

Stability Studies on Colloidal Suspensions of Polyurethane Nanocapsules

K. Bouchemal*, S. Briançon, F. Couenne, H. Fessi, and M. Tayakout

Laboratoire d'Automatique et de Génie des Procédés, UMR-CNRS 50 07 CPE Lyon,
Université Claude Bernard Lyon 1, 43, Boulevard du 11 Novembre 1918, F-69622 Villeurbanne, Cedex, France

Generally nanocapsules suspensions are a colloidal system in a metastable state, there is aggregation due to attraction and repulsion forces between particles. The objective of this work was to bring the role of the polymeric membrane in the protection of the active drug against damaging caused by external agents and to select the monomer which leads to obtain stable formulation with the highest possible payload of the active drug. The stability testing involving visual aspect, particle size measurement, transmission electron microscopy (TEM) examination, and drug loss was conducted after 6 months of storage at different temperatures (4, 25, and 45°C). The colloidal suspensions of nanocapsules were obtained using the combined interfacial polycondensation and spontaneous emulsification, the technique was used to encapsulate α -tocopherol using polyurethanes polymers. It is a one step procedure: An organic phase composed of a water miscible solvent (acetone), lipophilic monomer (Isophorone diisocyanate IPDI), oil, and a lipophilic surfactant, is injected in an aqueous phase containing hydrophilic monomer (diol with various molecular weight: 1,2-ethanediol (ED), 1,4-butanediol (BD), and 1,6-hexanediol (HD)) and hydrophilic emulsifying agent. The water miscible solvent diffuses to the aqueous phase, the oil precipitates as nano-droplets, and the two monomers react at the interface, forming a membrane around the nanoemulsion leading to nanocapsules. A good physical stability of suspensions corresponds to absence of symptoms such as sedimentation or agglomeration, significant size change and α -tocopherol degradation due to external agents such as oxygen, temperature, and ultraviolet (UV) irradiation. The size of nanocapsules before storage was about 232 ± 3 , 258 ± 29 , and 312 ± 4 nm for ED, BD, and HD, respectively. After 6 months of storage, polyurethanes nanocapsules possess good stability against aggregation at 4 and 25°C. Comparing results obtained using different monomers, it reveals that the polyurethane based on HD offers good protection of α -tocopherol against damaging caused by the temperature and UV irradiation.

Keywords: Nanocapsules, Polyurethane, UV Irradiation, α -Tocopherol, Stability.

1. INTRODUCTION

In recent years nanoparticles have received considerable attention due to the importance of its potential applications; related to their ability to protect the encapsulated drug and to deliver it at its site of action with the desired kinetics. The attraction of nanoparticles for application in personal care and cosmetics as well as in health care is due to numerous advantages: First, nanoparticles are suitable for a controlled delivery of active ingredients through the skin because the solid matrix of the nanoparticles immobilize the active ingredient leading to differences in release kinetics and allowing controlled delivery of actives. Jenning and co-workers¹ have demonstrated that retinol encapsulated in solid lipid nanoparticles (SLN) can be effectively

delivered to the upper skin layers and prolonged delivery was obtained with nanoparticles loaded by retinol in comparison with a nano-emulsion. Moreover, nanoparticles possess a high specific surface area and therefore adhesive properties; the formation of a film of nanoparticles reduces the water evaporation and increases the occlusive effect.² Finally, the fluidity nature of the system (at reasonable oil concentrations) as well as the absence of any thickeners may give them a pleasant aesthetic character and skin feels,³ the physico-chemical stability of the suspension during long term storage is one of the key properties of the system.

In this paper, the stability of polyurethane based nanocapsules was studied; nanocapsules were obtained using the combined interfacial polycondensation and spontaneous emulsification technique.^{4,5} This is an original technique which offers numerous advantages compared to the classical interfacial polycondensation⁶⁻⁸

*Author to whom correspondence should be addressed.

Kawthar.bouchemal@u-psud.fr

Université Paris-Sud 11, UMR CNRS 8612. Faculté de Pharmacie, 5, Rue J.B. Clément. 92296 Châtenay Malabry, France.

