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PREPARATION AND CHARACTERISTICS OF GELATIN MICROSPHERES

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ABSTRACT

Gelatin microspheres are prepared by emulsification of a aqueous gelatin solution in a oily phase containing a surfactant, gelation by cooling, dehydration by isopropanol and cross-linking by formaldehyde. The pH, the gelatin concentration in the aqueous solution and the surfactant concentration in the oily phase have some influence on the size distribution of unloaded and loaded microspheres and on the drug contents of microspheres.

INTRODUCTION

Gelatin, a natural macromolecule is widely used to prepare microcapsules by coacervation. However, some works report processes based on emulsification, gelation and dehydration which can be used to prepare micropar-

ticules or nanoparticules with gelation. Tanaka and al.[9] have dispersed and gelated gelatin in an oily phase and dehydrated it by isopropanol before cross linking by formaldehyde. They obtained micropellets which are subtained release dosage forms. Goto and al.[3] prepared microcapsules of sulfonamides with this process. Haschida and al.[4,5,6], Sezaki and al.[8] used the same processes to prepare microspheres of about 1,6 µm which can deliver antineoplasic agent to lymphatics. By controlling the extent of emulsification, Yoshioka and al.[10] prepared two kinds of spheres : nanospheres (280 nm) and microspheres (15 µm).

In this work, we report a detailed method to prepare gelatin microspheres and we attempt to examine the influence of some parameters such as : pH and concentration of gelatin solution, stirring rate during gelation, surfactant concentration in the oily phase, on the size distribution of microspheres or on their drug contents.

MATERIALS

Gelatin : Pig Skin, 260 Bloom, isoelectric point 8.9-9 (Rousselot, France) ; mineral oil : viscosity 220 cst (Esso, Primol 352), sorbitan trioleate : HLB 1.8 (Arlacel 85, I.C.I. France), metronidazole (1H-imidazole, 1-ethanol-2-methyl-5-nitro-) (Specia).

METHODS

The method used for the preparation of microspheres was developped from that described by Tanaka and al.[9] The different steps are represented in the following scheme (figure 1). Aqueous gelatin solution is prepared a day before use, distilled water is added for gelatin swelling, the mixture is heated at 55°C to obtain a sol, pH is ajusted with NaOH N or HCl N and the sol is settled in a refrigerator until use.

Aqueous gelatin solution at 55°C is slowly added (10 minutes) with stirring to mineral oil containing sorbitan trioleate. Then the vessel is stepped in ice water (0°-50°C) and kept under controlled continued stirring 1 h 30 to gelify the gelatin microdrops. Dehydration is obtained by addition to the dispersion of isopropanol (5°C) under gently stirring. Only isopropanol and acetone can be used to separate the microspheres from the oily phase and to obtain a free flowing powder, when mixtures of water and alcohol or ether are used, the microspheres are agglomerated. Microspheres are then resuspended in isopropanol containing formaldehyde (1 ml/g of microspheres) as the cross-linking agent. Excess carbonyl reagent is removed by adding isopropanol, pelleting the microspheres by centrifuging and decanting the supernate. The microspheres are washed two times in this manner and subsequently stored

