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Influence of the casting solvent on the physico-chemical properties of 5-fluorouracil loaded microspheres

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Abstract

Ethylcellulose microspheres containing 5-fluorouracil (5-FU) were prepared by an oil-in-oil evaporation/extraction method. Three drug/polymer ratios (1:1, 1:2 and 1:3) were utilized. The solvent of the dispersed phase was either acetone or ethanol. In vitro dissolution studies showed that the 5-FU release was dependent both on the drug/polymer ratio and on the nature of the casting solvent. The drug release rate was fast when ethanol was used; it was slower with acetone and could last 7 days when the drug/polymer ratio was 1:3. The influence of the solvent was also evident on the characterization of the microspheres. Indeed, ethanol had a plasticizer role and consequently the matrix structure of the microspheres was more porous than with acetone.

Keywords: 5-Fluorouracil; Ethylcellulose microspheres; Casting solvent

1. Introduction

5-Fluorouracil (5-FU) is an antimetabolite of the pyrimidine analog type that is widely used alone or in combination chemotherapy regimens for the treatment of advanced gastrointestinal tract cancer, breast cancer and several other types of cancer (Pinedo and Peters, 1988). Because of its short biological half-life and its poor oral absorption, it is an appropriate candidate for microencapsulation.

Many biodegradable or non-biodegradable polymers have been already used to encapsulate 5-FU (Hazrati and DeLuca, 1989; Ghorab et al., 1990). In the pharmaceutical field, ethylcellulose is a widely used polymer to formulate oral controlled release delivery systems by coating either small particles or tablets and in the preparation of microparticles (Deasy, 1984). In addition, ethylcellulose microcapsules have been administered intravascularly for chemoembolization in humans (Kato et al., 1981). The non-biodegradability of this particular polymer prevents a rapid revascularization of the embolized area. On the other hand, 5-FU is generally administered by infusion

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and today there is no available oral dosage form. The interest of choosing ethylcellulose to coat 5-FU particles is that ethylcellulose microspheres could be used either as a modified drug release dosage form for an oral administration or as a chemoembolization agent after sterilization.

The preparation and the characterization of ethylcellulose microspheres containing 5-FU were previously described (Zinutti et al., 1994). These microspheres were prepared by a solvent evaporation/extraction method using light mineral oil as the continuous phase and acetone as the organic solvent. Since ethanol is a more acceptable solvent for pharmaceutical purposes, we have studied the influence of the organic solvent, i.e. ethanol versus acetone, on the physico-chemical properties of the microspheres.

2. Materials and methods

2.1. Materials

Ethylcellulose 20 mPa·s (Aldrich, Saint-Quentin Fallavier, France) and light mineral oil (Cooper, Melun, France) were used as provided. 5-FU (batches BA 909022 and BA 331920), kindly supplied by Roche Laboratories (Neuilly-sur-Seine, France), was passed through a 125-μm sieve before use. Acetone was normapur grade (Prolabo, Paris, France) and 95% ethanol was purchased from Carlo Erba (Rueil Malmaison, France). Sorbitan trioleate and polyoxyethylene monolaurate sorbitan were obtained from Seppic (Paris, France). The other organic solvents and chemicals used for microsphere preparation and analytical procedures were purchased from commercial suppliers and used without further purification.

2.2. Microsphere preparation

5-FU ethylcellulose microspheres were prepared according to a solvent evaporation/extraction method. Light mineral oil (80 g) containing 2.5% (w/w) sorbitan trioleate was used throughout all the study as the continuous phase. Three drug/polymer ratios 1:1, 1:2 and 1:3 corresponding to theoretical drug contents of 50, 33 and 25% (w/w), were studied. Ethylcellulose was dissolved either in acetone or 95% ethanol. For the drug/polymer ratios 1:2 and 1:3, ethylcellulose (2 and 3 g) was dissolved in 27 and 40 ml of the organic solvent, respectively. For the drug/polymer ratio 1:1, 20 ml of acetone or ethanol were used to dissolve 1 g of ethylcellulose. 5-FU was suspended in the dispersed organic phase. The resulting oil-in-oil emulsion was agitated continuously at 500 rev./min under atmospheric pressure for 16 h. The stirrer (Heidolph, Bioblock Scientific, Strasbourg, France) was a variable speed stirring motor fitted with a three-blade stirring shaft. For the microspheres prepared with acetone, the temperature was maintained at 25°C to allow the evaporation of the solvent, while for microspheres casted from ethanol the temperature was fixed at 30°C since its boiling point is higher than that of acetone. The microspheres were collected by filtration, washed three times with petroleum ether and dried in an oven at 50°C for at least 24 h. The microspheres were passed through a series of sieves to obtain a 200- to 400-μm fraction.

