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Original scientific paper

THE INFLUENCE OF STIRRING RATE IN EMULSION SOLVENT EVAPORATION METHOD ON BIOPHARMACEUTICAL PROPERTIES OF MICROPARTICLES CONTAINING ACETAMINOPHEN

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Acetaminophen is widely used for managing musculoskeletal pain, particularly arthritis in elderly individuals, and for reducing fever associated with colds, flu, and infections. Cellulose-based microparticles were prepared using the acetone/liquid paraffin solvent system through the emulsion solvent evaporation technique. The objective of this study was to assess the influence of stirring rate on encapsulation efficiency, *in vitro* drug release, release kinetics, and microparticle morphology. Drug loading ranged from 13 % to 15 %, while encapsulation efficiency ranged from 67 % to 77 %. A directly proportional relationship between stirring speed and encapsulation efficiency was observed. Drug release followed the Higuchi model, with the release mechanism predominantly Fickian diffusion. In one trial, non-Fickian diffusion was observed. Microphotographs revealed the formation of both spherical and angular microparticles.

Key words: stirring rate; acetaminophen; microparticles; emulsion solvent evaporation technique; biopharmaceutical properties

INTRODUCTION

Interest in designing a microencapsulation process for active pharmaceutical ingredients (APIs) to achieve controlled drug release mechanism has grown significantly in recent decades. Sustained-release formulations are highly desirable in medicine and pharmacy as they reduce the frequency of administration, leading to improved patient compliance. Consequently, polymeric microparticles have been extensively investigated as a controlled drug delivery system.

For the encapsulation of hydrophilic drugs, the double emulsion method, followed by organic solvent evaporation or extraction, is most commonly employed [1, 2]. However, research studies using O_1/O_2 emulsions for microencapsulation have also been conducted [3]. Compared to spray-drying, the emulsion-solvent evaporation method (ESE) allows for the preparation of microparticles with controlled particle size [4]. However, careful selection of starting materials and preparation conditions is necessary to achieve higher encapsulation efficiency (EE), low residual solvent content, and acceptable extraction of the oil phase from the microparticle surface.

To obtain microparticles with desired properties using the ESE method, the following parameters are commonly varied: viscosity of the dispersed phase, volume ratio of dispersed phase to continuous phase, drug quantity in the dispersed phase, emulsifier content, stirring rate, temperature, and pressure [5]. The effects of solvent removal rate have been investigated in Poly(L-lactide) acid (PLLA) and poly(lactic-co-glycolic acid) (PLGA) microspheres [6, 7]. Additionally, the effect of ambient or reduced ambient pressure on solvent evaporation rate in the ESE method has been reported [8]. It has been observed that as the stirring rate increases, the average particle

size of microparticles decreases, as confirmed by relevant publications [9, 10]. However, no data correlating the influence of process parameter stirring time on particle shape, morphology, and surface topography was found at the time of literature review.

Due to the increasing prevalence of musculoskeletal pain (MSP), there is a growing demand for pharmacological treatments worldwide [11, 12]. Non-steroidal anti-inflammatory drugs (NSAIDs) with or without opioids are commonly administered for both acute and chronic MSP management [13]. However, in elderly populations, patients with comorbidities or contraindications may experience adverse effects, leading to the prescription or recommendation of acetaminophen as a therapy. Acetaminophen possesses analgesic and antipyretic properties with minimal anti-inflammatory activity, alongside minor gastrointestinal, renal, and vascular side effects. It has long been one of the most commonly administered drugs, both over-the-counter (OTC) and by prescription, for pain and fever [14].

Therefore, the aim of this study was to prepare, investigate, and characterize a hydroxypropyl cellulose (HPC)-based microsystem as a potential dosage form with controlled release of acetaminophen following oral administration.

MATERIALS AND METHODS

Materials

The active pharmaceutical ingredient (API) N-(4-hydroxyphenyl) acetamide (acetaminophen or paracetamol), hydroxypropyl cellulose as a polymer matrix, and Polyoxyethylene (80) sorbitan monooleate were obtained from Zhejiang Kangle Pharmaceutical Co., Ltd., Nippo Soda, and Sigma Aldrich, respectively. Liquid paraffin, n-hexane, and acetone were purchased from Alkaloid AD. All other chemicals used were of analytical grade.

