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NYLON MEMBRANE FORMATION IN BIOCATALYST MICROENCAPSULATION: PHYSICOCHEMICAL MODELLING

DENIS PONCELET

Biotechnology Research Institute, National Research Council Canada, 6100 Royalmount Ave., Montreal, Que., H4P 2R2 (Canada)

BRIGITTE PONCELET DE SMET and RONALD J. NEUFELD

Department of Chemical Engineering, McGill University, 3480 University St., Montreal, Que. H3A 2A7 (Canada)

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Summary

A simple model of nylon membrane formation was developed for enzyme or biological cell microencapsulation. The model which involves physicochemical effects was shown to be useful for optimizing membrane formation and maintaining high activity of the encapsulated biocatalyst. Simulations were conducted to define the effect of the determinant parameters (pH, solvent, temperature, diamine) on membrane characteristics and formation rate. The membrane thickness was shown to be independent of the microcapsule diameter (25–400 μm) when the pH is controlled during formulation (pH 9.5). At pH levels less than 9.5 and hexanediamine concentrations less than 200 *mM*, thin membranes resulted and pH values dropped considerably during formulation. The use of an acidic buffer and a diamine with lower dissociation constant and higher solubility in the organic solvent, permitted nylon membrane formation at lower pH levels, potentially resulting in less inactivation of the biocatalyst during immobilization. The membrane thickness was seen to be strongly dependent on the solvent polarity, however the solvent selection is limited by the toxicity to the biocatalyst. Control of temperature was needed for reproducibility of the membrane thickness.

Introduction

Microcapsules consist of particles or liquid droplets with diameters ranging from 1 to 1000 μm contained within a 20 to 500 nm polymeric membrane [1]. Applications are found in medicine [2,3] (artificial kidney; artificial blood), pharmacology [4] (controlled drug release), agriculture [5] (long term sustained release for fertilizers and pesticides) and bioprocessing [6] (immobilization of biological cells, preventing washout and minimizing shear damage [7]). The membrane provides protection from environmental denaturation of the encapsulated material [2,6], storage of volatile or unstable products for

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extended periods [8], and capabilities for controlling the release of microencapsulated contents to the external medium [2,5].

Wittbecker and Morgan [9] found that nylon (polyamide) may be obtained by interfacial polymerization between a diacid chloride in an organic phase and a diamine in an aqueous phase:

$$n(NH_2\text{-R-}NH_2) + n(ClOC\text{-R}'\text{-}COCl) \rightarrow (NH\text{-R-}NH\text{-}CO\text{-R}'\text{-}CO)_n + 2n\ HCl$$

Chang [1,2] has applied this polycondensation reaction to the microencapsulation of urease in hemolysate solution:

Equal volumes of hemolysate and alkaline solution of 1,6-hexanediamine (carbonate buffer, pH 9.8) are mixed together and emulsified in chloroform–cyclohexane solution containing an emulsifying agent. Interfacial polymerization occurs at the interface of the aqueous drops when an organic solution of sebacyl chloride is added to the emulsion. The reaction is stopped by dilution of the organic phase and the microcapsules are finally transferred in aqueous phase by aid of a surfactant.

A few variations on this procedure have been proposed [10,11] leading to differences in size distribution, membrane thickness and mechanical resistance of the microcapsules.

The control of any process involving microencapsulation requires control of the membrane properties and its formation. The interfacial polymerization process is well known but in the context of nylon production [12]. Several papers deal with membrane formation in microencapsulation [10,13], however a systematic approach is needed to better understand nylon membrane formation in microencapsulation.

Microencapsulation within a nylon membrane is initiated at pH levels of 9.5 or higher. The high concentration of monomers, buffer and filler compounds predominating in the microcapsule core lead to a high osmotic pressure and ionic strength. The organic solvent mixture (chloroform–cyclohexane) is toxic to a number of encapsulated biological materials [14,15]. These conditions are often not suitable for medical and food applications and may promote enzyme denaturation or cell death.

A simple phenomenological and mathematical model of nylon membrane formation was developed in the present study to better understand the controlling parameters. Improvements in microencapsulation efficiencies, membrane properties and maintenance of biocatalyst activities during the formulation procedure may be anticipated with a better understanding of the interfacial polymerization process in organic/aqueous two phase systems.

Modelling of membrane formation

The condensation between the organic acid chloride and the primary amine is an extremely rapid reaction (10^2–10^4 mol-sec^{-1}) [9]. Morgan and Kwolek

[12] demonstrated that polymerization occurs at the exterior face of the membrane exposed to the organic phase. Thus the limiting process is the transfer of the diamine from the microcapsule core through the membrane.

As the membrane by itself constitutes a barrier to the diamine transfer, diffusion is significantly slower in the membrane than in the microcapsule core [16,17]. The diamine concentration, C_{DA}, is then assumed to be uniform throughout the microcapsule. Similarly, physicochemical equilibrium and homogeneity were assumed in the microcapsule core. The pH may thus be computed from thermodynamic laws.

The dispersion of the aqueous phase within the organic solvent is achieved by high mechanical mixing. Homogeneity is then assumed in the bulk of the organic phase. Since polymerization occurs on the external surface, mass transfer of the diacid chloride is not considered.

Figure 1 illustrates the concentration profile of the diamine monomer from the interior aqueous phase, $[DA]_a$, to the exterior organic solvent phase, $[DA]_o$.

As a consequence of the fast polymerization rate, the diamine concentration at the outer membrane side and in the organic phase, $[DA]_o$, remains low and can be assumed equal to 0 (Fig. 1). Thus, partition of the diamine between the membrane and the organic phase was not considered.

