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Research paper

# Characterization and physical stability of fast-dissolving microparticles containing nifedipine

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**Abstract**

The suitability of a poly(sodium methacrylate, methyl methacrylate) (NaPMM), a novel mucoadhesive material, to prepare fast-dissolving microparticles containing nifedipine (NIF) in the range of 25–75% w/w was verified. Microparticles made of a low-viscosity hydroxypropylmethylcellulose (HPMC), were also prepared to compare the NIF release profile and bioadhesive properties. The release test was carried out in oversaturation conditions. The physical state of microparticles was also investigated. The formulation stability was evaluated over a 3-month period in long-term and accelerated conditions. The presence of amorphous NIF within freshly prepared microparticles was attributed to interactions between the drug and both polymers. NaPMM conferred to microparticles suitable mucoadhesive properties and significantly increased NIF dissolution rate in comparison to HPMC. Nevertheless, NIF apparent solubilities obtained by NaPMM microparticles were lower than those obtained by the HPMC set. After 3-month storage in the case of HPMC microparticles, NIF dissolution rate and supersaturation degree significantly decreased due to drug crystallization. As far as NaPMM microparticles are concerned, neither NIF dissolution rate nor apparent solubility significantly changed.


*Keywords:* Nifedipine; Mucoadhesion; Solid state stability; Microparticles; Poly(sodium methacrylate, methyl methacrylate); Supersaturation

## 1. Introduction

In recent years the interest in bioadhesion has inspired the development of novel bioadhesive polymers for mucosal delivery. Mucoadhesive polymers present the potential to prolong both the residence time and the extent of contact on the absorbing mucosa, resulting in an enhancement of drug absorption.

Poly(sodium methacrylate, methyl methacrylate), NaPMM, obtained by neutralization with alkali of a pharmaceutical approved polymethyl methacrylate, has been proposed as a low-swellable mucoadhesive material to design drug delivery systems intended for buccal administration of active ingredients [1,2]. Mucoadhesion has been attributed to interactions between the (C=O) of the esteric group of NaPMM and mucin via hydrogen bond formation which could be responsible for the properties of the copolymer [3]. Additionally, NaPMM was demonstrated to be a suitable carrier to prepare microparticles containing amorphous piroxicam. The microparticulate systems significantly improved the drug apparent solubility and exhibited satisfactory mucoadhesive properties [2].

In the preparation of mucoadhesive fast-dissolving systems, the selection of a suitable polymeric carrier represents a critical issue since it is supposed to assure mucoadhesive properties, improve the drug apparent solubility and dissolution rate. These properties can be deeply influenced by possible interactions between the drug and the polymer.

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Sublingual NIF is widely used in the management of moderate and severe hypertensive emergencies both in adults [4–6] and children [7–9]. NIF is administered as a solution using liquid oral dosage forms or squeezing out the fluid content of a soft gelatin capsule beneath the tongue. Microparticulate systems appear to be an interesting approach to improve dosage accuracy, residence time beneath the tongue as well as patient compliance.

In the present work, NaPMM microparticles containing different amounts of NIF were prepared and their performances were compared to those of microparticles made of Methocel® E5, a low viscosity hydroxypropylmethylcellulose (HPMC). This polymer was selected because of its compatibility with NIF and inhibitory effect on NIF crystallization in supersaturated solutions [10,11]. The effects of drug loading and the type of polymer were assessed on NIF release profile and mucoadhesion. In particular, the *in vitro* drug release test was carried out in oversaturation condition to discriminate possible differences among microparticulate delivery systems in terms of NIF dissolution rate and apparent solubility [12,13]. The physical state of the pure NIF and NIF loaded within microparticles was investigated using differential scanning calorimetry (DSC), X-ray diffraction and ATR-FTIR spectroscopy. The formulation stability was evaluated in long-term and accelerated conditions over a 3-month period.