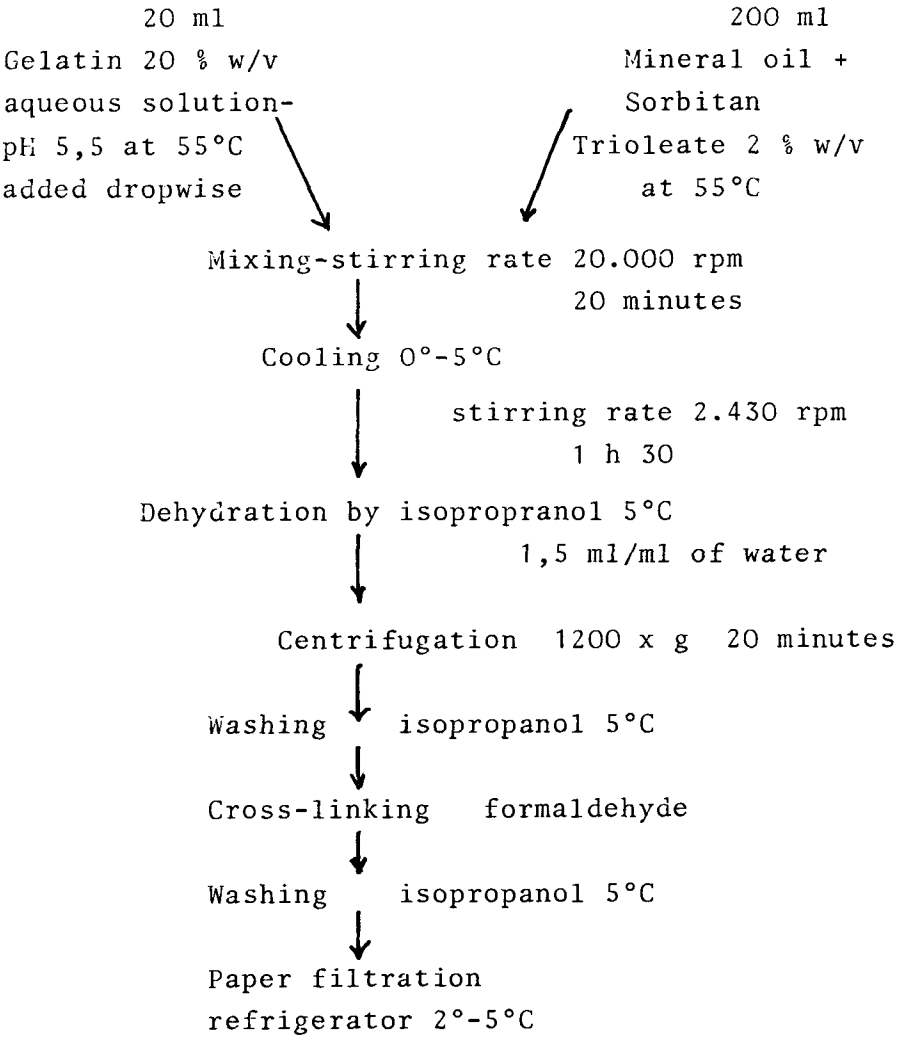


FIGURE 1
Microspheres preparation scheme

24 h in the refrigerator at 2-5° to get free from isopropanol.

To prepare loaded microspheres, metronidazole is dispersed in the gelatin solution at 55°C under stirring and the oily phase containing 5 per cent v/v of

surfactant. Quantities of metronidazole and gelatin used were 1:1.

A Coulter Counter model TA II was used to determine microspheres size distributions. Samples were dispersed in an electrolyte solution containing 0.9 % w/v sodium chloride and Monarox by sonification for 10 minutes prior to each experiment.

Apparent specific surface areas were calculated from the particule size distributions. The microspheres density was considered as 1.

The surface characteristics of microspheres were examined by means of a scanning electron microscope.

To determine total drug content, microspheres were settled in HCl (3N) at 55°C during 20 minutes. The mixture was filtered, diluted with water and essayed spectrophotometrically at 275 nm. Percentage of total metronidazole was expressed in g/100 g of microspheres.

To determine free drug content, microspheres were dispersed in HCl (0.1N) during 3 minutes. The mixture was filtered diluted with water and essayed at 275 nm. Percentage of free drug was expressed in g/100 g of microspheres.

Encapsulated drug is definited as : total drug content menor free drug content.

RESULTS AND DISCUSSION

Gelation and dehydration of gelatin give microspheres of size range 6 to 14 μm.

Photo 1 shows unloaded microspheres and Photo 2 loaded microspheres, both obtained at pH 5.5. With this pH, we can observe agglomerates of particules near the microspheres, those agglomerates are noted for unloaded and loaded microspheres.

Loaded microspheres prepared at pH 9 are presented on Photo 3, at pH 9 microspheres are individualized, the size is more homogenous but the surface aspect looks still irregular. Photo 4 shows unloaded microspheres prepared at pH 9.

The pH of the Aqueous Gelatin Solution Influences Size Distributions

. The size distributions of gelatin microspheres, standard deviations, and apparent specific surface areas are reported in Table 1.

. For unloaded microspheres, at pH 11, gelatin gives agglomerates which do not disperse in isopropanol for washing and no microspheres were formed. The smallest unloaded microspheres are obtained at pH 7, for more acidic or alcalin pH values, the microspheres are bigger. The values of apparent surface areas follow a curve too, the highest value is obtained at pH 7. Gelatin viscosity varies with pH, maximum exist for acid and alcalin pH and for high viscosities dispersion of gelatin droplets will not be as efficient as for pH 7 and pH 5.5.