2.3. Viscosity determination

The viscosities of light mineral oil containing 2.5% (w/w) sorbitan trioleate and polymer solution were determined using a coaxial cylinder viscometer (Brookfield digital viscometer DV-II +, Stoughton, USA).

2.4. Physico-chemical characterization of the microspheres

2.4.1. Determination of 5-FU content

Duplicate samples of 5-FU microspheres (50 mg) were dissolved in 20 ml of chloroform. Then, 20 ml of distilled water were added and the mixture was shaken for 30 min followed by centrifugation to recover the aqueous phase. Under these conditions, 5-FU was totally recovered in the aqueous phase, which was further assayed by spectrophotometry at 266 nm according to the USP XXII method (US Pharmacopeia XXII, 1990).

2.4.2. Scanning electron microscopy (SEM)

The microspheres were coated with a thin film of gold palladium and then examined by scanning electron microscopy (JEOL T. 330A).

2.4.3. Differential scanning calorimetry and X-ray diffraction studies

Differential scanning calorimetry (DSC) and X-ray diffraction analysis were performed in order to characterize the physical state of both the drug and the polymer in the microspheres.

DSC was carried out on a Mettler 30 calorimeter (Mettler Toledo, Viroflay, France). The samples (5–10 mg) were heated from 20 to 300°C at a rate of 10°C/min under nitrogen. X-Ray diffraction studies were carried out on a Diffractinel using Co-Kα radiation (Diffractinel CP 120, Inel).

2.5. In vitro drug release

A weighed quantity of 200- to 400-μm microspheres (200 mg) was suspended in 100 ml phosphate buffer (pH 7) containing 0.75% (w/v) polyoxyethylene monolaurate sorbitan. The dissolution medium was stirred at 200 rev./min. All the experiments were carried out at 37°C. Aliquots of the dissolution medium (2 ml) were periodically removed and assayed by spectrophotometry at 266 nm after adequate dilution. The dissolution medium was maintained at a constant volume by adding to the flask the volume of dissolution medium which had been removed.

3. Results and discussion

The solvent evaporation/extraction techniques are commonly used in the field of microencapsulation to extend the release of drugs. The classical solvent evaporation/extraction method in which a water-immiscible organic polymer solution is emulsified into a continuous aqueous phase, is limited to the encapsulation of water-insoluble drugs. Recently, modified methods were developed for the encapsulation of water-soluble drugs. For example, an aqueous continuous phase saturated with drug (Bodmeier and Mc Ginity, 1987) or a water-in-oil-in-water emulsion (Ogawa et al., 1988) can be used. Likewise, an oil-in-oil solvent evaporation/extraction process whereby the continuous aqueous phase is replaced by an oil phase has also been used to encapsulate water-soluble drugs (Jalil and Nixon, 1989; Bodmeier et al., 1994). Since 5-FU is a water-soluble drug, the latter technique was retained to prepare microspheres.

In addition, ethylcellulose microspheres prepared from two casting solvents acetone and 95% ethanol were compared in terms of release profiles and morphology.

3.1. Microsphere preparation

The continuous phase was light mineral oil, containing 2.5% (w/w) sorbitan trioleate, with a viscosity of 34 mPa·s. In order to prepare microspheres, we used a constant quantity of 5-FU (1 g). For the drug/polymer ratios 1:2 and 1:3, the volumes of the 7.5% (w/v) ethylcellulose acetone solution were 27 and 40 ml, respectively. This polymer solution had a viscosity of 134 mPa·s. Under these conditions, it was possible to obtain a dispersion of the ethylcellulose solution in spherical droplets in the external phase. In order to keep the same proportions and to adjust the drug/polymer ratio to 1:1, 13.5 ml of a 7.5% ethylcellulose solution had to be used. However, when this solution was added to the continuous phase, it was not possible to get spherical particles. Indeed due to a lower acetone volume, the casting solvent partitioned more rapidly from the polymer phase into the light mineral oil phase and consequently the polymer precipitated prior to the fine dispersion of the organic polymer solution. To obtain spherical particles it was necessary to decrease the viscosity of the polymer solution in order to facilitate the emulsion process and consequently decrease the concentration of ethylcellulose to 5% (w/v). In these conditions, the viscosity of the polymer solution was 34 mPa·s and the volume 20 ml.

