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Research paper

W/O/W multiple emulsions with diclofenac sodium

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Abstract

The disperse oil droplets of W/O/W multiple emulsions contain small water droplets, in which drugs could be incorporated, but the structure of these emulsions is also the reason for possible instability. Due to the middle oil phase which acts as a 'semipermeable' membrane the passage of water across the oil phase can take place. However, the emulsions have been produced in a two-step-production process so not only the leakage of encapsulated drug molecules out of the inner water phase during storage but also a production-induced reduction of the encapsulation rate should be considered. The aim of this study was to ascertain how far the production-induced reduction of the encapsulation rate relates to the size of inner water droplets and to evaluate the relevance of multiple emulsions as drug carrier for diclofenac sodium. Therefore multiple emulsions were produced according to a central composite design. During the second production step it was observed that the parameters pressure and temperature have an influence on the size of the oil droplets in the W/O/W multiple emulsions. Further experiments with different W/O emulsions resulted in W/O/W multiple emulsions with different encapsulation rates of diclofenac sodium, due to the different sizes of the inner water droplets, which were obtained in the first production step.

Keywords: W/O/W multiple emulsions; Central composite design; Production process; Droplet size; Encapsulation rate; Diclofenac sodium

1. Introduction

Multiple emulsions are complex systems which contain small droplets in their disperse phase. As phase distribution W/O/W or O/W/O emulsions are possible. Multiple emulsions were observed as a laboratory curiosity in former times [1] but these systems have now been evaluated systematically [2–4] and could prove to be suitable as an interesting delivery system with potential for biopharmaceutical application [5–7] and as vehicles for various drugs [8–13].

To summarize, the aspects of application are

- protection of the organism against substances with local irritating properties or bad taste
- depot-function of the inner phase and controlled release
- protection of drugs against destabilizing influences from the organism [14,15]

However, an adequate system stability is necessary, because the claimed advantages of the multiple emulsion as innovative formulation for drug delivery can only be made use of with an intact vesicular structure. Due to the middle oil phase which acts as a 'semipermeable' membrane, the passage of water across the oil phase can take place. This leads to either swelling or shrinkage of the internal droplet, depending on the direction of the osmotic gradient. Many studies have attempted an analysis of the possible mechanisms of instability [2,16,17].

The aim of this study was to examine the second production step in the formulation of W/O/W multiple emulsions and to investigate the influence of the process parameters on the droplet size and encapsulation rate. First, the second emulsifying step with the dispersion of a W/O emulsion in an outer water phase provides the vesicular structure of multiple emulsion. So for production of multiple emulsions it is necessary to reduce the loss of the inner water phase during the process to gain a high encapsulation rate of the active ingredient. Thus a further aim was to investigate the influence of the inner W/O emulsion on the encapsulation rate. The hypothesis was that the second production step can only lead to multiple emulsions low loss with fine inner water droplet of

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the primary W/O emulsion. Diclofenac sodium, already used in multiple emulsions [13], was encapsulated in the inner water phase. Furthermore, its relevance of encapsulation in the inner water phase of a multiple emulsion should be estimated.

2. Materials and methods

2.1. Materials

Miglyol 812 (MCT) was supplied by Condea Chemie GmbH (Witten, Germany). Cremophor®WO7, a hydrogenated castor oil which has 7 mol ethylene oxide added to it, was obtained from BASF AG (Ludwigshafen, Germany), Tween®80 and Arlacel®P135 were obtained from Uniqema (Everberg, Belgium). Diclofenac sodium was supplied by Heumann Pharma GmbH (Feucht, Germany).

2.2. Methods

2.2.1. Emulsion preparation

The emulsions were prepared with a two-step-procedure. In the first step a W/O emulsion was formulated using 30% water phase and 70% oil phase containing 30% of the lipophilic surfactant. Both phases were heated separately, mixed together after reaching the production temperature and then pre-emulsified using an Ultra-Turrax® T25 (Janke and Kunkel, Staufen, Germany) for 1 min. The final emulsification was carried out with a high pressure homogenizer (Micron Lab 40, APV Gaulin, Lübeck, Germany). In the second step the primary W/O emulsion was dispersed in an outer water phase containing 1.5% hydrophilic surfactant. For pre-emulsification also an Ultra-Turrax was used and finally the multiple emulsion was prepared with the high pressure homogenizer.