Methods

Preparation of microparticles

The hydroxypropyl cellulose-based microparticles were prepared using the ESE technique. Briefly, the API was dissolved in acetone, and the polymer was dissolved in the drug solution using a magnetic stirrer until a viscous solution was formed. The resulting drug-polymer solution was extruded using a needle and syringe into an oil phase of liquid paraffin and surfactant (at a constant volume). The emulsion was stirred with a mechanical stirrer at three levels (low – 700 rpm, medium – 900 rpm, and high – 1100 rpm stirring rate) for four hours at ambient temperature to allow the evaporation of the organic solvent from the internal phase. Separation of the obtained microparticles was performed by decantation and filtration. Acetaminophen microparticles were left to stand still for 10–15 minutes to allow particle settlement under gravity. The microparticle residues were washed with n-hexane as a solvent to remove traces of oil. Drying of the microparticles was performed for 24 hours at room temperature.

Characterization of microparticles

Determination of yield of microparticles

The yield (%) of dried HPC microparticles was calculated as the ratio of dried microparticles to the total theoretical amount of starting ingredients.

Determination of encapsulation efficiency of acetaminophen

Microparticles were dissolved in methanol and water (45:55, *v*/*v* %) to achieve a final sample concentration of 0.02 mg/ml. The drug content was analyzed spectrophotometrically at 243 nm (UV spectrophotometer Pharo 600, Merck). The encapsulation efficiency was expressed as the percentage of entrapped acetaminophen in the polymer matrix with respect to the theoretical acetaminophen concentration.

In vitro drug release study

In vitro release from the microparticles was carried out using a magnetic stirrer for four hours at a stirring speed of 50 rpm and a temperature of 37±0.5°C in phosphate buffer pH 6.8, simulating the duodenal medium. The initial concentration of the microparticles was 0.2 mg/ml. Samples of the dissolution media (2 ml) were taken at predetermined time intervals and replaced by fresh buffer. The content of released drug was analyzed spectrophotometrically at 245 nm (UV spectrophotometer Pharo 600, Merck).

ATR-IR spectroscopy

ATR-IR spectra of pure polymer, API, and microparticles prepared at different operating conditions were measured using a Varian 660 FT-IR spectrometer, Varian Inc., equipped with an ATR module with a ZnSe crystal and a low-pressure clamp. The spectra were recorded in the region of 4000–550 cm^{-1}. All spectra were averaged from 16 scans per spectrum, and the resolution was set to 4 cm^{-1}.

Morphology of microparticles

The morphology of the microparticles was analyzed using a Zeiss Axioscope 5 microscope with a Zeiss Axiocam 208 color camera and a reflected light source, at a magnification range of 2.5x-50x.

RESULTS AND DISCUSSION

Methods

Preparation of microparticles

The stirring rate during the hardening phase of emulsified droplets in preparing HPC microparticles is considered an important factor affecting microparticle characteristics. Figure 1 illustrates the morphology of the acetaminophen-loaded microparticles obtained at different stirring rates. Spherical morphology of the HPC-loaded microparticles was observed when the highest investigated mixing speed was applied to remove solvent for forming microparticles. In contrast, angular and nonspherical morphology of the microparticles were observed when a low mixing speed was applied to form microparticles. The obtained microparticles were free-flowing granules, although some agglomeration can be observed from the micrographs (Figure 1). No sharp differences were noted in terms of surface porosity and smoothness.

