 kriterine uygun, dar partikül büyüklüğü dağılımı ve iyi akış özellikleri göstermelerinin yanı sıra; etilselüloz N100 ve her iki polimerin yüksek oranları ile hazırlanan mikroküreler, daha düzgün küreler şeklinde elde edilmiştir. Böylece, etilselüloz açısından değerlendirildiğinde; dispersiyon ortamındaki polimerin oranı ve viskozitesinin, etkin madde açığa çıkışında belirleyici etkenler olduğu bulgusuna ulaşılmıştır. Mikrokürelerin yüzeyini teşkil eden etilselüloz N10 ve N100 materyalleri, çözünme hızı profillerinde farklılığa neden olmuştur. Etilselüloz N100 ve her iki polimerin yüksek oranları ile hazırlanan mikroküreler, daha düşük bir ani salım etkisi ile daha yavaş bir salım fazı göstermiştir. Bu nedenle, indometazinin etilselüloz mikrokürelerinden salımı herhangi bir kinetik model ile değerlendirilememiştir.

Anahtar Kelimeler: *Emülsiyon-çözücü buharlaştırma yöntemi, Etilselüloz, İndometazin, Mikroküreler, Modifiye salım*

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INTRODUCTION

Indomethacin is a typical nonsteroidal anti-inflammatory agent (NSAIA) that also exhibits analgesic and antipyretic activity. Indomethacin may cause serious adverse effects and should not be used as a simple analgesic or antipyretic (1). Indomethacin is a poorly soluble, highly permeable (Class II) drug, its oral absorption is often controlled by the dissolution rate in the gastrointestinal tract.

The object of the present study is, to prepare indomethacin microparticles by the encapsulation of drug particles in gastroresistant polymer film of ethylcellulose, which can act as a physical barrier and can keep the corrosive drug particles out of contact with the gastric mucosa, thus minimizing stomach irritation. Another purpose of choosing ethylcellulose is its water-insoluble character. So the drug release can be modified and the prolonged action of this poor soluble antirheumatic agent in the normal acid pH 1.2 of the stomach can be prevented (2,3). It is of interest to investigate the effect of ethylcellulose matrix on the in vitro release rate from microspheres, by comparing two different viscosity grades, ethylcellulose N10 and N100 (having a viscosity of approximately 9 cP and 100 cP, respectively). This study was also carried out also to find the differences between microspheres in regard to particle size distribution, bulk and tapped densities, surface topography, tangent of angle of repose, compressibility index, Hausner ratio and flow rates and to determine the optimal formulation for indomethacin drug delivery.

EXPERIMENTAL

Materials

Indomethacin (Eczacıbaşı-Zentiva Health Products Co., Turkey), ethylcellulose N100 (Aqualon® N100, Hercules GmbH, Germany), ethylcellulose N10 (Aqualon® N10, Hercules GmbH, Germany), solid paraffin (Sincan Plastik, Turkey), liquid paraffin, acetone, polysorbate 20 and n-hexane (Merck, Germany), polysorbate 40 and polysorbate 60 (J.T. Baker, England), polysorbate 80 (Acros Organics, England) were the reagents and chemicals used all of analytical grade.

Preparation of indomethacin microspheres

Indomethacin-loaded microspheres were prepared by the emulsion solvent evaporation technique. Acetone was used as the polymer solvent, light mineral oil as oil phase and n-hexane to wash away the paraffin oil. To prepare microspheres with various drug to polymer ratios (w/w) such as 1:1, 1:2, 1:3, accurately weighed amount of indomethacin was dissolved in acetone solution (w/v) of ethylcellulose, either N10 or N100 also depending on the desired ratio. The drug to polymer ratio was varied keeping the amount of drug and solvent constant in all cases, but changing the type and amount of polymer was performed to obtain an organic layer consisting of indomethacin, acetone and ethylcellulose respectively 2:75:2; 2:75:4; 2:75:6 (g/ml/g). The oil phase, which was prepared by respectively dispersing of previously melted solid paraffin and one of the polysorbate 20, 40, 60 or 80 as the emulsifying agent in liquid paraffin, consisted of a light mineral oil dispersion (w/v) with a constant composition in all cases, composing of solid paraffin, liquid paraffin and one of the varied types of polysorbates in 2:100:2 (g/ml/g). The oil phase was poured into the organic phase under constant stirring at 700 rpm with a propeller stirrer, and after emulsion formation, acetone was completely removed by evaporation during the approximately 3 h stirring period at 1000,1500 or 2000 rpm stirring rates at room temperature. In preformulation studies it was observed that, the effect of higher stirring rates predominated on prepared particle size of microspheres, so the effect of 1000 rpm stirring rate was not evaluated in formulation studies. The light mineral oil was decanted and the

microspheres were collected, washed three times with 100 ml of n-hexane at room temperature, afterwards the microspheres were separated by filtration and air dried for 12 h (4,5). The codes and differences between microsphere formulations with process variables are shown in Table 1.
Characterization of indomethacin microspheres

