Abstract

Objectives. To develop a combined once-daily sustained release microencapsulated dosage form of nimesulide (NSD) and serratiopeptidase (SPD) with different physicochemical properties using ethyl cellulose (ETC) as the release-controlling factor and to evaluate the drug release parameters in accordance with various kinetic release models.

Material and Methods. In order to achieve the intended sustained-release profile, microparticles were prepared using a non-solvent addition coacervation technique. An in-vitro dissolution analysis was conducted using a USP dissolution apparatus type II. The temperature of the dissolution medium (phosphate buffer pH 6.8 plus sodium lauryl sulphate 1%, 900 mL) was maintained at 37°C ± 1°C with a stirring rate of 75 rpm.

Results. The physico-chemical parameters of the formulated microparticles were assessed and the results were found to be within acceptable limits. Various kinetic models were used to analyze the dissolution data to investigate the mode of drug release. The dissolution behavior was found to be a complex anomalous pattern of drug release, best explained by the Higuchi expression. ETC had no significant chemical interaction with the drugs. All the batches of microcapsules showed good stability and reproducibility.

Conclusions. The non-solvent addition coacervation technique is a promising method to develop a combined once-daily sustained release microencapsulated dosage form of nimesulide and serratiopeptidase with different physicochemical properties using ethyl cellulose as release-controlling factor (Adv Clin Exp Med 2011, 20, 5, 605–611).

Key words: nimesulide; serratiopeptidase; ethyl cellulose; microencapsulation.

Streszczenie

Cel pracy. Opracowanie złożonej, podawanej raz dziennie, o przedłużonym uwalnianiu, w mikrokapsułkach postaci nimesulidu (NSD) i serratiopeptidazy (SPD) o różnych właściwościach fizykochemicznych, z zastosowaniem etylocelulozy (ETC) jako czynnika kontrolującego uwalnianie i ocenę wskaźników uwalniania leku według różnych kinetycznych modeli uwalniania.

Materiał i metody. W celu osiągnięcia zamierzonego profilu o przedłużonym uwalnianiu, przygotowano mikrokapsułki za pomocą techniki koacerwacji bez dodatku rozpuszczalnika. Analizę rozpuszczania in vitro przeprowadzono z użyciem urządzenia do rozpuszczania USP typu II. W środowisku uwalniania (bufor fosforanowy o pH 6,8 oraz laurylosiarczan sodu 1%, 900 ml) utrzymywano temperaturę 37°C ± 1°C z szybkością mieszania 75 rpm.


Słowa kluczowe: nimesulid, serratiopeptidaza, etyloceluloza, mikrokapsułkowanie.
Many different peroral modified release (MR) formulations have been developed in the last twenty years. The aim of MR formulations is to deliver the drugs at an established rate, in order to minimize dose frequency, improve patient compliance and achieve more uniform circulatory drug levels, thus improving the therapeutic effectiveness of the medication [1, 2]. A variety of polymers have been introduced for developing MR formulations. One of these is ethyl cellulose (ETC), a lipophilic non-biodegradable polymer which is increasingly used in the fabrication MR products due to its attractive properties: excellent compressibility and a compatible physico-chemical nature offering minimal toxicity [3, 4]. ETC was therefore selected for the formation of a matrix to control drug release.

Nimesulide (NSD) is a nonsteroidal anti-inflammatory drug. It is acidic in nature due to the presence of a sulfonanilide group [5]. NSD is able to inhibit the activity of cyclo-oxygenase-2, which ultimately limits the production of harmful prostaglandins and enhances the availability of useful prostaglandins [6]. NSD is categorized as a class-II drug in the biopharmaceutic classification scheme, since it exhibits very sparing solubility (0.01 mg of NSD is soluble in 1 ml of water) along with high permeability [7], due to which formulators consider NSD a problematic drug that is difficult to formulate as an oral drug delivery product with high bioavailability.

Current research trends in pharmaceutical technology are focused on the development of multi-unit MR formulations like nano- and micro-particles. Multi-unit formulations are capable of distributing the drug more evenly in the alimentary canal for more even availability of drug to the systemic circulation [3–4, 8–9].

Proteolytic enzymes such as serratiopeptidase (SPD) have been proposed as good analgesic and anti-inflammatory agents that are rapidly absorbed in the intestinal region [10–13]. However, the acidic contents of stomach cause SPD to biodegrade, which results in its very low oral bioavailability [14]. This situation necessitates the development of formulations that deliver the drugs in the intestine, such as enteric-coated preparations. In the study, NSD and SPD loaded ETC microparticles were prepared for oral delivery in combined form, filled in hard gelatin capsules and subjected to various quality control tests in triplicate. A survey of the literature showed no sustained-release formulation for SPD or for combined NSD-SPD. Thus, the present study may be considered a pioneer achievement.

