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Cinnamaldehyde loaded-microparticles obtained by complex coacervation: Influence of the process parameters on the morphology and the release of the core material

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ABSTRACT

Encapsulation by complex coacervation includes several steps that have been developed in order to attain a better control over the whole process and to achieve an important delayed effect. This has been carried out for the encapsulation of cinnamaldehyde (CN) by the classical gelatin/acacia gum pair of coacervating polymers. This preparation was performed in different conditions (stirring speeds, cooling rate, emulsion and coacervate time, use of surfactant, and polymer concentration) in order to investigate their effect on the encapsulation efficiency and drug release kinetics. Optical microscopy studies showed spherical microcapsules. The yield of the encapsulation attains more than 88% of all prepared microcapsules. The mean Sauter diameter (d_{32}) of obtained microparticles was in the range from 124 to 200 μm . The microspheres were also characterized by the FTIR method; showing the presence of core and polymers in the microparticles. The release of cinnamaldehyde was performed in heterogeneous medium (Water / ethanol, v/v: 30/70) at $25 \pm 0.5^\circ\text{C}$ using UV-Vis analysis. It was demonstrated that the drug release followed the Fickian diffusion mechanism. The data were best fitted to the Fick's law with high correlation coefficients (R^2).

Keywords: *Cinnamaldehyde; Microcapsules; Complex Coacervation; Fick's Diffusion.*

1. INTRODUCTION

Several formulations of drug delivery are developed including spherical dosage forms [1], tablets [2], microparticles [3-5], nanoparticles [6] and liposomes [7]. These systems are formulated with different carrier polymers to allow modulation of the pharmacokinetics of the active agent and the achievement of a controlled release of the drug within a defined therapeutic period [8-10]. Microencapsulation is one of the most numerous techniques used to prepare drug delivery systems. It is used in order to achieve various goals: to protect sensitive ingredients from oxidation or hydrolysis, to mask unpleasant taste and to transform liquid droplets into solid particles [11, 12].

The process of coacervation is a physicochemical method of the microencapsulation; it may occur in polymer solutions containing a few tenths, or even a few hundredths, of a percent of the polymer, in which case the polymer concentration in the coacervate drops may be as high as several dozen percent. For this reason, coacervation is used as a means of concentrating and fractionating native and denatured biopolymers (in particular, water-soluble proteins) and synthetic polymers [13].

The coacervation method may be either simple or complex. Simple coacervation is the result of the interaction of a dissolved polymer with a low-molecular substance as gelatine with alcohol or sodium sulphate [14]. Complex coacervation consists in the precipitation of a hydrophobic substance, a coacervate, and its deposition at the surface of oil droplets so as to build a wall of polymer material [15-17].

Complex coacervation is a phase separation phenomenon induced by electrostatic interactions between polymers of opposite

electrical charge, most often hydrophilic biopolymers. Gelatin/acacia gum couple is the most widely used pair of biopolymers for this technique [18-22].

Encapsulation by complex coacervation has been used in different fields such as pharmaceutical, food, chemical, and cosmetics for the controlled release of several types of cores such as flavours, drugs, colorants and paints with a wide range of applications.

The aim of this paper is to encapsulate cinnamaldehyde using Gelatin/gum acacia system. This active agent is a natural compound isolated from the stem bark of *Cinnamomum cassia* [23, 24]. It has been shown various activities such as peripheral vasodilatory, antitumor, antifungal, cytotoxic and mutagenic. Cinnamaldehyde is developed as food antimicrobial agent due to its demonstrated activity against both gram-positive and gram-negative bacteria, including organisms that are of safety concern [23]. Cinnamaldehyde has been reported to inhibit the growth of *Clostridium botulinum* [25], *Staphylococcus aureus* [26], *Escherichia coli* [27] and *Salmonella enterica* serovar Typhimurium [28, 29]. Furthermore, cinnamaldehyde also found to show cyclin dependent kinases (CDKs) inhibition activity. It can inhibit the proliferation of several human cancer cell lines including those established from breast, ovarian lung and colon carcinomas and leukaemia's [30, 31].

We want to report herein, first the preparation and characterization of cinnamaldehyde-loaded microparticles with different processes parameters by complex coacervation and then, their release study in heterogeneous medium at 25°C .

