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PREPARATION AND CHARACTERIZATION OF OILCONTAINING MICROPARTICLES

S. Keipert, P. Melegari
Humboldt-Universität zu Berlin, FB Pharmazie
Goethestraße 54, O-1120 Berlin

ABSTRACT

Oilfree as well as oilcontaining microparticles based on gelatin and gelatin-Acacia-systems respectivly are produced by preparing a "multiple emulsion" using the simple coacervation. The characterization of the particles points out relationships between the content of polyunsaturated fatty acids and the mean diameter, the oil content and the shape of the surface. Both, the ratio between oil-colloid-emulsion and external phase and the viscosity of this phase have some influence on the mean size of the microparticles.

INTRODUCTION

There are many possibilities of using fatty oils in pharmacy. On the one hand they can serve as auxiliary materials, e.g. as softener or vehicle especially to realize a retard release; on the other hand oils are used in dermatology due to their therapeutic effects. This refers especially to olive-oil. Fish-oil is making its way into the prophylaxis of coronary heart diseases

and artherosclerosis. The disadvantages of oils, however, are the bad manufacturing parameters. The preparation of microparticles is one possibility of changing the technological properties of oil. Many patents and articles /1-6/ show the interest in the production of an oil containing free flowing powder. In most of these cases the complex coacervation is described as method for production. But the reproducibility of this method is not satisfactory because of numerous influencing factors /7/.

The object of the present work is the production of gelatin based microparticles containing Miglyol^{R}, olive-oil, peanut-oil and fish-oil using a simple method. Two kinds of the simple coacervation technique are introduced in this article. The characterization of the final particles includes the determination of the mean size, the oil content and the analysis of the particle surface.

MATERIALS

Gelatin: isoelectric point= 5,5 /8/(Laborchemie Apolda); Acacia (Laborchemie Apolda); peanut-oil (Ankerwerk Rudolstadt); olive-oil (Ankerwerk Rudolstadt); Miglyol^{R} 812 (Dynamit Nobel AG, Troisdorf); fish-oil: mackarel oil (Institut of Deep Sea Fishery, Rostock); liquid paraffin (Leuna Werke, Merseburg).

All other substances were analytical reagent or purity.

METHODS

Preparation of the Microparticles

In the development of a method to produce oilfree and oilcontaining microparticles the results of the preexperiments described method 1 and 2 as the best techniques.

Method 1

Figure 1 represents the preparation scheme for microparticles. "Colloid-phase" in method 1 means an aqueous gelatin solution (10% w/w) for oil free systems and a mixture of aqueous gelatin solution (10% w/w) and Acacia solution (10% w/w) in the ratio 2:1 for oil containing systems respectivly. The exact composition of the colloid-phase for the particles is demonstrated for each result.

The primary emulsion is formed by three parts colloid-phase and one part oil at 60°C using an Ultra-Turrax (Janke & Kunkel AG, Staufen). Under stirring (Laborrührer LR 40, Prüfgeräte, Medingen) this o/w-emulsion is added quickly to the same quantity of decane (60°C). Then the "multiple emulsion" system is cooled down to gelify the droplets. After 15 min of stirring isopropanol is added to soldify the particles. In this stage of preparation the speed of stirring can be reduced. After separation by gravitation-filtration the microparticles are washed twice with isopropanol and then air-dried.

Method 2

The method used for the preparation of these microparticles (figure 2) is a modification of Chemtob's method /9/.

An o/w-emulsion is prepared by homogenizing three parts of colloid-phase - in this case aqueous gelatin solution (10% w/w) - and one part oil phase of the same temperature (40°C). The primary emulsion is added slowly to paraffin (40°C) of the double quantity. The reducing of the temperature constant stirring guarantees the optimal gelifying of the gelatin microdrops. The addition of isopropanol leads to dehydration, linked with a further solidifying of the microparticles. The normal filtration

