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A comparative study of ketoprofen-loaded microparticles prepared using emulsion-congealing and solvent evaporation techniques

HADJER SEBAIHI[1], WASSILA BENSALAH[2], KARIMA BADIS[1], MERINE HAOUARIA[1]*

[1]*Laboratory of Macromolecular Physical and Organic Chemistry, Faculty of Exact Sciences, University of Djillali Liabes, Sidi Bel-Abbes, Algeria,* [2]*Laboratory for the Application of Organic Electrolytes and Polyelectrolytes (LAEPO), University of Tlemcen, B.P. 119, 13000 Tlemcen, Algeria.*

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Abstract: Ketoprofen (Ket) is a commonly used non-steroidal anti-inflammatory drug (NSAID) with analgesic and anti-inflammatory properties. However, its poor aqueous solubility and short biological half-life limit its therapeutic efficacy and patient compliance. Controlled-release microparticles offer a strategy to prolong drug release and improve bioavailability. In this study, we prepared ketoprofen-loaded microparticles using two microencapsulation techniques: emulsion/congealing with beeswax and solvent evaporation with cellulose acetate butyrate (CAB). We then tailored co-matrices containing hydrophobic components (PMMA and PCL) and hydrophilic components (HPMC and β-cyclodextrin) to modulate drug release. Microparticles based on beeswax, particularly when combined with PMMA, exhibited slower release due to reduced matrix permeability. Including hydrophilic excipients in beeswax-based microparticles accelerated the release of ketoprofen by promoting water penetration and drug solubilisation. By contrast, the incorporation of hydrophilic excipients into CAB-based microspheres slightly decreased drug release, probably because a denser matrix structure formed during solvent evaporation. These results demonstrate that the encapsulation method and matrix composition both critically influence ketoprofen release kinetics, providing guidance for the rational design of controlled-release drug delivery systems.

Keywords: ketoprofen; encapsulation techniques; beeswax micropellets; cellulose acetate butyrate microspheres; controlled release.

INTRODUCTION

Ketoprofen (Ket), a non-steroidal anti-inflammatory drug (NSAID), is widely used in clinical practice for its analgesic, antipyretic, and anti-inflammatory properties.[1] In particular, it is prescribed to relieve symptoms associated with

* Corresponding author. E-mail: merine_houaria@yahoo.fr
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chronic conditions such as osteoarthritis, rheumatoid arthritis, ankylosing spondylitis, and dysmenorrhea.[2] Despite its clinical efficacy, ketoprofen has several pharmaceutical limitations, most notably its poor water solubility and short biological half-life (approximately 2-3 hours).[3] These pharmacokinetic properties result in a rapid decline in plasma concentration following administration, necessitating frequent dosing to maintain therapeutic levels.[4] Such a regimen may result in decreased patient compliance and increased risk of adverse effects, including gastrointestinal irritation-a common concern with NSAIDs.[5,6]

To overcome these limitations, oral controlled release (CR) formulations have been extensively studied as a solution to prolong therapeutic effect, reduce dosing frequency and improve patient adherence.[7-9] Controlled release systems offer the added benefit of minimizing peak-trough fluctuations in plasma drug concentrations, thereby improving therapeutic outcomes and reducing side effects.[9] Among the various approaches being explored, multiparticulate drug delivery systems such as microspheres, microcapsules, micropellets, tablets and granules have received significant attention due to their potential to offer customizable release profiles, ease of administration, and better gastrointestinal tolerability compared to monolithic dosage forms. [6,8,10-16]

The success of these systems is highly dependent on the selection of appropriate polymer matrices, which dictate the release kinetics and stability of the encapsulated drug.[17] Both hydrophilic and hydrophobic polymers have been used to formulate matrix-based or membrane-coated delivery systems. Hydrophilic polymers, particularly cellulose derivatives such as hydroxypropyl methylcellulose (HPMC), hydroxypropyl cellulose (HPC), cellulose acetate (CA), carboxymethyl ethyl cellulose (CMEC), ethyl cellulose (EC), and methyl cellulose (MC), have attracted considerable interest due to their swelling and gel-forming capabilities in aqueous media.[18,19] These properties allow them to control water penetration and drug diffusion, which are essential mechanisms for sustained drug release.[20-25]

On the other hand, hydrophobic polymers such as poly(lactic-co-glycolic acid) (PLGA),[26] poly(ε-caprolactone) (PCL),[21,22] and polymethyl methacrylate (PMMA)[21] are widely used for their biodegradability, biocompatibility, and ability to retard water penetration, thereby prolonging drug release. These materials are particularly valuable for the formulation of microspheres intended for long-term therapeutic use.

In addition to polymers, cyclodextrins (CDs)-a class of cyclic oligosaccharides composed of α-(1,4)-linked glucopyranose units-have been extensively studied for their ability to form inclusion complexes with poorly water-soluble drugs, thereby enhancing their aqueous solubility, dissolution rate, and absorption profile.[3,21,22,27] CDs possess a hydrophobic inner cavity and a hydrophilic outer surface, making them ideal candidates for complexation-based

drug delivery.[27,28] The incorporation of drug-CD complexes into solid oral dosage forms such as tablets or capsules can further enhance bioavailability and enable the development of controlled delivery systems, especially when used in conjunction with appropriate matrix-forming agents.

Among the various encapsulation techniques, the emulsion-solvent evaporation method has been widely used in pharmaceutical development to produce polymer-based microspheres. This technique typically involves dissolving both the drug and the polymer in a volatile organic solvent, followed by emulsification into an aqueous phase and subsequent evaporation of the solvent.[6,8] The resulting microspheres are able to encapsulate the drug in a stable matrix, providing controlled and sustained release over an extended period of time. This method is particularly suitable for poorly water-soluble drugs, where oil-in-water (O/W) emulsions and water-insoluble polymers are commonly used. The process is relatively simple, inexpensive and does not require sophisticated equipment, making it an attractive option for pharmaceutical manufacturing.