2. EXPERIMENTAL SECTION

2.1. Materials. The following materials were used: bovine type B gelatin (GE) (Sigma-Aldrich, USA), gum acacia (GA) (Merck Chemicals, Germany), trans-cinnamaldehyde (Sigma-Aldrich, USA), glutaraldehyde (25% v/v, Sigma Aldrich, USA), tween20 (Sigma-Aldrich, USA), acetic acid (Wacker Chemie AG, Germany), NaOH (Wushi, China)

2.2. Experimental part. The microencapsulation method is based essentially on the procedure described in the literature [21, 32]. The simultaneous desolvation of two water-soluble polyelectrolytes with opposite charge (GE and GA) is used. A pH modification of the reaction medium induced an electrostatic attraction of these polymers. Once the coacervation is formed around the microdroplets of cinnamaldehyde, the temperature is decreased to 5 °C in order to obtain a gelling of the coating. The crosslinking of microcapsules obtained is carried out by adding glutaraldehyde in order to solidify the particles.

The process of microencapsulation by complex coacervation was realized in cover cylindrical glass reactor (volume of 1000 mL, external diameter = 80 mm) under mechanical stirring with four-bladed turbine impeller (blade length = 50 mm, blade width = 08 mm) by a homogenizer (IKA RW20 digital, UK) as described in the following steps:

- **Emulsion:** the solution of gelatin (GE) (40g, 1%) is introduced into the reactor containing the solution of gum acacia (GA) (40g, 1%) at 40 °C, the mixture is allowed to stir at 500 rpm. After 15 min, cinnamaldehyde (CN) is gradually added to the reactor. The pH of the mixture is 6.2.
- **Coacervation:** After 30 min, the pH is decreased to 3.9 by adding 2.4 mL of acetic acid solution CH₃COOH (1M). The stirring speed is reduced to 250 rpm.
- **Consolidation:** After 45 min, 0.08 mL of 25% glutaraldehyde was introduced to consolidate the membranes by crosslinking. The temperature of mixture was reduced to 5°C by cooling water for 45min ($\Delta T / \Delta t = 1.2 \text{ }^\circ\text{C}/\text{min}$). The mixture is left stirring at 5 ° C. for 1 hour.
- **Alkalinization:** The crosslinking is improved by alkalinization of the reaction medium at pH = 8.9 by adding 2.6 mL of sodium hydroxide solution (1M), the temperature is then raised to 40 ° C, under stirring.
- After 15 hours, the pH was lowered from 8.9 to 4.5 to form the microcapsules, by adding 4.7 mL of acetic acid solution (1M). The observations of each step were released by optical microscope (OPTIKA 4083. B1). They are presented in figure1.

3. RESULTS SECTION

3.1. Characterization of the coacervated microcapsules.

3.1.1. Encapsulation efficiency: The drug amount in microcapsules was determined by Soxhlet extraction using isopropanol as solvent: 1g of drained and dried microcapsules was introduced into a Soxhlet cartridge. Extraction was carried out with 200 mL of isopropanol under reflux for 6 hours (the heating is fixed to obtain 7 cycles/hour). The resulting solution was analyzed by UV-VIS spectroscopy (Shimadzu UV-2401 PC,

The microcapsules prepared were named TA1; the other formulations were carried out by following the same protocol of TA1 with modifying process parameters to see their effect on the morphology of the microcapsules as well as the release of the active agent from these microcapsules. The process parameters are listed in Table 1.

Table 1. The microencapsulation process parameters CN by complex coacervation.

Code	Process parameters
TA1	As cited above
TA2	$\Delta T / \Delta t = 0.340^\circ\text{C}/\text{mn}$ in consolidation step
TA3	$\Delta T / \Delta t = 0.180^\circ\text{C}/\text{mn}$ in consolidation step
TA4	$\Delta T / \Delta t = 0.148^\circ\text{C}/\text{mn}$ in consolidation step
TA5	The prepared solutions of gelatin and gum acacia have the concentration 4% for 20g and we dilute the system in coacervation step before adding 2.4 mL of solution CH ₃ COOH (1M) with 100g of Tween20 solution (0.25%).
TA6	Emulsion is carried out for 15mn
TA7	Coacervation is carried out for 10mn