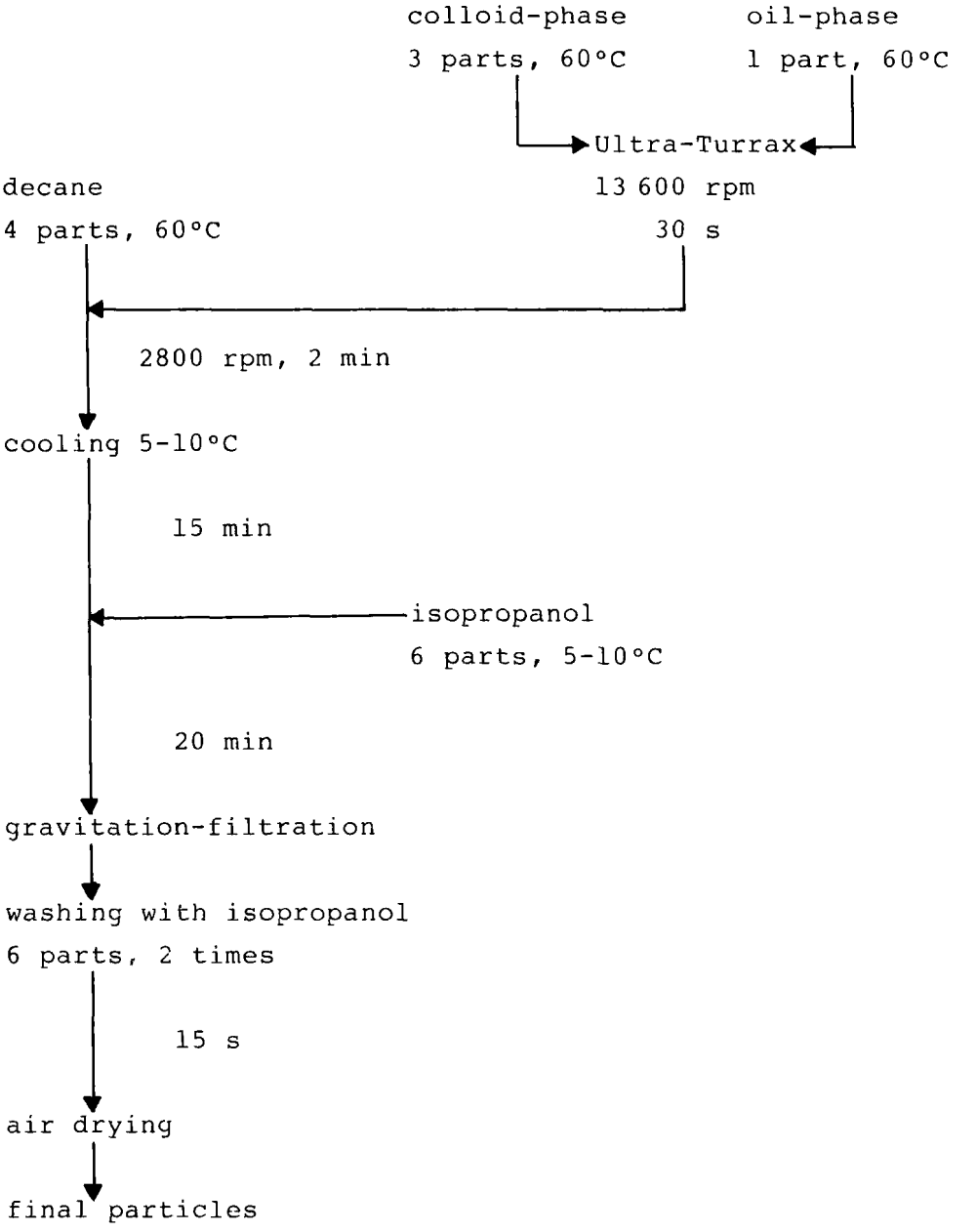

FIGURE 1

Preparing method for microparticles using a external phase with low viscosity (method 1)

