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A New Process for Drug Loaded Nanocapsules Preparation Using a Membrane Contactor

Catherine Charcosset and Hatem Fessi
Laboratoire d'Automatique et de Génie des Procédés, Villeurbanne Cedex 69 622, France

ABSTRACT In this paper, we describe a new process for the preparation of drug loaded nanocapsules using a membrane contactor which may be scaled up for industrial applications. Nanocapsules are prepared according to the nanoprecipitation method. The organic phase (solvent, polymer, oil, and drug) is pressed through the pores of an ultrafiltration membrane via the filtrate side. The aqueous phase (water and surfactant) circulates inside the membrane module, and sweeps away the nanocaspules forming at the pore outlets. Two model drugs are selected for the preparation of drug loaded nanocapsules: indomethacin and vitamin E. It is shown that indomethacin loaded nanocapsules with a mean diameter of 240 nm and vitamin E loaded nanocapsules with a mean diameter of 230 nm are obtained with a 150,000 daltons ultrafiltration membrane, a transmembrane pressure of 3 bar, and a crossflow rate of 1.7 $m.s^{-1}$. High fluxes are also obtained (around 0.6 $m^3/h.m^2$), leading to the preparation of 1.8 10^{-3} m^3 drug loaded nanocapsules in 8 min. The advantage of this membrane contactor compared to other processes for drug loaded nanocapsules preparation is shown to be its scale-up ability.

KEYWORDS Membrane reactor, Membrane contactor, Drug loaded nanocapsule, Nanoparticle

INTRODUCTION

Nanoparticles are solid colloidal particles ranging in size from about 10 to 1,000 nm. They consist of macromolecular materials and can be used as drug carriers. Nanoparticles is a collective name for nanospheres and nanocapsules. Several review articles have highlighted the ability of such nanoparticles to reduce associated adverse effects of various drugs (Allémann et al., 1993a; Couvreur et al., 2002). Some of the commonly reported methods of preparing nanoparticles from biodegradable polymers include solvent evaporation (Song et al., 1995), salting out procedure (Allémann et al., 1993b), nanoprecipitation (Fessi et al., 1989; Govender et al., 1999), and monomer polymerization (Hincal & Kas, 2000).

The present study investigates the preparation of drug loaded nanocapsules using a membrane contactor, according to the nanoprecipitation method.

Address correspondence to Catherine Charcosset, Laboratoire d'Automatique et de Génie des Procédés, UMR CNRS 5007, UCB Lyon 1, ESCPE-Lyon, 43 Bd du 11 Novembre 1918, Villeurbanne Cedex 69 622, France; Fax: 04-72-43-16-99; E-mail: charcosset@lagep.univ-lyon1.fr

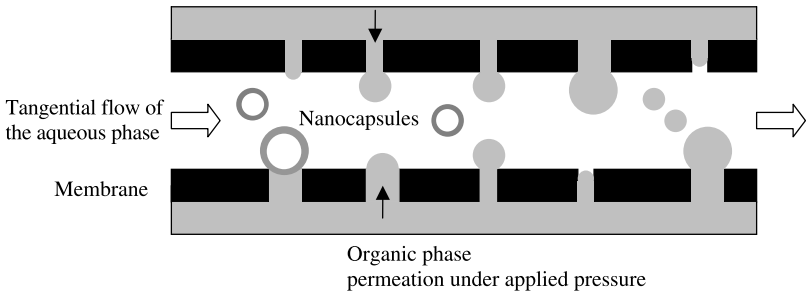

FIGURE 1 **Schematic Drawing of the Membrane Contactor for the Preparation of Nanoparticles.**

Nanoprecipitation is an easy and reproducible method involving dispersion of preformed polymers (Fessi et al., 1989; Govender et al., 1999). It is based on the interfacial deposition of a polymer following displacement of a semi-polar solvent miscible with water from a lipophilic solution. The organic phase (solvent, polymer, eventually oil, and drug) is added dropwise under moderate stirring into the aqueous phase (water and surfactant).

A schematic drawing of the nanocapsules preparation with a membrane contactor is shown in Fig. 1. The organic phase is pressed through the membrane pores allowing the formation of small droplets. The reaction occurs between the droplets of the organic phase and the aqueous phase flowing tangentially to the membrane surface. The organic phase contains the solvent, polymer, oil, and drug, and the aqueous phase contains water and surfactant. This membrane contactor for nanoparticles preparation was investigated previously for the preparation of nanospheres, with two methods: the nanoprecipitation and the interfacial polymerization methods (Charcosset & Fessi, 2005). The membrane contactor was shown to allow large scale production of nanoparticles. In this study, we investigate the preparation of drug loaded nanocapsules with the membrane contactor. Two drugs (indomethacin and vitamin E) are chosen as model drugs for the preparation of drug loaded nanocapsules. Also, the influence of process parameters (aqueous phase velocity and organic phase pressure) on nanoparticles size and organic phase flow rate is reported.