2.2.2. Emulsion characterization

Both, the oil and the water droplets of the multiple emulsions were characterized by a laser diffraction analyzer (HELOS, Sympatec, Clausthal, Germany) with a 20 mm lens which permits a detection of particles between 0.18 and 35 μm. The mean particle size of the oil droplets (which contain water droplets) of the multiple emulsions was measured by photon correlation spectroscopy PCS using laser light scattering (Malvern spectrometer RR 102, Malvern, UK) with helium–neon laser $\lambda = 632.8$ nm (Siemens, Germany). The application of two methods for particle sizing is necessary because PCS enables only moderately narrow distributions to be analyzed and is very precise in the small particle size range. However, if the system shows a broad particle size distribution and large particles only the use of laser diffraction is suitable.

Ultrafiltration (Ultrafree®-4, Millipore, Eschborn, Germany) was used to detach a little outer water phase which was analyzed with HPLC (Kontron Instruments, Neufahrn, Germany) to determine the amount of active ingredient and to calculate the encapsulation rate. To reduce distribution phenomena not only a W/O/W but also a reference emulsion which contains the active ingredient in the outer water phase was produced. This emulsion was used as reference for determination of the encapsulation rate.

Table 1
Production conditions for the emulsions

Emulsion no.	Pressure (bar)	Temperature (°C)
1	200	30.0
2	200	50.0
3	700	30.0
4	700	50.0
5	100	40.0
6	800	40.0
7	450	25.9
8	450	54.1
9	450	40.0
10	450	40.0

2.2.3. Experimental design

To investigate the influence of different production parameters on droplet size and the encapsulation rate of multiple emulsions an unvaried W/O emulsion was used. Diclofenac sodium as active ingredient was dissolved in 30% inner water phase in a concentration of 5 mg/ml. The homogenization conditions in the first step were 500 bar, 45 °C and 5 cycles.

In the second step a central composite design was used and the parameters pressure and temperature were varied as mentioned in Table 1. The droplet size (oil droplets which contain water) and the encapsulation rate of totally 10 emulsions was determined after 1, 3 and 5 cycles to investigate the influence of production parameters. For the statistical analysis the program Statistica® was used.

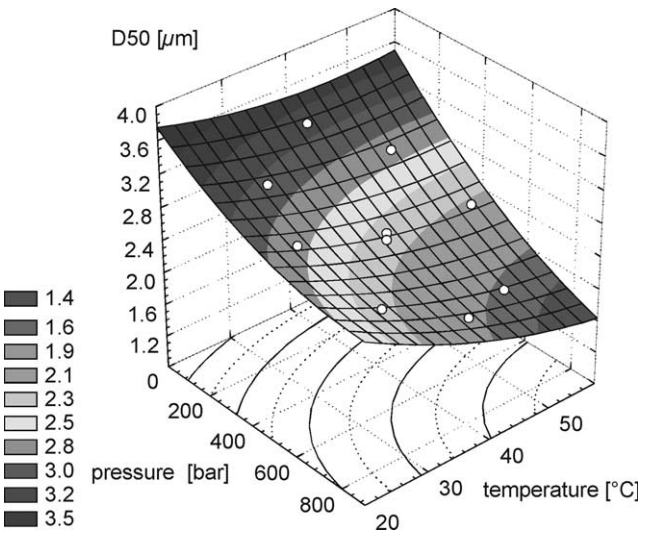

Fig. 1. D50-values after 1 homogenization cycle ($R^2 = 0.9763$).