## 2. Materials and methods

### 2.1. Materials

Micronized crystalline nifedipine (NIF) was kindly donated by TEVA Chemicals (I). According to manufacturer's data sheet, the mean diameter of particles measured by light scattering was 10 μm; due to NIF instability upon light exposure, all the operations were carried out under light protected conditions.

Poly(sodium methacrylate, methyl methacrylate) (NaPMM), molar proportions of the monomer units 1:2, molecular weight 135,000 Da, was prepared as previously described [1]. Briefly, NaPMM was obtained by adding NaOH pellets to 3% w/w Eudragit®S100 (Röhm, G) aqueous suspension untill complete salification. The required amount of NaOH was calculated after titration on the basis of Eur. Ph. method [14]. The final solution (pH 7.1) was dried by spray-drying.

Methocel® E5 (HPMC, substitution: % methoxyl 28–30; % hydroxylpropyl 7–12; nominal viscosity 2% in water: 5 cP) was provided by Colorcon (I). HPMC used in the solid state characterization as well as mucoadhesion evaluation was dissolved and spray-dried as described in Section 2.2.

Crude (type II) mucin from porcine stomach was purchased from Sigma Chemical Co (USA). Microcrystalline cellulose (Avicel® PH 101) was obtained from FMC BioPolymer (USA). All solvents were of analytical grade.

### 2.2. Preparation of NIF loaded microparticulate systems

Two sets of microparticulate systems made of NIF/NaPMM and NIF/HPMC were designed. For each series the drug/polymer ratios were at 25/75, 50/50 and 75/25% w/w, respectively (Table 1). Microparticles were prepared by spray-drying (Lab-Plant model SD04, UK). The feed was obtained by dissolving NIF in a predetermined volume of acetone and the polymer in a prefixed volume of water; the concentration in each solution was fixed to obtain the desired NIF/polymer ratio in a clear 3% w/v feed. The microparticles were obtained by spraying the feed through a standard nozzle with inner diameter of 1 mm. The process parameters were set as follows: inlet temperature: 80 °C; outlet temperature: 48–50 °C; feed flow rate: 11 ml/min. All the microparticles were stored a week at 25 °C before being assayed (time 0).

### 2.3. Particle size and morphology

Accusizer 770 (PSS Inc., USA) using the technique "Single Particle Optical Sensing" was used to determine the size distribution of microparticles. Particle sizes were expressed as volume mean diameters.

The surface morphology and shape of placebo and drug loaded microparticles were analyzed by using SEM (JEOL JSM 5500LV, J).

### 2.4. Solid state characterization

#### 2.4.1. ATR-FTIR spectroscopy

About 15.0 mg sample was place on a diamond crystal mounted in ATR cell (Perkin-Elmer, US). FTIR measurements were performed with Spectrum™ One spectrophotometer (Perkin-Elmer, USA). The spectra were recorded

Table 1
Theoretical composition (%, w/w), drug content and residual moisture content of NIF microparticles

| Formulation No. | Theoretical composition (%, w/w) | | | Drug content (%) | Moisture content (%, w/w) |
|---|---|---|---|---|---|
| | NIF | NaPMM | HPMC | | |
| 1 | 25 | 75 | – | 26.5 ± 0.4 | 9.09 |
| 2 | 50 | 50 | – | 49.4 ± 0.7 | 2.27 |
| 3 | 75 | 25 | – | 74.4 ± 1.1 | 0.67 |
| 4 | 25 | – | 75 | 25.0 ± 0.3 | 1.47 |
| 5 | 50 | – | 50 | 50.0 ± 0.1 | 1.70 |
| 6 | 75 | – | 25 | 70.1 ± 0.2 | 1.06 |

at 2 $cm^{-1}$ resolution and 16 scans were collected over the wavenumber region 4000–650 $cm^{-1}$. The analyses were performed on NaPMM, HPMC, NIF, physical mixtures and microparticles. The NIF/NaPMM and NIF/HPMC physical mixtures were prepared by mixing the components in a mortar.