MATERIALS AND METHODS

Reagents

The solvents acetone, acetonitrile, and tetrahydrofuran were purchased from VWR (Fontenay-sous-Bois, France). The surfactant Tween 20 was supplied by Sigma-Aldrich. The oil Mygliol was obtained from Condea (Hamburg, Germany) and vitamin E from Coletica (Neuilly-sur-Seine, France). The polymer used was polycaprolactone 10,000 (Sigma-Aldrich). Indomethacin was supplied by Sigma-Aldrich as a dried power. Ultra-pure water prepared with an Alpha Q System (Millipore, Saint-Quentin-en-Yvelines, France) was used throughout the experiments.

Experimental Set-Up

The experimental set-up used for the experiments is shown in Fig. 2. It includes a pump (PCM, Vanves, France), a Micro Carbosep/Kerasep crossflow filtration device (Rhodia Orelis, Saint-Maurice-de-Beynost, France) equipped with two manometers placed at the module inlet and module outlet, and a valve placed at the module outlet. The aqueous phase is stirred continuously with an impeller RW 20 (Ika-Werk, Staufen, Germany). The organic phase is placed in a pressurized vessel (Millipore), equipped with a manometer, connected to a nitrogen bottle and to the membrane module on the filtrate side. The experiments are conducted at 22°±1°C.

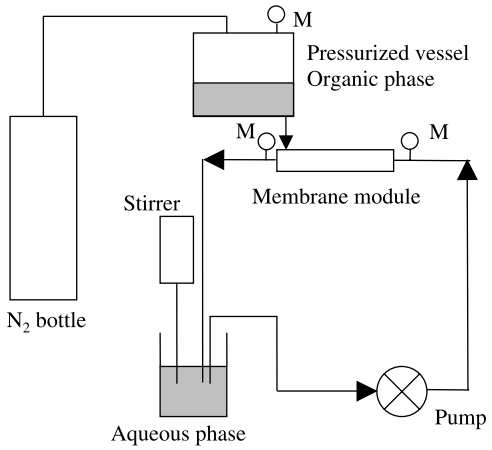

FIGURE 2 **Experimental Set-up for the Preparation of Nanoparticles. M: Manometer.**

The membrane used is a 150,000 daltons cutoff Kerasep ceramic membrane with an active ZrO_2 layer on an Al_2O_3–TiO_2 support (Rhodia Orelis, France). The membrane length is 0.4 m, the inner diameter 6 10^{-3}m and the outer diameter 1 10^{-2} m. Therefore, the active membrane surface area is 7.5 10^{-3} m^2.

Nanoparticles Preparation

The formulations used for the nanoparticles preparation are reported in Table 1. Formulation 1 is used for the preparation of unloaded nanospheres, formulation 2 for the preparation of indomethacin loaded nanocapsules, and formulation 3 for the preparation of vitamin E loaded nanocapsules.

The following protocol is used for the experiments. The aqueous phase is stirred continuously and circulates tangentially to the membrane surface. The organic phase is placed in a pressurized vessel. At time $t = 0$, the valves connecting the pressurized vessel to the nitrogen bottle and to the filtrate side of the membrane module are opened. The aqueous phase immediately turns milky with bluish opalescence as a result of the formation of nanoparticles. The experiment is stopped when air bubbles start to appear in the tube connecting the pressurized vessel to the membrane module. The mean dispersed phase flux (J) is calculated as the organic phase volume (V) divided by the reaction time (Δt) and the membrane surface (A):

$$J = V/(\Delta t \cdot A)$$

At the end of the experiment, a sample in the nanoparticles preparation is taken for size analysis.