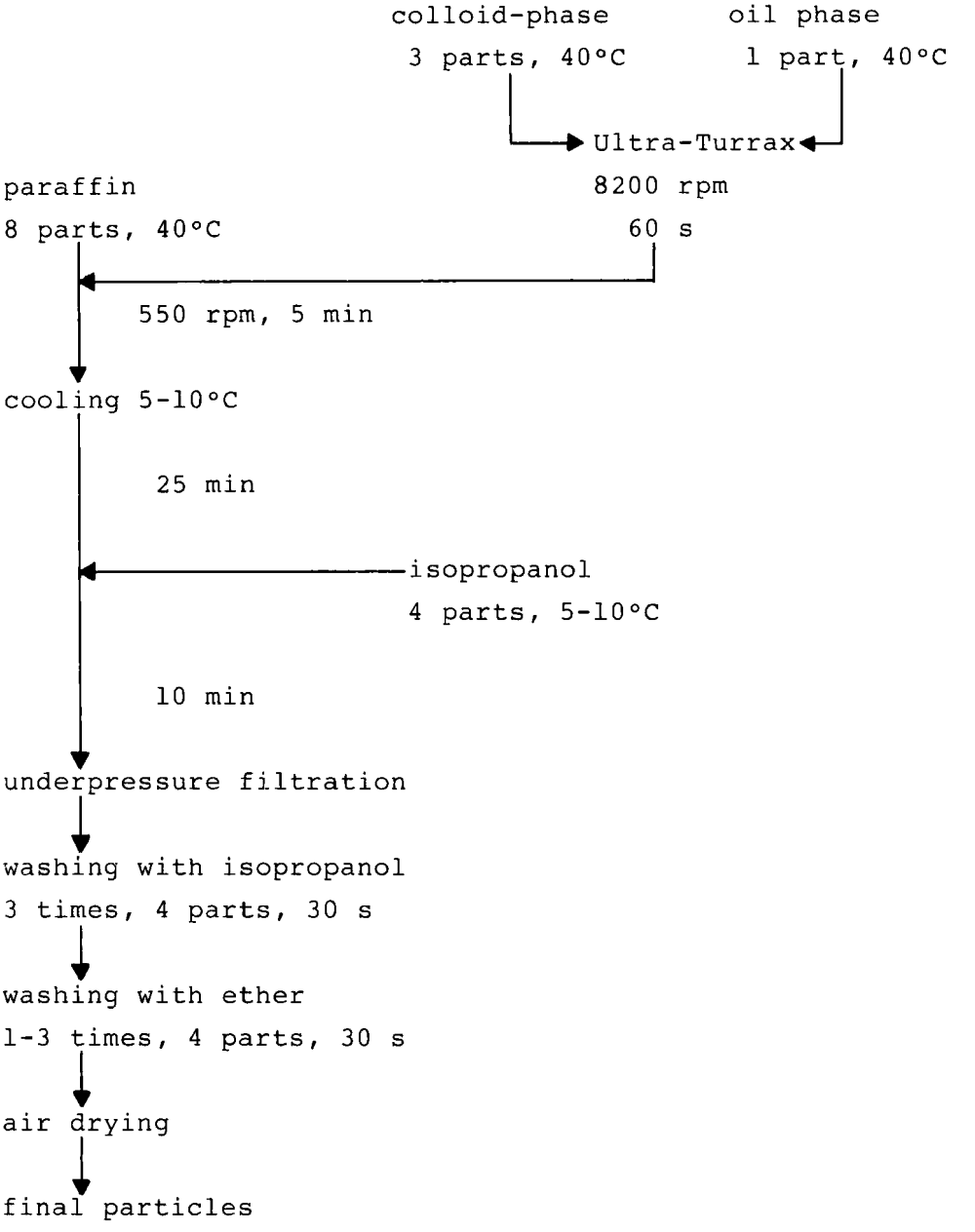

FIGURE 2

Scheme for preparation of microparticles using an external phase with high viscosity (method 2)

as used in method 1 can not be used here because of the small particle size and the higher viscosity of the external phase. After separating the microparticles by underpressure filtration they are first washed three times with isopropanol and then with ether once (Miglyol) or three times (peanut-oil) respectively in dependence on the kind of oil. The washing of the particles with ether is necessary to remove all rests of the lipophilic external phase from the particle surface.

Determination of the Particle Size

Sieve fractionation is used for determining the size distribution of the microparticles. The mean diameters of the particles are calculated using the double logarithmic network of Rosin-Rammler-Benett. If the particles are to small for sieving, their size is determined by microscop (Carl Zeiss Jena).

Determination of the Oil Content

Because there was not any relevant method for determining the oil content of microparticles described in literature it was necessary to develop a special technique providing the required precision.
0,500 g microparticles are shaken in 5 ml trypsin solution (0,1 % w/v in phosphate buffer pH = 7,8) at 40°C for one hour. After adding 1 ml concentrated hydrochloric acid the dispersion is extracted three times by an ether-petrolether-absolute ethanol-mixture (8:3:1) /10/. The organic phase is collected, washed with 20 ml water three times and dried with sodium sulfate. After filtering this phase into calibrated flasks and removing the solvents the flasks are stored in the exsiccator for 8 hours. Then the oil content is determined by weighting.