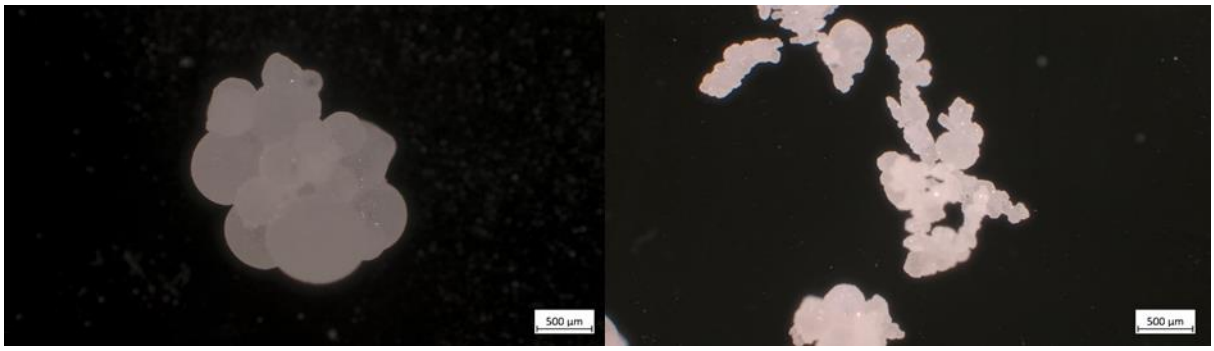

Figure 1. Micrographs of microparticles fabricated at different stirring rates F-1 (1100 rpm) and F-2 (700 rpm)

The physical state of the drug, polymer, and microparticles, as well as drug encapsulation in the polymer matrix, were investigated by ATR-IR spectroscopy. The FT-IR spectrum (Figure 2) showed the characteristic band of phenolic alcohol of acetaminophen at 3321 cm^{-1}, C=O belonging to an amide group at 1651 cm^{-1}, C=C (aromatic) at 1609 cm^{-1}, and N–H (amide) at 1561 cm^{-1} [15].

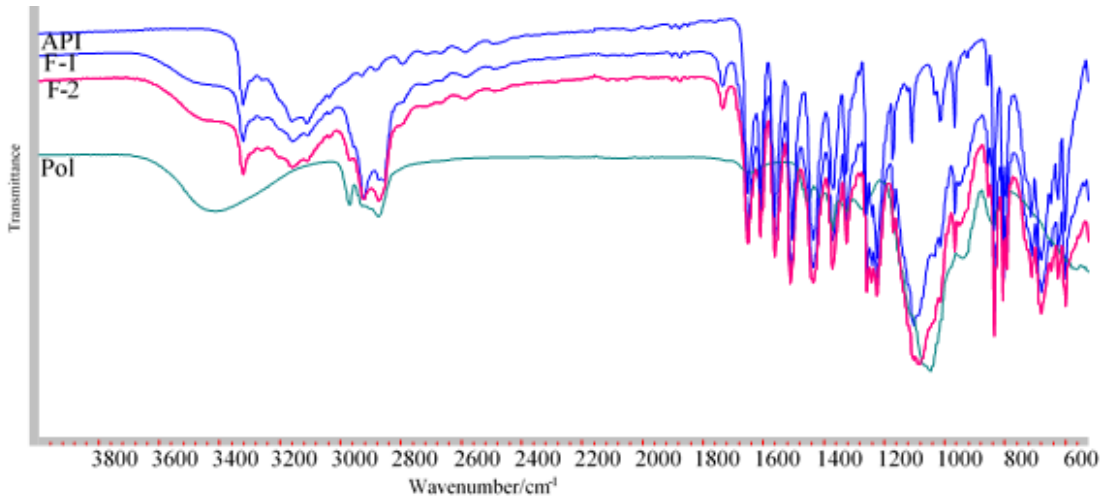

Fig. 2. FT-IR spectra of API, polymer (Pol) and microparticles F-1 and F-2

In the case of HPC, a broad band on the HPC spectrum was observed at 3411 cm^{-1}. The peaks in the region 2970–2874 cm^{-1} represent symmetric and asymmetric C-H stretching. The sharp peaks at

1373 cm^{-1} and 1069 cm^{-1} reflect C-H asymmetric deformations and C-O-C stretching, respectively. The peak at 1644 cm^{-1} is associated with the adsorbed water [16]. Absence of this peak is observed in the microparticles.

In the FTIR spectra of microparticles, there were no notable shifts in the characteristic bands for acetaminophen in the microparticles, indicating compatibility of the components. The new appearing band at 1735 cm^{-1} in the microparticles represents C=O stretching, arising from the residues of the used emulsifier. A slight increase in the intensity of the characteristic bands for acetaminophen in the trials (F-1 *vs*. F-2) at 3321 cm^{-1} and 1651 cm^{-1} can be observed, due to higher API content (14.7 % *vs*. 13.0 %) in the microparticulate delivery system. The FTIR data of HPC (control and microparticles) suggested that C-O-C stretching peaks were observed at a higher wavenumber (1069→1085 cm^{-1}) compared to control. The increase in wavenumber of the stretching peak might be attributed to a change in the bond strength [17].