However, the use of organic solvents raises potential safety and environmental concerns. To overcome these limitations, alternative encapsulation techniques such as the emulsion/ congealing technique have been explored. This technique is based on the melting of lipophilic materials (e.g. natural waxes or fats) in which the active pharmaceutical ingredient (API) is either dissolved or dispersed. The melted mixture is emulsified in an aqueous phase and then cooled to form solid microparticles.[12-15] This solvent-free process is environmentally friendly, cost effective, suitable for thermolabile compounds and offers a simple and scalable process with good reproducibility.

Solid lipid microparticles (SLMPs), based on natural or synthetic waxes, represent another class of lipid-based drug delivery systems that have shown promise for sustained release and protection of sensitive drugs from chemical degradation. These systems are increasingly being used as excipients due to their biocompatibility, low toxicity and ability to provide controlled drug release profiles. Beeswax, a natural lipid with a long history of pharmaceutical and cosmetic use, is particularly attractive due to its GRAS (Generally Recognized As Safe) status, low cost and availability from renewable sources.[29]

Despite the considerable potential of multiparticulate drug delivery systems, particularly those utilizing lipid or polymer-based encapsulation techniques, limited comparative studies have been conducted to evaluate their respective efficacy in modulating the release of poorly water-soluble drugs such as ketoprofen.[10]

In this study, we investigated and compared two different microencapsulation techniques for the formulation of ketoprofen-containing controlled-release microparticles:

1. The emulsion-congealing technique using beeswax from the Tessala region of Sidi Bel Abbes (Algeria) as the primary lipid matrix.

2. The emulsion-solvent evaporation technique using cellulose acetate butyrate (CAB) as the main encapsulating polymer.

To modulate the drug release profiles and improve the physicochemical properties of the microparticles, various hydrophobic and hydrophilic excipients, including, PCL, PMMA, HPMC and β-cyclodextrin (β-CD), were incorporated in the matrix formulations at different ratios. The study aims to evaluate the effect of encapsulation technique and matrix composition on the morphology, particle size, drug entrapment efficiency, and in vitro drug release kinetics of the prepared microspheres. This work contributes to the growing field of advanced oral drug delivery systems by providing insight into the comparative performance of polymeric and lipid-based microspheres for the sustained release of poorly water-soluble drugs.

EXPERIMENTAL

Chemicals

Ketoprofen (MW: 254.29) was obtained from APM Company (Sult, Jordan). Cellulose acetate butyrate, with a viscosity of 0.1 Pa·s in a 5% w/w solution prepared in a toluene/ethanol mixture (v/v = 4:1), was supplied by Merck (India). Beeswax was kindly provided as a gift sample by Tessala, SBA (Algeria). Tween 80, hydroxypropylmethylcellulose (HPMC), β-cyclodextrin (β-CD), and polycaprolactone (PCL, Mw = 70,000–90,000) were all purchased from Sigma-Aldrich (USA). Dichloromethane (DCM, >98% purity) was used as the organic internal phase. A simulated gastric fluid (pH 1.2) was prepared by dissolving 2 g of NaCl and 60 mL of hydrochloric acid solution (1 M) in 1 L of deionized water. The phosphate buffer solution at pH 7.4 was prepared by mixing 250 mL of potassium dihydrogen phosphate solution (KH_2PO_4, 0.2 M) with 195.5 mL of sodium hydroxide solution (NaOH, 0.1 M), and adjusting the final volume to 1 L with deionized water.

Materials and equipments

Infrared (IR) spectra were recorded using a Bruker Alpha FT-IR spectrometer equipped with a platinum ATR single-reflection diamond module. X-ray diffraction (XRD) patterns of the pure drug, polymeric carriers and microsphere formulations were obtained using a Rigaku MiniFlex 600 diffractometer (MiniFlex acquisition system, $\lambda = 1.541$ Å) over a 2θ range of 5° to 70° and analysed for comparative purposes. The carbon-13 nuclear magnetic resonance (^{13}C NMR) spectra of the polymers were recorded on a Bruker spectrometer operating at 300 MHz.

Viscometric measurements were conducted using a Cannon-Fenske KPG-type capillary viscometer, with the temperature maintained at (25 ± 0.1) °C using a thermostatic water bath. The average molar mass (Mv) of the PMMA fractions was determined via intrinsic viscosity measurements using the Mark–Houwink equation.

The mean particle diameter and size distribution of the microspheres were calculated based on optical microscopy observations (Optika 4083.B1) by counting over 500 individual microparticles at the appropriate magnification. The number average diameter (d_{10}), the average surface diameter (d_{32}), the weight average diameter (d_{43}) and the particle size distribution (δ) were calculated from the expressions given below:

$$d_{10} = \frac{\sum n_i d_i}{\sum n_i} \quad (1)$$

$$d_{32} = \frac{\sum n_i d_i^3}{\sum n_i d_i^2} \quad (2)$$

$$d_{43} = \frac{\sum n_i d_i^4}{\sum n_i d_i^3} \quad (3)$$

$$\delta = \frac{d_{43}}{d_{10}} \quad (4)$$

The morphology of the ketoprofen-loaded microspheres was examined further using scanning electron microscopy (SEM) with a Hitachi TM 1000 microscope.

Ketoprofen release kinetics were monitored using a double-beam UV-Vis spectrophotometer (Shimadzu UV-2401) equipped with thermostated cells in a simulated gastric medium (pH = 1.2), which was maintained at 37 ± 0.1 °C.

Synthesis and characterization of polymethylmethacrylate (PMMA)

Polymethylmethacrylate (PMMA) was obtained by a radical polymerization, under nitrogen atmosphere, in anhydrous tetrahydrofuran (THF) as solvent, at 90°C, and in the presence of initiator: 0.5% of benzoyl peroxide during 4 hours.

In two glass polymerization tubes, five grammes of monomer (MMA), 0.5% by mass of benzoyl peroxide and 3 ml of THF are introduced into each tube. After degassing with nitrogen, the polymerization tube is immersed in a bath of oil set at 90°C.

Polymers are generally mixtures of homologs that differ in molecular weights. Fractionation is a means of separating the different molecular weights of the polymer. The poly-molecularity index is a quantity that provides information on the heterogeneity of the macromolecule.

In our case, the fractionation process involves adding the polymer solution to a non-solvent (precipitating agent) to precipitate the polymer.[30] This method is based on the principle that longer polymer chains precipitate first, followed by progressively shorter chains. A total of eight fractions (F1–F8) were obtained.