Surface Characteristics

After a special preparation of the samples the particle surface and pore diameters are examinated by means of a scanning electron microscop (REM BS 300, Tesla, ČSFR).

Measurement of Viscosity

The viscosimeter of Ubbelohde is used for the determination of the viscosity of the colloid-phases, the o/w-emulsions and the external phases at preparation temperature (40°C respectively 60°C).

Determination of Interfacial Tension

The primary emulsion is adjusted to the corresponding temperature and covered with the specific lighter external phase (decane or paraffin). The interfacial tension between the phases is determined by a LAUDA-tensiometer (Meßgeräte-Werk LAUDA Dr. R. Wobser KG, Königshofen) at a temperature of 40°C.

Determination of the Fatty Acid Composition of the Oils

After preparing the methylester of the fatty acids the oils are analized by gas chromatografy (MGC 4000, Chromatron, Berlin).

RESULTS AND DISCUSSION

The following differences between the two preparation methods can be pointed out for the judging of the results :

- Method 2 works with a higher viscous external phase in contrast to method 1 (paraffin = 12,14 mPas; decane = 0,86 mPas; 40°C).

- The higher viscosity of the external phase in method 2 is the reason for decreasing the stirring speed (method 1: 2800 rpm; method 2: 550 rpm).
- The ratio between the primary emulsion and the external phase runs up to 1:1 in method 1 and 1:2 in method 2.

Both methods reproducibly produce almost spheric particles. Their properties depend on the preparation factors and the kind of containing oil (table 1 and 2).

Particle Size

One of the most important criteria for the characterization of microparticles is the particle size.
If the producing temperature of method 2 is increased from 40°C to 60°C there is not any influence on the mean diameter d_m, so that a comparison of the mean sizes between method 1- and 2-particles is allowed.
Decane-paraffin-mixtures as external phase and aqueous gelatin solution as "primary emulsion" are used in order to testing the influence of the external phase-viscosity on the particle size (method 2) - viscosity decreases with increasing decane quantity.
The results (figure 3) show a possible relation between the paraffin quantity in the mixture and the size of the microparticles. An influence on viscosity and interfacial tension can not be excluded.
The influence of the quantity ratio of colloid-dispersion to external phase is examinated by using aqueous gelatin solution only. Figure 4 shows a continious decrease of the mean particle size with a growing quantity of the external phase paraffin (method 2). Whereas the use of decane (method 1) leads to a curve with a

TABLE 1

Results of the gelatin-Acacia-microparticles (method 1)

oil		parameters of: primary emulsion		final particles			
kind	fa-content /%/	σ 40°C /mN·m/	η 60°C /mPas/	d_m /mm/	oil content /%/	pore-characterization number	diameter/µm/
Miglyol^R	0	32,41	16,09	0,4310	61,45	+++	0,014-1,73
olive-oil	4,5	32,62	16,05	0,4950	56,02	+++	0,080-1,67
peanut-oil	20,9	28,18	16,10	0,3410	55,59	+	0,019-0,30
fish-oil	46,9	18,12	15,84	0,1930	54,10	(+)	0,007-0,04

fa-content content of polyunsaturated fatty acids

d_m mean diameter

+++ many

\+ few

(+) very few

σ interfacial tension between primary emulsion and decane

η viscosity

TABLE 2

Results of gelatin-microparticles (method 2)

oil	parameters of: primary emulsion		final particles			
kind	fa-content /%/	σ 40°C /mN·m/	η 40°C /mPas/	d_m /mm/	oil-content /%/	pore-characterization number	diameter/µm/
Miglyol^R	0	20,91	33,16	0,1030	59,80	+++	0,05-0,17
peanut-oil	20,9	17,60	27,35	0,0930	55,03	(+)	-