Indeed, classical interfacial polycondensation leads to capsules with a mean size diameter varying from 10 to 200 μm . An important quantity of mechanical energy is needed to obtain capsules with lower size diameter. Thus, there is a risk of monomer hydrolysis by reaction with water and the active agent should diffuse to the continuous phase, decreasing the yield of encapsulation. The interfacial polycondensation combined to spontaneous emulsification technique, allows the spontaneous formation of a nano-emulsion immediately followed by the polymer interfacial formation and the nano-suspension is obtained without supplying high energy quantity. In this paper, nanocapsules are loaded by α -tocopherol, this active agent is widely used as an antioxidant in many medical and cosmetic applications,^{9,10} but is rapidly degraded, because of its oxygen, heat, and light sensitivity.¹¹ The encapsulation of α -tocopherol in polymer nanocapsules should protect it from the degradation factors and enhance its efficacy in different kind of cosmetic formulations. Since all of its formulation has to avoid contact with air, heat, or light, experiments attempt to reproduce the samples aging using accelerated conditions.

The objective of this study was twice: The first was to determine the protective effect of the polymer membrane

on α -tocopherol against external factors; the drug degradation was measured after 6 months of storage at 4, 25, and 45°C and after UV irradiation. The second objective of this work was to study the colloidal stability of the nanocapsules suspension, through the evolution of the particle size after 6 months of storage at different temperatures. Visual samples examination and TEM microscopic observations were also conducted, to improve the size distribution measurement, and detect aggregation and creaming. The colouring of the sample can also be evocative of the α -tocopherol degradation as it will be seen below.

2. EXPERIMENTAL DETAILS

2.1. Materials

The monomers: 1,2-ethanediol ($\text{HO}-(\text{CH}_2)_2-\text{OH}$), 1,4-butanediol ($\text{HO}-(\text{CH}_2)_4-\text{OH}$), 1,6-hexanediol ($\text{HO}-(\text{CH}_2)_6-\text{OH}$) as hydrophilic monomer and isophorone diisocyanate ($\text{C}_{12}\text{H}_{18}\text{N}_2\text{O}_2$) as lipophilic monomer, and the solvent acetone were obtained from Sigma, France. The active agent α -tocopherol was obtained from Engelhard (Coletica), France. The emulsifiers (Span[®] 85, Tween[®] 20) were supplied by SEPPIC, France.

2.2. Nanocapsules and Nano-Emulsion Preparation

The methodology for obtaining nanocapsules by combined spontaneous emulsification and interfacial polycondensation was described in previous study.^{4,5} It is a one step procedure, the organic phase composed of a

solvent (acetone 40 ml), lipophilic monomer (IPDI 10^{-3} mol), oil (α -tocopherol 400 mg), and a lipophilic surfactant (86 mg Span[®] 85), is injected in an aqueous phase containing hydrophilic monomer with various molecular weight (ED, BD, HD 10^{-2} mol), hydrophilic emulsifying agent (136 mg Tween[®] 20) and 80 ml of water. The water miscible solvent diffuses to the aqueous phase, the oil precipitates as nano-droplets, and the two monomers react at the interface, forming a membrane around the nanoemulsion leading to nanocapsules. The same technique was used to obtain the nano-emulsion (NE) but without using monomers.¹² For all preparations, the totality of the solvent (acetone) as well as 40 ml of the water is removed by evaporation during 45 min under reduced pressure. The chemical structure of the polymer was checked using Fourier Transform Infra-Red spectroscopy (FT-IR) and nuclear magnetic resonance spectroscopy (¹H-NMR). The characterization of the chemical structure of the polyurethane polymer is informative about the performance of the encapsulation experiments, and it allows the quantitative identification that the right polymer was formed.^{4,5}

2.3. Samples Preparation for the Stability Studies

The suspensions stability was studied using an environment support of 25% butylene glycol (5 ml) and of 0.1% microbicide (20 μl of parabens) and distilled water (13 ml) during 6 months, 2 ml of each preparation was preserved in the environment support at different temperatures; 4°C in the refrigerator, 25°C and 45°C in a thermoregulated room protected from light. Nanocapsules colloidal stability studies include the macroscopic observations for detecting the presence of agglomerations, the size distribution and the mean size measurements, the observations of the produced nanocapsules using TEM. The chemical stability implies the dosage of α -tocopherol contained in the nanocapsules and nano-emulsion before and after storage. The protection role of the polymeric membrane on the α -tocopherol stability in the nanocapsules against damaging caused by UV irradiation was also investigated through α -tocopherol dosage after UV irradiation of the nanocapsules.

