

Effect of surfactant level on morphology, encapsulation efficiency, and drug release of procaine-loaded ethylcellulose microspheres

Ilham Abdelmalek, *Synthesis and catalysis Laboratory, Ibn Khaldoun University, Tiaret, Algeria.*

Ilham.abdelmalek@univ-tiaret.dz

Nacira benabdellah, *Synthesis and catalysis Laboratory, Ibn Khaldoun University, Tiaret, Algeria,*

naciraikramhadjer@gmail.com.

Mansour Debdab, *Synthesis and catalysis Laboratory, Ibn Khaldoun University, Tiaret, Algeria,*

mansour.debdab@univ-tiaret.dz.

Meryem Mouffok, *Physical and Organic Macromolecular Chemistry Laboratory, Exacts Science*

Faculty, Sidi Bel Abbes, Algeria, meriemresearchdoc@gmail.com.

Abderrezzak Mesli, *Physical and Organic Macromolecular Chemistry Laboratory, Exacts Science*

Faculty, Sidi Bel Abbes, Algeria, abderrezzak-mesli@netcourrier.com.

Abstract— This study evaluated the effect of Tween-80 concentration on the properties and release behavior of procaine-loaded ethylcellulose microspheres prepared by the oil-in-water solvent evaporation method. Three formulations (1%, 2%, and 4% Tween-80) were tested, with the lowest concentration (T1) showing the highest drug loading (23.48%) and encapsulation efficiency (71.15%). FTIR confirmed successful encapsulation without chemical interaction. Increasing Tween-80 reduced particle size, while SEM showed T1 formed regular, spherical microspheres, unlike the aggregated T2 and T3. In-vitro release was fastest for T1, indicating that low Tween-80 concentration favors optimal microsphere formation, higher drug retention, and improved release.

key words: Drug release study, O/W encapsulation, Procaine, Tween80 concentration effect.

I. INTRODUCTION

Procaine is a classical local anesthetic belonging to the amino-ester class, originally synthesized by Alfred Einhorn and introduced for clinical use under the trade name Novocain [1]. Beyond its conventional use in infiltration anesthesia, dental procedures, and minor surgeries, procaine has attracted renewed scientific interest for non-anesthetic properties, including antioxidant, cytoprotective and possibly geroprotective effects, as suggested by recent experimental studies [2]. Despite these potentials, the clinical utility of procaine is limited by drawbacks such as relatively short duration of action and rapid metabolic degradation in plasma by pseudocholinesterase [3]. To overcome such limitations, modern drug-delivery strategies have explored various encapsulation systems as well as polymeric nanoparticles, liposomes or microspheres to improve stability, control release, and prolong local anesthetic action [4, 5]. The incorporation of procaine hydrochloride into hollow poly-(DL-lactide) (PLA) microcapsules by an emulsification/freeze-drying method has been shown to yield controlled release over several days [6]. Similarly, incorporation of procaine hydrochloride into hollow poly-(DL-lactide) (PLA) microcapsules by an emulsification/freeze-drying method has been shown to yield controlled release over several days [7]. This approach can significantly improve patient outcomes by providing a more consistent delivery of the anesthetic, reducing the need for frequent dosing [8]. another recent work about the inclusion of procaine in cyclodextrin, revealed that procaine forms a stable 1:1 inclusion complex with β -cyclodextrin, enhancing its solubility and bioavailability in pharmaceutical applications [9]. These advancements highlight the importance of

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S. B. Author, Jr., was with Rice University, Houston, TX 77005 USA. He is now with the Department of Physics, Colorado State University, Fort Collins, CO 80523 USA (e-mail: author@lamar.colostate.edu).

T. C. Author is with the Electrical Engineering Department, University of Colorado, Boulder, CO 80309 USA, on leave from the National Research Institute for Metals, Tsukuba, Japan (e-mail: author@nrim.go.jp).

optimizing drug delivery systems for local anesthetics, ultimately aiming to enhance therapeutic efficacy and patient comfort in clinical practice.

Microspheres are widely used as drug delivery systems due to their ability to control release and improve drug stability. In this study, procaine-loaded ethylcellulose microspheres were prepared using the oil-in-water solvent evaporation method, with Tween-80 as a surfactant. The effect of surfactant concentration on microsphere properties, including particle size, morphology, drug loading, and release behavior, was systematically investigated. The results provide insight into how formulation parameters influence microsphere performance. Ultimately, the study aims to identify the optimal conditions for achieving efficient drug encapsulation and controlled release.