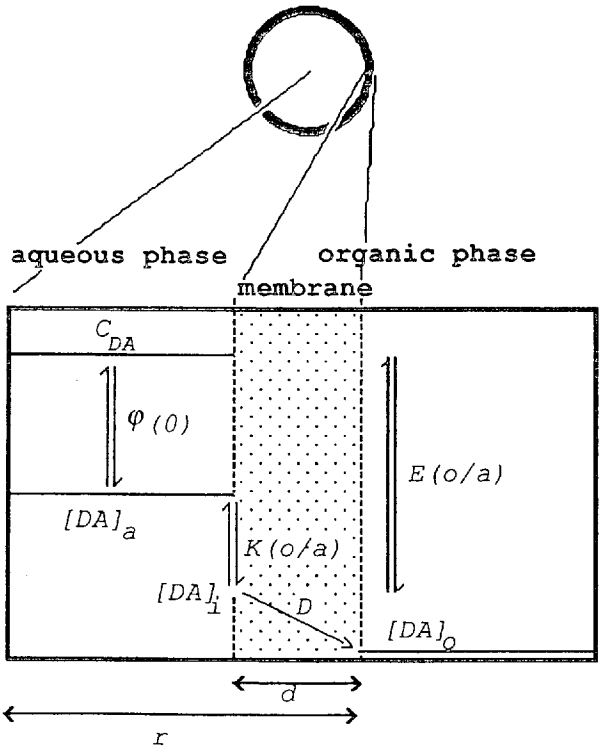

Fig. 1. Schematic evolution of the diamine concentration through the microcapsule (r: distance from the microcapsule center).

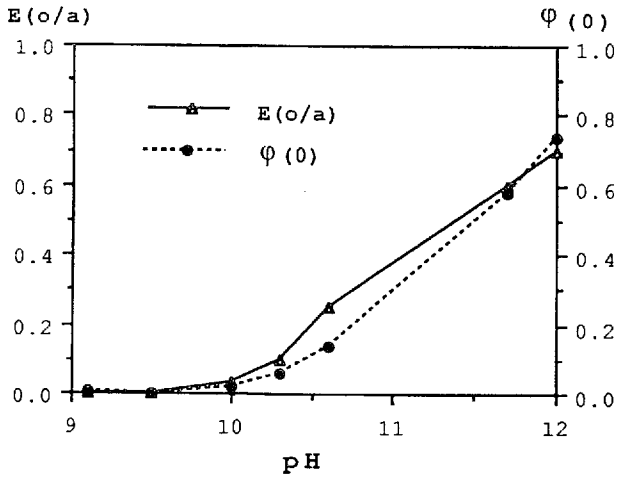

Fig. 2. Total hexanediamine partition coefficient, $E(o/a)$, between water and chloroform, and neutral hexanediamine fraction, $\varphi(0)$, as function of pH ($E(o/a)$ computed from [9], $\varphi(0)$ computed following [17], $T=20°C$).

The internal membrane interface corresponds to the interface between the aqueous and organic phases. The diamine partition is generally considered at the equilibrium and described by the total diamine partition coefficient, $E(o/a)$, or the ratio of diamine concentrations at the inner membrane side, $[DA]_i$, to the total diamine concentration in the aqueous phase, $[DA]_a$, as illustrated in Fig. 1 (step change of diamine concentration at the organic and aqueous interface) [12,18]:

$$E(o/a)=\frac{[DA]_i}{C_{DA}} \tag{1}$$

Morgan and Kwolek [12] demonstrated that the total diamine partition coefficient, $E(o/a)$ varies with pH. Moreover, Fig. 2 shows that the total diamine partition coefficient, $E(o/a)$ is proportional to the acid–base partition function of the diamine in the aqueous phase, $\varphi(0)$, which represents the fraction of the diamine in the neutral form [19]:

$$\varphi(0)=\frac{[DA]_a}{[DA]_a+[HDA^+]_a+[H_2DA^{2+}]_a}=\frac{[DA]_a}{C_{DA}} \tag{2}$$

where C_{DA} is the total diamine concentration in the aqueous phase. $\varphi(0)$ can be computed from thermodynamic constants over the range of pH considered [19].

To avoid the pH dependence, it is more useful to describe the diamine partition by the neutral diamine partition constant, $K(o/a)$:

$$K(o/a)=\frac{[DA]_i}{[DA]_a}=\frac{[DA]_i}{\varphi(0)\,C_{DA}}=\frac{E(o/a)}{\varphi(0)} \tag{3}$$

Second and third terms are obtained by combination with eqns. (1) and (2). $E(o/a)$ is equal to $K(o/a)$ when $\varphi(0)$ is equal to 1 or when the diamine is entirely in the neutral form. It is then assumed that only neutral diamine may be transferred to the organic phase.

Considering the fast reaction compared to slow diamine diffusion through the membrane, the molar nylon production rate was assumed equal to the diamine transfer rate through the membrane. The diamine consumption rate per unit membrane surface, q, is equal to the product of the diamine diffusion constant, D, and the neutral diamine concentration gradient through the membrane (quasi-steady state Fick's diffusion):

$$q = D\frac{[DA]_i - [DA]_o}{\delta} \text{ (mol-m}^{-2}\text{-sec}^{-1}\text{)} \tag{4}$$

where δ is the membrane thickness and the index i and o refer respectively to the internal and external membrane face (Fig. 1).

Microcapsules have very thin membranes ($\delta \approx 20$–500 nm) in contrast to their diameter ($d \approx 20$–$1000\ \mu$m) [20]. The external surface area of the membrane, A, was assumed constant during the membrane formation and the curvature of the membrane was not taken into consideration in eqn. (4).