Scanning electron microscopy (SEM)

Shape and surface characteristics of the indomethacin-loaded microspheres were investigated and photographed using scanning electron microscopy (SEM, JEOL JSM-840A). The samples were mounted on an aluminum stage using adhesive carbon type and placed in a low humidity chamber for 12 h prior to analysis. Samples were coated with gold-palladium for 60 sec under an argon atmosphere using sputter coater in a high vacuum evaporator equipped with an omni-rotary stage tray. Images were taken at an acceleration voltage of 20 kV and magnifies of 33-150.

Determination of particle size distribution
Sieve analysis

Seperation of the microspheres into various size fractions was carried out using a mechanical sieve shaker. A series of five standard stainless steel sieves (Erweka, DIN 4188) of 1.000, 0.500, 0.250, 0.125 mm of meshes were arranged in order of decreasing aperture size. About 10 g of drug loaded microspheres were placed on the uppermost sieve. The sieves were shaken for a period of 10 min, then the particles on each screen were weighed (6). The procedure was carried out six times for each product.

Laser diffractometry

The particle size distribution of microspheres was determined by laser diffractometry using Sympatec Laser Diffraction Particle Sizer (Sympatec HELOS, HO728). Filtered and degassed purified water was used as a carrier fluid. About 1 mg of microspheres was dispersed in purified water in the sample unit and circulated 1000 times per min. Each determination was carried out in triplicate.

Measurement of bulk and tapped densities

The loose bulk density (ρ_b) and the tapped density (ρ_t) were measured in a 10 ml cylinder by carrying out 20 taps.The difference between these two volumes was calculated from the established Equations 1 and 2.

$$\rho_b = (M)/(V_0) \tag{1}$$
$$\rho_t = (M)/(V_f) \tag{2}$$

ρ_b and ρ_t, bulk density and tapped density respectively, in g per ml;
M, the weight of microspheres, in g;
V_0, unsettled apparent volume, in ml;
V_f, the final tapped volume, in ml.

The changes in the packing arrangement of microspheres subjected to the tapping procedure were expressed as the compressibility index (Carr) and Hausner ratio, by using Equations 3 and 4.

$$\text{Compressibility Index (Carr)\%} = 100 \times \frac{(V_0 - V_f)}{V_0} = 100 \times \frac{(\rho_t - \rho_b)}{\rho_t} \tag{3}$$

$$\text{Hausner Ratio} = \frac{V_0}{V_f} = \frac{\rho_t}{\rho_b} \tag{4}$$

Characterization of powder flow

To determine the tangent of angle of repose and flow rates, 30 ml of microspheres were poured into a conical flask having a 0.9 cm diameter and placed 10 cm above the surface. After letting the microspheres flow freely from the height of 10 cm to the surface, the tangent of angle of repose was determined by using the Equation 5. Flow rates of the microspheres were determined with the same equipment mentioned above, letting 15 g of the microspheres flow freely until the flask got emptied, at the end calculating the discharged mass per time (g/sec) (7,8). Both of the procedures were repeated at least 10 times and the results were taken as the mean.

$$\tan(\alpha) = \frac{h}{r} \tag{5}$$

Where, α is the angle of repose, determined by measuring the height (h) and radius (r) of the powder cone.