#### 2.4.2. Thermal analysis

The residual water content of microparticles was determined by thermogravimetric analyses using a TGA 2050 thermogravimetrical analyzer (TA Instruments, USA). Samples of approximately 20 mg were heated in a platinum crucible at 5 K/min under a nitrogen atmosphere and the loss of weight was recorded.

DSC data were recorded by using a DSC 2010 TA (TA Instruments, USA). The samples were sealed in aluminum pans and heated in inert atmosphere (70 ml/min $N_2$). The reference was an empty pan. The equipment was calibrated with an indium sample. Samples of NIF microparticles were scanned at 10 K/min from 30 to 200 °C under nitrogen purging (70 ml/min).

#### 2.4.3. Powder X-ray diffraction

Powder X-ray diffraction spectra of NIF, NaPMM, HPMC, their physical mixture and drug loaded microparticles were collected by using a Rigaku DMAX powder diffractometer (Rigaku, J) with Cu-Kα radiation and a monochromator on the diffracted beam. The analyses on NIF microparticles were repeated after 1 and 3 months of storage.

### 2.5. Drug content

NIF content of the microparticles was determined by an HPLC method [15]. HPLC system was an HP1100 Chemstation (Hewlett Packard, USA). Samples of microparticles were exactly weighted in order to get 7 mg of NIF and dissolved in 100 ml of mobile phase and aliquots of 1 ml were diluted 1:10 in mobile phase. The results were expressed as means of three determinations.

*Chromatographic conditions*. Column: Lichrospher 100 RP-18 E, 5 μm, 125 × 4.0 mm ID; mobile phase: acetonitrile/methanol/water (25/25/50, % v/v/v); flow rate = 1.3 ml/min; wavelength = 230 nm; temperature = 37 °C; injection volume = 10 μl. The experimental conditions were set up in order to avoid interferences of the other components.

### 2.6. Determination of equilibrium solubility

To determine drug solubility, NIF was added to deionized water (20 ml) until a heterogeneous system (solid sample and liquid) was obtained. The solution containing solid excess of NIF was stirred for a period of 30 min at a temperature of 60 ± 2 °C. Then, the suspension was cooled down at 37 ± 2 °C and stirred vigorously allowing it to achieve thermodynamic equilibrium. After a period of 48 h, three aliquots of 0.5 ml were taken out and diluted with fresh acetonitrile. The NIF concentration in the saturated solution was measured by HPLC assay.

### 2.7. In vitro drug release

Comparison of release profile of the pure crystalline NIF with drug loaded microparticles was performed a week after preparation (time 0). Microparticles were mixed with microcrystalline cellulose in ratio 1:2 to improve flowability and avoid aggregation. The mixture was exactly weighed in order to obtain an amount of NIF corresponding to 4-fold drug solubility in water (4.7 ± 0.2 mg/l).

*Experimental conditions*. Dissolution medium: 500 ml purified water; temperature: 37 ± 0.5 °C; paddle speed: 50 rpm; wavelength: 235 nm (UV spectrophotometer Beckman DU640, USA). The results were expressed as means of three determinations. This wavelength was selected as the two polymers did not interfere.

The increase of NIF apparent solubility at 37 ± 0.5 °C was expressed as degree of supersaturation (SD) [12,13] according to the following equation:

$$SD = C_{max}/C_s$$

where $C_{max}$ represents the maximum concentration of NIF in the dissolution medium during testing and $C_s$ is NIF equilibrium solubility in water at 37 °C.

### 2.8. In vitro mucoadhesive test

The texture analysis was performed as previously described [1] using mucin as the adherent substrate [16–18]. Briefly, NaPMM, HPMC and NIF loaded microparticle compacts weighing 170 mg were obtained applying a compression force of 10 tons for 30 s by means of a hydraulic press (Glenrothes, UK) equipped with a flat faced punches (11.28 mm die diameter).