II. EXPERIMENTAL PART

A. Chemicals

Procaine hydrochloride ($\geq 99\%$ purity) was purchased from Sigma-Aldrich (St. Louis, MO, USA) and used as the model local anesthetic drug. Ethyl cellulose (viscosity 10 cP) was obtained from Merck (Darmstadt, Germany) and served as the polymeric matrix for microsphere formation. Tween 80 (polyoxyethylene sorbitan monooleate, pharmaceutical grade) was supplied by Sigma-Aldrich (St. Louis, MO, USA) and used as a surfactant to stabilize the emulsion. Dichloromethane used as the organic solvent was purchased from Merck (Darmstadt, Germany). All chemicals were used as received without further purification.

B. Microspheres preparation

The procaine hydrochloride (PR) was encapsulated using O/W solvent evaporated method described in different papers [10, 11]. The formulation consists initially to prepare the organic phase by dispersing ethylcellulose (EC) and active agent (PR) in dichloromethane (DCM), this solution was emulsified with the continuous phase prepared by dissolving Tween 80 in distilled water. The mixture was agitated mechanically with 750rpm of speed at room temperature. The solvent was evaporated and the microspheres were washed with distilled water and dried at 40°C. The composition of the prepared microparticles is classified in table I.

TABLE I
COMPOSITION OF THE PREPARED MICROSPHERES.

Code	Organic phase	aqueous phase
T1	1.2 g EC, 0.6g PR, 100mL DCM	1% Tween 80, 100mL H ₂ O
T2	1.2 g EC, 0.6g PR, 100mL DCM	2% Tween 80, 100mL H ₂ O
T3	1.2 g EC, 0.6g PR, 100mL DCM	4% Tween 80, 100mL H ₂ O

C. Characterization

The encapsulation efficiency and the distribution sizes: The percentage of active agent in prepared microparticles was

determined by dissolving 0.1g of microparticles in 100mL of pH=1.2. The sealed bottle was stirring for 24h at room temperature and the resulting solution was analyzed by UV-VIS spectroscopy (Shimadzu UV-2401 PC, Shimadzu, Japan) at $\lambda_{\max} = 227$ nm.

The drug loading (DL%) and the encapsulation efficiency (EE%) of the microspheres were determined using the following equations:

$$\% \text{ DL} = (\text{Drug mass in microspheres} / \text{Mass of microspheres}) \cdot 100 \quad (1)$$

$$\% \text{ EE} = (\text{Actual drug loading} / \text{Theoretical drug loading}) \cdot 100 \quad (2)$$

The diameters of microparticles were determined by optical microscopy method using an image analyzer OPTIKA 4083. B1. The diameters were determined as described by Kaczmarzski and Bellot [12]. using the following equations: 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: $d_{43} = \sum n_i d_i^4 / \sum n_i d_i^3$, Dispersion^a = d_{43} / d_{10} .

The Fourier Transform Infrared Spectroscopy (FTIR): The FTIR spectra of prepared complex and particles were recorded using an FTIR-8300 Shimadzu spectrophotometer (Shimadzu, Japan).

SEM: Surface morphology of complex and microparticles was characterized by SEM using Quanta 200 at the Center for Development of Advanced Technologies (Algeria).

D. Drug release study:

The in vitro drug release study was carried out using a sealed reactor equipped with a glass capillary tube. Initially, 100 mg of the prepared microspheres were dispersed in 100 mL of simulated gastric fluid (pH 1.2). The mixture was maintained at a constant temperature of 37 °C and continuously stirred at a controlled rate to ensure homogeneity. At predetermined time intervals, 1 mL aliquots of the release medium were withdrawn for analysis. The withdrawn samples were immediately replaced with an equal volume of fresh medium to maintain sink conditions. The concentration of procaine released was determined using UV-Visible spectrophotometry at $\lambda_{\max} = 227$ nm. The cumulative percentage of drug released was calculated and plotted as a function of time to evaluate the release profile of the microspheres.