Determination of drug loading of microspheres

Assay of the microspheres was carried out spectrophotometrically (Shimadzu, 1202 UV visible) by using methanol as common solvent of indomethacin and ethylcellulose (9). Accurately weighed (20 mg) microspheres were dissolved in 50 ml of methanol, filtered by using Whatman No. 42 filter with a pore size of 2.5 μm, following suitable dilutions with methanol the content of indomethacin was assayed spectrophotometrically at 318 nm, the detected wavelength of maximum absorbance of indomethacin in methanol. Ethylcellulose did not interfere in methanol at this wavelength. The encapsulation efficiency was calculated as follows (10, 11):

$$\text{Encapsulation efficiency} = \text{Actual drug content} \times 100\% / \text{Theoretical drug content} \tag{6}$$

Yield of microspheres

The yield of the microspheres was expressed as percentage of the weight of the dried microspheres at room temperature compared to the theoretical amount (12). Percent yield is calculated by using the Equation 7.

$$\text{Percent yield} = \frac{\text{the amount of microspheres obtained (g)}}{\text{the theoretical amount (g)}} \times 100 \tag{7}$$

In vitro dissolution studies

The USP 28 – NF 23 paddle method (dissolution apparatus: Aymes, D96D) was used to determine the release of indomethacin from the microspheres. Accurately weighed (25 mg) sieved microspheres with 0.250-0.500 mm particle size were immersed in 900 ml of pH 7.2 USP 28 – NF 23 phosphate buffer dissolution medium acting as a simulated intestinal fluid in which indomethacin is slightly soluble in comparison to pH 1.2 simulated gastric fluid. The system was adjusted to ensure sink conditions and agitated at 50 rpm in a thermostated water bath at 37±0.1° C. Aliquots (5 ml) of the dissolution medium were withdrawn at predetermined time intervals, filtered by using Whatman No. 42 filter and were replenished immediately with the same volume of fresh medium. Withdrawn samples were assayed spectrophotometrically at 320 nm, the detected wavelength of maximum absorbance of indomethacin in pH 7.2 phosphate buffer (Shimadzu, 1202 UV visible). Ethylcellulose did not interfere with indomethacin absorption in pH 7.2 phosphate buffer at this wavelength. All experiments were conducted in six replicates.

All experimental data were compared statistically using the one-tailed independent sample means t-test. In all cases P< 0.05 was accepted to denote significance.

Kinetic models and the analysis of the release profiles

The best curve fit of the release data was tested with the mathematical models of zero-order kinetics (Equation 8), first-order kinetics (Equation 9), Hixon-Crowell cube-root model (Equation 10), Higuchi square-root model (Equation 11) and RRSBW kinetics (Rosin-Rammler- Sperling-Bennett- Weibull model) (Equation 12) (13-16). The statistical results were computed with a standard calibration curve of the drug.

$$w = w_0 - k_0 \times t \tag{8}$$
$$\ln w = \ln w_0 - k_1 \times t \tag{9}$$
$$w_0^{1/3} - w^{1/3} = k_4 \times t \tag{10}$$
$$Q = k \times t^{1/2} \tag{11}$$
$$M_t = M_\infty \times [1 - \exp\frac{-(t-t_0)^\beta}{\tau_D}] \tag{12}$$

The abbreviations are as follows:

w_0: Initial amount of drug
w: Undissolved amount of drug
Q: Released amount of drug
M_t: The dissolution (%) at time 't' (min)
M_∞: The dissolution (%) at infinite time
k_0: Zero order dissolution rate constant (mg/min)
k_1: First order dissolution rate constant (min^{-1})
k_4: Hixon-Crowell rate constant (min^{-1})
k: Higuchi rate constant ($min^{-1/2}$)
β: Shape parameter of the curve
t_0: The lag-time of the dissolution (min)
t: Time (min)
τ_D: Time (min) when 63.2 % of M_∞ has been dissolved.

RESULTS AND DISCUSSION

In an attempt to modify the indomethacin release from the microspheres, the formulations in Table 1 were prepared in which the increasing amounts of ethylcellulose either N10 or N100 types were added to the fixed weight of indomethacin with stirring rates and surfactants as variables. Optimization and proper control of all these variables were essential for the formation of discrete and spherical microspheres.

Table 1. Formulation parameters of prepared indomethacin-loaded ethylcellulose microspheres

Formulation	Polymer	Drug to Polymer Ratio	Stirring Rate (rpm)	Surfactant
F1	Ethylcellulose N10	1:1	1500	Polysorbate 80
F2	Ethylcellulose N100	1:1	1500	Polysorbate 80
F3	Ethylcellulose N10	1:2	1500	Polysorbate 80
F4	Ethylcellulose N100	1:2	1500	Polysorbate 80
F5	Ethylcellulose N10	1:3	1500	Polysorbate 60
F6	Ethylcellulose N100	1:3	2000	Polysorbate 40

The shape of indomethacin crystals is irregular (Figure 1.A). SEM images of the microspheres showed that they possess a grossly spheroidal form, when compared to the active drug particles. The higher the viscosity and concentration of the selected polymer, the more spherical in shape microspheres can be obtained with less micropores on the surface. Comparing the surfaces of microspheres (Figures 1.B- 1.E), it could be observed that shape and porosity of microspheres were influenced by ethylcellulose viscosity. For this reason, F6 microspheres prepared with the highest amount of ethylcellulose N100, have more regular particles.