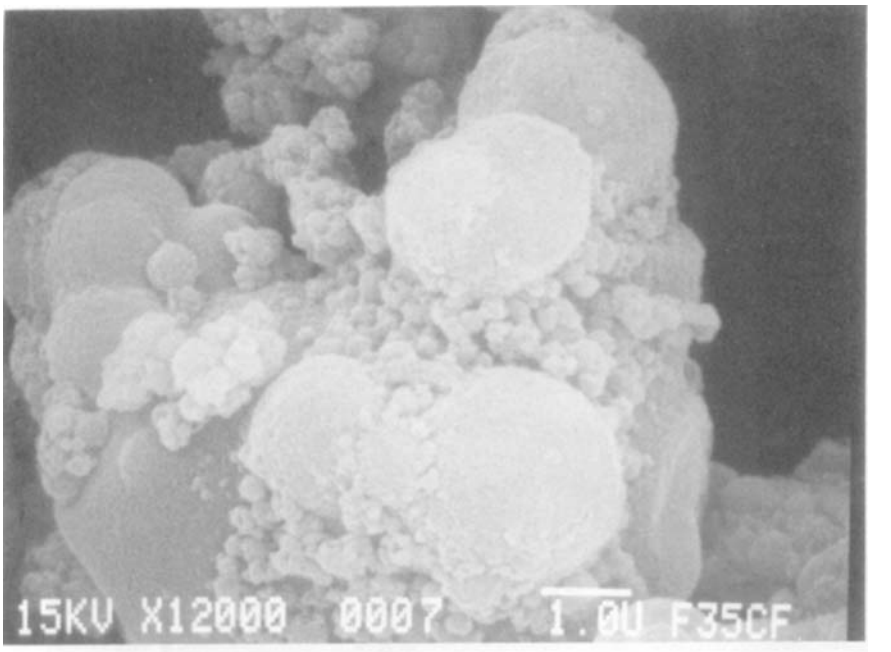
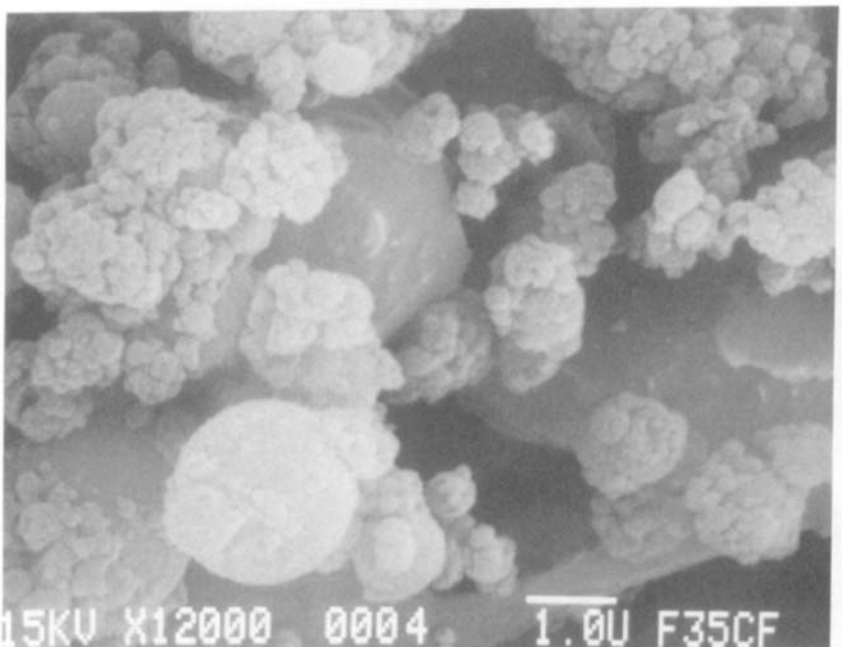