σ interfacial tension between primary emulsion and paraffin

other abbreviations see table 1

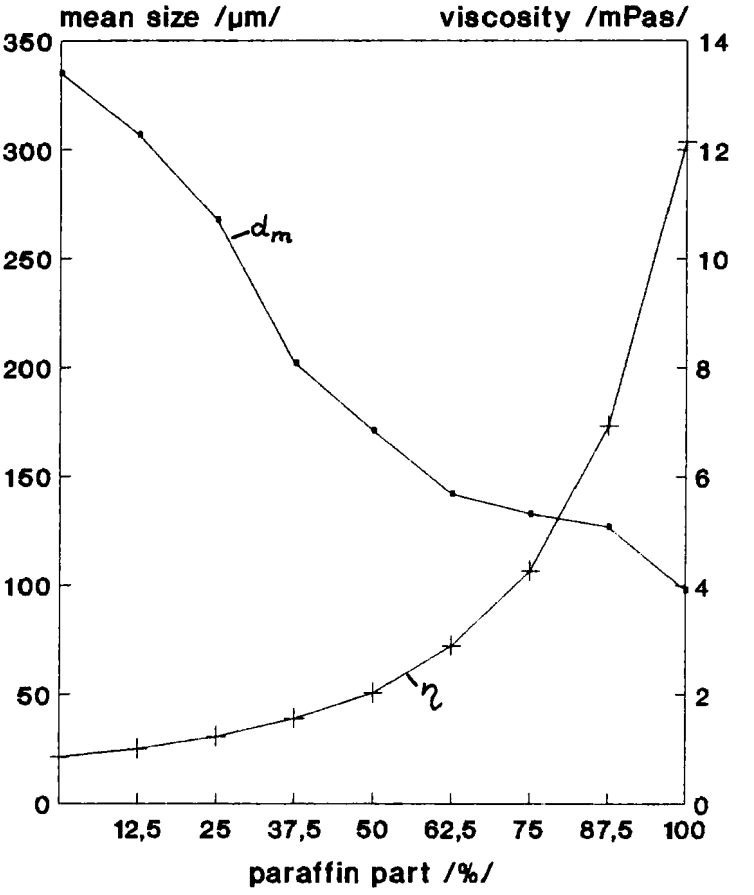

FIGURE 3

Influence of the particle size by the viscosity of the external phase

break. The minimum (30 ml decane) is the optimal phase ratio of the "multiple emulsion". This quantity of the external phase is sufficient to cover the total surface of the o/w-droplets and to prevent their confluence. The reason for the increasing particle size with higher decane-quantity may be the displacement of the droplets in the low viscous external phase by the stirrer instead of their further dividition.

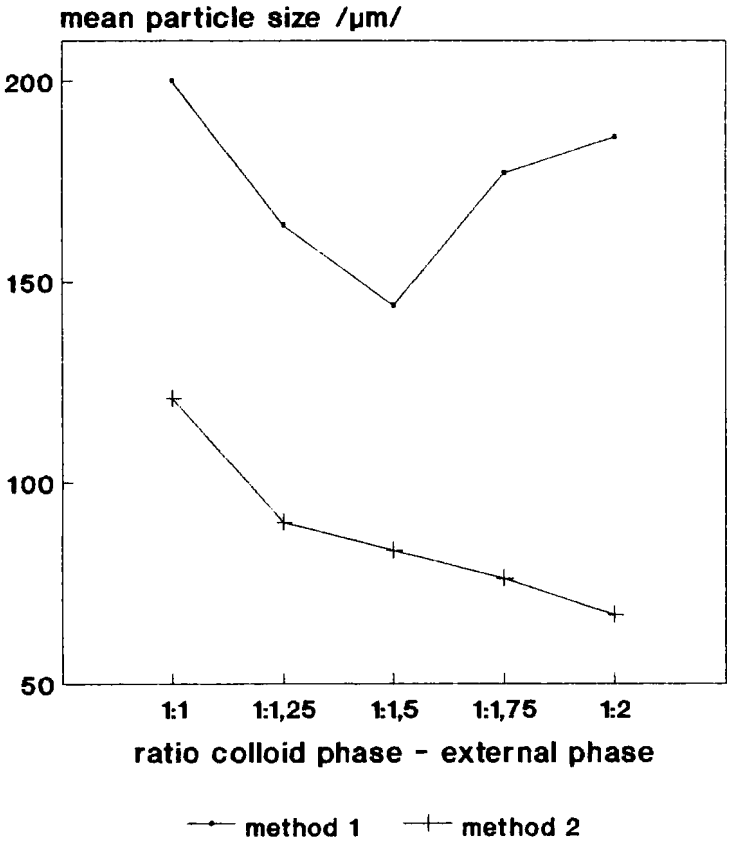

FIGURE 4
Relation between colloid-phase-external phase-ratio and particle size

The comparison between Miglyol^{R} and peanut-oil containing microparticles of methods 1 and 2 shows that the mean diameter of method 2-particles runs up to only a quarter of method 1-particles in spite of a lower stirring speed. One reason for this phenomenon is the different interfacial tension of the respective primary emulsion to the external phase.