Experimental fractionation protocol

The polymer was solubilized in 20 ml of chloroform and then poured into a beaker. A volume of heptane was poured into a burette and added progressively (drop by drop) to the solution under continuous stirring until the appearance of a turbidity. After a few hours of ripening, the haze is dissolved by varying the temperature. The solution is left to stand for several hours.

The concentrated phase is separated by decantation; then dissolved in a small amount of solvent and finally isolated by pouring the solution into pure precipitant (the volume of precipitant is 3 times that of the solution). The solid obtained after vacuum filtration is oven-dried at 40°C until the weight is constant. The volume of supernatant (reduced by evaporation) is treated again with an additional amount of precipitant to obtain a new fraction. This process is repeated until a large quantity of precipitant has no effect. The final solution was then concentrated under reduced pressure, the precipitant poured off and the last fraction isolated. Note that the first fraction was further fractionated to give 2 further fractions PMMA (F1, 1) and PMMA (F1, 2). After drying all the fractions. A yield of 92% was obtained. The aim of our study is to investigate the effect of molecular weight on the release of ketoprofen by focusing on fractions F1.1, F3 and F4.

The eight fractions of white aspects were characterized:

IR, $\bar{\nu}$ (cm^{-1}): 1735,73 : C=O (ester) ; 1041.72: C-O (ester) ; 2953 : -C-H, stretch ; 1452,75: $-CH_3$(bending);

^{1}H NMR(300MHz): OCH_3: 3,568ppm; $-CH_2$ 2,143ppm; $-CH_3$: 1,224ppm.

^{13}C NMR (300MHz, $CDCl_3$), δ (ppm): 178 (C=O); O-CH_3 (52); 46(C: tertiary carbon) 31 ($-CH_2-$); 17(C-CH_3);

$M_{V(F1)}$ =52177 g/mol ($M_{V(F1,1)}$= 59319 g/mol, $M_{V(F1,2)}$ =43677 g/mol), $M_{V(F2)}$ = 42788 g/mol, $M_{V(F3)}$ =36323 g/mol; $M_{V(F4)}$ =15434 g/mol, $M_{V(F5)}$ =10894 g/mol, $M_{V(F6)}$ =8721 g/mol, $M_{V(F7)}$ =715 g/mol,

Preparation of microparticles

Encapsulation using the emulsion/congealing technique is carried out according to the following procedure:

- First, dissolve 0.75g of Tween® 80 in 150 mL of distilled water, stirring vigorously and heating to 90°C. This temperature is kept constant.

- In a second step, depending on the formulation, an appropriate amount of beeswax or a mixture of beeswax (C.A.) with polymethylmethacrylate (PMMA) at different fractions (F1, F2 and F3), β-cyclodextrin (β-CD) and hydroxypropyl methylcellulose (HPMC) were used. These polymers were added as additives to control the release of ketoprofen.

The mixture is melted in a water bath. The appropriate amount of ketoprofen (Ket) is added to the molten mixture. The composition of various formulations is given in Table 1.

Finally, the molten mixture was poured into the hot aqueous solution containing Tween® 80 under precisely regulated mechanical stirring at 800 rpm. Agitation was maintained until the emulsion had cooled to room temperature for 20 min. The solid-state micropellets obtained were vacuum-filtered and washed three times with distilled water, then dried at room temperature.

Microspheres produced by the emulsion/solvent evaporation process are prepared according to the following procedure:

One or more polymers (depending on the formulation) and an appropriate amount of ketoprofen were dissolved in 30 mL of dichloromethane (DCM). The resulting organic solution was then poured into 150 g of deionized water containing 0.75 g of Tween 80, which served as the external aqueous phase.

The resulting O/W emulsion was stirred under mechanical agitation 800 rpm for 3 hours at room temperature until the solvent evaporated. The resulting microspheres were collected by filtration, washed several times with deionized water and dried under vacuum in a desiccator for at least 48 hours.

The initial composition of the various microspheres prepared by the two encapsulation processes is summarized in Tables 1 and 2.

TABLE I. Experimental conditions for microspheres formulations prepared by emulsion/ congealing technique

	Ket : CA (33:67)
µP2	Ket : CA : PMMA (F1) (33:60:07)
µP3	Ket : CA : PMMA (F3) (33:60:07)
µP4	Ket : CA : PMMA (F4) (33:60:07)
µP5	Ket : CA : βCD : HPMC (25:25:25:25)
µP6	Ket : CA : βCD (25:50:25)
µP7	Ket : CA : HPMC (25:50:25)

Stirring speed/min^{-1}: 800rpm; Number of blades: 4

TABLE II. Experimental conditions for microspheres formulations prepared by emulsion /solvent evaporation process

Lot	Composition (matrix: drug)
µS1	Ket : CAB (33:67)
µS2	Ket : CAB : PMMA (F1) (33:60:07)
µS3	Ket : CAB : PMMA (F3) (33:60:07)
µS4	Ket : CAB : PMMA (F4) (33:60:07)
µS5	Ket : CAB : βCD : HPMC (25:25:25:25)
µS6	Ket : CAB : βCD (25:50:25)
µS7	Ket : CAB : HPMC (25:50:25)

Stirring speed/min^{-1} : 800rpm; Number of blades: 4

Determination of drug loading, encapsulation efficiency and microparticles yield

Two protocols were followed to determine the ketoprofen content in the microparticles prepared by the two methods studied:

Ketoprofen was extracted by weighing 15 mg of micropellets that were prepared using the emulsion/congealing technique with different polymers. This amount was dispersed in 10 ml of phosphate buffer solution (pH 7.4) and stirred for 10 minutes at 70 °C. After filtration, the solution was analysed using a UV-visible spectrophotometer at 262 nm (16107 Lmol^{-1}cm^{-1}) to determine the ketoprofen content. Each determination was performed in triplicate.

On the other hand, ketoprofen content of the microspheres prepared by the emulsion /solvent evaporation process is determined by the extraction of 10 mg of microspheres dissolved in 10 ml of absolute ethanol under magnetic stirring for 24 h. The solution is examined at 250 nm (2711 Lmol^{-1}cm^{-1}) to determine the Ket content. Each determination is carried out in triplicate.