Assuming $[DA]_o = 0$, eqn. (4) combined with eqn. (3) (second term) becomes:

$$q = D\frac{K(o/a)\varphi(0)C_{DA}}{\delta} \text{ (mol-m}^{-2}\text{-sec}^{-1}\text{)} \tag{5}$$

Assuming a constant diameter, d, the volume, V, of the microcapsule is equal to:

$$v = \frac{\pi d^3}{6} \text{ (m}^3\text{)} \tag{6}$$

and the membrane surface area, A, is:

$$A = \pi d^2 \text{ (m}^2\text{)} \tag{7}$$

The consumption rate of diamine inside the microcapsules is thus given:

$$\frac{dC_{DA}}{dt} = -\frac{qA}{V} = -\frac{6D}{d}\frac{K(o/a)\varphi(0)\ C_{DA}}{\delta} \text{ (mol-m}^{-3}\text{-sec}^{-1}\text{)} \tag{8}$$

From the stoichiometry of the nylon formation equation, it follows that chloride ion formation equals:

$$\frac{dC_{Cl}}{dt} = \frac{12D}{d}\frac{K(o/a)\varphi(0)\ C_{DA}}{\delta} \text{ (mol-m}^{-3}\text{-sec}^{-1}\text{)} \tag{9}$$

The nylon membrane density may be expressed as the concentration of po-

lyamide dimeric unit (one molecule of diamine plus one molecule of diacid chloride) per liter, symbolized by $[Dimer]_m$. This concentration is obtained by dividing the nylon concentration, expressed in $g\text{-}l^{-1}$, by the molecular weight of one polyamide dimeric unit. The nylon concentration in the membrane, $[Dimer]_m$, was assumed constant under all polymerization conditions.

The change in the membrane thickness, δ, is given by the diamine consumption rate (eqn. (5)) divided by the nylon concentration in the membrane, $[Dimer]_m$:

$$\frac{d\delta}{dt}=\frac{q}{[Dimer]_m}=\frac{D}{[Dimer]_m}\frac{K(o/a)\varphi(0)\,C_{DA}}{\delta}\ (m\text{-}sec^{-1}) \qquad (10)$$

Equations (8), (9) and (10) are relatively complex and non-linear (considering pH effects on the acid base partition of the diamine). This equation set cannot be solved analytically and the system must be integrated by a numeric method. The integration was done following Euler's method after discretization of eqns. (8), (9) and (10). The computation of the pH value and the diamine partition, $\varphi(0)$, was performed according to [19]. Simulations demonstrated that integration must be performed with a maximum step change of 5% for the membrane thickness.

Results

Except when specified, the parameter values used in the following simulation were those presented in Table 1. The values were chosen based on microcapsule formation procedures described in the literature (initial concentrations [1]) or published data (partition coefficients [12]; dissociation constants [21,22]). In the case of the diffusion constant, data were not available. Its

TABLE 1

Parameter value use into simulation[a]

Microcapsule diameter, d	200	μm
Nylon membrane concentration, $[Dimer]_m$	0.57×10^{-3}	$mol\text{-}m^{-3}$
Diamine diffusion constant, D	1.3×10^{-12}	$m^2\text{-}sec^{-1}$
Diamine partition constant, $K(o/a)$	79×10^{-3}	
Initial diamine concentration, C_{DA}	400	mM
Initial chloride concentration[b], C_{Cl^-}	718	mM
Initial pH value	9.5	
Temperature, T	20	°C
Diamine: hexanediamine-1,6		
No buffer, pH constant		

[a]Value or conditions considered for simulation except when specified.
[b]pH and diamine concentration dependent.

value was chosen to result in membrane thickness values similar to those found in the literature [23,24].

Figure 3 illustrates the thickness of the nylon membrane as a function of the reacted diamine. Microcapsules ranging from 25 to 500 μm diameter have a reported membrane thickness of about 200 nm [24]. Under these conditions, Fig. 3 illustrates that only a few percent of the diamine is consumed in membrane formation. More than 90% of the diamine remains unreacted. If the diamine were totally consumed in the reaction, microcapsules of 200 μm would have a membrane thickness approaching 60 μm.

Figure 4 presents a typical evolution of the membrane thickness, δ, with time, t, at either constant or freely varying pH. The membrane formation rate,

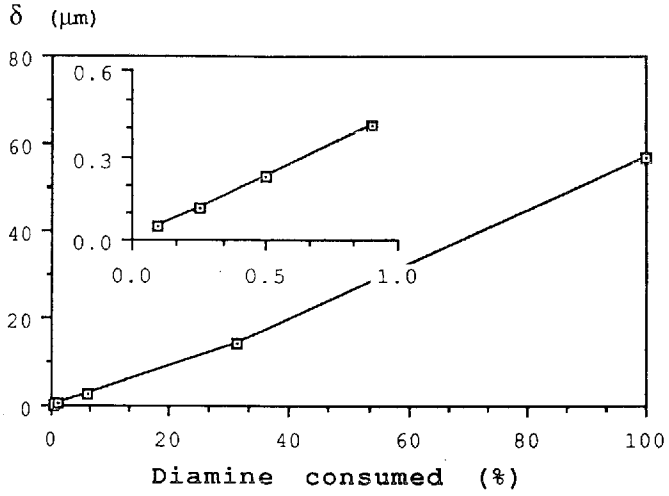

Fig. 3. Influence of the diamine consumption, ΔC_{DA}, on the nylon membrane thickness, δ (see Table 1 for polymerization conditions).

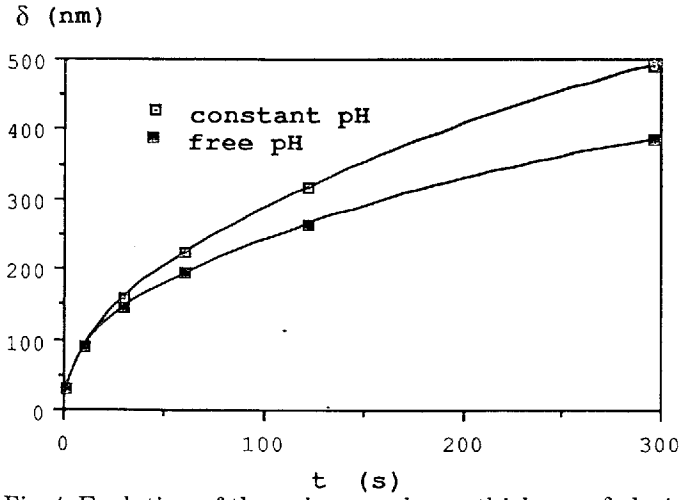

Fig. 4. Evolution of the nylon membrane thickness, δ, during the polymerization at constant and freely varying pH (see Table 1 for polymerization conditions).