Material and Methods

Material

The nimesulide and serratiopeptidase samples used in the study were received as a gift from PharmEvo Pharmaceuticals, Pakistan. Ethyl cellulose (22 cp) was procured from Sigma, USA. Dichloromethane was purchased from BDH Chemicals Limited, UK. Methanol and n-hexane were purchased from Merck, Germany. Mineral oil was obtained from Acros Organics, USA. All the materials were of analytical grade and were used as purchased.

Preparation of the Microparticles

The non-solvent addition coacervation technique was used to prepare SPD-ETC and NMD-ETC microparticles, employing relatively safe solvents and mediums such as dichloromethane (DCM) and mineral oil as the solvent and non-solvent, respectively. First, 1 g of ETC was dissolved in 15 ml of DCM with continuous magnetic stirring (Velp, Italy) at 700 rpm and then 1 g of the drug is dispersed in the ETC solution. Phase separation
was achieved by adding mineral oil, and ultimately a microparticulate suspension was achieved with a milky color. This was followed by triple washing with n-hexane, air- and oven-drying for 48 hours at 45°C, and then storage in tightly closed containers until the characterizations were done. The dried microparticles were filled into hard gelatin capsules (size 000) so that each capsule contained 200 mg of NMD and 20 mg of SPD, which are the maximum daily doses of the drugs. For stability studies, equal-weight quantities of microparticles were placed in nine air-tight amber bottles and stored at 25°C ± 0.1 (in an oven, Memmert, Germany), 40°C ± 0.1 (in an oven) and 40°C ± 0.1/75% relative humidity (in an oven). Every month, one bottle was taken, analyzed for the drug contents and used for the dissolution study.

**In Vitro Release Study**

The *in vitro* dissolution study was conducted using a USP dissolution apparatus type II. The temperature of the dissolution medium (phosphate buffer pH 6.8 plus sodium lauryl sulphate 1%, 900 mL) was maintained at 37°C ± 1, with a stirring rate of 75 rpm. One capsule filled with microparticles of NSD and SPD was placed in each dissolution vessel (n = 6). At 0, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12 and 24 hours, 5 mL of samples were withdrawn with an automatic sample collector (Pharma Test, Germany). The volume of the dissolution fluid was adjusted every time to 900 mL. Samples were assayed spectrophotometrically at λ<sub>max</sub> = 404 nm (NSD) and λ<sub>max</sub> = 230 nm (SPD) in a double beam UV-visible spectrophotometer (Shimadzu UV-1601, Japan) [15]. The drug concentration was calculated using a standard calibration curve on a range of 5–30 μg/mL for both drugs. The regression coefficients for NSD and SPD were 0.992 and 0.984, respectively.

**Determination of Production Yield and Encapsulation Efficiency**

The production yield (PY %) was calculated by weighing the raw materials (WR) and the microparticles obtained (WM) and using following formula:

\[
\text{PY\%} = \frac{\text{WM}}{\text{WR}} \times 100.
\]

Encapsulation efficiency was assessed by dissolving the weighed quantities of microparticles from each batch in 10 ml of methanol to dissolve the ethyl cellulose coating. Then an equal volume of phosphate buffer pH 6.8 was added and moderately heated to evaporate the methanol. Phosphate buffer pH 6.8 was added in sufficient quantity to bring the final volume up to 900 mL; this was followed by filtration and analysis at 230 and 404 nm for SPD and NSD, respectively. Encapsulation efficiency was calculated using equation 1 and 2 [16]:

\[
\text{DL\%} = \frac{\text{AD}}{\text{AM}} \times 100,
\]

where DL %, AD and AM are drug loading, the amount of drug in microspheres, and the amount of microspheres, respectively.

\[
\text{EE\%} = \frac{\text{AL}}{\text{TL}} \times 100,
\]

where EE %, AL and TL represent encapsulation efficiency (%), actual drug loading (%) and theoretical drug loading (%), respectively.

**Micromeritic Properties of the Microparticles**

To assess the flowability characteristics of the microparticles, the angle of repose was evaluated. It has been proposed that particles exhibit good flow characteristics if the angle of repose is under 50, and vice versa [17]. The angle of repose was assessed by passing the microparticles through a sintered glass funnel with an internal diameter of 27 mm, resulting in the formation of a heap. The height (h) of this heap and the radius (r) of the cone base were measured. The following equation was used to assess the angle of repose (θ):

\[
\theta = \tan^{-1} \left( \frac{h}{r} \right).
\]