The different equations below make it possible to determine the drug loading (*DL*) and the Percentage yield (Y %) of microencapsulation.

$$\text{Ket.}_{\text{loaded}} = \frac{\text{Ket. mass in microparticles}}{\text{mass of microparticle}} \text{X } 100 \quad (5)$$

$$\text{Ket.}_{\text{EE}} = \frac{\text{Ket.actual drug load}}{\text{Theoretical drug load}} \text{X } 100 \quad (6)$$

$$\text{PY} = \frac{\text{microparticle recovered(practical mass)}}{\text{mass of carrier and drug used in the formulation (theoretical mass)}} \text{x}100 \quad (7)$$

In vitro ketoprofen (Ket) release measurements

A suitable glass dissolution reactor immersed in a bath regulated at 37 ± 0.5 °C equipped with a filter tube to allow removal of the solution without microparticles was adopted for the *in vitro* dissolution tests of ketoprofen from the formulations obtained by the two microencapsulation processes.

Appropriate amounts of formulations containing 25 mg of ketoprofen were placed in the 1000 ml dissolution reactors, filled with 900 ml of simulated gastric fluid at pH 1.2 at 37°C and a stirring speed of 500 rpm. Aliquots of the medium of 3 mL were taken periodically at predetermined time intervals, and analyzed by UV spectroscopy at the appropriate wavelength of the gastric medium: λ max= 264 nm (15130 L mol^{-1}cm^{-1}). The removed volume was replaced with an equal volume of fresh pre-warmed medium (37 ºC ± 0.5 ºC). Drug release kinetics for each batch were performed in duplicate and the average readings were used for calculation.

The corresponding drug release profiles were represented by plots of cumulative percent drug release (calculated from the total amount of Ket contained in each formulation) versus time.

Two mathematical models recording the Higuchi's and the Korsmeyer-Peppar's equations were developed, to elucidate drug transport processes and predict the resulting drug release kinetics.

RESULTS AND DISCUSSION

Microspheres characterizations

Excipients are essential components of nearly all pharmaceutical dosage forms. The formulation of a stable and effective solid dosage form depends on the selection of appropriate excipients, which are added to facilitate drug administration and protect it from degradation.

In this context, fourteen microparticles were analysed for their shape, surface morphology, drug entrapment, and size (mean diameter). Fourteen formulations loaded with ketoprofen and various polymers were developed using two microencapsulation processes: emulsion/congealing and solvent evaporation. Different proportions of polymer were used under the same experimental conditions, resulting in varying sizes (average diameter) and surface morphologies, as well as different levels of drug entrapment. Table 3 provides information on the drug loading results (ket loaded %), Entrapment efficiency, percentage practical yield (yield/%), and size distribution

TABLE III. Microencapsulation results for the prepared microparticles.

Lot	DL%	EE %	Yield	D_{10}/µm	D_{32}/µm	D_{43}/µm	δ
µP1	19.49	58.40	33.58	324.26	423.85	453.86	1.4
µP2	47.02	96	72.68	327.52	509.68	544.86	1.66
µP3	30.21	84	68.24	402.58	699.11	795.74	1.97
µP4	25.33	81.11	56.76	325.30	436.91	469	1.44
µP5	15.67	47	30	162.11	197.64	213.58	1.36
µP6	27	80	41	227.56	289.74	308.87	1.36
µP7	21.48	64.44	52	141.03	186.92	205.93	1.46
µS1	13.63	27.27	35.02	135.01	164.96	178.82	1.32
µS2	36.22	72.43	48.21	155.94	186.80	200.23	1.28
µS3	19.82	39.65	39.03	138.36	158.1	168.23	1.22
µS4	18.04	36.07	30.42	162.80	180.08	187.51	1.16
µS5	16.43	32.86	31	95.36	102.73	106.62	1.12
µS6	20.00	40.00	43.06	117.20	130.24	137.24	1.12
µS7	17.00	34.00	28.48	99.12	107.13	110.91	1.12

DL – drug loading , EE % : Entrapment efficiency , Yield % - Percentage practical yield

The drug content in all formulations ranged from 15.67 % to 47.02 % for micropellets (µP1-µP7) and from 13.63 % to 36.22% for microspheres (µS1 -µS7), the encapsulation efficiency (EE) varied from 47 % to 96 % for micropellets and from 27.27% to 72.43% for microspheres , while the practical yield ranged from

30 % to 72.68 % for micro pellets and 28.48 % to 48.21% for microspheres. The loading efficiency and yield were found to be dependent on te technique of encapsulation and the nature of polymer used in the formulation.

The results clearly show that PMMA matrices combined with β-cyclodextrin achieve a higher encapsulation rate, yield and efficiency than beeswax using the emulsion–congealing technique, and than CAB using the solvent-evaporation method. This improved performance is due to the hydrophobic nature of PMMA, which restricts water from penetrating the molten phase containing the beeswax and active ingredient.[21] This reduces the loss of the drug during microencapsulation. Overall, these findings demonstrate that PMMA is more effective than beeswax or CAB at retaining ketoprofen.

Comparing microparticles µP2, µS2, µP3, µS3, µP4 and µS4, which contain PMMA with different viscometric masses, reveals an increase in loading efficiency (ketoprofen loading percentage), yield and encapsulation efficiency as the viscometric mass of the different fractions increases (F1: 59,319 g/mol; F2: 36,323 g/mol; F3: 15,434 g/mol). This confirms the hypothesis that the hydrophobic nature of PMMA limits the transfer of ketoprofen into the aqueous phase. The high molar mass of the entangled structure of PMMA fraction F1 further reduces the solubility of ketoprofen in water, which explains the higher encapsulation rates observed in µP2 (47.02%) and µS2 (36.22%).

On the other hand, it was found that the micropartricles containing CDs with CA or CAB depending on the case µP6 and µS6, presented a high drug content compared with the microparticles containing CA or CAB alone (µP1 and µS1). β-CDs are fairly soluble in water; they can form water-soluble complexes with lipophilic guests hiding in the CD cavity improving drug entrapment.[22,31,32]

The low yield observed in the formulation (µP5, µS7, µS5) may be attributed to the water solubility of HPMC and β-CD which could result in their transfer to the external phase.[22,33] Furthermore, the reduced yield observed in formulations µS5 and µS7, which contain the CAB polymer and the HPMC–β-CD/HPMC co-matrix, may be attributed to the migration of fine microparticles during the filtration process.