2.4. Particle Size and Size Distribution

The particle size distribution of the prepared nanocapsules was measured in an aqueous suspension by static laser light scattering on a LS 230 COULTER[®] granulometer. The LS 230 measures particle size distribution using the principle of laser diffraction. To measure the particle size distribution, 0.5 ml suspension is introduced in the measure compartment (125 ml of purified water). The results are presented as volume fraction distribution.

2.5. Nanocapsule Morphology

The shape and the morphology of the produced nanoparticles were investigated by transmission electron microscopy observations (Topcon[®] EM002B, 200 kV).

As usually, samples were prepared by placing a drop of preparation on a collodion support on cooper grids,¹³ followed by negative staining with an aqueous solution of sodium phosphotungstate, 14 phosphotungstic acid¹⁵, or uranyl acetate, in this investigation, we use a solution of phosphotungstic acid.

2.6. UV Irradiation

20 ml of each sample were diluted in 20 ml of water; 1 ml of each preparation was deposited on a plaque containing 12 holes (3 essays for each sample: ED, BD, HD, and nano-emulsion) and exposed to UV irradiation using irradiator (Suntest CP+Atlas). Irradiation conditions were: 250 Watt, dose: 2600 kJ·m⁻² at 37°C. The water evaporation was limited because samples were hermetically closed using adaptable top and parafilm. After irradiation, samples were collected and α -tocopherol concentration in the capsules was determined according to the method described below.

2.7. α -Tocopherol Dosage

2.7.1. Sample Preparation

The evaluation of drug loading in nanoparticles needs the separation of the free form of the drug from the encapsulated one. The separation technique most widely used by researchers is ultracentrifugation.^{16–18} In the present study, total α -tocopherol concentration ($T\alpha$) was determined after dissolution of nanoparticles suspension in isopropanol followed by 30 min of ultrasounds agitation. Free α -tocopherol ($F\alpha$) was determined after separation of loaded-nanoparticles from the aqueous medium by an ultracentrifugation technique (Optima™ Ultracentrifuge, BEKMAN-COULTER Instruments, USA). Ultracentrifugation of 4.5 g was carried out in Beckman Optima™ tubes (11 ml) housed in a MLA-80 rotor. All tubes were centrifuged at 45 000 rpm for 20 minutes at 20 °C.

The supernatant contains loaded nanocapsules; the rest of the centrifuged preparation representing the aqueous phase contains the free (non-encapsulated) α -tocopherol. The concentration of α -tocopherol in loaded nanoparticles ($L\alpha$) was obtained as follows: After centrifugation, the supernatant was isolated, the volume was completed to 4.5 g using (Tween® 20/water) mixture at 0.33% (w/w) of Tween® 20 in water. This washing operation was reproduced two times to eliminate α -tocopherol remaining at the surface of nanocapsules. Then, 10 mg of the washed nanocapsules were digested in 20 ml of isopropanol followed by 30 min of ultrasounds agitation. α -tocopherol concentration was determined by HPLC analysis and the yield of encapsulation (Y) was calculated as follows (Eq.(1)).

$$Y = (L\alpha/T\alpha) * 100 \quad (1)$$

$L\alpha$: α -tocopherol concentration in loaded nanoparticles.

$T\alpha$: Total concentration of α -tocopherol introduced in the preparation.

The α -tocopherol standard solution was prepared using isopropanol as solvent.

2.8. HPLC Analysis

The samples were assayed using HPLC analysis, a ChromoQuest thermoquest France SA instruments was used with a Lichrospher RP 18-e (5 μ m, 125×4 mm). The mobile phase consisted of methanol. The flow rate was 1 ml/min. A 40- μ l sample size was injected. α -tocopherol was detected using a variable wavelength UV detector at 285 nm and results were quantified through the use of the equation derived from the slope of the standard curve prepared for α -tocopherol at 285 nm.

3. RESULTS AND DISCUSSION

The principle of the polyurethane film formation by interfacial polycondensation is well described in different reviews and publications, it is generally applied to the formation of microcapsules.^{19–22} With the general process described in previous articles^{4,5} polyurethane nanocapsules about 200–350 nm were obtained, all samples presented homogeneous aspect with a white color. The size distribution of polyurethane nanocapsules obtained from ED, BD, and HD is showed in Figure 1 and the mean size and the yield of encapsulation before storage are represented in Table I. The size distributions are very similar and their mean particle sizes are 232±3, 258±29, and 312±4 nm, respectively.⁴

The aim of the present work was to select the monomers leading to the production of a stable formulation with the highest possible protection of α -tocopherol. After 6 months of storage at 4°C, 25°C, and 45°C, the visual aspect of the nano-emulsion did not change; neither creaming nor sedimentation could be observed, the preparation of nanocapsules remained white and fluid but the colouring of the nano-emulsion changed from white to yellow and brown at 25°C and 45°C, respectively.