TABLE 1 Formulations Used for the Nanoparticle Preparations

Organic phase:	Aqueous phase:
Formulation 1: Nanospheres	
Acetone: 0.6 l	Water: 1.2 l
Polycaprolactone: 15 g	Tween 20: 2.04 g
Formulation 2: Indomethacin loaded nanocaspules	
Acetone: 0.6 l	Water: 1.2 l
Polycaprolactone: 15 g	Tween 20: 2.04 g
Indomethacin: 600 mg	
Mygliol: 30 g	
Formulation 3: Vitamin E loaded nanocapsules	
Acetone: 0.6 l	Water: 1.2 l
Polycaprolactone: 15 g	Tween 20: 2.04 g
Vitamin E: 15 g	

The microfiltration membrane is then regenerated. The washing is performed by flushing the membrane module with 1 10^{-3} m^3 tetrahydrofuran at 20°C during 5 min (transmembrane pressure 0 bar), 0.5 10^{-3} m^3 tetrahydrofuran in the pressurized vessel (pressure 3 bar), and finally with 15 10^{-3} m^3 pure water. The membrane permeability is measured at the beginning of each experiment. A new cycle of cleaning is performed if the permeability is found smaller than 80% of its initial value.

Nanoparticles Characterization

Nanoparticles were characterized for their size using laser light scattering measurements on a Zetasizer 3000 HS (Malvern Instruments, France).

The drug amount loaded in the nanoparticles was determined by the following method with 0.5 ml of the nanoparticle preparation centrifuged in an Ultracentrifuge Optima™ (Beckman) at 60,000 rpm at 20°C during 1 h. The nanoparticle supernatant was then removed and dissolved in 20 mL acetonitrile. The light absorption of these preparations was measured on a spectrophotometer (Safas, Monaco) at 319 nm for indomethacin loaded nanocapsules and at 295 nm for vitamin E loaded nanocapsules. Drug entrapment efficacy is calculated as the ratio of the final drug content in the nanoparticles to the initial drug content, expressed in percent. This ratio is of great relevance for industrial applications, since it indicates the proportion of drug lost during the manufacturing process.

RESULTS

Preparation of Nanospheres

Unloaded nanospheres were prepared according to the nanoprecipitation method and Formulation 1. The 150,000 daltons cutoff membrane was used, with a crossflow velocity of 1.7 $m.s^{-1}$ and an organic phase pressure of 3 bar. The reproducibility of nanoparticle size was estimated on 3 measurements to be ±5% and the reproducibility of the organic phase fluxes on 3 measurements ±5%. The morphological analysis of the colloidal suspension showed a unimodal size distribution, with a polydispersity index close to 0.15. Six months of stability study indicates no marked differences in the nanocapsules size.

TABLE 2 Characteristics of Nanoparticles Preparations Using a Membrane Contactor

Formulation	Nanoparticles mean diameter (nm)	Organic phase flow rate ($m^3/h.m^2$)	Drug entrapment efficacy (%)
Formulation 1: Nanospheres	290	0.40	—
Formulation 2: Indomethacin loaded nanocapsules	240	0.45	73
Formulation 3: Vitamin E loaded nanocapsules	230	0.60	100

The organic phase was pressed through the membrane pores allowing the formation of nanoparticles with 290 nm mean diameter and an organic phase flowrate of 0.40 $m^3/h.m^2$ (Table 2). This flowrate corresponds to the preparation of 1.8 10^{-3} m^3 of nanoparticles in 12 min, which confirms the potentiality of this process for industrial applications. An extrapolation to membranes with larger surface area (i.e., Kerasep membrane module with a membrane area of 0.34 m^2) would lead to the preparation of 0.082 m^3 of nanoparticles in the same time. This high flux is explained by the organic phase formulation for which the main component is the solvent, which leads to a low viscosity and to favorable interfacial tensions in membrane pores.

Preparation of Indomethacin Loaded Nanoparticles

Indomethacin, well-known for its anti-inflammatory properties, was chosen as a model drug for the preparation of drug nanocapsules (Ammoury et al., 1990). Indomethacin loaded nanocapsules were prepared according to Formulation 2 with the nanoprecipitation method. The 150,000 dalton membrane was used with a crossflow velocity of 1.7 $m.s^{-1}$ and an organic phase pressure of 3 bar. This method yields to the formation of spherical nanocapsules which consist of an oily cavity (where indomethacin is dissolved) surrounded by a thin wall formed by polycaprolactone interfacial deposition. The indomethacin loaded nanocapsules with 240 nm mean diameter have been obtained with an organic phase flowrate of 0.45 $m^3/h.m^2$ (Table 2). This flowrate corresponds to the preparation of 1.8 10^{-3} m^3 indomethacin loaded nanocapsules in 11 min, which confirms again the potentiality of this process for industrial applications. The drug entrapment efficacy is found equal to 73%.