Effect of the encapsulation process is notable on the drug loading, encapsulation efficiency and yield. Microencapsulation by the emulsion/ congealing technique gives promising results and presents a drug loading which reaches a value of 47.02% p.a for µP2 compared to the solvent evaporation method of microencapsulation.[25]

Solid lipids nano or micro particles SLNs are considered promising drug carrier systems, particularly with the aim of giving a sustained release profile to active substances.[34]

In fact, common ingredients include solid lipids, surfactants and water. The term lipid is used in a broad sense and includes triglycerides, partial glycerides,

fatty acids, steroids and waxes (e.g. beeswax as in our case). [They have a better biocompatibility because they're made up of lipids similar to physiological lipids, which reduces toxicity. In addition, SLNs are physico-chemically stable and can be easily produced on a large industrial scale, and the raw materials and production costs are relatively low.[35]

Emulsions can be used as precursors for the preparation of solid lipid particles since lipids, which are solid at room temperature, can be heated 5 to 10 °C above its melting point to obtain a liquid lipid. In the first step, the lipophilic drug is dissolved in molten lipids. The lipids are then emulsified with a hot surfactant solution using a high shear homogenisation. The resulting hot O/W emulsion is cooled to room temperature, and the droplets solidify in the form of solid lipid particles. The microparticles efficiently entrapped ketoprofen due to the appropriate matrix structures of the lipophilic materials, which allowed for the encapsulation of lipophilic drugs.

Optical microscopic analysis was carried out on various microparticle samples. Observations revealed that microgranules were predominantly irregular in shape, whereas microspheres exhibited a generally spherical morphology with varying sizes. The mean particle diameter was measured, and the number-based, surface-based and volume-based mean diameters were calculated from a data set of 500 individual microparticles. Depending on the formulation, the Sauter mean diameter (d_{32}) ranged from 196.92 µm to 699.11 µm for micropellets and from 102.73 µm to 186.80 µm for microspheres. The average polydispersity index (PDI) was 1.52 for micropellets and 1.19 for microspheres, indicating a narrower size distribution and greater uniformity for the latter. Under identical processing conditions - including a stirring speed of 800 rpm and the use of Tween as surfactant - the solvent evaporation method produced smaller, more spherical, more homogeneous and less dispersed microspheres. In contrast, the thermal gelation method produced larger micropellets with more irregular shapes and greater batch-to-batch variability.

The surface and morphology of the microparticles were further examined by scanning electron microscopy (SEM), representative images of which are shown in Figure 1. Microspheres prepared by thermal gelation (µP1, µP4) showed irregular shapes with a pronounced tendency to agglomerate (aggregate formation). In contrast, microspheres obtained by solvent evaporation were mostly spherical with rough and highly porous surfaces, indicating that drug release is likely to occur through these channels. In addition, microspheres formulated with CAB/BCD (µS6) were also spherical but had smooth surfaces with a slightly collapsed appearance.

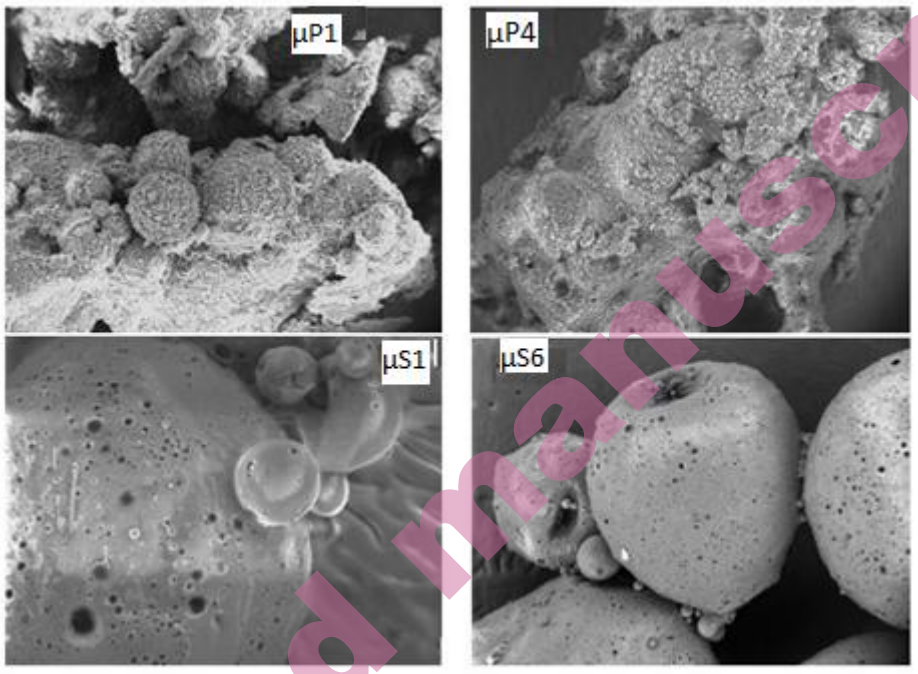

Fig. 1. SEM micrographs of the surface and the morphology of ketoprofen loaded microparticles prepared

The infrared spectra of the micropellets (µP1-µP7) and microspheres (µS1-µS7) were compared with those of the polymeric matrices and the active ingredient, ketoprofen. As an illustration, Figure 2 presents the spectra of samples µP6 and µS6 along with those of their respective matrices. Comparative analysis showed that the microparticles exhibited certain characteristic bands of ketoprofen, albeit with relatively low intensities, which can be attributed to the low drug loading. Moreover, none of the spectra of the formulations showed the appearance of new bands, suggesting the absence of chemical interactions between ketoprofen and the polymeric excipients (CA, CAB and β-CD). The comparison between the IR spectra of the starting materials and that of the µP6 and µS6 microparticles (Fig.2) confirms the presence of the active ingredient in the formulation, as evidenced by the O-H stretching vibration band observed around 2916 cm^{-1} and the C=O stretching vibration of the carboxylic acid group of ketoprofen around 1735.42 cm^{-1}. The simultaneous presence of characteristic bands from both ketoprofen and the polymers, without any additional bands, indicates good compatibility between the components and chemical stability of the active ingredient in the formulations studied.