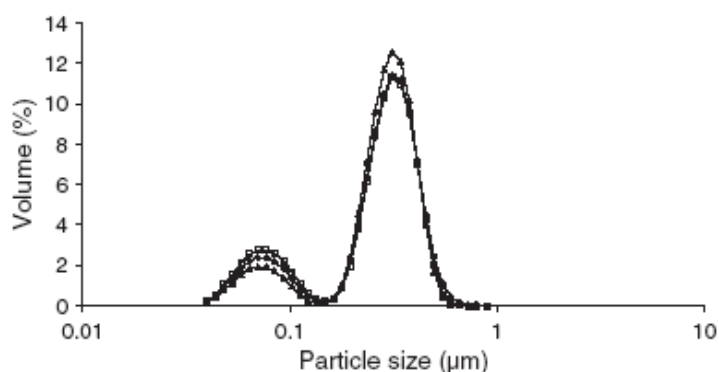


Fig. 1. Size distributions of α -tocopherol nanocapsules with polyurethane polycondensates obtained from ED (\square), BD (\diamond), and HD (\blacktriangle).

Table I. Granulometric analysis of the nanocapsules prepared from different diols as measured by small-angle light scattering and α -tocopherol contents in polyurethane nanocapsules before conservation.

Diol	Diol molecular weight (g · mol ⁻¹)	Mean size (nm)	Yield of encapsulation of α -tocopherol (% w/w)
HO-(CH ₂) ₂ -OH 1,2-ethanediol	62.07	232 ± 3	85
HO-(CH ₂) ₄ -OH 1,4-butanediol	90.12	258 ± 29	87
HO-(CH ₂) ₆ -OH 1,6-hexanediol	118.17	312 ± 4	89

The change of the colouring is due to active agent degradation as it will be seen below. The slow variation of size is mainly manifested by the growth of the larger population at the expense of the smaller, strongly suggesting Ostwald ripening as a mechanism.²³

3.1. Size Determination

The size of nanocapsules is usually found to be between 100 and 500 nm and depends on several factors: The nature and the initial concentration of monomers²⁴ and of the loaded drug, the amount of surfactants, the ratio of organic solvent to water, the concentration of oil in the organic solution, or the rate of diffusion of the organic phase into the aqueous phase. In this study focused on the influence of the storage conditions, the evolution of the particle mean was studied. Before conservation, the particle mean size was about 232±3, 258±29, and 312±4 nm for ED, BD, and HD. In previous works,⁴ After 6 months of storage, the particle mean size remains stable at 4°C but increases about few nanometers at 25°C (Table II).

At 45°C, the particle size increases significantly with the presence of a second population of capsules about 1800 nm. This population represents 10% (volume fraction) of the total sample examined in the granulometer; it is probably due to capsules agglomeration. On Figure 2 (2-1 and 2-2), TEM observations show that capsules are still spherical but the sample is heterogeneous in size.

Table II. Granulometric analysis after conservation of the nanocapsules suspensions prepared with different oils as measured by small-angle light scattering.

Temperature of the storage (°C)	Diol	Small size population (nm)	Large size population (nm)	Volume fraction of the large size population (%)
4	ED	239 ± 4	None	0
	BD	261 ± 11	None	0
	HD	320 ± 9	None	0
25	ED	300 ± 6	None	0
	BD	319 ± 16	None	0
	HD	341 ± 8	None	0
45	ED	375 ± 9	1839 ± 19	10
	BD	376 ± 7	1837 ± 20	10
	HD	270 ± 11	1808 ± 14	11