Comparing the oil containing microparticles (method 1), a revers proportional relation is observed between the content of polyunsaturated fatty acids and the mean

size. This content increases in the following order: olive-oil < peanut-oil < fish-oil. The mean diameter of the particles decreases in the same succession. It is interesting that fish-oil-particles are the smallest. Miglyol^{R} - a partialsynthetic oil - has an exceptional position because of its oil composition (saturated fatty acids with short chain lengths).

The interfacial tension between the o/w-emulsion and decane shows the same tendency to the content of fatty acids like the mean size. The multiple bonds of the fatty acids act as "hydrophilic" molecular parts. That means the "amphiphilic character" of the lipids grows with increasing content of multiple bonds.

If σ is lower, e.g. in emulsions containing fish-oil, a smaller droplet diameter is obtained in the preparation of the "multiple emulsion", which has a direct influence on the size of the final particles.

Oil Content

Although there are no essential differences between the oil content of the particles of method 1 and 2 it can be noted that an increasing content of polyunsaturated fatty acids is associated with a decreasing tendency of the incorporated oil quantity (table 1). The oil content bases on the size of the final particles depending directly on the composition of the oil. The size of oil microdrops in the primary emulsion amounts to a maximum of 2 µm. During the dispersion in the external phase the o/w-emulsion is divided mechanically and some oil drops are located free on the surface and can be washed out by decane or paraffin respectively isopropanol. Therefore a slightly falling tendency of the microparticle-oil content is observed with increasing content of polyunsaturated fatty acids.

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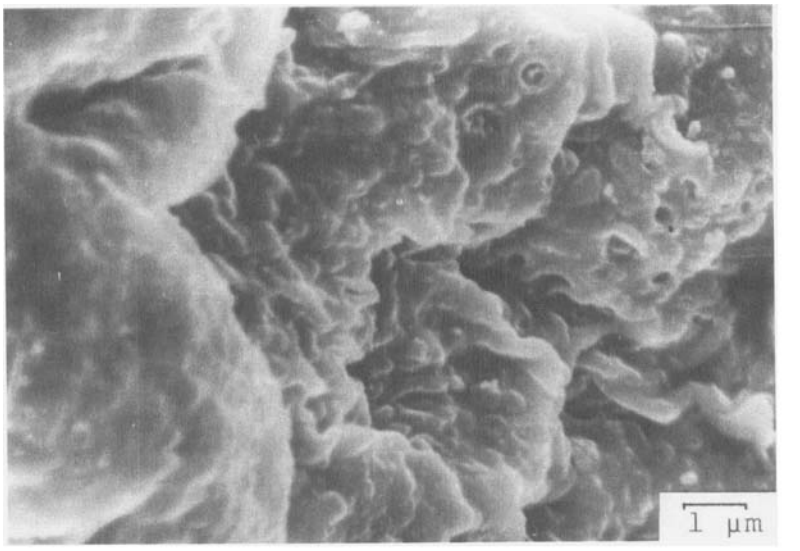