(A)

(B)

(C)

(D)

(E)
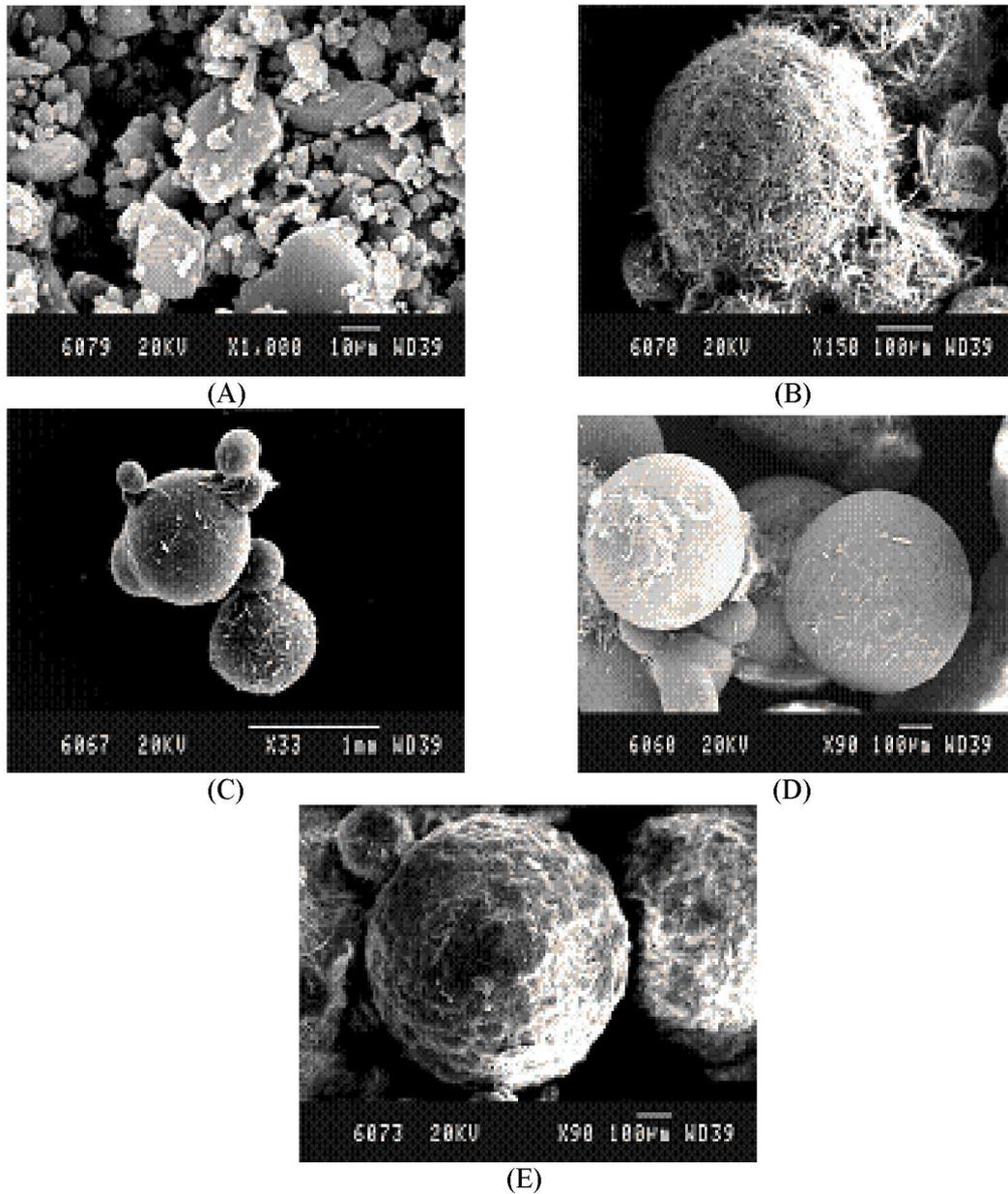
Figure 1. Scanning electron micrographs of indomethacin and indomethacin loaded ethylcellulose microspheres. Key, (A) Indomethacin drug particles, (B) F3, (C) F4, (D) F5, (E) F6.