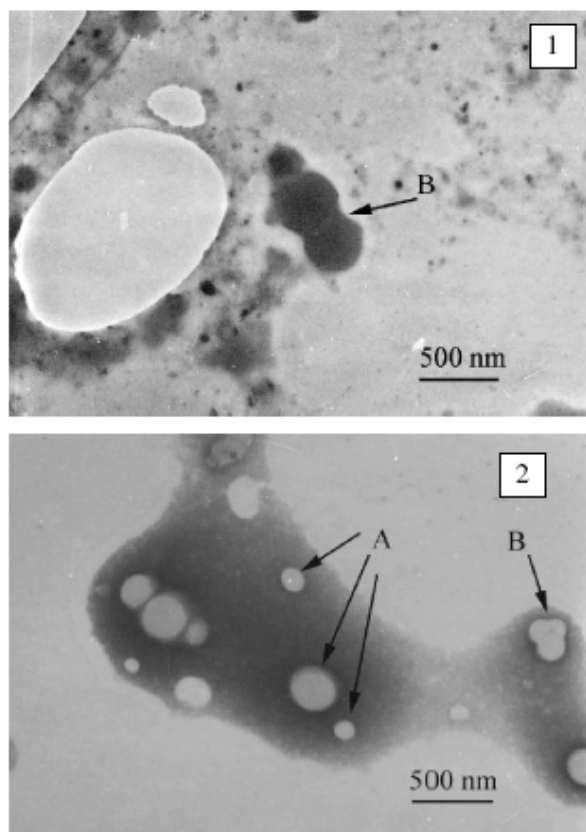


Fig. 2. Transmission electron microscopy positive (1) and negative observation (2) of 1,6-hexanediol based nanocapsules after 6 months at 45°C. Non agglomerated (A) and agglomerated (B) nanocapsules.

Two populations of nanocapsules are detected: A population of non agglomerated capsules (A) with an average size diameter less than 400 nm representing the majority of the particles and agglomerated nanocapsules (B) constituting the other population. These results are in harmony with the size distribution, which reveal two populations of capsules. The size and the homogeneity of the samples are important especially for cosmetic uses when capsule size is limitative for their permeation through the skin. Nevertheless; this problem can be resolved because the two populations of capsules could be separated using filtration if necessary.

To select samples for cosmetic applications, besides the size of capsules, the active agent stability is a very important criterion, for this reason, the encapsulated active drug concentration after the storage was determined by the method previously described in paragraph 2.6 and compared to results obtained with the nano-emulsion.

3.2. α -Tocopherol Dosage

The percentage of degraded α -tocopherol (X) in the samples was calculated from the yield of encapsulation before and after storage (Y and Y') as follows (Eq.2). Only the concentration of the active drug in form of α -tocopherol was determined, and no metabolites molecules obtained after chemical reactions such as oxidation.²⁵ In this work, the term "degradation" designs all α -tocopherol metabolites, so the yield of encapsulation after storage corresponds to the quantity of intact active drug really present

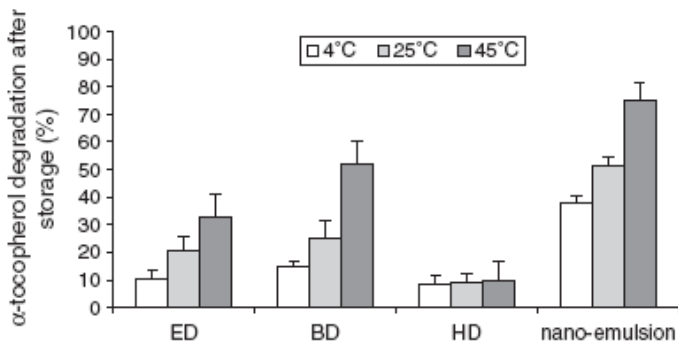


Fig. 3. Percentage of α -tocopherol degradation after 6 months of storage of nanocapsules at different temperatures.

in the nanocapsules or in the nano-emulsion. The results are represented on Figure 3.

$$X = ((Y - Y')/Y) \cdot 100 \quad (2)$$

Y : Yield of encapsulation before storage.

Y' : Yield of encapsulation after storage.

At 4 °C, the degradation of the encapsulated α -tocopherol is less than 15% (10%, 15%, and 8% for ED, BD, and HD, respectively), while 38% of α -tocopherol contained in the nano-emulsion is degraded. Even when stored in a refrigerator, α -tocopherol will be subject to chemical reactions with atmospheric oxygen. In comparison with the nano-emulsion, nanoparticles protect the active ingredient against chemical degradation by the surrounding dispersing medium e.g., chemical reactions with the environmental factors, even at 4°C. The percentage of α -tocopherol degradation is more important at 25°C than 4°C even for nanocapsules, 20%, 25%, and 9% of active agent was degraded (ED, BD, and HD, respectively). At 25°C and 45°C the nano-emulsion is subject to an important degradation (51% and 75%, respectively).