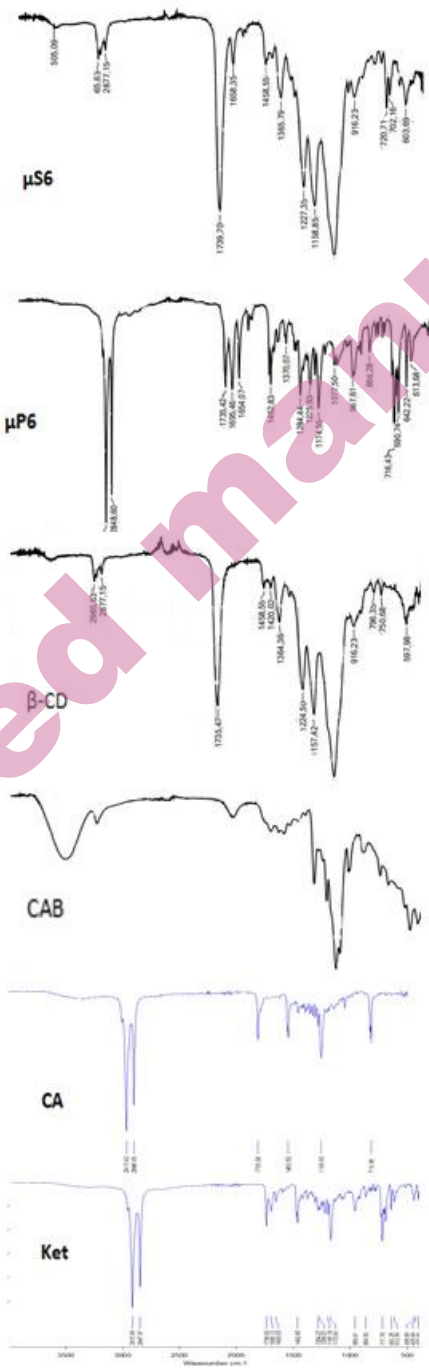

Fig. 2. Infrared spectra of Ket, microparticles (μS6, μP6), and matrices (β-CD, CAB, CA)

Figure 3 shows the X-ray diffraction (XRD) patterns of microspheres µP1 and µS2, together with those of the active ingredient ketoprofen and the polymeric matrices CA, CAB and PMMA. It should be noted that ketoprofen and beeswax are semi-crystalline compounds, whereas CAB and PMMA have amorphous structures. Accordingly, the Bragg reflections observed in the µP1 profile can be attributed to the crystalline phases of ketoprofen and CA. In particular, the crystalline peaks of CA are well resolved in µP1, indicating the presence of a highly crystalline material. In contrast, the diffraction pattern of µS2 shows no detectable peaks associated with ketoprofen, suggesting that the drug is present in an amorphous state within the µS2 microspheres.

The nature of surfactant used has been shown to have a significant effect on the transfer of the drug to the external phase, thereby affecting its entrapment efficiency.[36] Surfactants, known for their ability to reduce surface and interfacial tension, are often added to pharmaceutical formulations to improve drug solubility.[37] Surfactants with a hydrophilic-lipophilic balance (HLB) above 15 have been identified as particularly effective solubilising agents.[38] Accordingly, the µp formulations were developed using Tween 80, a hydrophilic surfactant with an HLB of 15, which is ideal for forming stable emulsions with lipid components such as beeswax.[39] In addition, Tween 80 contributed to a significant reduction in microparticle size, as HLB values have a significant effect on droplet size.[40] Similar results were reported by Brahmi et al. who also observed the formation of small droplets when using Tween 80[25]. This discussion focuses specifically on the role of Tween 80 in our system, emphasising its direct impact on emulsion stability and microparticle size, rather than on general surfactant theory.

In vitro dissolution of ketoprofen from microspheres formulations

A dissolution study of the active pharmaceutical ingredient was conducted in simulated gastric fluid (pH 1.2). Simultaneously, an *in vitro* drug release evaluation was carried out on various formulations produced by both manufacturing methods, employing beeswax as the primary matrix for micropellets and CAB for microspheres. Hydrophobic and hydrophilic excipients, including, PCL, PMMA (with varying molecular weights), HPMC and β-cyclodextrin (β-CD), were incorporated in different proportions, depending on the specific formulation. Drug release profiles from the microparticles, assessed after 360 minutes in the simulated gastric medium, are presented in Figures 4.

The *in vitro* release of ketoprofen from microparticles (µP1-7 and µS1-7) is influenced by several factors, in particular the encapsulation method, matrix type and formulation composition. Table 4 shows the evaluation of the ketoprofen release rates from the microparticles after 30, 120 and 360 minutes. Initially, a comparative study was conducted on four micropellet batches (µP1–µP4) and four microsphere batches (µS1–µS4). The primary matrix used for the micropellets was

beeswax (CA), and the primary matrix used for the microspheres was CAB. All formulations included PMMA as a co-matrix with different molecular weights.