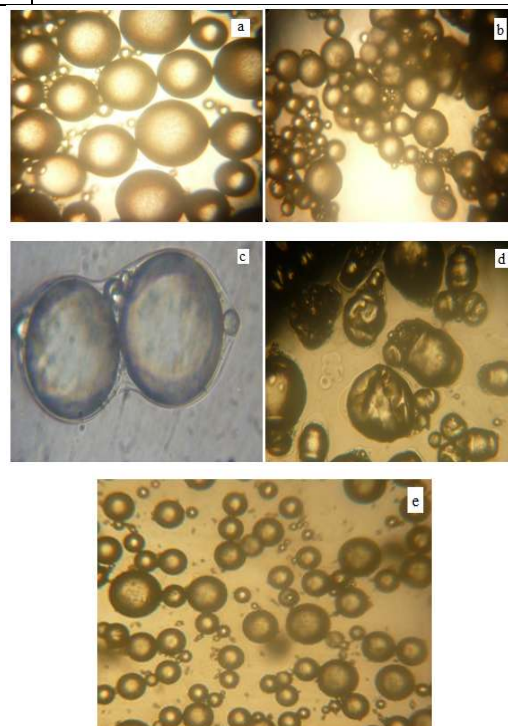


Figure 1. Stages of microencapsulation by complex coacervation (a: Emulsion, b: Coacervation, c: Consolidation, d: Alkalinization, e: The obtained microcapsules).

Shimadzu, Japan) at $\lambda_{\text{max}} = 305 \text{ nm}$. The drug concentration was determined from the standard curve. The encapsulation efficiency (Yield) of the microcapsules was calculated using the equation (1).

$$\text{Yield}\% = \left(\frac{\text{Masse of active agent in microparticles}}{\text{Initial masse of actif agent}} \right) \times 100 \quad (1)$$

3.1.2. Mean diameter and size distribution of the particles. The mean particle size of the active agent microcapsules was determined by optical microscopy (OPTIKA 4083. B1). At least

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500 microspheres were analyzed for each preparation and the mean diameter was calculated. Each sample was measured in triplicate. The particle-size distribution was calculated from several equations [33] (See Figure 2).

3.1.3. FTIR Spectroscopy. The microcapsules were characterised by infrared spectroscopy. The infrared spectra of cinnamaldehyde, gelatin, gum acacia, and the corresponding microparticles were compared. The FTIR spectra were recorded using an FTIR-8300 Shimadzu spectrophotometer (Shimadzu, Japan).

3.1.4. Morphology. An optical microscope (OPTIKA 4083. B1) was used to obtain images from moist microparticles. A sample (TA2) was dried by lyophilisation and then analyzed using Scanning Electron Microscopy (Quanta 200 FEI, France) at Bordeaux Center Imaging (University Bordeaux-1). The sample was mounted on a double-scotched carbon film fixed on a metal support.

3.2. In vitro drug release studies. The in-vitro release study from the microcapsules was carried out using a cylindrical double-wall glass reactor equipped with a fritted glass extremity immersed in the solution. This allows the ascent of the solution without passage of microparticles. The release kinetics of the active agent from microcapsules were followed by using an UV-Vis spectrometer 2401PC SCHIMADZU (The apparatus was calibrated at the maximum wavelength of cinnamaldehyde $\lambda_{max}=305\text{nm}$). 100mg of drained and dried microparticles was soaked in 100 mL of heterogeneous solution (Water / ethanol, v/v: 30/70). The dispersion medium was stirred magnetically at a rotation speed of 500 rpm at $T=25^\circ\text{C}$; the dosage of released active agent is obtained on taking out 1mL of solution containing the support (reading of the optical density).

3.3. Results and discussion. The microcapsules containing cinnamaldehyde were prepared by complex coacervation in order to study the effect of parameters on the formulation of different samples, and to control the concentration of drugs in living microparticles. The obtained microcapsules have a different morphology as indicated in Table 2.

Table 2. The yield and the morphology of obtained microcapsules.