3.3. UV Irradiation

Exposure of the body surface to UV irradiation produces free radicals in the skin, generally leading to premature aging. The inclusion of an antioxidant agent such as α -tocopherol is important to protect the skin against irradiation damaging. In this study, nanocapsules and nanoemulsion loaded by α -tocopherol were exposed to UV irradiation, and the percentage of damaged α -tocopherol was calculated from the yield of encapsulation before and after irradiation in accordance to the technique of α -tocopherol dosage described above. Figure 4 represents the evolution of the α -tocopherol percentage of degradation after UV irradiation, it is clear that the capsule wall protects the active agent against UV damaging and the efficacy of the protection is proportional to molecular weight of the hydrophilic monomer.

Comparing the results obtained using different monomers; it appears that the polyurethane based on HD offers good protection of α -tocopherol against damaging caused

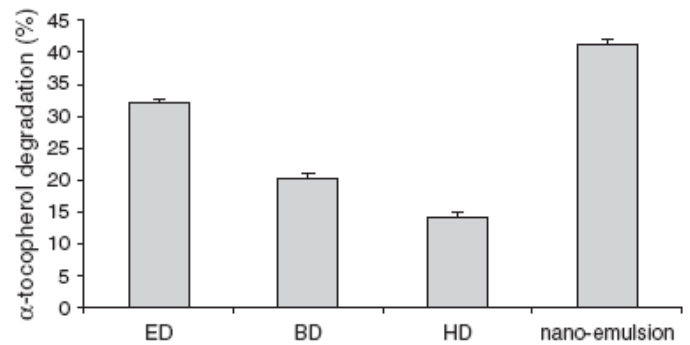


Fig. 4. Percentage of α -tocopherol degradation after UV irradiation.

by the temperature and UV irradiation. These results are in close relation with the physical and chemical properties of the obtained polymeric membrane: Actually, parameters such as mechanical properties and flexibility of the polymer, membrane porosity, and capsule thickness have a direct influence on drug stability in the delivery system. Therefore, the physicochemical evaluation of a nanoparticulate polymeric membrane is technically difficult to achieve owing to the very small size of the particle in the final colloidal suspension product. For this reason, an experimental study of the polymers on plane geometry will help us to extract information about the relation between initial properties of the monomers and the final properties of the polymer. Such as described in Bouchemal and coworkers paper,²⁴ mathematical modelling of the nanocapsule formation present another solution to estimate the wall thickness and to establish a correlation between the monomer molecular weight and the membrane properties such as the thickness and the polymer swelling, porosity, flexibility, and resistance to the storage conditions. The model described allows to predict correctly the thickness of the capsule membrane and to simulate the influence of the monomers concentrations. It gives the possibility to increase the membrane thickness and so to increase its protection role by varying the initial concentrations of the monomers. These results indicate that the use of initial high concentration in lipophilic monomer can significantly increase the thickness rate of the wall of the capsule; the initial concentration of hydrophilic monomer has effects only on the final time of reaction.²⁴

4. CONCLUSION

Tocopherol is one of the most important and essential natural antioxidant, which can protect biological membranes against lipid peroxidation as radical injury, but its sensibility to the temperature and UV irradiation, is limitative of the efficiency of preparations based on α -tocopherol. In this paper, the stability of α -tocopherol was increased after its encapsulation in polyurethane nanocapsules. After nanocapsules characterization, it reveals that drug loaded carriers such as nanoparticles are an attractive opportunity to increase the stability of the formulations (e.g. size) and

to limit the chemical degradation due to external agents such as oxygen, temperature, and UV irradiation. After 6 months of storage, polyurethanes nanocapsules possess good stability against aggregation at 4°C and 25°C. The storage of α -tocopherol at higher temperatures leads to an important degradation for the nano-emulsion (75% at 45°C) in comparison with nanocapsules (32%, 52%, and 10% at 45°C for ED, BD, and HD, respectively). Dosage before and after experiments storage reveals that the membrane of the capsule presents a protection barrier against active agent degradation due to the temperature and UV irradiation, this protection is more important when the monomer molecular weight is increased. Quite a satisfactory system regarding the nanometric size and the stability requirements was the IPDI-HD system containing the polyurethane membrane formed from IPDI and HD. Despite the fact that these results are interesting, some aspects would require further work; a more precise characterization of the polymers, in particular chemical degradation due to the conservation conditions would help much in understanding their resistance to storage. A complementary study on the influence of molecular weight of the monomers on capsule properties should be fulfilled in order to control the membrane thickness and so to increase its protection role.²⁴

Acknowledgments: The authors are grateful to Prof. BLANCHIN M.G and Teodorescu V.S (Claude Bernard University Lyon-1, "Département de Physique des Matériaux," France) for their help for the microscopic observations. Thanks to BONNET I and PERRIER E from Engelhard (Coletica) Company for financial supporting.