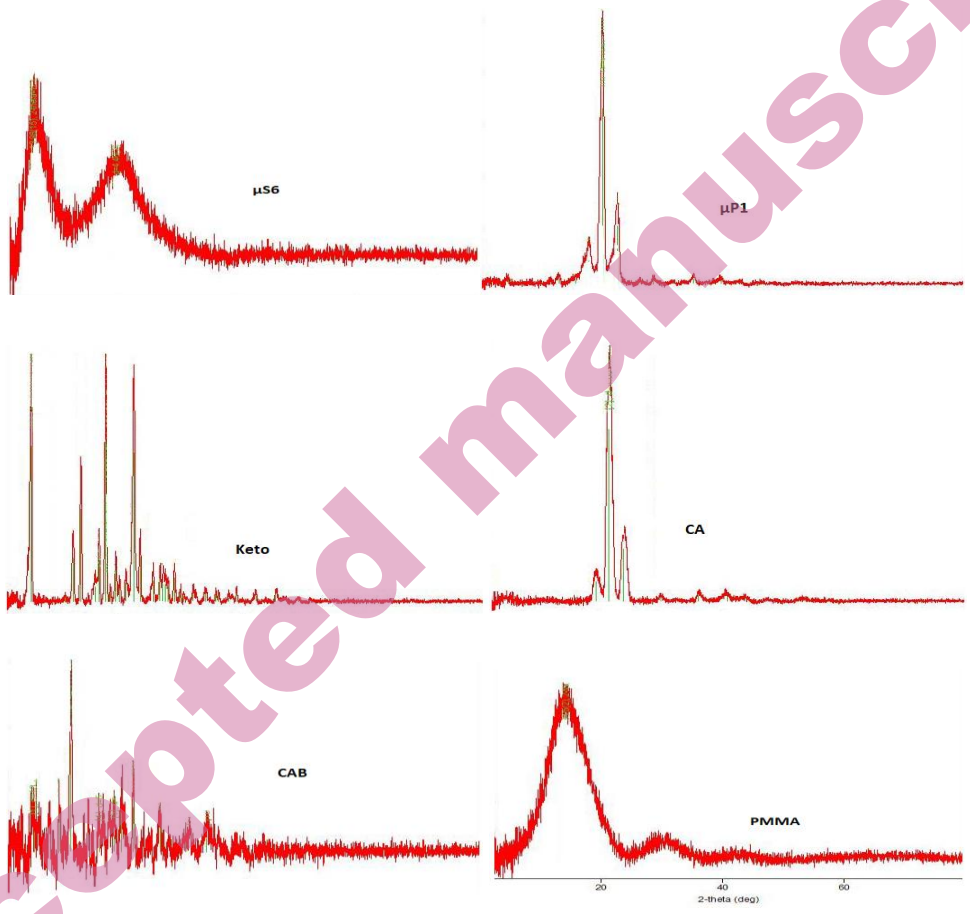

Fig. 3. DRX of Ket, microparticles (µS6, µP1), and matrices (CA, CAB, PMMA)

The results showed that drug release from beeswax-based micropellets (µP1) was lower, reaching only 8.23% after 2 hours, while CAB-based microspheres showed higher release, with the control batch (µS1) releasing 36% over the same period. In contrast, PMMA-containing microsphere batches (µP2-µP4, corresponding to F1, F2, and F3) released 5.26%, 4.13%, and 8.48%, respectively. Similarly, microspheres containing PMMA showed reduced release: 21.5%, 34%, and 28.3% at 2 hours.

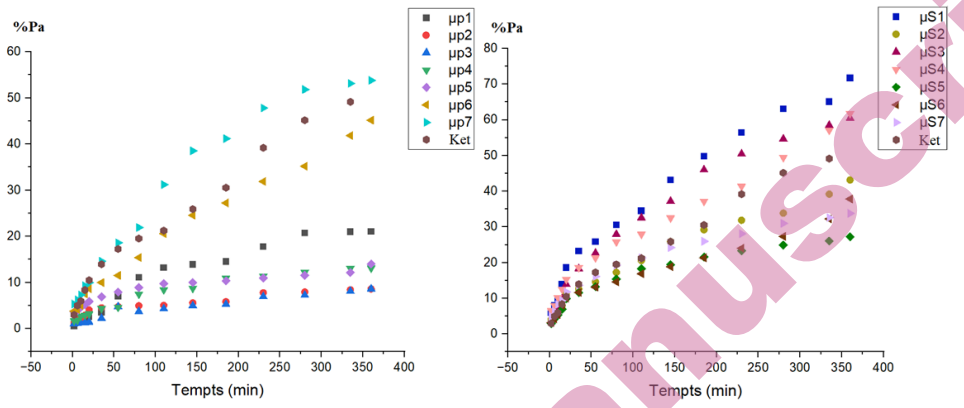

Fig. 4. Drug release profiles from the microparticles, assessed after 360 minutes in the simulated gastric medium

TABLE IV. Percentage of ketoprofen released after 30 mn, 120 mn and 360 mn at pH 1.2.

lots	Ket	μP1	μP2	μP3	μP4	μP5	μP6	μP7	μS1	μS2	μS3	μS4	μS5	μS6	μS7
30mn	13.05	3.13	4.21	1.9	4.1	6.3	9.6	14.2	23	12.3	18	18.2	11	11.2	13.2
120mn	17.91	8.23	5.26	4.13	8.48	9.8	21.5	33	36	21.5	34	28.3	18.5	17.6	23.3
360mn	47.16	21.04	8.59	8.62	13.11	14	45.16	53.8	71.73	43.2	60.6	61.8	27.3	37.9	24.24

After 360 minutes, the cumulative release from microspheres reached 21.04% for the control (μP1), and 8.59%, 8.62%, and 13.11% for μP2 (F1), 3 (F2), and 4 (F3), respectively. For microspheres, the corresponding values were 71.73%: μS1, 43.2%: μS2 (F1), 60.6%: μS3 (F2), and 61.8%: μS4 (F3).

These results confirm that the presence of PMMA, due to its hydrophobic nature, limits the penetration of the aqueous medium and slows the diffusion of ketoprofen from both micropellets and microspheres. Furthermore, the drug release rate was inversely correlated with the molecular weight of PMMA, as demonstrated by the similar profiles of F1 (Mv = 59319 g/mol) and F2 (Mv = 36323 g/mol) and the comparatively higher release of F3 (Mv = 15434 g/mol).

In addition, the encapsulation method significantly influenced the drug release. Micropellets prepared by thermal gelation showed a more pronounced delayed release behavior, which was attributed to the hydrophobicity of beeswax, which further reduces water permeability and drug diffusion.

The incorporation of hydrophilic excipients such as HPMC and β-cyclodextrin (β-CD) led to a progressive increase in ketoprofen release from micropellets (μP5–μP8), likely due to improved wettability and water penetration in the hydrophobic beeswax matrix. In contrast, microspheres μS5–μS8, also containing HPMC and β-CD, showed slightly slower release rates compared to μS1–μS4. This difference may be related to the structural characteristics of the microspheres formed by the solvent evaporation method, where the addition of co-matrices could result in a

denser internal structure or reduced porosity, thus slowing the diffusion of the drug.