Microspheres presented a narrow distribution of particle size and generally fallen into the 0.250-0.500 mm range.

Ethylcellulose type and its added amount in formulations had a major effect on the organic phase viscosity, influencing the particle size distributions of gathered up microspheres shown by sieve analysis results in Table 2. The viscosity of the organic phase due to the concentration of polymer inside was effective on solvent diffusion and emulsification, while the shearing rate during stirring was kept constant. The geometric mean diameters of microspheres were found to be dependent on polymer concentration dispersed in the organic phase. But a variation in the initial drug loading up to 33.33 % (1:1 drug to polymer ratio) did not produce any significant change in mean particle size as can be seen from the laser diffraction patterns of formulations prepared with 1:2 and 1:3 drug to polymer ratios corresponding to 25.0 % and 20.0 % drug loadings (Figures 2.A- 2.D). The data obtained for particle size distribution by laser diffractometry were analyzed statistically with t-test; the difference was not significant (P> 0.05). Particle size distributions of F1 and F2 coded microspheres were not determined additionally by laser diffractometry, because of their particle size range has been already found by sieve analysis.

The stirring rate became an important factor to disperse the inner phase into the outer phase, since the viscosity of the outer phase was built up by the polymer amount, while the drug, surfactant and solvent amounts were kept constant. The speed of stirring affected mostly low viscosity ethylcellulose N10 emulsion phases, causing fractions with decreased particle size as could be seen from the sieve anaysis results of microspheres coded as F1, F3 and F5. The difference by t-test, was found to be significant (P< 0.05); but the change in particle size distribution was not proportional to polymer content.

The surfactant provides an interaction between the drug substance and solvent. This ensures the superiority of dispersion medium conditions, influencing the size of emulsion droplets. Although the type of surfactant was not dominant on particle size, polysorbate 80 tended towards microsphere formations with smaller average diameters as compared with the presence of other types, observed in preformulation studies performed with constant drug to polymer ratio and stirring rate. So in our formulations we included mostly polysorbate 80. Besides the other variables, polysorbate 40 and 60 have influence on the formations of microspheres F5 and F6.

Table 2. Sieve analysis results of microspheres

Formulation		Sieve Size (mm)				
		>1.00	1.00-0.500	0.500-0.250	0.250-0.125	0.125>
F1	(a)	0	1.16	87.39	9.66	0.62
	(b)	±0	±0.02	±0.03	±0.03	±0.01
F2	(a)	0	1.21	73.44	22.07	0.73
	(b)	±0	±0.02	±0.10	±0.10	±0.01
F3	(a)	0	0.26	26.97	61.63	9.62
	(b)	±0	±0.01	±0.05	±0.32	±0.36
F4	(a)	1.07	15.60	69.27	13.19	0.25
	(b)	±0.01	±0.29	±0.15	±0.19	±0.01
F5	(a)	1.88	16.98	57.72	21.41	1.00
	(b)	±0.02	±0.25	±0.22	±0.06	±0.02
F6	(a)	0.12	4.08	83.46	7.92	0.07
	(b)	±0	±0.24	±0.31	±0.31	±0.01