The microparticles were evaluated by assessing their particle size and micromeritic properties, including tapped bulk density, percentage compressibility index (% CI: a crucial parameter used for the assessment of flowability) and true density (d). The diameter of dried microparticles was measured from the scanning electron micrographs. The density of the microparticles was determined by immersing them for three days in a 0.02% Tween 80 solution placed in a metal mesh basket, then using the submerged particles to determine density by the displacement method, using n-hexane as a non-solvent [4]. Tapped densities and % CI were evaluated by the tapping method, using the following equations:

\[
\text{Tapped density} = \frac{\text{Mass of microparticles}}{\text{Volume of microparticles after tapping}}
\]

Compressibility index (%) = \[1–\frac{V}{V_o}\] × 100,
where V and Vo are, respectively, the volumes of the sample after and before the standard tapping. A 10 mL measuring cylinder was used for the tapping. The initial volume of the microparticles was noted, followed by continuous tapping at a rate of 100 taps/min on a hard surface; tapping was stopped when no further change in the volume was observed.

**Interaction Study**

The drug-polymer interaction was studied by FTIR spectroscopy (MIDAC M2000, USA). Active SPD and NSD and their microparticles were evaluated by the KBr disc method. The background spectrum was recorded before studying each sample. The FTIR spectrum of each sample was taken in the range of 500–4500 cm⁻¹ [16].

The X-ray powder diffractometric analysis of the microparticles and their individual components was conducted using a D8 Discover (Bruker, Germany) to detect any possible alteration in the crystallinity of NMS during microencapsulation. The sample scanning in a diffraction angle range from 10° to 70° was conducted using the following experimental conditions: Cu-Kα radiation 1.5406 Å (source), 4°/min scan speed, scintillation detector, primary slit 1 mm, secondary slit 0.6 mm [16].

**Results and Discussion**

The non-solvent addition coacervation technique was used to prepare NSD and SPD microparticles. NSD and SPD were dispersed in dichloromethane (the solvent) and mineral oil was used as a non-solvent to induce coacervation. The election of ETC as a coating material was made on the basis of its safety, stability, hydrophobicity and compactness among hydrophobic polymers [18].

The microparticles of NSD were light yellow, free flowing and spherical in shape when observed under a scanning electron microscope, while the SPD microparticles were white in color. However, the microparticles of both drugs were aggregated. The mean particle size for NSD and SPD microparticles was 32 μm and 43 μm, respectively. The entrapment efficiency of the microparticles was 89% and 85% for NSD and SPD, respectively. The rest of the micromeritic characteristics are given in Table 1. Weight variation of the capsule shells filled with microparticles was within the permitted compendial limits.

The full drug release profiles (Fig. 1) were evaluated kinetically, and the goodness of fit with various models was investigated: the zero order model, the first order model, the Higuchi model, the Hixson-Crowell model and the Korsmeyer-Peppas model. The one with the highest coefficient of determination $R^2$ was deemed the appropriate model.

The release profiles of all the microparticles were best explained by the Higuchi model, with the highest linearity ($R^2 = 0.97$ and $0.98$ respectively for SPD and NSD), followed by the zero order model ($R^2 = 0.88$ and $0.94$ respectively) and the first order model ($R^2 = 0.49$ and $0.52$ respectively). This indicates that the drug release is controlled by diffusion through pores in the coating and not through the swollen polymer [19]. The $n$-values, calculated by the Korsmeyer-Peppas model, were in the range of 0.75–0.62, which confirmed the release of NMS from ETC microparticles via non-Fickian/anomalous diffusion. The erosion seems to be the result of drug release. Judging from the structure of the microparticles, diffusion seems to be the predominant mechanism (Fig. 2).

There were no significant differences in the release profiles of the various batches of microcapsules, which indicates that the processes used in their manufacture are reliable and reproducible ($p > 0.05$, $f_1 < 0.28$, $f_2 > 99.67$). It was also noted there was no alteration in the release behavior for up to three months of storage. In addition, there were no changes in the physical characteristics of the microparticles, indicating that NSD and SPD were stable in the ETC microparticles for up to three months.

<table>
<thead>
<tr>
<th>Formulations (Właściwości)</th>
<th>NSD</th>
<th>SPD</th>
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</thead>
<tbody>
<tr>
<td>Bulk density (g/mL)</td>
<td>0.231</td>
<td>0.243</td>
</tr>
<tr>
<td>Tapped density (g/mL)</td>
<td>0.243</td>
<td>0.261</td>
</tr>
<tr>
<td>Hausner’s ratio</td>
<td>1.052</td>
<td>1.075</td>
</tr>
<tr>
<td>Compressibility index (%)</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td>Angle of repose</td>
<td>23.47°</td>
<td>21.78°</td>
</tr>
<tr>
<td>Packing rate (C)</td>
<td>0.13</td>
<td>0.15</td>
</tr>
<tr>
<td>Yield (%)</td>
<td>96</td>
<td>94</td>
</tr>
<tr>
<td>Entrapment efficiency (%)</td>
<td>89</td>
<td>85</td>
</tr>
<tr>
<td>Particle size (μm)</td>
<td>32</td>
<td>43</td>
</tr>
<tr>
<td>$t_{50%}$ (M ± S.D) (hrs)</td>
<td>5</td>
<td>4</td>
</tr>
</tbody>
</table>

*Average of three batches ± SD.