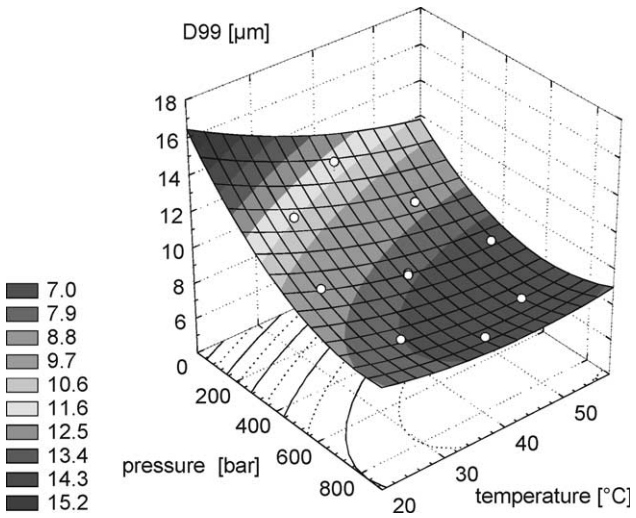

Fig. 2. D99-values after 1 homogenization cycle ($R^2 = 0.9628$).

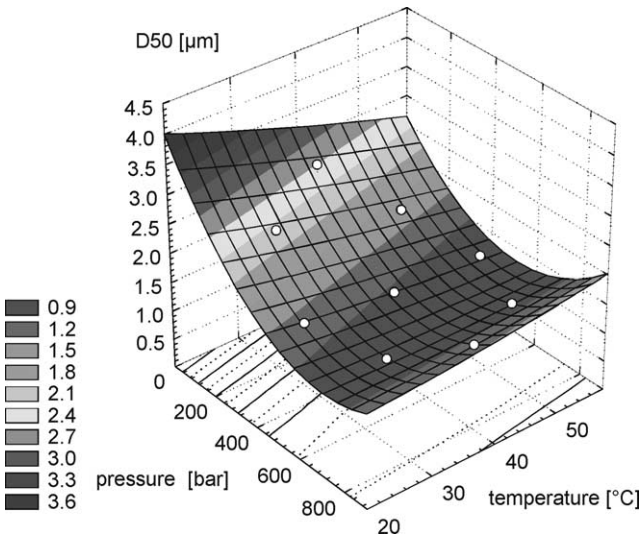

Fig. 4. D50-values after 3 homogenization cycles ($R^2 = 0.9701$).

3. Results and discussion

3.1. Determination of droplet size

3.1.1. Results after one cycle

The results after one cycle in Figs. 1–3 show that low pressure or temperature lead to large oil droplets. With increasing pressure and temperature the droplet size decreases. Regarding the extent of droplet size reduction the influence of pressure is higher than the temperature. Furthermore, it can clearly be seen that the size of droplets can only be decreased up to a pressure of 450 bar and a temperature of 45 °C. Any further increase of these parameters does not give smaller droplets. The diagram of PCS radius and D99-values illustrates this observation whereas the D50-values (Fig. 1) can be decreased over the total pressure and temperature range. So no further droplet reduction can take place above values of 450 bar and 45 °C but the droplet size distribution can still be influenced and leads to changes in the D50 values.

3.1.2. Results after three cycles

After the third cycle at low pressure and low temperature the largest droplets were obtained. In comparison to the results after the first cycle a distinct reduction of particle size could be observed after the third cycle. So an increase of cycle numbers results in droplets with lower size. The characteristics of Figs. 4–6 show, as also observed after the first cycle, that pressure has more influence on droplet size reduction than temperature. In comparison to the first cycle it is noticeable that the mentioned plateau with no further reduction of droplet size when increasing temperature or pressure could also be observed at the D50 value level in Fig. 4. As the PCS values in Fig. 6 show similar characteristics at this pressure range and temperature the smallest particles and a similar narrow distribution can be