Code	Yield %	Morphology of microcapsules
TA1	92 ± 0.85	Individual spherical microcapsules, individual with a fine-looking membrane (aggregates: 1%)
TA2	93 ± 1.25	Individual spherical microcapsules with a clearly visible membrane (aggregates: 0%)
TA3	92 ± 1.12	Aggregated microcapsules (aggregates: 18%)
TA4	92 ± 0.97	Aggregated microcapsules (aggregates: 26%)
TA5	96 ± 1.03	The microcapsules are very small and individual with a thin membrane (aggregates: 1%)
TA6	90 ± 1.45	Spherical microcapsules with a thick membrane. (aggregates: 0%)
TA7	89 ± 0.43	Individual spherical microcapsules with a visible membrane (Aggregates: 0%)

The optical microscopy analysis of various samples was carried out. The study indicated that the surface of the microcapsules were spherical with different sizes. The mean

diameter of the microcapsules was kept in average 90 to 200 μm (Figure2). It can be noted that smaller microspheres particle sizes were obtained with addition of emulsifier (tween20). It is well known that the surfactant reduces the surface tension of continuous phase, and prevents the coalescence and agglomeration of drops by stabilizing the emulsion [34]. The presence of Tween20 favors the formation of uniform small spherical microcapsules. The value of the dispersion^a is less than 1.6, indicating adjacent sizes of obtained microcapsules (Figure3 & Figure4). The observation by scanning electron microscopy of the freeze-dried microcapsules (TA2) shows that they have an ovoid shape, and the "gum acacia-gelatin" matrix well surrounds the encapsulated "cinnamaldehyde" agent. The membrane appears wrinkled (Figure5).

The percentage of polymer was chosen according to the work of Xiao J-X et al [35]. The authors showed that this percentage allows the total encapsulation of the active agent. The agitation rate was kept constant in the first step (emulsion) for all samples because it affects the size of the microdroplets and the size of the microcapsules [36]. Thus, the decrease the rotation speed was chosen in the second step. Accordingly, the size of the microdroplets in the emulsion step and the size of microcapsules were similar. The same result is obtained if the speed is kept constant in both steps. The size is maintained when the surface of the cinnamaldehyde microdroplets was completely covered by matrices.

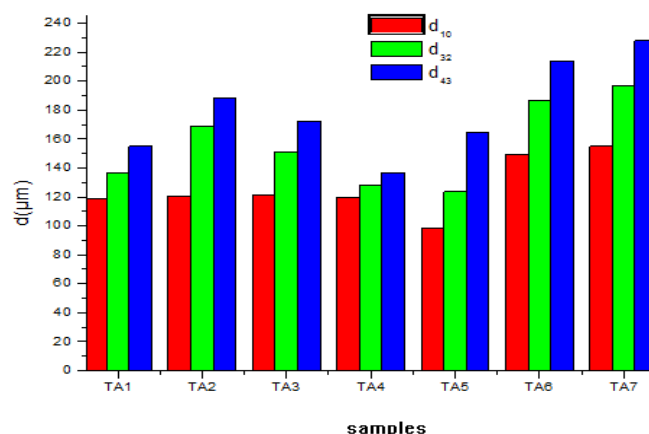


Figure 2. Size distribution of different microcapsules (the arithmetic mean: $d_{10} = \sum n_i d_i / \sum n_i$, the volume-surface mean, $d_{32} = \sum n_i d_i^3 / \sum n_i d_i^2$, the volume-moment mean: $d_{43} = \sum n_i d_i^4 / \sum n_i d_i^3$. Where, $\sum n_i$, i is an index of the population, and d_i is the particle diameter of population i).

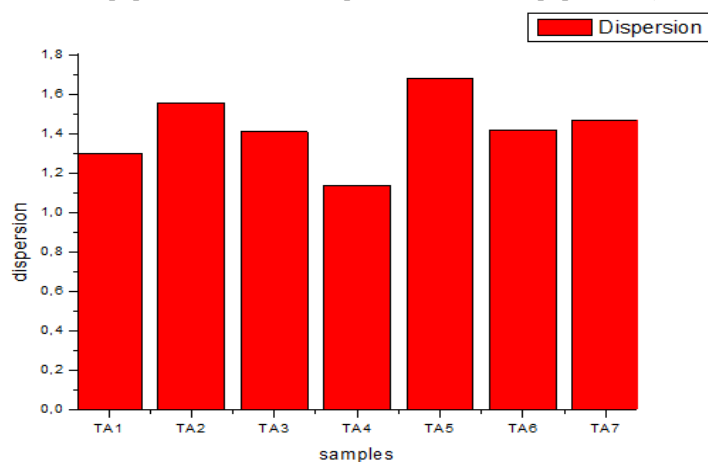


Figure 3. Dispersion of different microcapsules (Dispersion^a = d_{43}/d_{10})