The testing material compacts were attached to the mobile steel punch by cyanoacrylate glue. Mucin compacts of 130 mg were obtained applying a compression force of 10 tons for 60 s. The compact was attached by cyanoacrylate glue to a steel plate fixed at the bottom of the tensile apparatus and hydrated with 80 μl deionized water upon 5 min to obtain a jelly surface layer. Upon making contact between the polymeric compact and the hydrated mucin, a constant force of 1.3 N was imposed for 360 s. The mucoadhesive performance was measured in terms of the detachment force required to separate a bioadhesive compact from the mucin (maximum detachment force, MDF) upon an elongation of 10 mm at the constant rate of 0.1 mm/s. The areas under the curve of the detachment force versus the elongation were also determined to represent the work or energy required to detach the two compacts. The stainless steel punch was used as negative control. A software-controlled dynamometer (AG/MCL, Acquati, I) with a 5 daN force cell was used. The results are expressed as means ± standard deviation ($n = 4$).

### 2.9. Physical stability testing

Physical stability testing on microparticles containing NIF/NaPMM and NIF/HPMC was conducted by storing the samples in tightly closed amber vials in long-term ($25 \pm 1$ °C, 60% RH, installed with a saturated solution of $KNO_2$) and accelerated ($40 \pm 1$ °C, 75% RH, installed with a saturated solution of NaCl) conditions. Changes in physical state of the samples after 1 and 3 months of storage were evaluated by SEM, DSC and X-ray diffraction. The *in vitro* drug release profile was also determined.

### 2.10. Statistical analysis

Tests for significant differences between means were performed by Student's *t*-test or one-way ANOVA by using the software SPSS 11 (Spss Inc., USA). Differences were considered significant at the $p < 0.05$ level.

## 3. Results and discussion

### 3.1. Morphology characterization of microparticles

The particle-size distribution of the NIF loaded microparticles ranged from 8.6 to 11.5 μm and the mean diameters overlapped with that of commercial NIF.

In general, the spray-dried microparticles displayed a characteristic raisin-like morphology (corrugated particles) due to low permeability to solvents during the crust formation in drying step [2]. However, the effect was more pronounced with the HPMC microparticles compared to the NaPMM microparticles (Fig. 1a and b). This feature did not appear to be affected by the drug/polymer ratios.

### 3.2. Solid state characterization

#### 3.2.1. NaPMM series

In the diffractograms of physical mixture the main bands of NIF were clearly detectable over the broad pattern of the amorphous polymer according to the drug/NaPMM ratio (data not shown). In the microparticle diffractograms, the characteristic peaks of crystalline drug were not detectable at the lowest NIF content, and the X-ray diffraction patterns of the microparticles (Fig. 2d) were comparable to the spectrum of raw NaPMM (Fig. 2e). Increasing the NIF/NaPMM ratios, peaks associated with crystalline NIF (Fig. 2a) were detectable over the amorphous baseline (Fig. 2c and b). The ATR-FTIR spectroscopy revealed the molecular structure of microparticles since the position of the main bands of NIF crystalline form, namely the ν-(NH) peak at 3326 and the ν-(C=O) peak of the esteric groups and 1678 $cm^{-1}$, can be used to indicate changes in hydrogen bonding [19]. Indeed, in the spectrum of amorphous NIF the shifts of both ν-(NH) and ν-(C=O) peaks take place towards to free regions because of the breaking of intermolecular hydrogen bond