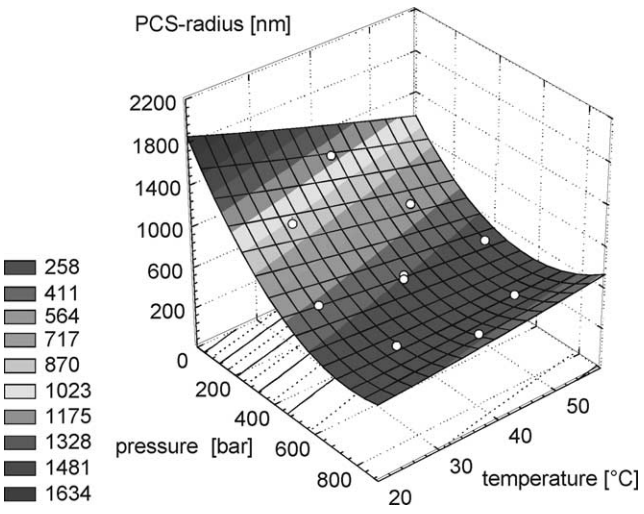

Fig. 3. PCS-radius after 1 homogenization cycle ($R^2 = 0.9398$).

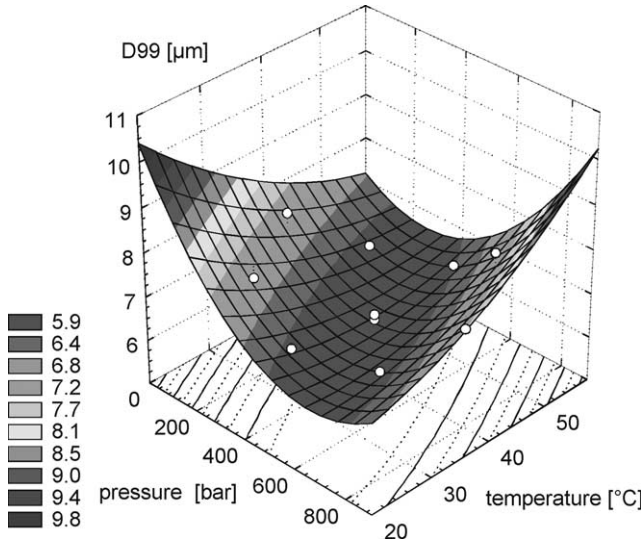

Fig. 5. D99-values after 3 homogenization cycles ($R^2 = 0.8688$).

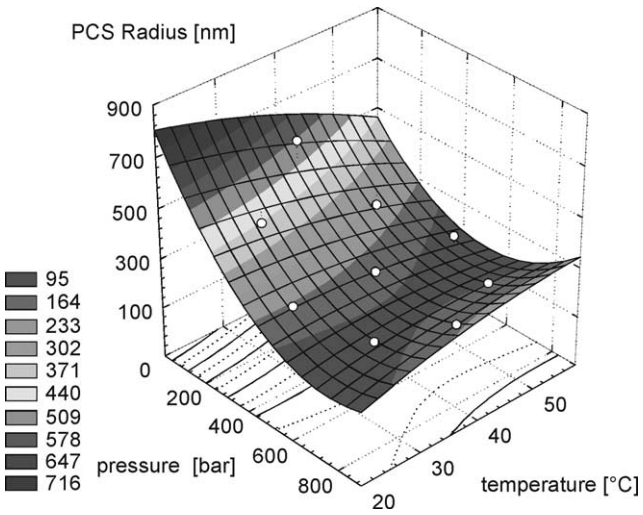

Fig. 6. PCS-radius after 3 homogenization cycles ($R^2 = 0.8995$).

found there. Above these values a further input of energy would not result in smaller droplets. In comparison to the results after the first cycle the D99 values (Fig. 5) are displaced to lower pressure and temperature and a further increase of energy input is accompanied by an increase of droplet size. This could be explained as follows: above a certain pressure/temperature combination the increase of production parameter even results in smaller droplets, but the amount of emulsifier is too low for sufficient stabilization of the new interface. A coalescence of some oil droplets takes place to compensate the energy excess and the droplet size increases. This phenomenon could mainly be observed regarding the D99 values which indicates that after the third cycle only a few larger oil droplets occur.