In order to establish the influence of the organic casting solvent on the dosage form characteristics, the microspheres obtained from ethanol were prepared according to the same conditions as these involving acetone. It should be noted that 5-FU

was slightly soluble in ethanol (5 mg/ml): as a consequence, 5-FU was partly dissolved and partly suspended in the ethanolic polymer solution. Nevertheless, it was possible to obtain microspheres in ethanol: however the microspheres aggregated and adhered to the filter during filtration which indicated the presence of residual solvent. To obviate this phenomenon, since acetone had a lower boiling point (56.2°C) than ethanol (78.5°C), the temperature during the evaporation step was increased from 25 to 30°C. Therefore, it was possible to ensure complete solidification of the microspheres.

Sanghvi and Nairn (1992) observed that the viscosity ratio of the dispersed phase to the continuous phase was an indicator for assessing the influence of viscosity on the particle size. These authors observed that the larger the viscosity difference between the two phases, the larger the microspheres size difference. In our study, for the microspheres with drug/polymer ratios 1:2 and 1:3 and prepared with acetone, due to an increased polymer concentration, the viscosity of the dispersed phase prior to mixing was larger than the continuous phase. As emphasized by Sanghvi and Nairn (1992), the granulometric distribution of the 1:2 and 1:3 drug/polymer ratio microspheres showed an increase in the particle size with regards to the 1:1 drug/polymer ratio (Fig. 1). This phenomenon was also observed when ethanol was employed as solvent. In the case of ethanol, the viscosities of the polymer solutions were larger than with acetone: 188 mPa·s for the 7.5% polymer solution and 50 mPa·s for the 5% polymer solution. However, the mean size of the microspheres prepared with ethanol was lower than with acetone (Fig. 1). It is therefore obvious that the viscosity ratio of the dispersed phase to the continuous phase is not the only critical parameter governing the microsphere mean diameter. The diffusion of the casting solvent to the oily phase is a more dramatic parameter than the viscosity difference between the two phases. In order to display the more rapid diffusion of acetone than ethanol towards the light mineral oil, we have carried out the following experiment: when an acetonic solution containing ethylcellulose was added, without agitation, over an oily phase formed by light mineral oil, the interface between the two phases was convex. After 24 h the organic solvent was found under the oily phase. If we replaced acetone by ethanol the interface was still convex but the curvature was less marked. After 24 h the ethanolic phase was always over the oil phase. It is therefore confirmed that acetone has a more rapid diffusion into the light mineral oil than ethanol.

The overall yields of the microencapsulation process were well above 80%. The drug incorporation efficiency which was defined as the ratio of the actual to the theoretical 5-FU content was greater than 92% for the acetone casted microspheres. The encapsulation efficiency was slightly less important in the case of ethanol as the dispersed phase (Table 1).

As 5-FU was insoluble in the continuous phase the different encapsulation efficiencies can be ex-