[a] % of the fraction of microspheres; [b] Standard deviation (S.D.); n=6
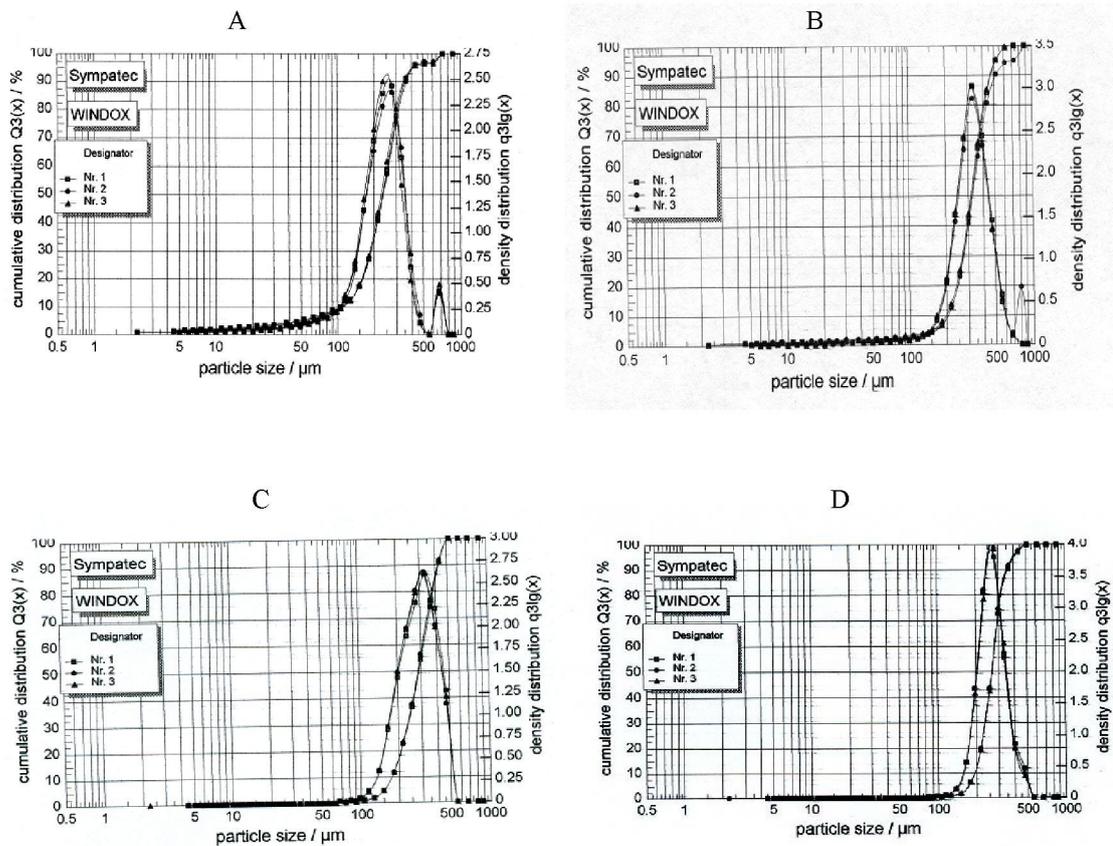
Figure 2. Laser diffraction patterns of particle size distribution of the microspheres with the average diameter ± S.D. (μm) (n=3). Key; (A) F3, 234.27 ± 4.38 μm; (B) F4, 325.70 ± 4.92 μm; (C) F5, 290.78 ± 3.23 μm; (D) F6, 266.74 ± 1.27 μm

Table 3 represents micromeritic properties regarding bulk and tapped densities, percentages of compressibility and Hausner ratios, angles of repose and flow rates of the microspheres. The tapping procedure was carried out until difference between bulk and tapped volumes was found to be less than 2 %. No significant difference between the bulk and tapped densities was found (P> 0.05), concluding that the microspheres have similar shapes. Due to the greatest tapped and bulk density, F6 microspheres have better uniformity of particle size with more spherical shapes. Compressibility index and Hausner ratio are indirect measures of bulk density, size and shape, surface area, moisture content and cohesiveness of microspheres. All microspheres showed good flow characteristics according to USP 28-NF 23 criteria, with Hausner ratio less than 1.18 and percentage of compressibility less than 15. In consideration of compressibility index and Hausner ratio, F6 microspheres were appeared predominant in flowability. Characterizing the flow property, angle of repose values of all microspheres did not exceed 30-35° and the microspheres were accepted as free-flowing. Regarding the flow rates, F6 microspheres flowed faster, confirming its powder properties.

Table 3. Micromeritic properties of indomethacin-loaded microspheres

Formulation	Bulk density (ρ_b) (g/cm^3) ±S.D.[a]	Tapped density (ρ_t) (g/cm^3) ±S.D.[a]	Compressibility index (Carr) (%) ±S.D.[a]	Hausner Ratio ±S.D.[a]	Angle of repose (°) ±S.D.[b]	Flow rate (g/sec) ±S.D.[b]
F1	0.35 ±0.01	0.39 ±0.01	10.35±1.21	1.12 ±0.02	30.23 ±2.17	4.12 ±0.17
F2	0.27 ±0.01	0.31 ±0.01	12.35±0.76	1.14 ±0.01	27.95 ±1.76	3.91 ±0.12
F3	0.26 ±0.01	0.29 ±0.01	11.70±0.86	1.13 ±0.01	30.08 ±0.89	3.29 ±0.09
F4	0.34 ±0.01	0.38 ±0.01	9.92±0.50	1.11 ±0.01	25.31 ±1.47	4.02 ±0.13
F5	0.30 ±0.01	0.33 ±0.01	8.07±2.18	1.09 ±0.03	33.15 ±0.91	4.05 ±0.14
F6	0.56 ±0	0.60 ±0.01	6.44±0.81	1.07 ±0.01	20.34 ±1.51	6.68 ±0.23