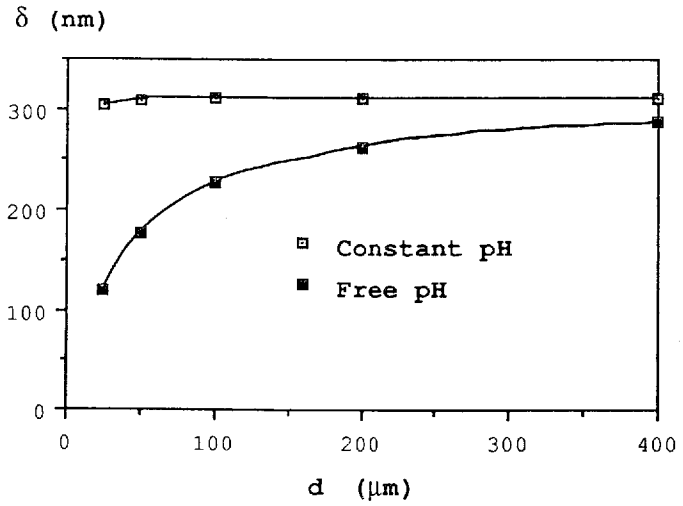

Fig. 5. Variation of nylon membrane thickness, δ, with microcapsule diameter, d, at constant and freely varying pH (see Table 1 for polymerization conditions).

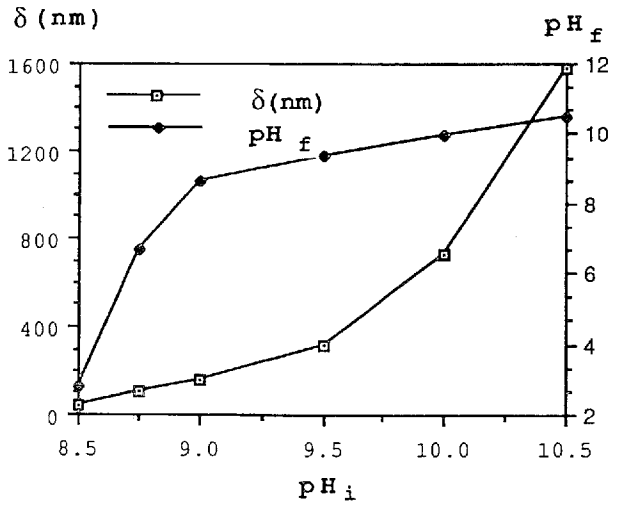

Fig. 6. Impact of the initial pH, pH_i, on the membrane thickness, δ, after 2 min of polymerization (constant pH) and on the final pH after formation of 250 nm membrane (freely varying pH, see Table 1 for other polymerization conditions).

$d\delta/dt$, (slope) decreases as the membrane thickness increases. This effect is more pronounced when the pH is not held constant.

When the pH is maintained constant, the membrane thickness, δ, after 2 min of polymerization, is independent of the microcapsule diameter, d, for microcapsules having a diameter larger than 50 μm (Fig. 5). If the pH is not maintained constant, the membrane thickness decreases as the diameter decreases.

Figure 6 shows that the initial pH, pH_i, strongly influences the membrane thickness, δ, (2 min of polymerization and at constant pH). When the pH is

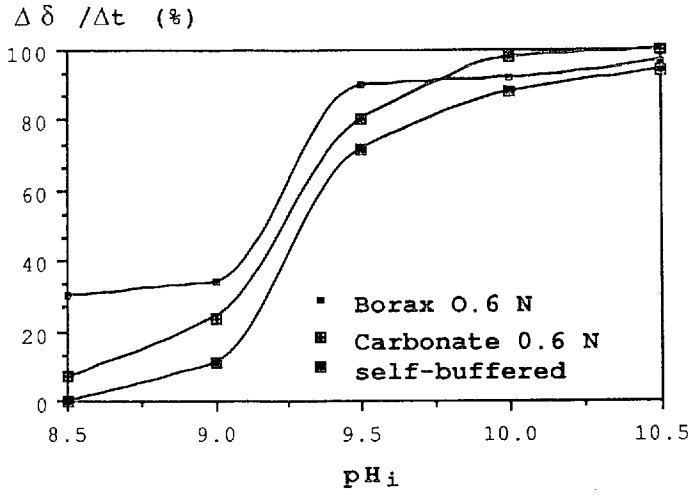

Fig. 7. Mean membrane formation rate, $\Delta\delta/\Delta t$, as function of the initial pH, pH_i, for different acid-base buffers (see Table 1 for other polymerization conditions; the mean rate at constant pH is considered as 100%).

allowed to vary, the final pH, pH_f, after formation of a 250 nm nylon membrane drops to low values when the initial pH, pH_i, is lower than 9. The presence of an acid buffer is thus required to avoid high acidity within the microcapsules.

The mean rate of membrane formation, $\Delta\delta/\Delta t$, as a function of the initial pH value, pH_i, is plotted in Fig. 7, using different acid–base buffers. In all cases, a 250 nm membrane is formed ($\Delta\delta = 250$ nm). The mean membrane formation rate, $\Delta\delta/\Delta t$, is considered equal to 100% when pH is held constant. The membrane formation rate, $\Delta\delta/\Delta t$, remains unchanged until the initial pH, pH_i, decreases to less than 9.5. The use of borax buffer results in a higher value of the membrane formation rate, $\Delta\delta/\Delta t$, at lower initial pH, pH_i.