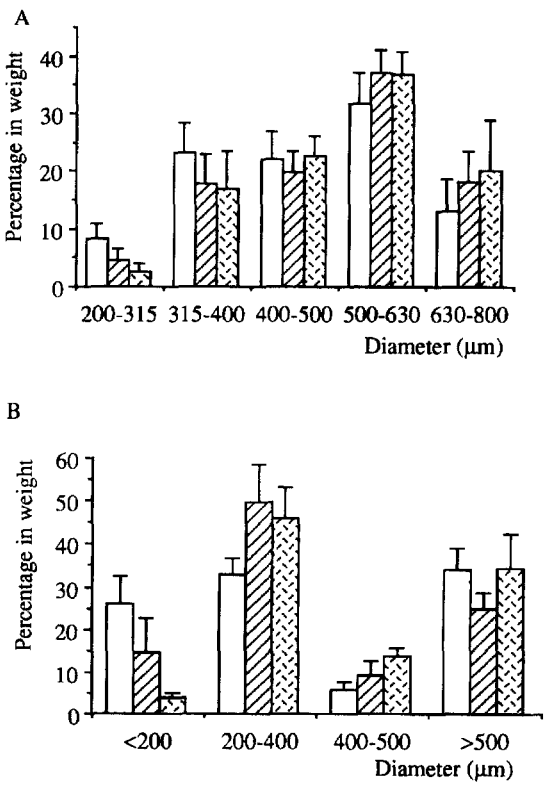

Fig. 1. Size distribution of 5-FU microspheres prepared from acetone (A) and ethanol (B) with drug/polymer ratio of 1:1 (□), 1:2 (▨) and 1:3 (■).

Table 1
Effect of the casting solvent type on the 5-FU encapsulation efficiency (n = 3)

Drug/polymer ratio	Encapsulation efficiency (%) ($\pm$ SD)	
	Acetone	Ethanol
1:1	98.0 $\pm$ 1.5	77.0 $\pm$ 4.6
1:2	93.3 $\pm$ 0.9	90.0 $\pm$ 0.5
1:3	92.4 $\pm$ 1.3	86.0 $\pm$ 0.4

plained by the rate of precipitation of ethylcellulose. This agrees well with Bodmeier and Mc Ginity (1988) proposal since the only difference between the acetone and ethanol procedures is the rate of precipitation of the polymer. These authors observed that the successful entrapment of a drug within the microspheres is associated to three main parameters: first a fast rate of precipitation of the polymer from the organic phase, second a low solubility of the drug in the continuous phase and finally a high concentration of the polymer in the organic phase. In our case, these three mentioned conditions are met for the two solvents. However, as mentioned above, the polymer precipitates faster in acetone which explains the higher encapsulation efficiency with this solvent.

3.2. Drug release studies

We have previously reported that 5-FU was slowly released from the 400- to 500-μm granulometric fraction of ethylcellulose microspheres prepared with acetone and containing a theoretical drug content of 25% (drug/polymer ratio 1:3) (Zinutti et al., 1994). Indeed, the time for the release of 90% of the drug (T_{90}) was around 7 days. For the microspheres with a drug/polymer ratio of 1:1 and 1:2 the T_{90} were 1 and 3 days respectively. On the other hand, the microsphere size had only a small effect on the 5-FU release, since the drug release profiles were similar for the 400–500 μm and 200–400 μm fractions for the drug/polymer ratios of 1:1, 1:2 and 1:3.

When ethanol replaced acetone the drug release profiles were dramatically different: the release rates of 5-FU were much faster than the rates observed with acetone (Fig. 2). The drug release profiles of microspheres with drug/polymer ratio 1:1 and 1:2 are very close and do not display any statistical difference ($P < 0.05$, ANOVA). For the drug/polymer ratio of 1:3, 90% of 5-FU were released within 24 h. Ghorab et al. (1990) also observed that 5-FU was rapidly released from ethylcellulose microspheres prepared with ethanol as the casting solvent. This difference in the release profiles of 5-FU might be explained by the structure of the polymer matrix, thus reflecting the importance of the dispersed casting solvent.

3.3. Microsphere structure

The observation by SEM showed that the drug-loaded microspheres prepared with acetone were spherical in shape with particle sizes ranging from 200 to 800 μm in diameter (Fig. 3). When they were obtained from ethanol, the microspheres were smaller with a trend to agglomerate (Fig. 3). When compared to microspheres prepared from acetone, the microspheres prepared using ethanol are somewhat less spherical and more irregular.