The variation of stirring rate had a significant effect on the encapsulation of acetaminophen in HPC microparticles, as indicated by the FTIR spectra. The parameter encapsulation efficiency evaluates the successfulness of a drug delivery system (entrapped drug into the carrier). Based on the provided data in Table 1, we may recognize that the investigated drug carrier in our research exhibits satisfactory encapsulation efficiency ranging from 67.4 % to 76.6 %. A value for encapsulation efficiency approaching 65 % was considered suitable.

Table 1. Properties of acetaminophen microparticles

Formulation code	Stirring rate [rpm]	Yield [%]	Bulk density [gcm^{-1}]	Encapsulation efficiency [%]	Theoretical drug content [*w*/*w* %]	Amount of entrapped drug [*w*/*w* %]
F-1	1100	87.3	0.484	76.6	20.0	14.7
F-3	900	70.4	0.349	72.6	20.0	14.0
F-2	700	83.1	0.456	67.4	20.0	13.0

A proportional dependence is observed between stirring rate and encapsulation efficiency. These results agree with previous publication [9]. Yet, an inverse dependence has been observed in encapsulation of simvastatin in a polymeric blend of PLA and PCL [18]. No dependence between bulk density and stirring rate can be noticed.

Figure 3 exhibits the cumulative release profiles of acetaminophen from microparticles that have been produced at various stirring rates. As indicated by Figure 3 and Table 2, acetaminophen *in vitro* release from the microparticle formulations showed an initial burst effect that may be attributed to the presence of drug particles on the surface of the microparticles.

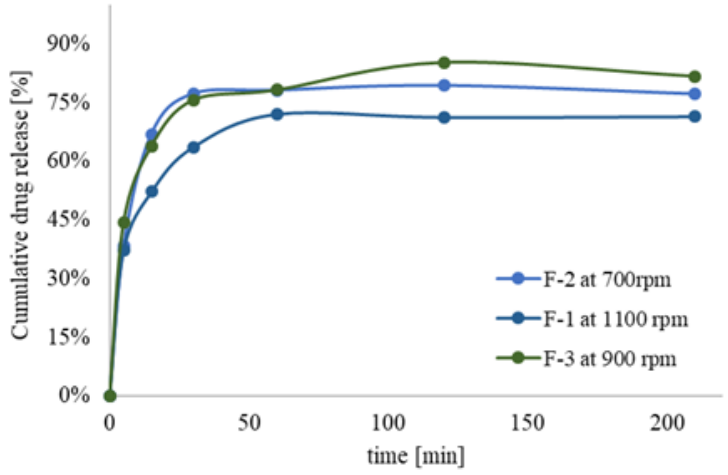

Figure 3. Cumulative percent release of acetaminophen from HPC-based-microparticles prepared at various stirring rates

The lowest initial burst effect was exhibited by F-1 formulation, whereas the highest initial burst effect was exhibited by F-2. Our microparticles were prepared by O_1/O_2 emulsion, in which the hy-

drophilic drug does not tend to migrate to the polar solvent, thus concentrating on the polymeric surface and leading to a burst effect. Nonetheless, the burst effect can be explained by a nonuniform encapsulation of the drug as a result of a nonstable emulsion during the preparation step "solvent removal". Such instability may cause migration of the encapsulated drug molecules toward the microparticle's surface, thus contributing to the initial burst effect [3]. Microparticles with the highest drug encapsulation efficiency (F-1) showed the lowest dissolution rate for all three specification points (Table 2). The specification points were selected based on USP monographs for Acetaminophen tablets and capsules with sustained release [19]. An inverse proportional dependence is observed between stirring rate and released drug at the 15th minute. The acetaminophen microparticles can be filled in gelatin capsules for peroral administration. According to the USP Pharmacopoeia, not less than 80 % in four hours should be released.

Table 2. Acetaminophen *in vitro* release from polymeric microparticles

Formulation code	**F-1 [1100 rpm]**	**F-3 [900 rpm]**	**F-2 [700 rpm]**
Time [minutes]		**Drug release [%]**	
15	54.16	67.76	71.05
60	76.77	84.07	83.76
210	76.12	88.08	82.75

By varying the stirring rate along with polymer content, the acetaminophen release rate can be controlled. The polymer content is expected to contribute to forming a thick polymeric wall that will slow the penetration of the dissolution medium into the polymeric microparticles, thus reducing the drug release and prolonging a lag time [3].