The impact of the initial diamine concentration, C_{DA}, on the membrane thickness, δ, is presented in Fig. 8 (constant pH and 2 min of polymerization). The membrane thickness, δ, appears proportional to the initial diamine concentration, C_{DA}. Figure 8 also plots the final pH, pH_f, after 250 nm nylon membrane formation as a function of the initial diamine concentration, C_{DA} (pH free, unbuffered). To prevent the pH from dropping, diamine concentration higher than 200 mM must be used when no acidic buffer is present.

Figure 9 reports the mean membrane formation rate, $\Delta\delta/\Delta t$, versus the initial diamine concentration, C_{DA}, for different buffers. The introduction of an acidic buffer thus allows improvements in the performance of the nylon formation while maintaining the pH higher than 9.

The neutral diamine partition constant, K(o/a), varies with the diamine solubility in the organic solvent. This in turn affects the membrane thickness, δ. This relationship is illustrated in Fig. 10 for different organic solvents after two minutes of polymerization.

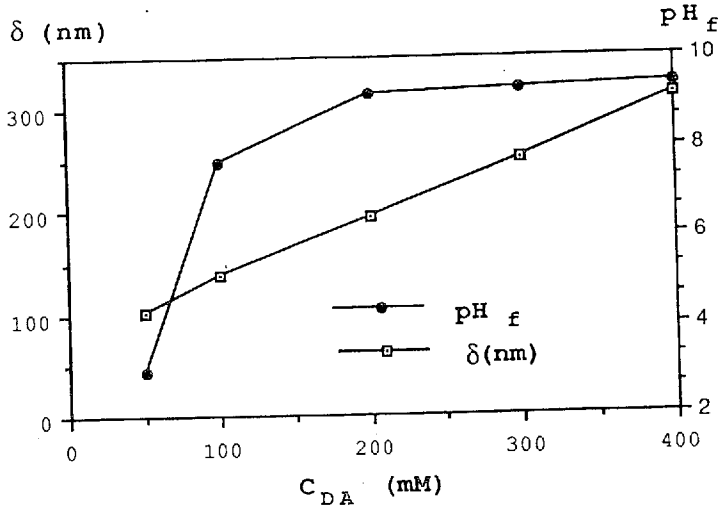

Fig. 8. Membrane thickness, δ, after 2 min (constant pH), and final pH after 250 nm membrane formation (free pH), as a function of initial diamine concentration, C_{DA} (see Table 1 for other polymerization conditions).

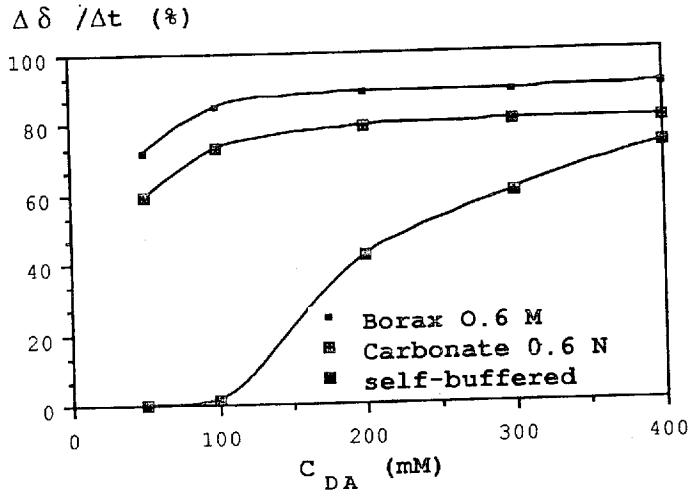

Fig. 9. Mean membrane formation rate, $\Delta\delta/\Delta t$, as a function of the initial diamine concentration, C_{DA}, for different acid-base buffers (100% corresponds to rate at constant pH, see Table 1 for other polymerization condition).

The partition constant, $K(o/a)$, is dependent on the choice of diamine. For example, when the carbon number, n, in the linear diamine molecule (H_2N–$(CH_2)_n$–NH_2) increases, the partition constant, $K(o/a)$, increases (Fig. 11). However, the neutral diamine fraction, $\varphi(0)$, simultaneously decreases (Fig. 11). Membrane thickness, δ, results from a compromise between these oppo-

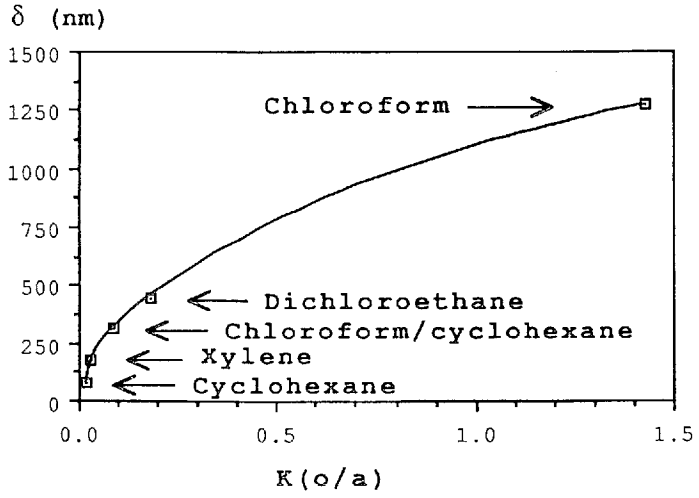

Fig. 10. Influence of neutral hexanediamine partition constant, $K(o/a)$, on the nylon membrane thickness, δ, (chloroform/cyclohexane reported here is a 1/4 v/v mixture; $K(o/a)$ values computed from Morgan [22]; see Table 1 for other polymerization conditions).