Indomethacin loaded nanocapsules were also prepared in a becher for a total volume of 180 mL. The aqueous phase (120 mL) was added dropwise to the organic phase (60 mL). The obtained indomethacin loaded nanocapsules have a mean diameter around 230 nm, which is close to the size obtained with the membrane contactor for the preparation of a larger amount ($\times 10$).

Preparation of Vitamin E Loaded Nanocapsules

Vitamin E was chosen as a second model drug for the preparation of drug loaded nanocapsules. This active agent is widely used as an antioxidant in many medical and cosmetic applications (Bouchemal et al., 2004). Vitamin E loaded nanocapsules were prepared according to formulation 3 with the nanoprecipitation reaction. The 150,000 dalton membrane was used with a crossflow velocity of 1.7 $m.s^{-1}$ and an organic phase pressure of 3 bar. This method yields to the formation of spherical nanocapsules which consist of an oily cavity surrounded by a thin wall formed by polycaprolactone interfacial deposition. Vitamin E is present only in the oily cavity; and the drug entrapment efficacy is equal to 100%. The 1.8 10^{-3} m^3 vitamin E loaded nanocapsules were prepared in 8 min (organic phase flux 0.60 $m^3/h.m^2$) (Table 2). The nanocapsule size is found equal to 230 nm.

TABLE 3 Influence of the Aqueous Phase Crossflow Velocity on the Nanoparticles Size and on the Organic Phase Flow Rate

Aqueous phase crossflow velocity (m/s)	Nanoparticles mean diameter (nm)	Organic phase flow rate ($m^3/h.m^2$)
1.1	294	0.28
1.65	272	0.19
3	241	0.21
6.5	251	0.17

Organic phase pressure = 3 bar.

TABLE 4 Influence of the Organic Phase Pressure on the Nanoparticles Size and on the Organic Phase Flow Rate

Organic phase pressure (bar)	Nanoparticles mean diameter (nm)	Organic phase flow rate ($m^3/h.m^2$)
3	272	0.19
4.5	262	0.28
6	261	0.53

Aqueous phase flow velocity = 1.7 $m.s^{-1}$.

Influence of Process Parameters

The two main parameters of the process are the aqueous phase crossflow velocity and the organic phase pressure. Their influence on nanoparticles size and organic phase flux was investigated in the case of the nanoprecipitation formulation 1 and for the 150,000 daltons ultrafiltration membrane. The influence of crossflow velocity was investigated for velocities between 1 and 6.5 $m.s^{-1}$ (Table 3). We observed that the nanoparticles size and the organic phase flux slightly decrease with an increase in crossflow velocity. It must be also underlined that the organic phase fluxes are smaller than those obtained previously, this fact being explained by the smaller initial membrane permeability.

The influence of the organic phase pressure is shown in Table 4 for a crossflow velocity of 1.7 $m.s^{-1}$, and pressures between 3 and 6 bar. The organic phase pressure has almost no effect on the nanoparticles size. On the other hand, it greatly influences the organic phase flux, increasing increases from 0.2 to 0.5 $m^3/h.m^2$. In case of industrial applications, a high pressure may be advised as it does not change the nanoparticles size but decreases considerably the processing time. This low influence of process parameters on nanoparticles size suggests that the nanoprecipitation phenomena (interfacial deposition of the polymer following displacement of a semi-polar solvent) are preponderant on membrane phenomena (formation of droplets through membrane pores).

CONCLUSIONS

The present study investigates the preparation of drug loaded nanocapsules involving the nanoprecipitation method and a membrane contactor. The organic phase (solvent, polymer, oil, and drug) is introduced through the membrane pores via the filtrate side, to form droplets which react with the aqueous phase (water and surfactant). The nanocapsules formed at the pore outlets are swept away by the aqueous phase flowing tangentially to the membrane surface. The membrane pores (having a diameter close to 60 nm for this ultrafiltration membrane) behave as parallel capillaries for the introduction of one phase in the other phase for the preparation of nanocapsules. Such a membrane process for the introduction of one phase in the other phase has been introduced recently and is termed "membrane contactor" (Drioli et al., 2003; Sirkar et al., 1999). It has been used previously for the preparation of emulsions ("membrane emulsification") (Charcosset et al., 2004; Joscelyne & Trägårdh, 2000) and for the preparation of precipitates (Chen et al., 2004; Jia et al., 2003). The main drawback of the membrane emulsification process is the low fluxes obtained, which does not allow industrial applications. In case of preparation of drug loaded nanocapsules, the membrane contactor offers the advantage of high fluxes, allowing the preparation of large volumes in small times, and therefore possible process scaling-up.

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