3.1.3. Results after five cycles

Also after the fifth homogenization cycle at low pressure and low temperature the largest droplets were obtained. This observation can only be hold true for the D50 values (Fig. 7)

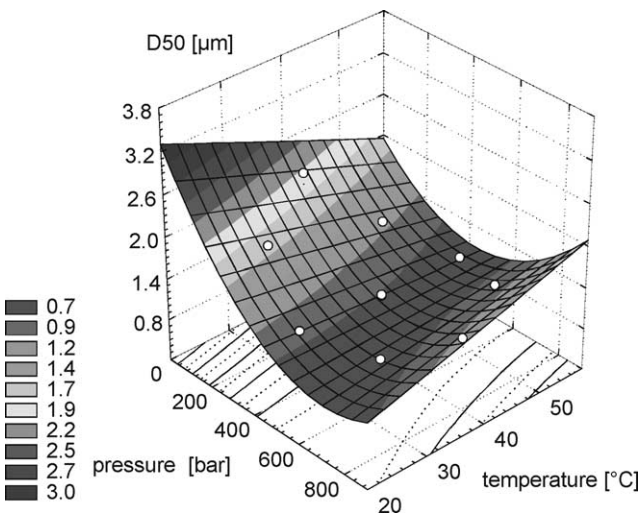

Fig. 7. D50-values after 5 homogenization cycles ($R^2 = 0.9253$).

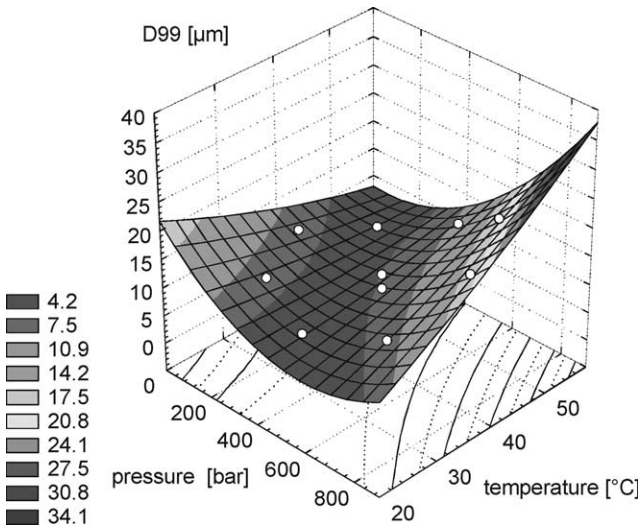

Fig. 8. D99-values after 5 homogenization cycles ($R^2 = 0.9742$).

and PCS radius. With increasing pressure and temperature the droplet size has reached a minimum much earlier. The reduction of droplet size from third to fifth cycle is not so distinct as the reduction from the first to the third cycle. The results of the laser diffraction analysis, particularly the D99 values (Fig. 8) show that the influence of temperature increases with higher homogenization cycles. The phenomenon after the third cycle of droplet size magnification at high energy input could also be seen after the fifth cycle and now concerning also the results of the PCS measurement in Fig. 9 and the D50 values in Fig. 7. These results indicate an increasing amount of bigger oil droplets due to coalescence. Fig. 8 shows that this increase of droplet size especially takes place at high temperature and high pressure. At high temperature or high pressure only the emulsions were damaged to a lesser extent. So a certain input of energy has to be exceeded, then the coalescence begins. The temperature has less influence on the droplet size reduction than