III. RESULTS AND DISCUSSIONS

A. The encapsulation efficiency and the distribution sizes

The influence of Tween-80 concentration on drug loading, encapsulation efficiency, and particle size distribution was clearly reflected in the characteristics of the three formulations (table 2). Formulation T1, prepared with 1% Tween-80, exhibited the highest drug loading ($23.48 \pm 0.57\%$) and encapsulation efficiency ($71.15 \pm 0.27\%$), and the lowest dispersion value (1.30). Increasing the surfactant concentration to 2% in T2 resulted in a moderate reduction in drug loading ($19.58 \pm 0.64\%$) and a marked decrease in encapsulation efficiency ($59.33 \pm 0.51\%$), accompanied by a broader size distribution (dispersion = 1.46), suggesting partial droplet coalescence during emulsification. At 4% Tween-80 (T3), drug loading decreased further ($14.91 \pm 0.32\%$), although encapsulation efficiency partially recovered ($67.57 \pm 0.32\%$). Overall, these results demonstrate that increasing Tween-80 concentration facilitates drug diffusion into the

external phase, leading to reduced drug loading and variable encapsulation efficiency. A comparison of the d_{10} values shows that increasing Tween-80 concentration led to a reduction in the size of the smallest particles. For T1, d_{10} was 330.23 μm , which decreased to 315.71 μm for T2 and further to 252.31 μm for T3. This trend indicates that higher Tween-80 levels promote the formation of smaller particles, likely by improving the emulsification and stabilization of the dispersed phase during microsphere preparation. This observation is fully consistent with the work of Kumar et al. [13]. In their study, increasing the ratio of Tween-80 within the surfactant/co-surfactant mixture led to a progressive decrease in droplet size because Tween-80 rapidly reduced interfacial tension and enhanced droplet breakup during emulsification. They also reported that this effect occurs only up to an optimal concentration, after which additional Tween-80 no longer reduces particle size and may slightly affect stability.

TABLE II
THE ENCAPSULATION EFFICIENCY AND THE
DISTRIBUTION SIZES OF PREPARED
MICROPARTICLES

Code	Encapsulation Efficiency	diameters and dispersion
T1	DL% 23.48 $\pm$ 0.57 EE% 71.15 $\pm$ 0.27	$d_{10}(\mu\text{m})$: 330.23 $d_{32}(\mu\text{m})$: 404.91 $d_{43}(\mu\text{m})$: 432.12 Dispersion 1.30
T2	DL% 19.58 $\pm$ 0.64 EE% 59.33 $\pm$ 0.51	$d_{10}(\mu\text{m})$: 315.71 $d_{32}(\mu\text{m})$: 382.94 $d_{43}(\mu\text{m})$: 461.60 Dispersion 1.46
T3	DL% 14.91 $\pm$ 0.32 EE% 67.57 $\pm$ 0.32	$d_{10}(\mu\text{m})$: 252.31 $d_{32}(\mu\text{m})$: 342.25 $d_{43}(\mu\text{m})$: 361.96 Dispersion 1.43

The graph shows a progressive decrease in the d_{10} diameter of the microspheres as the concentration of Tween-80 increases from 1% to 4% (figure1). At low surfactant levels, the particles remain relatively large ($\approx$ 330–315 μm), indicating limited interfacial stabilization during emulsification. As Tween-80 concentration increases, the surfactant more efficiently lowers the interfacial tension and stabilizes newly formed droplets. Overall, the results demonstrate that higher Tween-80 concentrations enhance emulsion formation, promoting the production of finer microspheres during the solvent-evaporation process.

B. FTIR

The FTIR spectra of the three formulations (T1, T2, and T3), prepared with different drug-loading levels and encapsulation yields, show the characteristic absorption bands of both procaine and ethylcellulose. Procaine is identified by its aromatic C–H stretching at 3000 cm^{-1} , the aromatic C=C band near 1605 cm^{-1} , the strong ester C=O stretching around 1730 cm^{-1} , and the C–O–C vibration at approximately 1300 cm^{-1} ,

together with aliphatic C–H stretching at 2960 cm^{-1} . Ethylcellulose exhibits its typical ether bands in the 1050–1150 cm^{-1} region [14]. Variations in peak intensity among the formulations reflect differences in drug loading: the T2 formulation, which contains the highest amount of procaine, shows more intense procaine-related bands, whereas T1 and T3 display weaker or broader signals, consistent with lower drug content and deeper entrapment of the drug within the polymer matrix. The absence of notable peak shifts or new absorption bands across the spectra confirms that the encapsulation process does not induce chemical interactions between procaine and ethylcellulose, indicating that the drug is physically entrapped and that spectral intensity accurately mirrors the encapsulation efficiency of each formulation.