The microsphere cross-sections examined by SEM revealed that the ethylcellulose microspheres obtained from acetone had a relatively dense internal structure with only a few channels (Fig. 4). In contrast, for microspheres prepared from ethanol the internal structure appeared more

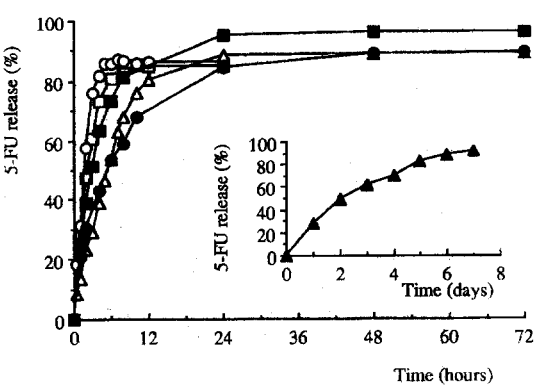

Fig. 2. Release profiles of 5-FU from ethylcellulose microspheres (200-400 μm) prepared from ethanol with drug/polymer ratio 1:1 (□), 1:2 (○), 1:3 (△) and from acetone with drug/polymer ratio 1:1 (■), 1:2 (●), 1:3 (▲).

Fig. 3. Scanning electron micrograph of microspheres (drug/polymer ratio 1:3) prepared from acetone (A) and ethanol (B).

porous (Fig. 4). This could be attributed to the slow diffusion rate of ethanol towards the oily phase and to a potential plasticizer role of ethanol.

Fig. 4. Scanning electron micrograph of a cross-section of microsphere prepared from acetone (A) and ethanol (B).

X-Ray diffraction studies, as well as DSC, give reliable information on the physico-chemical state of the ingredients composing the microspheres. The X-ray pattern obtained with microspheres prepared from an ethanolic solution of 5-FU and ethylcellulose presented the characteristic bands of a crystalline form of 5-FU. The 5-FU precipitated during the formation of the microspheres. In the case of microspheres obtained from acetone, the X-ray patterns suggest also that 5-FU forms crystalline domains dispersed in the amorphous polymer. The nature of the casting solvent does not affect the final physical state of 5-FU.

The thermal events observed with microspheres prepared from acetone or ethanol are presented in Table 2. For the microspheres obtained from acetone, the thermogramm shows an endothermic peak at 129°C corresponding to the glass transition temperature (T_g) of ethylcellulose (Dubernet et al., 1991). In addition, there is a large exothermic peak observed around 190°C which is due to the degradation of the polymer (Dubernet et al., 1991). The melting point of encapsulated 5-FU was 278°C, value close to the melting point of the pure drug (284.6°C).

In contrast, concerning the microspheres prepared from ethanol DSC charts show dramatic differences. The T_g of ethylcellulose was shifted to about 50°C. This low T_g-value indicates that ethylcellulose is plastified by the casting solvent.

Table 2
Thermal events observed in the microspheres thermograms prepared with acetone or alcohol

Preparation	T_g (°C)	Melting point (°C)
5-FU		284.6
Ethylcellulose	138	
Non-loaded microspheres (acetone)	130	
Microspheres 1:1 (acetone)	123	278.3
Microspheres 1:2 (acetone)	122	278.3
Microspheres 1:3 (acetone)	132	277.5
Non loaded microspheres (ethanol)	56	
Microspheres 1:1 (ethanol)	56	279.7
Microspheres 1:2 (ethanol)	59	273.1
Microspheres 1:3 (ethanol)	44	274.4

No matter what the drug concentration was, the T_g- of the polymer was strongly reduced. Therefore, the physico-chemical properties of ethylcellulose may be affected through an increase in chain mobility and consequently a less tortuosity of the matrix (Jenquin et al., 1990). This modification in the ethylcellulose properties is reflected by the major differences observed in the drug release kinetics.

4. Conclusion

The choice of solvent has a considerable influence upon the release rate of 5-FU from the ethylcellulose microspheres. Indeed, when ethanol was used for the preparation of the microspheres the solvent acted as a plasticizer for the polymer. Then the matrix structure was porous and the release rate of 5-FU was fast. In contrast, with acetone the microspheres had a denser and homogeneous structure and the 5-FU release can last 7 days when the drug/polymer ratio 1:3 was used. The ethylcellulose microspheres loaded with 5-FU will be evaluated in vivo. Keeping in mind that a too slow release profile, such as the one observed with the drug/polymer ratio 1:3 with acetone, is rather an inconvenient, the in vivo work will focus on the 5-FU microspheres displaying a rapid and intermediate release profile. This drug delivery system can be used as a new form of oral administration for 5-FU or in chemoembolization. However, for chemoembolization applications, we still have to control whether the sterilization of the microspheres by gamma irradiation alters the characteristics of the 5-FU microspheres.

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