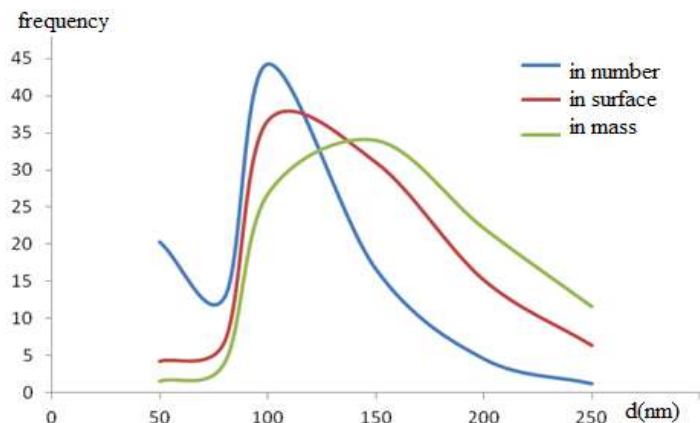


Figure 4. Size distribution curves of microcapsules TA1.

The emulsion time is an important parameter for the formulation of microparticles. Agnihotri N et al proved that the emulsification time must be more than 5 minutes [37]. The diameter of the microcapsules decreases when the emulsion time increases from 15 to 30 minutes. However, the decreasing time of coacervation does not lead to any variation in microparticle morphology. With decreasing cooling rate, the microcapsules were aggregated without any significant influence on the size of microparticles or the cinnamaldehyde content. This situation may suggest that deposition of the matrices on the cinnamaldehyde microdroplets is limited by the complete formation of the envelope.

The crosslinking agent used (glutaraldehyde) is a dialdehyde giving possible formation of "imine" bridges between the gelatin macromolecules. This reaction is more favorable in low acid medium because the amine functions are free. By carrying out the crosslinking in the basic medium, the microcapsules lost the previous spherical shape obtained in acid medium; and become ovoid with a wrinkled membrane. By varying the concentration of the polymer, the size of microparticles remains unchanged between 1% and 4%.

It should be noted that changing the cooling rate has no influence on the yield in TA1-TA4. However, when both the emulsion and coacervation times are reduced a low yield is obtained. This effect may be due to the incomplete process of emulsion or to a coacervate polymer deposit onto the microdroplets. A maximum yield is obtained after addition of the surfactant due to a more stable emulsion step (Table2).

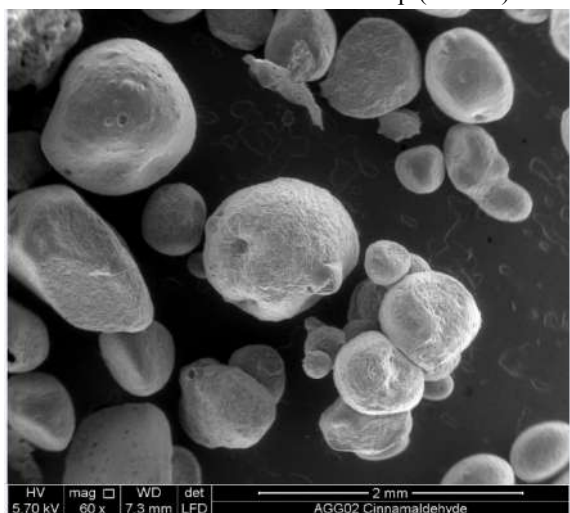


Figure 5. SEM of microcapsules (TA2).