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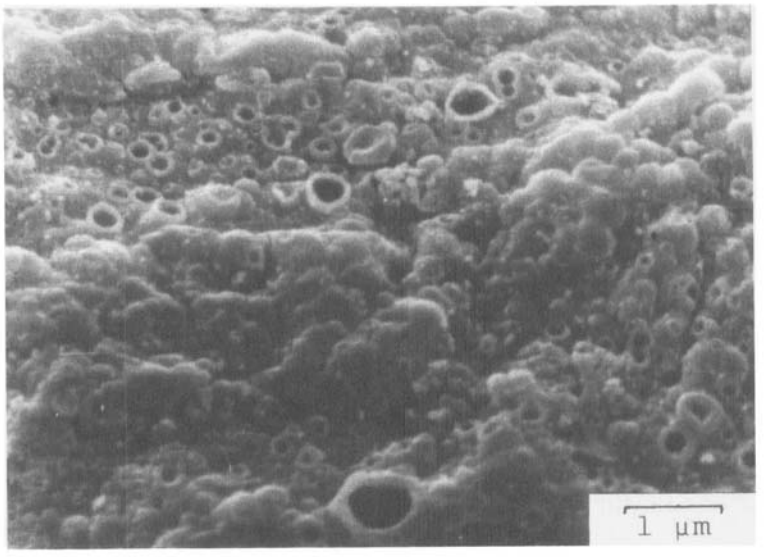
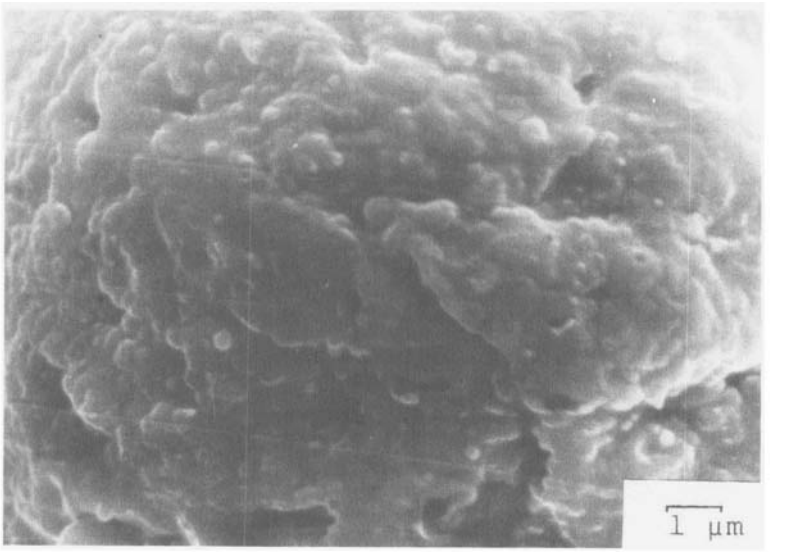


PHOTOS 1 - 4

Surface morphology of the gelatin-Acacia-oil-microparticles (method 1)

1 Miglyol^{R} (enlargement: 4500)
2 olive-oil (enlargement: 6000)
3 peanut-oil (enlargement: 3400)
4 fish-oil (enlargement: 9000)

3)



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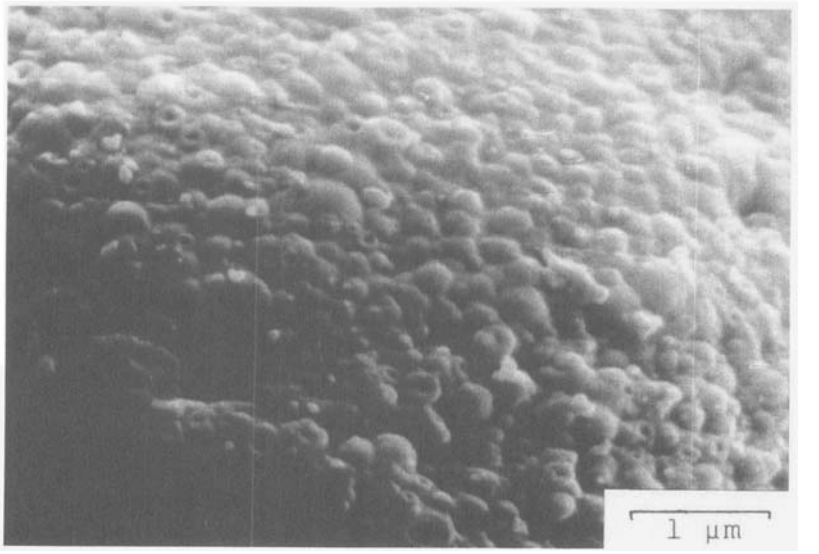

PHOTOS 3 & 4

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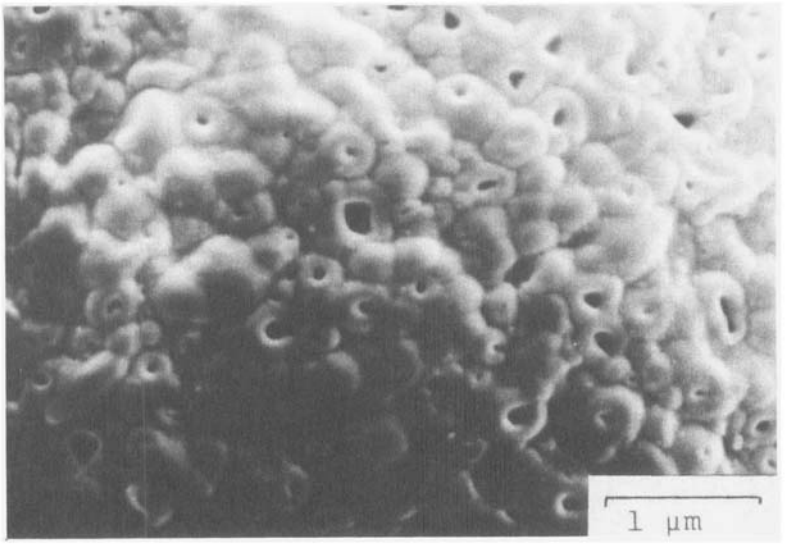