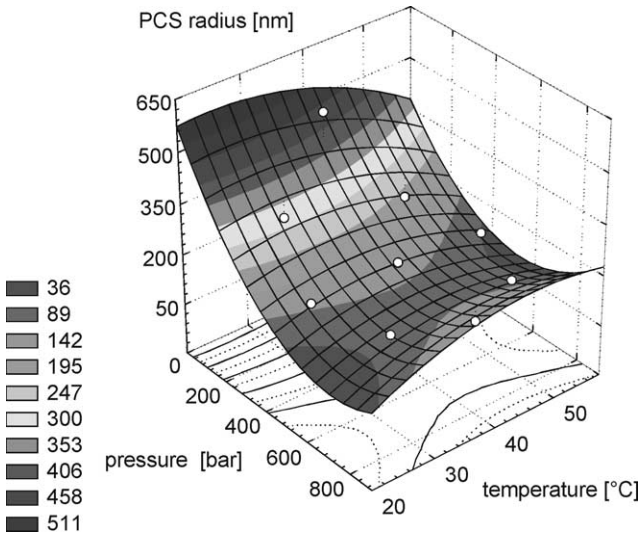

Fig. 9. PCS-radius after 5 homogenization cycles ($R^2 = 0.8509$).

the pressure; however, the influence of temperature and pressure on an increase of droplet size due to coalescence is the same, because the pressure is a direct parameter of the homogenization process. The temperature only decrease the viscosity or enhances the diffusion of the emulsifier to the new interface, thus temperature has no direct influence on droplet size reduction. Against this there is no correlation between coalescence of oil droplets and the homogenization process, because coalescence only occurs if the amount of emulsifier is not sufficient to stabilize the new interface, where the pressure is not influenced. However temperature directly influences the coalescence, due to its effect on viscosity of the disperse phase or the diffusion of the surfactant which are essential parameters for the emulsion stabilization.

3.2. Determination of the encapsulation rate

Intensification of the energy supply into the system results in smaller oil droplets. Therefore it could be expected that the inner water droplets (including the drug) in the oil droplets could be destroyed too and that the active drug migrates to the outer water phase. Regarding Figs. 10–12 this course of encapsulation rate could not be observed when pressure and/or temperature were increased. The variation coefficient calculated from the values of the centre-point ranged between 10 and 20% and the calculated surface plot does not show an acceptable correlation to the according model. A possible explanation could be that the encapsulation rate could not be influenced by the relation between the sizes of oil to inner water droplets but this is not probable. With this series of emulsions the inner W/O emulsion has not changed, so the size of the inner water droplets was constant. This would have the consequence that only the size of inner water droplets influence the encapsulation rate but the relation between oil to inner water droplets could be neglected. One last explanation could be the fact that the choice of diclofenac sodium as active ingredient and marker substance was not suitable. Due to its low saturation concentration of 6 mg/ml (in phosphate buffer pH 7.2) [18] and a distribution coefficient log *D* of 1.7 (octanol/water pH 6.8) [19] the detectable amount of diclofenac sodium in the water phase is very low and a high amount of drug is located in the oil phase. Probably this low concentration of diclofenac sodium in the outer water

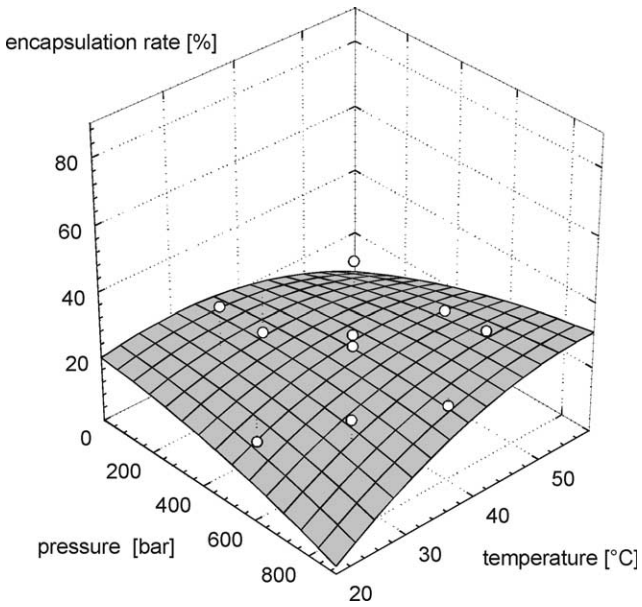

Fig. 11. Encapsulation rate of diclofenac sodium after 3 homogenization cycles.