These observations align with previous results on comparable polymeric matrices and microencapsulation processes, including studies from our laboratory and other research groups that have worked with beeswax-, PMMA- and CAB-based systems containing various active ingredients.[21-25,27] This comparison further highlights the relevance and consistency of our findings.

Release mechanisms and mathematical analysis

Two mathematical models, Higuchi and Korsmeyer-Peppas, were used to describe the release of the drug from polymeric matrices. Each batch was analyzed using the appropriate equations to determine the most appropriate model.

Higuchi

$$Q_t = K_H\sqrt{t} \quad (8)$$

Korsmeyer–Peppas

$$\frac{M_t}{M_\infty} = K_K t^n \quad (9)$$

The results, presented in TABLE V, show that both models fit the experimental data well, with correlation coefficients greater than 0.95. This suggests that the drug release is primarily controlled by a diffusion-controlled mechanism.

The values of the diffusion exponent n, close to 0.5 for formulations containing PMMA, HPMC and β-CD, indicate Fickian diffusion. For µP5 and µS7 batches, lower values of n correspond to quasi-Fickian diffusion, while the value of n = 0.705 for µP1 suggests an anomalous transport mechanism combining diffusion and matrix erosion.

Moreover, the nature of the matrix significantly affects the release behavior: the incorporation of PMMA significantly slows down the drug dissolution. These results confirm that *in vitro* drug release is influenced by the matrix composition, its molecular weight and the encapsulation method used.

TABLE V. Coefficients of correlation and dissolution rate constants of HCTZ from microspheres in simulated gastric fluid (pH 1.2)

N° lots	Higuchi		Korsmeyer- Peppas		
	r^2	K_H	r^2	LnK_K	n
Lot 1	0,954	1,285	0,965	-4,081	0,705
Lot 2	0,943	0,374	0,956	-2,413	0,324
Lot 3	0,975	0,435	0,964	-3,119	0,461
Lot 4	0,972	0,759	0,972	-2,671	0,461
Lot 5	0.966	0.530	0.979	-3.628	0.266
Lot 6	0.98	2.317	0.967	-4.006	0.517
Lot 7	0.985	3.103	0.971	-3.665	0.519
Lot 8	0.993	3.694	0.989	-3.306	0.497
Lot 9	0.99	2.131	0.985	-3.706	0.467
Lot 10	0.996	3.371	0.991	-3.798	0.568
Lot 11	0.978	2.934	0.982	-3.230	0.438
Lot 12	0.982	1.372	0.986	-3.817	0.437
Lot 13	0.964	1.698	0.979	-3.843	0.455
Lot 14	0.993	1.638	0.995	-3.355	0.385

CONCLUSION

The *in vitro* release of ketoprofen from microparticles is determined by a combination of formulation factors and manufacturing methods. Micropellets prepared by thermal gelation using beeswax as the primary matrix exhibited slower release profiles, especially when combined with hydrophobic excipients such as PMMA. The release rate further decreased with increasing molecular weight of PMMA, suggesting reduced matrix permeability. In contrast, the addition of hydrophilic excipients such as HPMC and β-cyclodextrin enhanced drug release from micropellets by facilitating water penetration and drug solubilization. However, in microspheres prepared by solvent evaporation using CAB, the same hydrophilic co-matrices resulted in a slight decrease in release rates compared to formulations without co-matrices. This may be due to denser or less porous structures formed during the solvent removal process. Overall, the results highlight that both the type of co-matrix and the encapsulation method have a significant impact on drug release behavior, with the solvent evaporation process generally producing more porous and faster releasing microspheres compared to the thermal gelation method. Based on these observations, the most promising system for achieving prolonged and controlled ketoprofen release is beeswax-based micropellets combined with high-molecular-weight PMMA.

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И З В О Д

КОМПАРАТИВНА СТУДИЈА МИКРОЧЕСТИЦА НАПУЊЕНИХ КЕТОПРОФЕНОМ ПРИПРЕМЉЕНИХ ТЕХНИКАМА ЕМУЛЗИЈЕ-ЗГУШЊАВАЊА И ИСПАРАВАЊА РАСТВАРАЧА

HADJER SEBAIHI[1], WASSILA BENSALAH[2], KARIMA BADIS[1], MERINE HAOUARIA[1]

[1]*Laboratory of Macromolecular Physical and Organic Chemistry, Faculty of Exact Sciences, University of Djillali Liabes, Sidi Bel-Abbes, Algeria,* [2]*Laboratory for the Application of Organic Electrolytes and Polyelectrolytes (LAEPO), University of Tlemcen, B.P. 119, 13000 Tlemcen, Algeria*.

Кетопрофен (Кет) је најчешће коришћени нестероидни антиинфламаторни лек (NSAID) са аналгетским и антиинфламаторним својствима. Међутим, његова слаба растворљивост у води и кратак биолошки полуживот ограничавају његову терапијску ефикасност и могућност придржавања употребе од стране пацијента. Микрочестице са контролисаним ослобађањем омогућавају стратегију за продужење ослобађања лекова и побољшање биорасположивости. У овој студији припремили смо микрочестице напуњене кетопрофеном користећи две технике микрокапсулације: емулзију / згушњавање пчелињим воском и испаравање растварача са целулозним ацетат бутиратом (ЦАБ). Затим смо прилагодили ко-матрице које садрже хидрофобне компоненте (ПММА и ПЦЛ) и хидрофилне компоненте (ХПМЦ и β-циклодекстрин) за модулацију ослобађања лека. Микрочестице на бази пчелињег воска, посебно у комбинацији са ПММА, показале су спорије ослобађање због смањене пропустљивости матрице. Укључивање хидрофилних помоћних материја у микрочестице на бази пчелињег воска убрзало је ослобађање кетопрофена промовисањем продирања воде и солубилизације лекова. Насупрот томе, укључивање хидрофилних помоћних материја у микросфере на бази ЦАБ-а незнатно је смањило ослобађање лека, вероватно зато што је гушћа структура матрице формирана током испаравања растварача. Ови резултати показују да метода енкапсулације и састав матрице критично утичу на кинетику ослобађања кетопрофена, пружајући смернице за рационални дизајн система за испоруку лекова са контролисаним ослобађањем.

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