Photo 1



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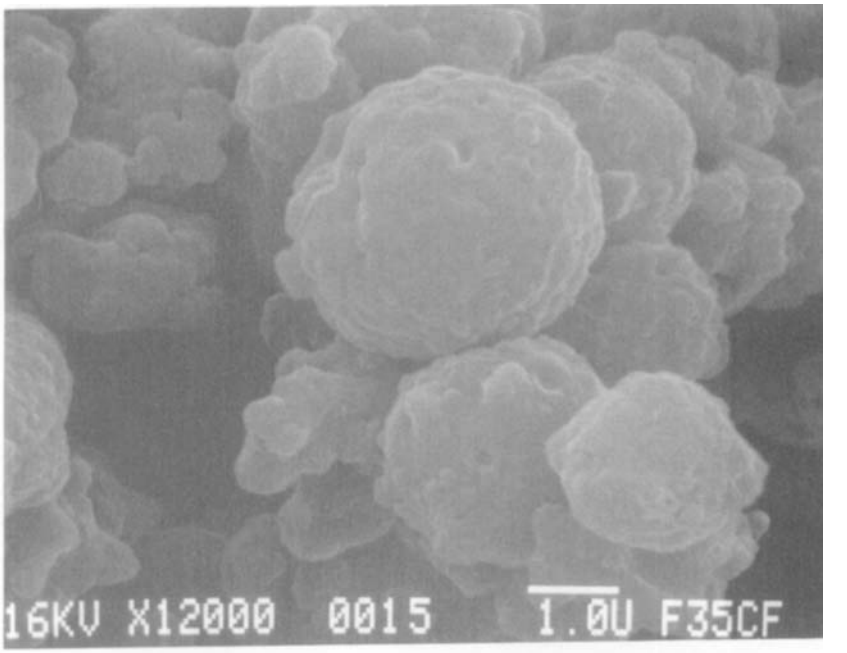

Photo 3

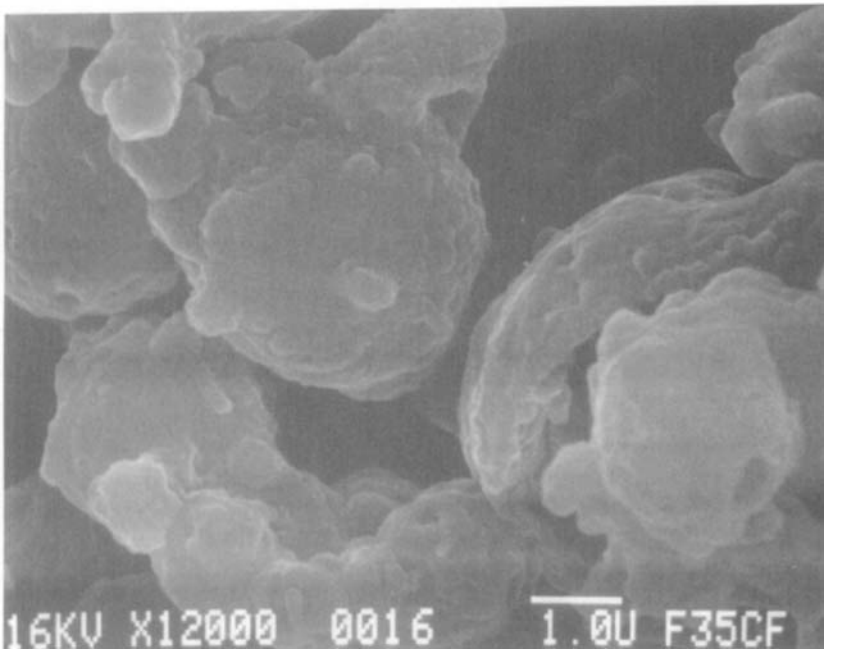

Photo 4

TABLE 1

Influence of the pH of the Aqueous Gelatin Solution on Size Distribution of Microspheres.

pH of the aqueous gelatin solution	Unloaded microspheres			Loaded microspheres		
	Mean diameter µm	Standard deviation	Specific surface areas cm^2/g	Mean diameter µm	Standard deviation	Specific surface areas cm^2/g
9	12,96	5,81	6 214	8,3	5,89	10,651
8	9,19	5,38	9 884	-	-	-
7	7,11	4,03	11 542	-	-	-
5,5	8,59	2,91	8 683	6,01	4,98	15,428
4,5	11,69	5,98	6 783	-	-	-
3,5	14,29	6,03	5 145	5,11	3,92	16,780

. The pH of aqueous gelatin solution modifies the size distribution of drug loaded microspheres. The smallest size is observed at pH 3.5 and larger size distributions are noted at other pH. The higher surfactant concentration (5 per cent, v/v) which was used to prepare loaded microspheres instead of 2 per cent for unloaded microspheres, has modified the size distributions of loaded microspheres even with acid and alcalin pH and the influence of pH on the viscosity of gelatin solution and on the size of gelatin droplets is not maintained.

The Surfactant concentration in the Oily Phase Interacts on the Size Distribution of Microspheres

The size distribution of unloaded microspheres decreases when the surfactant concentration in the oily phase is increased from 2 per cent to 5 per cent v/v (Table 2). Ishizaka and al.[7] using 0.1 to 5 per cent of surfactant observed a drop in size distribution when the surfactant concentration is the highest. But for a high quantity of surfactant such as 10 per cent v/v, we think that the concentration modifies the viscosity of the oily phase and the interfacial tension of the gelatin droplets inducing coalescence and larger microspheres.