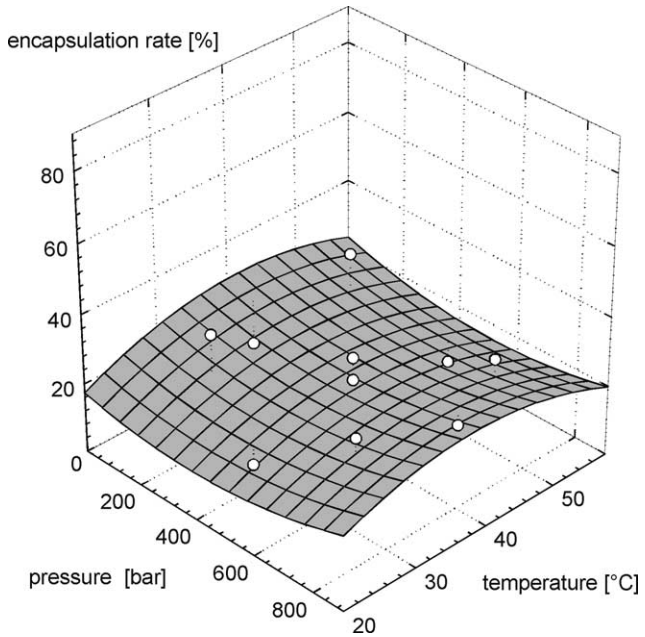

Fig. 10. Encapsulation rate of diclofenac sodium after 1 homogenization cycle.

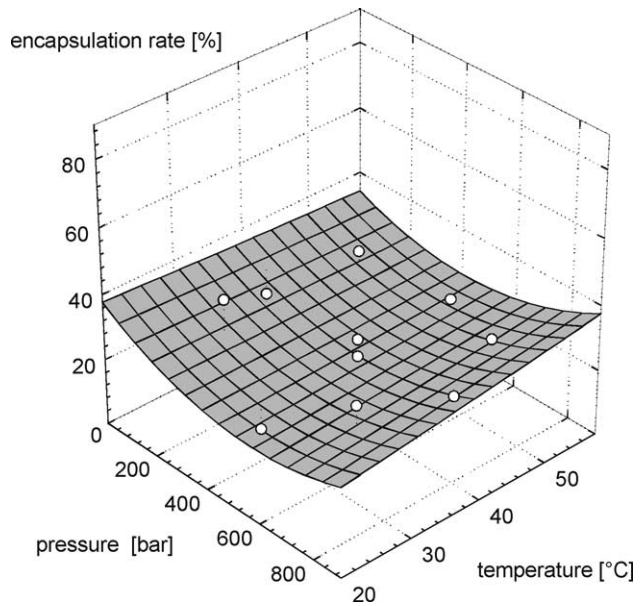

Fig. 12. Encapsulation rate of diclofenac sodium after 5 homogenization cycles.

phase prevents the detection of differences in the encapsulation rate in dependence to droplet size; they are covered by the variation of the sample preparation and the variations of the analytical method.

3.3. Influence of the inner W/O emulsion on the encapsulation rate of the W/O/W emulsion

It could be shown that increasing the production conditions during the second step results in smaller oil droplets which contain water but the encapsulation rate stayed nearly constant. With further trial it should be evaluated if different W/O emulsion with different sizes of inner water droplets would influence the encapsulation rate of diclofenac sodium in the W/O/W multiple emulsions. For the choice of different formulations especially W/O emulsions with a wide range of droplet size were chosen. This wide droplet size was obtained due to the use of W/O emulsifiers in the concentrations 10, 20 and 30% in the oil phase. Once again diclofenac sodium was dissolved in the inner water phase. The emulsions were produced at constant conditions in the first (45 °C, 500 bar, 5 cycles) and in the second (30 °C, 300 bar, 2 cycles) production step. The size of the water droplet in the W/O emulsion and also the size of oil droplets in the W/O/W emulsion was determined.