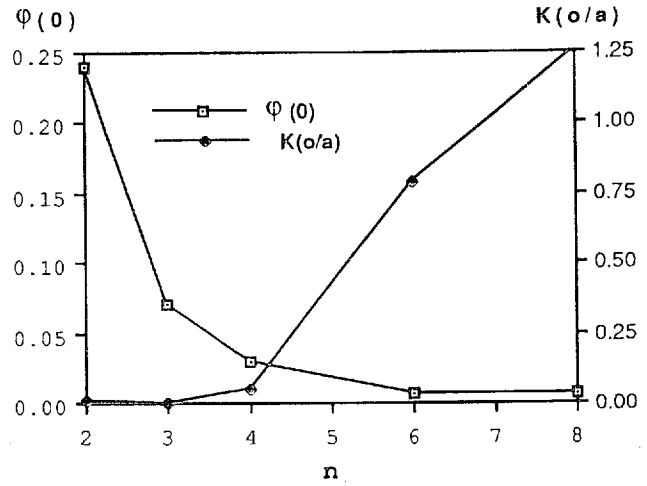

Fig. 11. Neutral partition constant, $K(o/a)$, and neutral chemical species fraction, $\varphi(0)$, for linear diamine series ($K(o/a)$ extrapolated from [20] and $\varphi(0)$ following [17]).

site effects as illustrated in Fig. 12. The diffusion constant, D, was assumed similar for all diamines.

The membrane thickness, δ, after 2 min of polymerization is strongly affected by the temperature, T, as reported in Fig. 13 (constant pH). This simulation takes into account the effect of temperature, T, on the diamine dissociation constants, K_a, and, then, on the acid–base partition, $\varphi(0)$. The diffusion constant, D, and the partition constant, $K(o/a)$, were considered independent of the temperature, T.

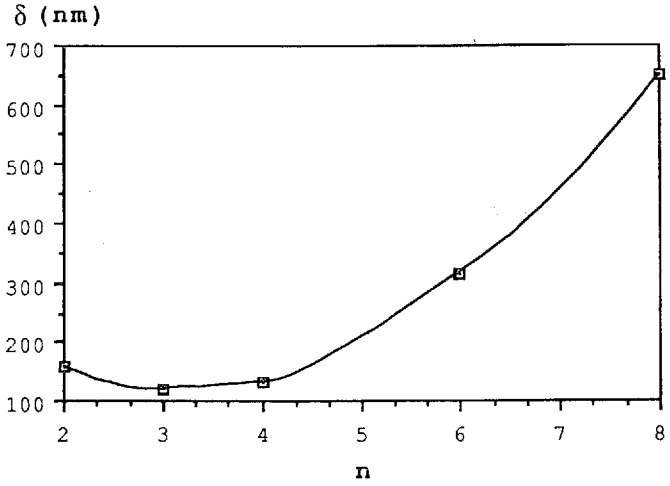

Fig. 12. Relation between the number of carbons in linear diamine, n, and the resulting microcapsule nylon membrane thickness, δ (see Table 1 for other polymerization conditions).

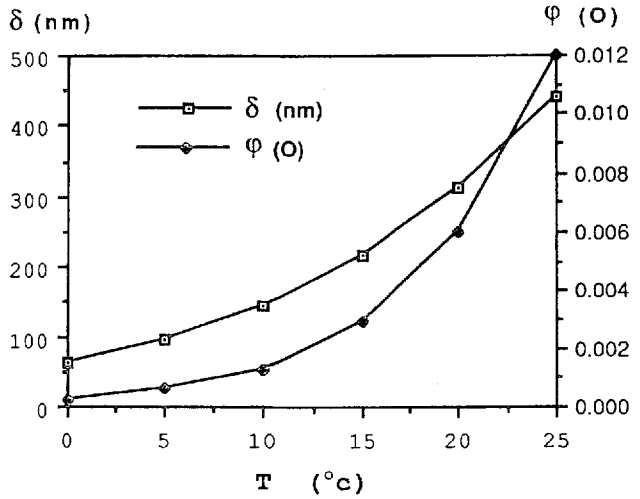

Fig. 13. Impact of the temperature, T, on the membrane thickness, δ, and the neutral diamine fraction, $\varphi(0)$ (see Table 1 for other polymerization conditions).

Discussion

A model has been presented to describe nylon membrane formation in microencapsulation. Only one parameter must be identified (diffusion constant, D) while other models [25] require multiple parameter identification (diffusion constant, D, liquid mass transfer coefficient, membrane swelling). The present model incorporates physicochemical effects which are determinant factors in the control of nylon membrane formation.

The first objective in developing this model was to better understand nylon membrane formation and how it is controlled. The analysis was restricted to a

few aspects of the problem (membrane thickness, diamine consumption, pH control) and did not take into account the control of nylon molecular weight or concentration in the membrane. Simulation was also limited to a 25 to 400 μm range of microcapsule diameters.

The second objective of this work was to optimize the microencapsulation conditions in order to maintain high biocatalyst activities. Resulting activities of encapsulated enzymes and bacterial cells are often reported to be low (less than 30% as compared to free biocatalysts) [14,15,17].

Several factors have not been considered in the model. Short nylon chains are soluble and may be carried away in the organic phase representing a loss of reactant. In addition, nylon membrane is generally formed in the presence of a protein (hemoglobin) or a polyamine (polyethyleneimine). These substances are partially incorporated into the membrane (up to 30%) and play an important function in the membrane structure [24,26] likely due to cross-linking with the membrane components [17,27]. The third factor not considered in the model is the fact that literature reported characteristics of the membrane (thickness, density) may not reflect variations through the membrane or variability within the sample. Finally, nylon polymerization is generally assumed to occur at the outer (organic) face of the membrane. More probably, reaction occurs in a thin layer at the outer side. Maturation (cross-linking, polymer chain union) occurs within the membrane.

Maximum membrane thickness, δ

The membrane formation rate, $d\delta/dt$, was shown to decrease with time, t, even when all other parameters including diamine concentration, C_{DA}, remain quasi constant (Fig. 4). Simulations show that the thickness of the membrane is roughly proportional to the square root of the partition constant, $K(o/a)$ (Fig. 10) or neutral diamine fraction, $\varphi(0)$. The membrane in fact constitutes a barrier to diamine diffusion and thus exerts a feedback effect on the membrane formation rate.