*Srednia trzech partii ± SD.

Table 1. Physico-chemical characterization of the microparticles (experiments performed in triplicate)

Tabela 1. Fizykochemiczne właściwości mikrokapsułek (eksperyment przeprowadzony 3 razy)
The FTIR spectra of the formulations showed all the characteristic peaks of the drugs, including C-H aromatic, S=O, C-O-C ether linkage, CH3 C-H bending, NO2, CH3 C-H stretching and N-H (Fig. 3). Thus, the spectroscopy results confirmed that there was no strong interaction of ETC with these drugs when they were encapsulated in ETC coating.

The X-ray diffractometry showed the amorphous and crystalline nature of nimesulide in both its encapsulated and free forms (Fig. 4). There was, however, a decrease in the signal intensity (i.e. the crystallinity) of the drugs in microparticle form as compared to the pure components, which is an indication of a slight shift from a crystalline nature to an amorphous one. This change increases the solubility of the drugs, which ultimately increases their bioavailability [16].

This study showed that the non-solvent addition coacervation technique employing dichloromethane and mineral oil as solvent and non-solvent, respectively, is an appropriate method for microencapsulate drugs with different physicochemical properties (such as SPD and NSD) in ETC coating. The variations observed in the entrapment efficiency, production yield, mean particle size and the drug release behavior of the formulations can be attributed to the nature of the drugs. This suggests the potential of ethyl cellulose microparticles as a suitable multi-unit sustained release drug delivery system, because it does not affect the nature of the drugs and there is no

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**Table 2. Goodness of Fit (using R²) for the mathematical models used to determine the drug release rate from NSD and SPD microparticles [Y-equation (Y = aX + b), determination co-efficient (R²), and release exponent (n)] (experiments performed in triplicate)**

<table>
<thead>
<tr>
<th>Model</th>
<th>Parameters (Wskaźniki)</th>
<th>Microparticles (Mikrokapsułki)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>NSD</td>
</tr>
<tr>
<td>Zero Order</td>
<td>Y-equation</td>
<td>6.61x + 14.65</td>
</tr>
<tr>
<td></td>
<td>R²</td>
<td>0.94</td>
</tr>
<tr>
<td>First Order</td>
<td>Y-equation</td>
<td>0.19x + 2.52</td>
</tr>
<tr>
<td></td>
<td>R²</td>
<td>0.52</td>
</tr>
<tr>
<td>Higuchi</td>
<td>Y-equation</td>
<td>27.53x – 6.28</td>
</tr>
<tr>
<td></td>
<td>R²</td>
<td>0.98</td>
</tr>
<tr>
<td>Korsmeyer-Peppa</td>
<td>Y-equation</td>
<td>0.62x + 2.98</td>
</tr>
<tr>
<td></td>
<td>R²</td>
<td>0.99</td>
</tr>
<tr>
<td>n (release exponent)</td>
<td></td>
<td>0.62</td>
</tr>
</tbody>
</table>

Fig. 1. Dissolution profile of NSD and SPD microparticles (experiments performed in triplicate)
Fig. 2. Scanning electron microscope images of the external structure of NSD and SPD microparticles

Ryc. 2. Zewnętrzna struktura mikrokapsułek NSD i SPD – obraz ze skaningowego mikroskopu elektronowego

Fig. 3. FTIR spectra of active nimesulide (A), active serratiopeptidase (B), ethylcellulose (C), microparticles of nimesulide (D) and microparticles of serratiopeptidase (E)

Ryc. 3. Widmo IR aktywnego nimesulidu (A), aktywnej serratiopeptydazy (B), etylocelulozy (C), mikrokapsułek nimesulidu (D) i mikrokapsułek serratiopeptydazy (E)

Fig. 4. X-ray diffractometer analysis of active nimesulide (A), active serratiopeptidase (B), ethylcellulose (C), microparticles of nimesulide (D), microparticles of serratiopeptidase (E)

Ryc. 4. Rentgenowska analiza dyfrakcyjna aktywnego nimesulidu (A), aktywnej serratiopeptydazy (B), etylocelulozy (C), mikrokapsułek nimesulidu (D) i mikrokapsułek serratiopeptydazy (E)
chemical bonding between these drugs and ethyl cellulose during the microencapsulation method. The *in vitro* characterization confirms this pioneer formulation as a suitable combined-drug delivery system for NSD and SPD. At the time of writing an *in vivo* characterization is in progress in the authors’ laboratory, and it will be presented after its completion.

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**References**


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