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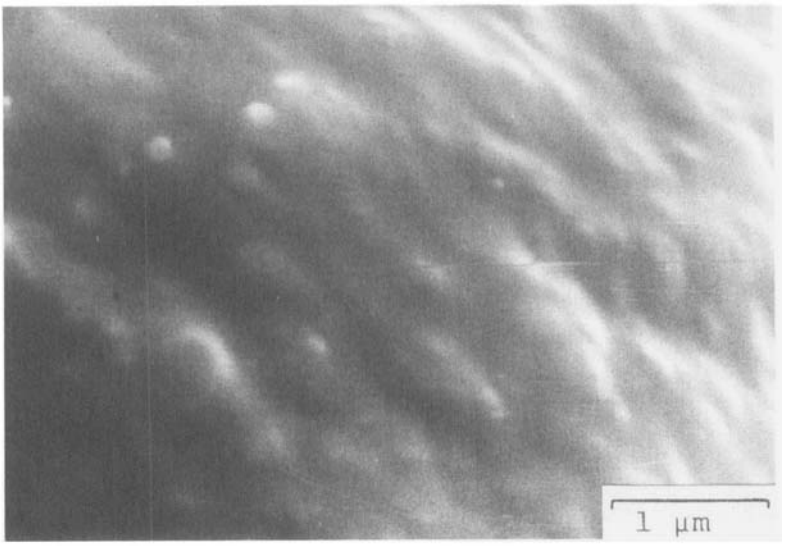

PHOTOS 5 & 6

Surface of the gelatin-oil-microparticles (method 2)

5 Miglyol^{R} (enlargement: 10 100)
6 peanut-oil (enlargement: 10 300)

Surface Morphology

Photos 1 to 6 make visible the surface of the microparticles. The photos of method 2-microparticles (photo 5 and 6) show a more regular surface than the particles of method 1 (photo 1 - 4). This phenomenon is based on the different interfacial tension of the comparable Miglyol^{R}- and peanut-oil-colloidphase-emulsions. The σ - values of the primary emulsion of method 2 run to only two thirds of the results of the adequate emulsions of method 1. The kind of external phase also plays a part in the shaping of the particles' surface.
Further the Miglyol^{R}-microparticles of method 1 (photo 1) show a bizarre surface and differ from the other particles containing naturally produced oil (photo 2-4). The photos of the particles containing vegetable or animal oil indicate a higher tendency of surface regularity with increasing content of polyunsaturated fatty acids. This phenomenon can be explained in relation to the interfacial tension, too.
The content of polyunsaturated fatty acids also influences the porosity of the microparticles. The number and the diameter of the pores decrease in the order olive-oil, peanut-oil, fish-oil. (It is not possible to differentiate between so called "pores" and washed out oil drops on the surface by REM-technique.) The results of the method 2-particles confirme this tendency.
Fish-oil-gelatin-Acacia-emulsion has a significantly lower viscosity than other emulsions. This result may explain - in connection with the relative small interfacial tension - the very regular surface of the fish-oil-microparticles of method 1 in contrast to the other particles.

CONCLUSION

The introduced methods of simple coacervation guarantee the reproducible preparation of both oil free and oil containing microparticles based on gelatin.
Preparation parameters like external phase-viscosity and ratio between the colloid- and external phase influence the particle size of the oilfree particles.
Incorporating natural oils in the microparticles the content of polyunsaturated fatty acids considerably influences their properties. Apart from mean size the shape of the particle surface, too, depends on the character of the oil. A revers proportional relation is observed between the content of the polyunsaturated fatty acids and the mean size respectively regularity of the particle surface.
The oil content can be determined exactly by the developed method. The oil content of the microparticles depends on the kind of preparation method and the mean size of the particles.

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