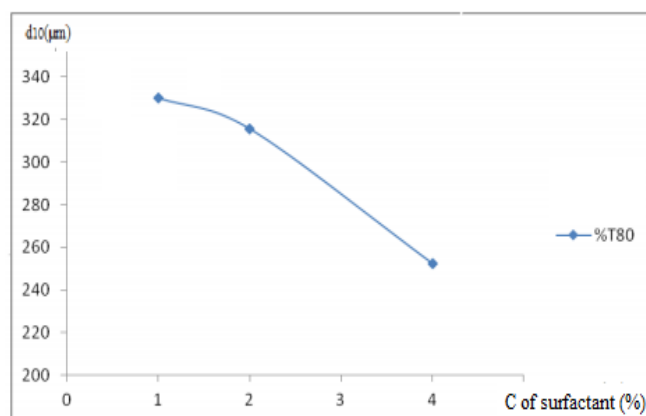


Fig.1 Variation of microspheres diameters as function surfactant concentration.

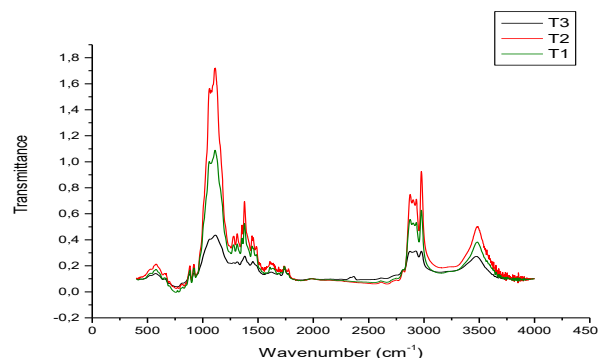


Fig.2 FTIR Spectra of prepared microspheres

C. SEM Micrograph

The SEM micrograph were carried out to know the morphology of different patches. The figure3 shows the SEM micrographs of the prepared microspheres. The morphology of the procaine-loaded ethylcellulose microspheres was strongly influenced by the concentration of Tween-80 used during emulsification. Formulation T1, prepared with 1% Tween-80, produced microspheres that were highly spherical, regular. In contrast, increasing the surfactant concentration to 2% (T2) and 4% (T3) resulted in microspheres that were less uniform in shape, exhibiting irregular contours and noticeable aggregation, consistent with their broader size distributions (dispersion = 1.46 and 1.43, respectively). These

morphological alterations align with the reduced drug loading observed for T2 and T3 and reflect the destabilization of droplets or enhanced particle–particle interactions at higher surfactant levels. Overall, the results indicate that lower Tween-80 concentrations favor the formation of well-shaped, non-aggregated microspheres, whereas higher concentrations promote non-uniformity and aggregation.