References and Notes

1. V. Jennings, M. Schäfer-Korting, and S. Gohla, *J. Control. Rel.* 15, 115 (2000).
2. V. Jennings, A. Gysler, M. Schäfer-Korting, and S. Gohla, *Eur. J. Pharm. Biopharm.* 49, 211 (2000).
3. O. Sonnevile-Aubrun, J.-T. Simonnet, and F. L'Alloret, *Adv. Coll. Interf. Sci.* 108–109, 145 (2004).
4. K. Bouchemal, S. Briançon, E. Perrier, H. Fessi, I. Bonnet, and N. Zydowicz, *Int. J. Pharm.* 269, 89 (2004).
5. K. Bouchemal, S. Briançon, H. Fessi, Y. Chevalier, I. Bonnet, and E. Perrier. *Mater. Sci. Eng. C* (2005), in press.
6. S.K. Yadav, C. Kartic, K. Khilar, and A.K. Suresh, *J. Membrane Sci.* 125, 213 (1997).
7. N. Zydowicz, P. Chaumont, and M.L. Soto-Portas, *J. Membrane Sci.* 189, 41 (2001).
8. K. Bouchemal, N. Zydowicz, S. Briançon, P. Chaumont, and H. Fessi, *J. Microencapsulat.* 20, 635 (2003).
9. A. Dingler, R.P. Blum, H. Niehus, R.H. Muller, and S. Gohla, *J. Microencapsulat.* 16, 751 (1999).
10. J.S. Trivedi, S.L. Krill, and J.J. Fort, *Eur. J. Pharm. Sci.* 3, 241 (1995).
11. M. Birringer, D. Drogan, and R. Brigelius-Flohe, *Free Radical Biology and Medicine* 31, 226 (2001).
12. K. Bouchemal, S. Briançon, E. Perrier, and H. Fessi, *Int. J. Pharm.* 280, 241 (2004).
13. A. Malaiya and S.P. Vyas, *J. Microencapsulat.* 5, 243 (1988).
14. B. Seijo, E. Fattal, L. Roblot-Treupel, and P. Couvreur, *Int. J. Pharm.* 62, 1 (1990).
15. S. Guinebretière, S. Briançon, H. Fessi, V.S. Teodorescu, and M.-G. Blanchin, *Mater. Sci. Eng. C, Biomim. Mater. Sens. Syst.* 21, 137 (2002).
16. R. Gaspar, V. Preat, and M. Rolland, *Int. J. Pharm.* 68, 111 (1991).
17. M.J. Alonso, C. Losa, P. Calvo, and J.L. Vila Jato, *Int. J. Pharm.* 68, 69 (1991).
18. V.H.K. Li, R.W. Wood, J. Kreuter, T. Harmia, and J.R. Robinson, *J. Microencapsulat.* 3, 213 (1986).
19. R. Arshady, *J. Microencapsulat.* 6, 13 (1989).
20. P.W. Morgan, Interfacial polymerisation. In *Encyclopaedia of Polymer Science*, 2nd edn., Wiley, New York (1987), Vol.3, p.231.
21. L.J.J.M. Janssen and K. te Nijenhuis, *J. Membr. Sci.* 65, 56 (1992).
22. L.J.J.M. Janssen and K. te Nijenhuis, *J. Membr. Sci.* 65, 69 (1992).
23. M. Porras, C. Solans, C. González, A. Martínez, A. Guinart, and J.M. Gutiérrez, *Coll. Surf. A: Physicochem. Eng. Aspects* 249, 115 (2004).
24. K. Bouchemal, F. Couenne, S. Briançon, H. Fessi, and M. Tayakout, *AiChE.* 52, 1 (2006).
25. M. Birringer, D. Drogan, and R. Brigelius-Flohe, *Free Radical Biology and Medicine* 31, 226 (2001).

Received: 21 December 2005. Revised/Accepted: 5 April 2006.