Assuming that all parameters in eqn. (10) may be held constant but the membrane thickness, δ, integration leads to:

$$\delta \leq \sqrt{\frac{D\,K(o/a)\varphi(0)C_{DA}t}{[Dimer]_m}} \quad (nm) \tag{11}$$

As will be discussed below, several parameter values decrease during the period of polymerization, leading to a smaller membrane thickness, δ, than expected by eqn. (11). This is illustrated in Fig. 3. When pH is free to vary, the neutral diamine fraction, $\varphi(0)$, decreases with time. The resulting membrane thickness, δ, is then lower than at constant pH. The membrane thickness provided by eqn. (11) constitutes a maximum value.

Membrane thickness, δ, and microcapsule size, d

A constant membrane thickness, δ, was observed for microcapsules larger than 50 μm when the pH is held constant (Fig. 5). Only a small fraction of the diamine is consumed during membrane formation (Fig. 3). The amount of diamine by unit of surface is then not a limiting parameter and the diamine concentration, C_{DA}, may be assumed constant during the membrane formation.

The constant membrane thickness, δ, (and probably other membrane characteristics) through a size distributed microcapsule sample would partially explain the reduction of the mechanical resistance to shear breakage with increasing size [7]. This may also explain the experimental results of Jay and Edwards [28] who have observed that the stiffness and tension of the membrane were independent of the microcapsule size.

Distribution of parameter values through a system generally results in only mean values or complex mathematical tools must be used in analysis. Accuracy of identification is often reduced [7]. Constant membrane thickness, δ, through a batch of size distributed microcapsules simplifies identification of parameters such as membrane thickness itself, or permeation constants.

When the pH is not held constant, the membrane thickness, δ, is a function of the microcapsule diameter (Fig. 5). Even if the consumption of diamine is very low, it affects the pH value and consequently the neutral diamine fraction, $\varphi(0)$ (Fig. 2). This effect is as important as the pH and the diamine concentration, C_{DA}, are low (Figs. 6 and 8). Buffering the medium under these conditions is necessary to control the membrane thickness, δ.

Diffusion constant, D, *and nylon membrane concentration* $[\text{Dimer}]_m$

Few data are available in the literature on the nylon concentration, $[Dimer]_m$, and the diffusion constant of the diamine through the membrane, D. Experimental observations show that these parameters are dependent on pH, diamine structure and solvent. However, it is difficult to draw conclusions about the range of values taken by these parameters and which laws they follow. The literature reports variations in the membrane structure and density through the membrane, particularly for thick membranes [26]. These properties are dependent on the polymerization rate, temperature, solvent [23] and monomers used [20]. However, these effects have not been described quantitatively.

Consequently, the diamine diffusion constant, D, and the nylon concentration, $[Dimer]_m$, were assumed constant through the membrane (linear decrease of $[DA]$ in membrane, Fig. 1, and direct proportionality between change in membrane thickness and diamine consumption, eqn (10)). Their values represent a mean, through the membrane as well as the sample.

Effect of the solvent on the membrane formation

Figures 6 and 12 report values of diamine partition constant, $K(o/a)$, between aqueous and organic phases for various organic solvents and various

diamines. Diamine partition constant, $K(o/a)$, covers a wide range of values and constitutes an important parameter, affecting the membrane thickness, δ, (Figs. 6 and 13) even if its effect is restricted to its square root value (eqn. (11)). The value of the diamine partition constant, $K(o/a)$, is also dependent on the nature and the concentration of emulsifier introduced in the organic phase [29].

Toxicity of the organic solvent on the biocatalyst is primarily related to its polarity [15,30] and then to its capacity to transfer the diamine. Modifying the organic phase composition to reduce toxicity will also alter the final characteristics of the membrane. However, increasing the emulsifier concentration or introduction of an organic chemical electroconductor, such as quaternary ammonium salts [31], may compensate the use of a less polar solvent.

Influence of the diamine concentration, C_{DA}, on the membrane thickness, δ

Equation (11) assumes that the membrane thickness, δ, is proportional to the square root of diamine concentration, C_{DA}. In contrast, Fig. 9 shows a linear dependence between membrane thickness, δ, and diamine concentration, C_{DA}. Since only the neutral molecule may pass into the organic phase, the real diamine concentration, affecting the membrane thickness, δ, is given by the product of the neutral fraction, $\varphi(0)$, and the total diamine concentration, C_{DA}. The neutral diamine fraction, $\varphi(0)$, is affected by the ionic strength, I, and by the concentration of the diamine in the aqueous phase. This leads to an impact of the diamine concentration, C_{DA}, stronger (Fig. 9) than expected (eqn. (11)). In addition, the complexation of the diamine by the carbonate group may reduce the effective diamine concentration.

As only a small proportion of the diamine is consumed in the membrane formation, it would be interesting to work with a lower diamine concentration to reduce the osmotic pressure and the ionic strength. However this will reduce the membrane formation rate (Fig. 10) and will require careful control of the pH.

Membrane formation and pH

Figure 7 shows that the initial pH value, pH_i, has a considerable impact on the membrane formation. Between pH 8.5 and 10.5, the resulting membrane thickness, δ, seems exponentially related to the initial pH value, pH_i. Use of diamine with higher acid dissociation constants, K_a (low pK_a), may reduce or even counterbalance the pH-reduction effect on the neutral diamine fraction, $\varphi(0)$.