S.D.: Standard deviation; [a] n=6; [b] n=10

No interaction or rejection was observed between indomethacin and ethylcellulose during the evaporation process and microsphere formation as indicated in Table 4. The drug loading was affected by neither polymer content nor stirring rate or surfactant variables, but was consistently and slightly lower than the theoretical loading, with high encapsulation efficiencies close to 100 % in all cases. Before the assay of indomethacin incorporated inside microspheres, any of indomethacin drug particle remained free on the surface of microspheres has been already washed away with n-hexane during removal of parafin. Absence of free drug particles was confirmed by SEM pictures. It was reported that ethylcellulose led to high encapsulation efficiencies, which might be related to a fast precipitation of ethylcellulose, resulting in reduced drug diffusion into the aqueous phase (10). Also the recovered amount of total microspheres demonstrated the adequacy of process variables during solvent evaporation.

Table 4. Drug loading capacity (indomethacin content), encapsulation efficiency and percent yield of prepared indomethacin-loaded microspheres

Formulation	Theoretical indomethacin content[a](%)	Measured indomethacin content[b](%)±S.D.[1]	Encapsulation efficiency (b/a x100)	Yield (%) ±S.D.[1]
F1	33.33	32.03±1.18	96.10	79.10±0.63
F2	33.33	33.05±0.51	99.16	87.25±0.59
F3	25.00	24.49±0.78	97.96	85.97±0.89
F4	25.00	23.86±0.82	95.44	87.32±0.94
F5	20.00	19.30±0.88	96.51	91.65±0.75
F6	20.00	19.58±0.79	97.90	79.59±0.53

[1]n=6

Various drug release patterns were obtained, which are biphasic i.e. an initial rapid drug release phase ('burst effect') was followed by a second, slower drug release phase. It could be seen from SEM pictures, that at thicker consistency of microspheres, less porous surface is obtained. These micropores may facilitate drug diffusion from microspheres during dissolution. Thus, the drug located closer to the surface, is accesible by the release medium, regarding to the coat thickness surrounding the drug particles (10). With increasing polymer amount and viscosity, the initial burst effect was significantly decreased in addition to the decrease in second drug release phase. The subsequent decreasing relatated to drug release rates can be attributed to the resulting decreased drug concentration gradients. In vitro drug release strongly depended on the type of polymer. This might be explained by the higher viscosity of the organic phase in the case of ethylcellulose. Different permeabilities of the drug within the polymers and/or drug distribution within the microspheres could be the reason of this fact. It has been reported that the release of the drug depends on viscosity grade of the ethylcellulose. With an increasing viscosity grade, the rate of release decreases. It has also been described that, in addition, the release rate depends on the overall viscosity of the system (17). The present study was able to confirm these findings; for F6 microsphere formations (1:3 drug to polymer ethylcellulose N100), which had the highest overall viscosity, the slowest release was observed.