The Gelatin Concentration of the Aqueous Solution Influence Size Distribution of Unloaded and Loaded Microspheres

TABLE 2
Influence of Surfactant Concentration on Size Distribution of Unloaded Microspheres

Surfactant concentration %	Mean diameter µm	Standard deviation
2	8,59	2,91
5	4,48	3,11
10	5,61	2,86

When the gelatin concentration of the aqueous solution is lowered the mean diameter and the standard deviation decrease too (Table 3) Ishizaka and al.[7] noted that the size distribution curve became narrower with decreasing albumin concentration. The modification of gelatin concentration changes the viscosity of the gelatin solution and the microspheres'mean diameter.

The stirring rate used during gelatin step does not interract markely with size distribution of microspheres.

Drug Contents of Microspheres are affected by the pH of Aqueous Gelatin Solution

. The total drug contents and the free drug contents are reported in Table 4.

. Total drug content is higher for pH 5.5 but free drug content too. At pH 9, only 16.6 per cent of total metro-

TABLE 3
Influence of Gelatin Concentration on Size Distribution of Microspheres

Gelatin w/v- %	Unloaded Mean diameter	Unloaded Standard deviation	Loaded Mean diameter	Loaded Standard deviation
10	6,16	3,39	5,83	3,87
20	7,15	3,67	6,01	4,98
30	8,10	3,89	11,03	3,19

TABLE 4
Influence of Gelatin Solution pH on Drug Contents

pH Gelatin solution	Total drug content %	Free drug content %	Encapsulated drug %
9	16.6	8.96	7.64
5.5	26.3	22.62	3.68
3.6	7	6.55	2.45

nidazole is recovered, however the free drug content is the lowest and half of the metronidazole remains in the microspheres. The apparent specific surface area (Table 1) is smaller for those microspheres prepared at pH 9, so we can think that drug is encapsulated and not only fixed on the surface. Levy and al.[1] observed with gelatin of isoelectric point 8.9, that microcapsules prepared at pH 9 offer better stability to enzymatic substances.

TABLE 5
Influence of Stirring Rate used during Gelation on Drug Contents

Stirring rate rpm	Total drug content %	Free drug content %	Encapsulated drug %
3550	10,5	7,55	2,95
3020	11,2	7,85	3,25
2430	26,3	22,62	3,68
1450	16,7	15,25	0,85

TABLE 6
Influence of Gelatin Concentration on Drug Contents

Gelatin w/v %	Total drug content %	Free drug content %	Encapsulated drug %
10	3.2	-	-
20	26.3	22.62	3.68
30	19.1	16.8	2.3

So, for microspheres prepared at pH 9, the gelatin wall is less porous and the drug entrapment is better.

Drug Contents of Microspheres are Influenced by the Stirring Rate used during the Gelation Step

The total drug contents (Table 5) are increased when the stirring rate applied during the gelation step is lowered but the free drug contents are increased too,

and the percent of encapsulated drug remains still very low. The best results are obtained at 2430 rpm.

Drug Contents of Microspheres change with the Concentration of the Gelatin Solution

The increase of gelatin concentration 10 to 30 per cent modifies the total drug contents (Table 6), but the free drug content use always very important and the encapsulated drug very low. The 20 per cent w/v gelatin solution gives the highest quantity of total metronidazole. Gelatin microspheres prepared with the 20 per cent w/v gelatin solution at pH 9 have a free drug content lowered and a encapsulated drug content more important (Table 4). Haschida and al.[4,5] used a 20 per cent w/v gelatin solution but did not speak about the drug content.

With all processes, total drug contents are very low and free drug contents very high. The high amount of free drug may be due to the fact that drug is entrapped in the interstices of microspheres surface and leaves it very quickly. Friedman and al.[2] obtained with albumin microspheres the same kind of results, drug was immediatly free, they thought that this was due to the spongy nature of those microspheres.

CONCLUSION

With gelatin pig skin isoelectric point 8.9, gelatin microspheres can be easily prepared by controlling pH and concentration of the gelatin solution and also surfactant concentration of the oily phase.

The pH of the gelatin aqueous solution does not interact markely with the size distribution of unloaded and loaded microspheres. The surfactant concentration in the oily phase is an important factor to reduce the size distribution and a concentration of 20 per cent w/v of gelatin in the aqueous phase at pH 9 is the best to encapsulated metronidazole.

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