The infrared spectrum of the microcapsules is compared with spectrum of free CN, polymer matrix GE and GA in Figure 6. The analysis showed the presence of similar characteristic bands of the polymers and CN in the CN-loaded microcapsules TA1 spectrum: the OH functions of GE and GA appeared at 3400 cm^{-1} , the C-N and N-H gelatin functions appeared at 1308 cm^{-1} and 1577 cm^{-1} , the C-OH and C-O-C acacia gum functions appeared respectively at 1200 cm^{-1} and 1125 cm^{-1} . We find also the characteristic bands of cinnamaldehyde: C=O, C-H and the aromatic vibration at 1671 cm^{-1} , 2748 cm^{-1} and 1626 cm^{-1} respectively.

The spectrum of the CN-loaded microspheres appeared as the sum of the spectra of pure CN and pair of polymers GE/GA.

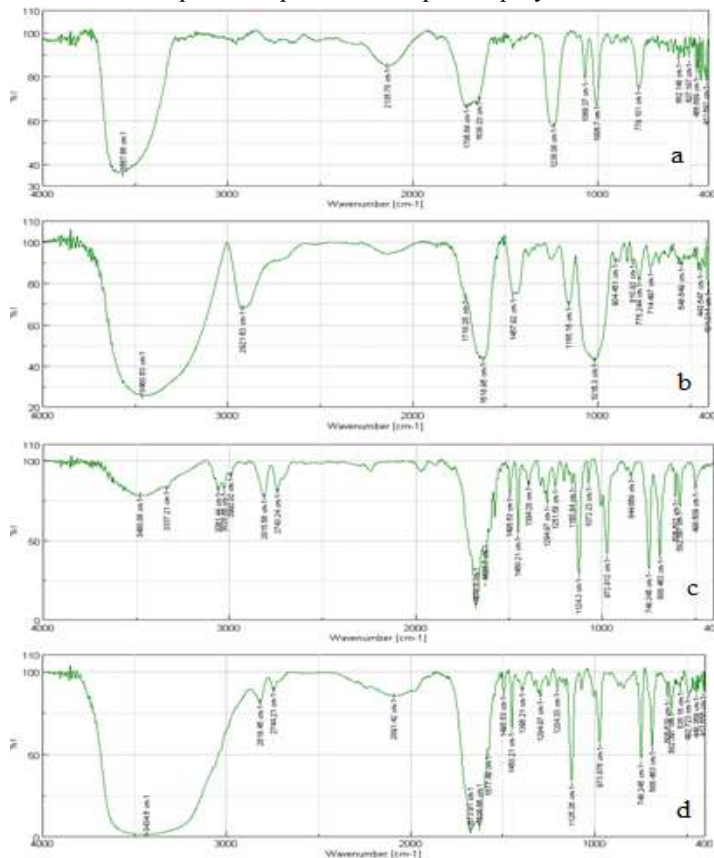


Figure 6. Infrared Spectra (a: gelatin, b: acacia gum, c: cinnamaldehyde, d: TA1).

The release of CN from the reservoir systems (microcapsules) was carried out in a water / alcohol mixture at $T=25\text{ }^{\circ}\text{C}$. The determination of the release concentrations were carried out by UV-VIS at λ_{max} of CN. Figure 7 shows CN release profiles from the prepared formulations (TA1-TA7).

In drug release curves of CN, two stages are noted: first, the percentage released of CN increases quickly and then, becomes constant. Different Drug delivery systems cannot be described by a classical kinetic equation. This process is related to a phenomenon of mass transfer controlled by diffusion according to the curves shown in Figure 8. Thus, a vertical tangent is observed at the beginning of the process. A linear effect is observed (at short time). Transfer of the CN from microcapsules to the medium represents the slowest step because in the reservoir systems the release of the active agent is linked to the width of membrane. The release is therefore governed by the diffusion of encapsulated material in the microcapsules.

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The influence of the parameters (cooling rate, coacervation time, addition of a crosslinker and emulsion time) is weak because the release of CN remains rapid in most cases.

The comparison of the release of CN from patches (TA1-TA4) shows that the release of CN decreases with the cooling rate of the process. This observation could be explained by a decrease in porosity caused by the lowering of the cooling rate and the diffusion difficulties of CN from coacervated microcapsules.

In the presence of the surfactant, we obtained an important released of CN, this is may be explained by the small size of corresponding microcapsules which leads to a large surface contact with the medium.