The *in vitro* release profiles were fitted with kinetic models to determine the mechanism of drug release [20, 21]. The fit parameters of zero order, first order, Higuchi model, and Korsemeyer-Peppas 60% are presented in Table 3. The n-value (graphically determined) of microparticles at different stirring rates was between 0.33 – 0.56, indicating that the drug release mechanism was Fickian diffusion (F-1 and F-3), whereas erosion and polymeric chain swelling occurred in F-2.

Table 3. Fitting parameters from kinetic assessment on drug release data

Formulation code	Zero order		First order		Higuchi		Korsemeyer-Peppas 60 %	
	D	R^2	K	R^2	K_H	R^2	n	R^2
F-1	0.0014	0.470	–6·106	0.470	0.028	0.664	0.33	0.999
F-3	0.0015	0.466	–7·106	0.467	0.030	0.659	0.36	1.0
F-2	0.001	0.289	–5·106	0.286	0.027	0.459	0.56	1.0

The assessment of release kinetics revealed that drug release from acetaminophen microparticles followed the Higuchi model. It was observed that, as the stirring rate increased, better compatibility with Q√t kinetics was displayed. Theoretically, it was expected that the prepared microparticles would be compatible with Q√t kinetics, being in a matrix structure [22]. This compatibility is an indicator that a matrix structure in the microparticulate form has been achieved rather than a solubility-controlled dosage form. Our findings are consistent with other investigations [3, 23–25].

CONCLUSION

HPC, which is a biodegradable polymer, was investigated in this study to produce acetaminophen-based microparticles with controlled release by a single ESE method. Microscopy analysis revealed spherical microparticles at the highest stirring rate. By UV-vis and FT-IR spectroscopy, the encapsulation of acetaminophen in HPC was confirmed. A direct dependency between encapsulation efficiency and stirring rate was observed. Yield

percentages may be improved by the scale-up process. An inverse proportional dependence is observed between stirring rate and released drug at the 15th minute. Drug release mainly follows Fickian diffusion, apart from one trial. By controlling the stirring rate, the solvent evaporates from the emulsion, so that the process of hardening and formation of HPC-based microparticles follows. Yet, further investigation into the influence of the following parameters: preparation temperature, reduction in ambient pressure, internal/external phase ratio, and emulsifier content has to be performed to attain a full screening-development study.

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ВЛИЈАНИЕТО НА БРЗИНАТА НА МЕШАЊЕТО НА РАСТВОРУВАЧОТ ВО МЕТОДОТ ЕМУЛЗИЈА-ИСПАРУВАЊЕ ВРЗ БИОФАРМАЦЕВТСКИТЕ СВОЈСТВА НА МИКРОЧЕСТИЧКИТЕ СО АЦЕТАМИНОФЕН

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Ацефаминофенот е широко употребуван во третирањето на мускулноскелетните болки, главно за третирање на артритисот кај повозрасни луѓе и за намалување на температурата при настинки, грип и инфекции. Целулозните микрочестички се подготвуваат со техниката емулзија-испарување на растворувачот со примена на системот ацетон/течен парафин. Целта на ова истражување беше да се евалуира влијанието на брзина на мешањето врз: ефикасноста на инкапсулацијата, *in vitro* ослободување на лекот, кинетиката на ослободувањето и морфологијата на микрочестичките. Инкапсулираниот лек вариираше од 13 % до 15 %, додека ефикасноста на инкапсулацијата се движеше во рангот од 67 % до 77 %. Беше утврдена правопропорционална зависност помеѓу брзината на мешањето и ефикасноста на инкапсулацијата. Ослободувањето на лекот го следи Хигучиевиот модел, а механизмот на ослободувањето на лек е доминантно Фиковата дифузија. За една проба не беше согледана Фикова дифузија. Од микрофотографиите може да се дојде до заклучок дека се добиени микрочестички со сферна и неправилна морфологија.

Клучни зборови: брзина на мешање; ацетаминофен; микрочестички; техника на емулзија-испарување на растворувач; биофармацевтски својства