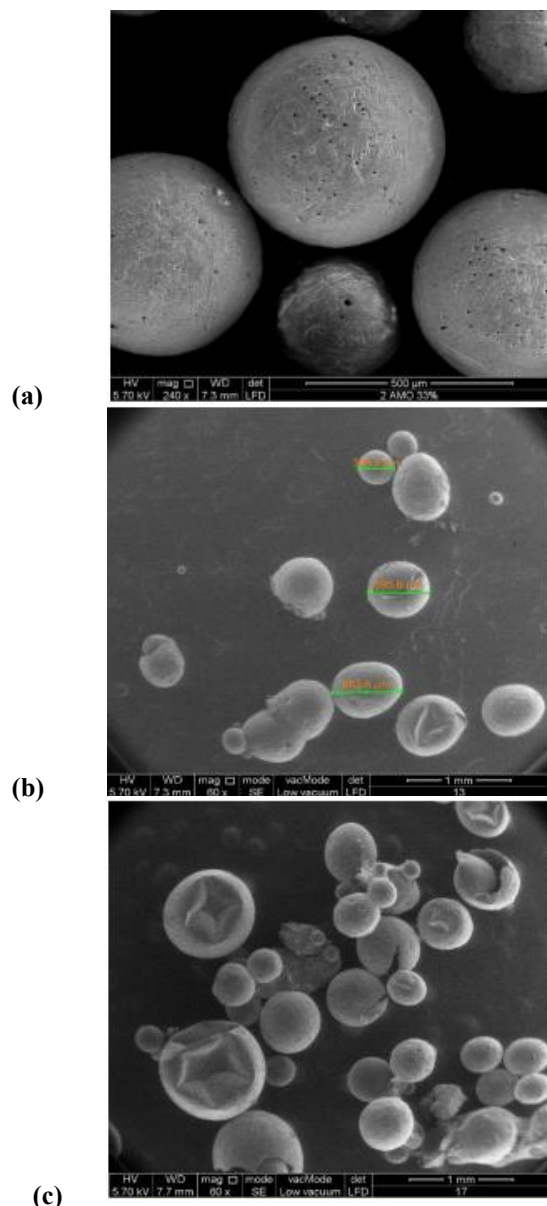


Fig.3 SEM micrograph of prepared microspheres a: T1, b: T2, C: T3

D. Drug release study:

As shown in figure 4, the release profiles of procaine from the three formulations (T1–T3) show a strong dependence on the Tween-80 concentration used during microsphere preparation. All formulations exhibit an initial burst release during the first minutes, attributed to surface-associated drug. However, the extent of this burst and the subsequent sustained-release phase differ markedly among the formulations. T1 shows the highest

release rate and greatest cumulative release, reaching approximately 38%, which correlates with its higher drug loading (23.48%) and smooth, spherical morphology. The more regular and porous internal structure likely facilitates faster diffusion of the drug through the polymer matrix. T2 presents an intermediate release profile, with cumulative release values slightly lower than T1 but still reaching around 35%. The moderate morphological irregularity and partial aggregation observed in this formulation may slow diffusion pathways, resulting in a release curve that increases rapidly but plateaus slightly below T1. T3 exhibits the slowest and lowest overall release, not exceeding 28% even at extended times. This reduced release can be explained by the lower drug loading (14.91%) and the presence of irregular, aggregated particles. The less uniform morphology and possible denser polymer arrangement may restrict diffusion, leading to slower drug liberation.

General, the results indicate that lower Tween-80 concentrations (as in T1) promote particle morphologies and internal structures that favor faster and more complete drug release, whereas higher surfactant levels (T3) produce irregular aggregated microspheres with reduced release efficiency. These findings confirm that Tween-80 concentration is a key parameter governing not only microsphere size and morphology, but also the in-vitro release kinetics of procaine.

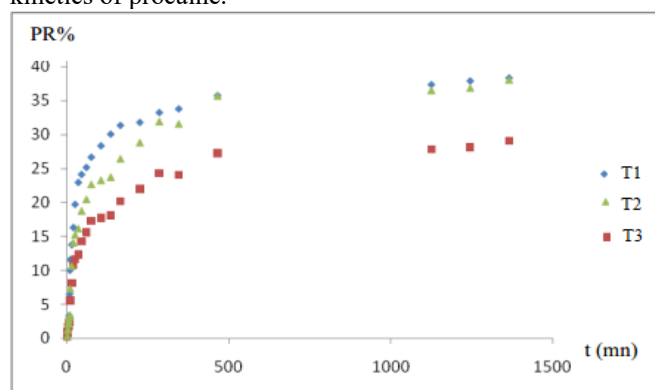


Fig.4. drug release study of PR% from prepared microspheres.

IV. CONCLUSION

The results of this study clearly demonstrate that the concentration of Tween-80 plays a decisive role in controlling the physicochemical properties and release behavior of procaine-loaded ethylcellulose microspheres. Using a low surfactant concentration (T1, 1% Tween-80) produced well-defined, spherical, and non-aggregated microspheres with the highest drug loading and encapsulation efficiency, which translated into the fastest and most complete release profile. Increasing the Tween-80 concentration to 2% (T2) and 4% (T3) led to progressively smaller but increasingly irregular and aggregated particles, accompanied by reduced drug loading and lower cumulative drug release. The less uniform morphology and denser internal structure of T2 and T3 likely contributed to their slower diffusion-controlled release. Overall, these findings highlight that an optimal surfactant concentration is essential for achieving both desirable

microsphere morphology and efficient drug release, with 1% Tween-80 providing the most favorable balance for procaine encapsulation in ethylcellulose microspheres.

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