Decreasing emulsion time to 15 min allowed to obtain great microcapsules. This is due to the shearing time of the microdroplets formed during the emulsion. As a result, the contact surface with the study medium is low in TA6 giving a low release percentage of CN. However, drug release profiles are not modified by changing the coacervation time.

From the different curves, it can be observed that all the releases have an important delayed effect due to their small diameters and the thinness of their membranes, thus facilitating the passage (by diffusion) of the active agent. The linearity of the percentage with the square root of the time confirms that the release of CN was controlled by diffusion inside the solid polymer particles dispersed in the solution according to the simplified Fick's equation for the short times (Table3)[38, 39]. However, it was not possible to get the diffusion coefficients due to unknown the membrane thickness, in the case of microcapsules.

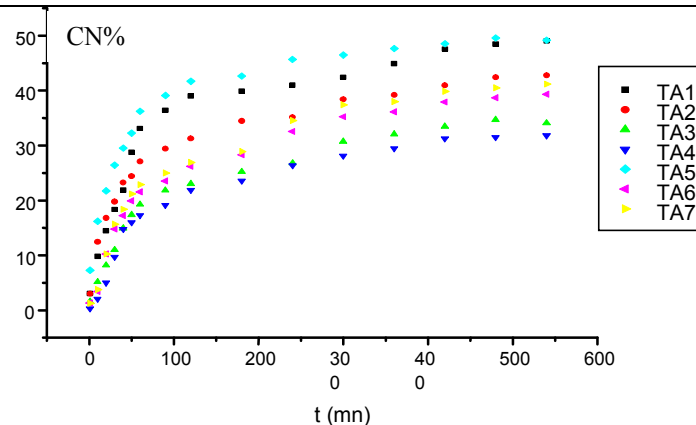


Figure 7. Cumulated percentage of CN released from TA1-TA7.

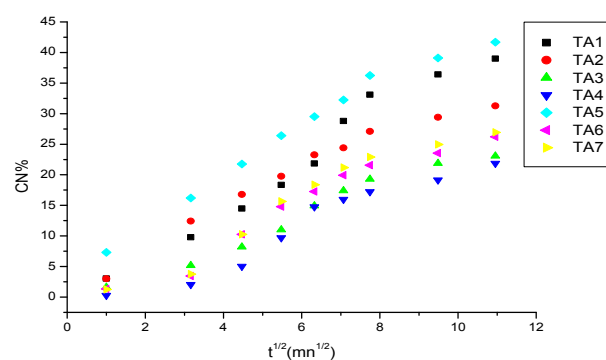


Figure 8. Cumulated percentage of CN released from TA1-TA7 as a function of square root of time.

Table 3. Release kinetic results of microcapsules.

Code	$CN\% = f(t^{1/2})$	R^2
TA1	$y = 4.24x - 3.01$	0.97
TA2	$y = 3.17x + 1.81$	0.97
TA3	$y = 2.62x - 2.20$	0.98
TA4	$y = 2.62x - 4.13$	0.93
TA5	$y = 3.10x - 0.65$	0.98
TA6	$y = 3.00x - 2.78$	0.95
TA7	$y = 3.21x - 3.18$	0.95

4. CONCLUSIONS

The present study emphasized the effects of formulation process parameters on the characteristics and in vitro release behavior of CN-loaded microcapsules prepared by microencapsulation using the complex coacervation method. This process presented high encapsulation efficiency, with spherical microparticles.

The concentration ratio of polymers matrix 1/1 with the presence of 0.25% tween20 as surfactant ensured the stability of the emulsion, and we obtained spherical microcapsules without aggregation with accelerating cool. The rotation speed of 250 rpm applied for 30 min makes it possible to obtain microcapsules with

average diameters of 90 μm . Releases of the hydrophobic core for moist samples were varied due to different formulation conditions. The release profiles showed a Fickian diffusion mechanism and the release rate could be controlled by adjusting the microencapsulation processing parameters that have significant effects on the particle size.

In the present study, we demonstrated that the microencapsulation provided a sustained release of cinnamaldehyde-induced apoptosis of human cancer cells, and we want to test those CN-loaded microparticles in living systems whether can be further developed into a chemotherapeutic agent.

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