In Fig. 13 it is obvious that multiple emulsions with a W/O emulsifier concentration of 30% have the highest encapsulation rate of diclofenac sodium. For emulsions with 20% emulsifier the encapsulation rate decreases to a value of about 40% and in the first emulsion with a concentration of 10% any active ingredient is encapsulated. In comparison to the relation of droplet size between inner water droplets and dispersed oil droplets (Fig. 14), for emulsions with 10% emulsifier the water droplets are bigger than the oil droplets. Due to this the inner droplets of the W/O emulsion will be destroyed during the second production step and it is evident that after the production diclofenac sodium as encapsulated substance is no longer entrapped and is exchanged into the continuous outer water

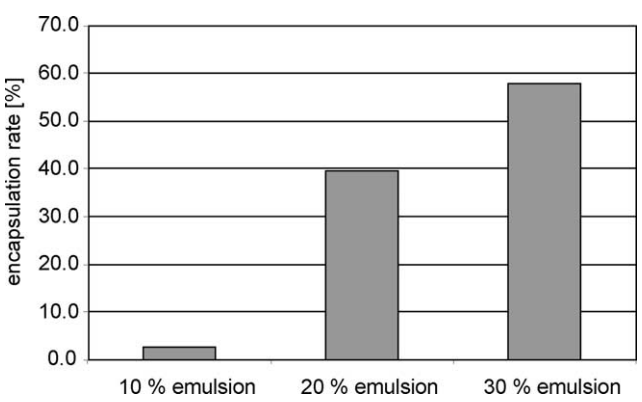

Fig. 13. Encapsulation rate of different W/O/W-emulsions.

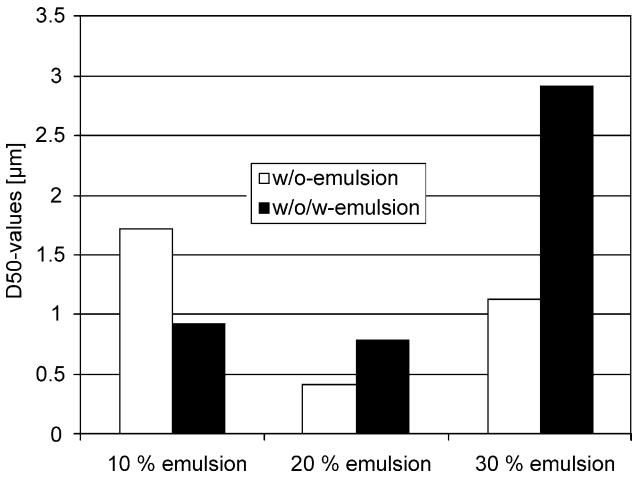

Fig. 14. Differences in droplet size between W/O- and W/O/W-emulsion.

phase. For 20% emulsions the relations are different, the oil droplets are bigger than the water droplets and an encapsulation rate can be determined, because after production some inner water droplets are still intact. If the distance of droplet size between water and oil droplets increases to significantly bigger oil droplets, the encapsulation rate would also increase, as clearly shown for the 30% emulsions. With these results it was shown that the encapsulation rate relates to the droplet size of the inner water.

4. Conclusion

It was shown that pressure and temperature as production parameter in the second step influence the size of oil droplets in the W/O/W multiple emulsion.

Using different W/O emulsions for the second emulsification step the finest W/O emulsion led to W/O/W emulsions with the highest encapsulation rate. To prevent a production-induced reduction of encapsulation rate the inner water droplets have to be much smaller than the oil droplets in the multiple emulsion.

Due to its low saturation concentration and its distribution into the oil phase there is no relevance to encapsulate diclofenac sodium in the inner water phase of a multiple emulsion. Due to the same reason diclofenac sodium is unsuitable as marker substance, if the inner W/O emulsion is left constant.

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