By examining the release profiles in detail, it can be seen that the rates were relatively fast and that more than 70 % of indomethacin was released in first minutes from microspheres prepared by lower amount and viscosity grade polymer (Figure 3. F1 – F3). Increasing the polymer ratio and its viscosity grade, drug release rates were modified and incomplete; especially for F6 microspheres, and the cumulative amount of drug released within 9 h was approximately 35 % (Figure 3. F4 – F6). It should be emphasized that the remaining indomethacin was actually located in the microspheres and did not undergo any degradation process during the kinetic experiment, which was reported to last almost 4 weeks and to depend on the retention capacity of the coating polymer (18). Incomplete dissolution rates of salbutamol sulphate from microcapsules have been also reported, because of impeding effect of ethylcellulose thickness surrounding the drug particles (5).
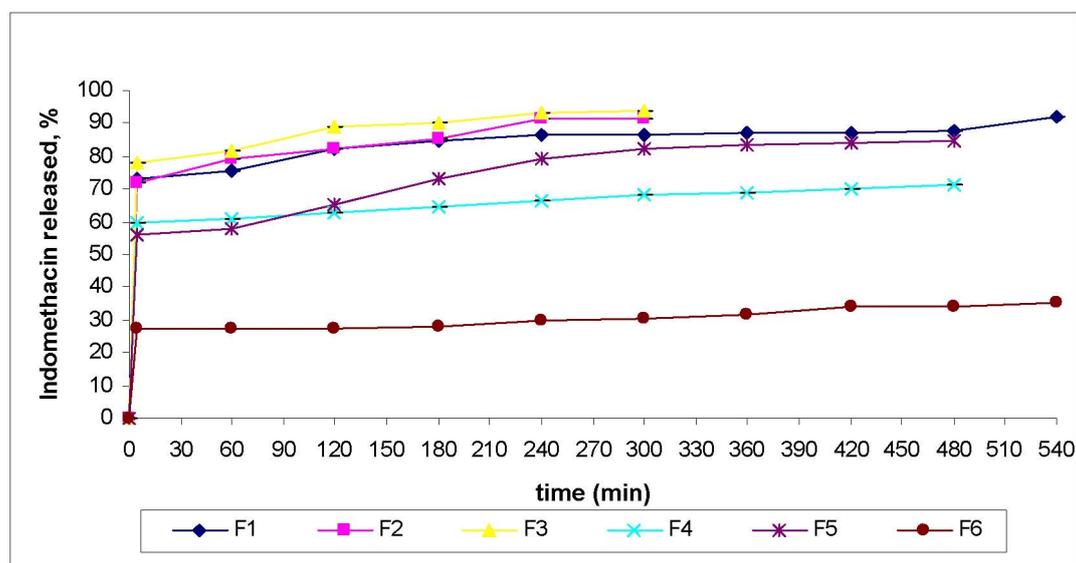
Figure 3. In vitro indomethacin release profiles in pH 7.2 phosphate buffer from different types of indomethacin-loaded microspheres.

In order to obtain meaningful information, the drug release models were fitted to five different kinetic models and the fit of the release data was assessed, as it is summarized in Table 5. Cumulative release profiles of indomethacin-loaded ethylcellulose microspheres can not fit closely to any of the kinetic model, so that no clear identification for the true individual release mechanism could be made in this specific case. Models with higher correlation coefficients were judged to be more appropriate, since there was no significant difference between either first-order and the square-root of time (Higuchi equation) release models or first-order and Hixson-Crowell cube-root release models, suggested to be the best kinetic models for correlation of in vitro activity.

Table 5. Release constants and correlation coefficients for linear relationship of the proposed combined kinetics

Formulation		F1	F2	F3	F4	F5	F6
Kinetics							
Zero Order	k_0	0.094	0.075	0.068	0.023	0.144	0.016
	r^2	0.762	0.635	0.679	0.983	0.849	0.926
First Order	k_1	6.2×10^{-3}	6.5×10^{-3}	4.7×10^{-3}	6.7×10^{-4}	5.6×10^{-3}	2.3×10^{-4}
	r^2	0.878	0.762	0.776	0.985	0.912	0.923
Hixson-Crowell	k_4	5.1×10^{-3}	4.8×10^{-3}	3.8×10^{-3}	7.3×10^{-4}	5.4×10^{-3}	3.2×10^{-4}
	r^2	0.843	0.720	0.749	0.985	0.892	0.925
$Q \rightarrow t^{1/2}$	k	1.608	1.351	1.391	0.618	2.617	0.370
	r^2	0.899	0.793	0.852	0.927	0.950	0.764
RRSBW	β	-0.111	0.183	-0.652	0.076	0.258	0.051
	τ_D	1833.38	0.545	23.67	75.22	17.39	4.8×10^{10}
	r^2	0.034	0.952	0.528	0.719	0.954	0.533

CONCLUSION

This study showed that during manufacture of the microspheres by solvent evaporation method, the viscosity and ratio of polymer in dispersion medium were the controlling factors of microsphere micromeritic parameters (19) and drug release. Surfactant type might play a role, but was not dominating. Microspheres prepared using ethylcellulose were found to be spherical, discrete and free-flowing. Microspheres had a Hausner ratio and a percentage compressibility sufficient for a direct tableting process (20). High encapsulation efficiency can be attributed to the probability for the drug to be entrapped within the microparticles based on the high solubility of indomethacin in organic phase when increasing the amount of ethylcellulose stepwise. The pores formed during preparation could be responsible for the initial fast release of drug. During this initial phase, the simultaneous swelling of polymer and the increase in distance that the drug must travel from has been completed and the drug release is thought to be dependent on the permeability of the swollen polymer.

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