Change of pH during polymerization may also drastically decrease the final membrane thickness, δ (Fig. 8). When the pH and the diamine concentration, C_{DA}, are high, the medium buffer capacity is sufficiently important to maintain the pH value and then the polymerization rate (Figs. 7 and 9). However, at lower values for the initial pH, pH_i, or the initial diamine concentration, C_{DA},

the final pH drops drastically (Figs. 6 and 8), resulting in a significant reduction of the membrane formation rate (Figs. 7 and 9, self-buffered curves). Under these conditions, an external acid–base buffer is required to hold the polymerization rate. The procedure for microcapsule nylon membrane formation is an extension of the initial procedure proposed by Wittbecker and Morgan [9] for preparing nylon. The latter is driven at pH 11 with carbonate as buffer. At this value of pH, the buffer capacity of carbonate is important (Fig. 8). However, at lower pH value (8.5–10), borate buffer presents a higher buffer capacity (Fig. 8). For even lower pH, other buffers such as tris(hydroxymethyl)methylamine ($pK_a = 8.3$) will be more appropriate.

Another method to provide a good buffer capacity to the medium would be to use a diamine with higher dissociation constants, K_a (low pK_a). This method would reduce the risk of interactions between the buffer and the diamine or involvement of the buffer in the polymerization process itself. Preliminary experiments have shown that nylon membranes obtained with borate buffer are thicker and more diffuse than with carbonate and tris buffers.

When using carbonate buffer at pH lower than 10, the pH adjustment (initial pH of the diamine solution is about 11–12) requires relatively concentrated HCl. Fast reaction between the falling acid drop and the carbonate solution leads to the formation of CO_2 bubbles resulting in a undefined final buffer quantity.

Temperature effect on the membrane formation

Temperature, T, does not explicitly appear in the membrane formation equation (eqn. (10)). However, most of the constants are temperature dependent. Only taking into account the impact of temperature, T, on the diamine dissociation constant, K_a, Fig. 11 shows that temperature has an important effect on the neutral diamine fraction, $\varphi(0)$, and consequently on the final membrane thickness, δ. The influence of temperature on diamine diffusion, D, and partition constant, $K(o/a)$, may intensify rather than compensate this effect.

Choice of an adequate diamine

The choice of the diamine strongly affects the membrane formation rate (Fig. 13) and resulting structure [20]. On one hand, lower dissociation constants, K_a, provide a better buffer capacity at lower pH and increase the neutral diamine fraction, $\varphi(0)$. As an example, in the linear diamine series, the dissociation constants, K_a, and, consequently, the neutral diamine fraction, $\varphi(0)$ decrease with the number of carbon atoms (increasing the pK_a). Preference will be given to diamine with amine functions separated by a short alkyl chain or linked by double bonding (phenyl group), or diamine containing electron captor groups. On the other hand, the diamine polarity decreases when the carbon number in a linear diamine series increases. Simultaneously, the partition diamine constant, $K(o/a)$, increases (Fig. 11). The impact on mem-

brane thickness would be a compromise between the diamine structure effect on the neutral diamine fraction, $\varphi(0)$, and on the neutral diamine partition constant, $K(o/a)$, (Fig. 13). Diamine selection may also have an impact on the membrane structure and consequently on the diamine diffusion constant, D, by itself and by the resulting membrane structure.

In order to maintain high biocatalyst activities, the solvent polarity, the diamine concentration and the pH value have thus to be lowered. However, this will result in a thinner nylon membrane. The key to solve this problem is an adequate diamine selection, i.a. diamine with high dissociation constants, K_a (low pK_a) associated with a high diamine partition constant, $K(o/a)$. Using cyclohexanediamine instead of hexanediamine may counterbalance a one unit decrease of pH.

One simple criterion to choose a diamine would be to select the highest diamine partition coefficient, $E(o/a)$ $(=K(o/a)\cdot\varphi(0))$ at the working pH value. Dissociation constants, K_a, are available from the literature but generally the partition constant, $K(o/a)$, is missing. Initial selection of diamines will then start from the former. Partition constant, $K(o/a)$, value may be evaluated from diamine structure. For example, cyclohexanediamine-1,2 has a similar pK_a value to ethanediamine (high neutral fraction, $\varphi(0)$, Fig. 12) but contains a four-carbon alkyl chain as hexanediamine (high partition constant, $K(o/a)$, Fig. 12). From these considerations, a 5–10 times thicker membrane may be expected in the case of cyclohexane diamine compared with hexanediamine.

Conclusions

This paper represents a first step in a larger study of the nylon membrane microcapsule formulation. Conclusions must be drawn carefully since the membrane formation process is more complex than the simple description presented here. However, the model developed in this study is an useful tool to program experiments and analyze their results.

Diamine selection, solvent polarity and initial pH value are the most determinant parameters on the membrane formation control. Attention must be taken to maintain the temperature and the pH value during the membrane formation. Selection of an adequate buffer system will help in this task.

Optimization of encapsulated biocatalyst activities are essentially dependent on the diamine selection. Higher values of the total diamine partition coefficient, $E(o/a)$, at the working pH, would permit increasing buffer capacity and compensate reduction of ionic strength, osmotic pressure (through diamine concentration), pH value and solvent polarity.

List of symbols

A	microcapsule membrane surface area (m^2)
C	total concentration into the aqueous phase* ($mol\text{-}m^{-3}$)
D	DA diffusion constant through the membrane ($m^2\text{-}sec^{-1}$)
d	microcapsules diameter (m)
$E(o/a)$	total diamine partition coefficient
$K(o/a)$	neutral diamine partition constant
q	DA consumption rate per membrane surface unit ($mol\text{-}m^{-2}\text{-}sec^{-1}$)
V	microcapsules volume (m^3)
$\varphi(i)$	fraction of diamine containing i protons [7]
[...]	form concentration in aqueous phase* ($mol\text{-}m^{-3}$)

Indices

a	interior aqueous phase
DA	diamine
Dimer	polyamide dimeric unit
i	inner membrane side
m	into the membrane
o	exterior organic phase and/or outer membrane side

*DA, HDA^+, H_2DA^{2+} are the three forms composing the diamine species [7].

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