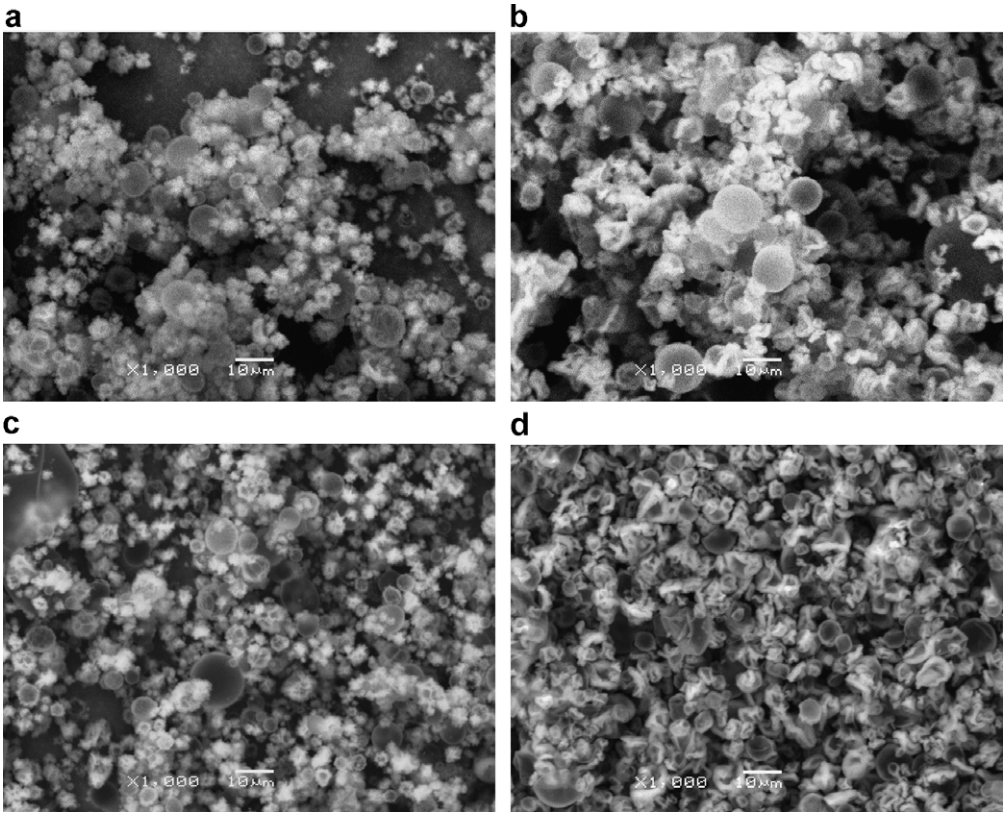


Fig. 1. Scanning electron microphotographs of NIF loaded microparticles: (a) formulation no. 1 and (b) formulation no. 4 after preparation; (c) formulation no. 1 and (d) formulation No. 4 after 3-month storage.

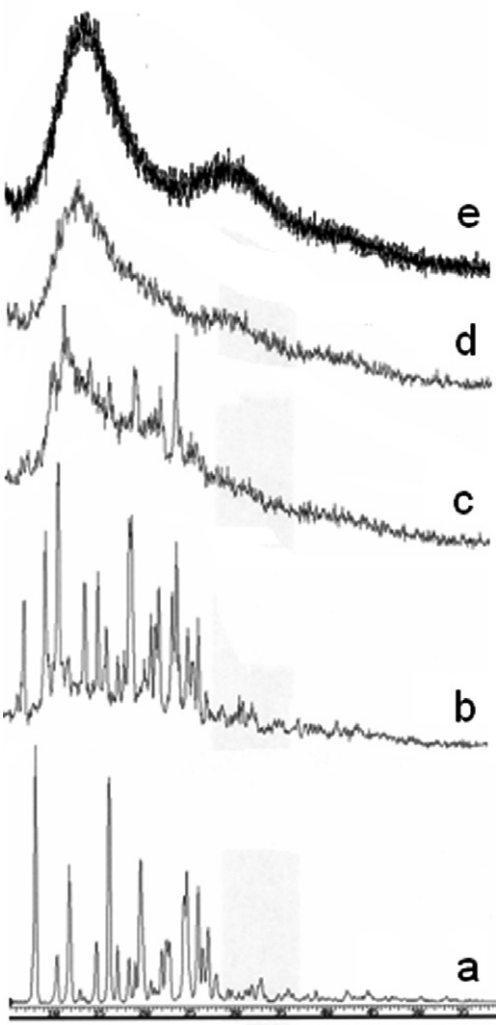


Fig. 2. X-ray diffraction patterns of (a) NIF, NaPMM microparticles containing (b) 75, (c) 50 and (d) 25%, w/w NIF (time 0) and (e) raw NaPMM.

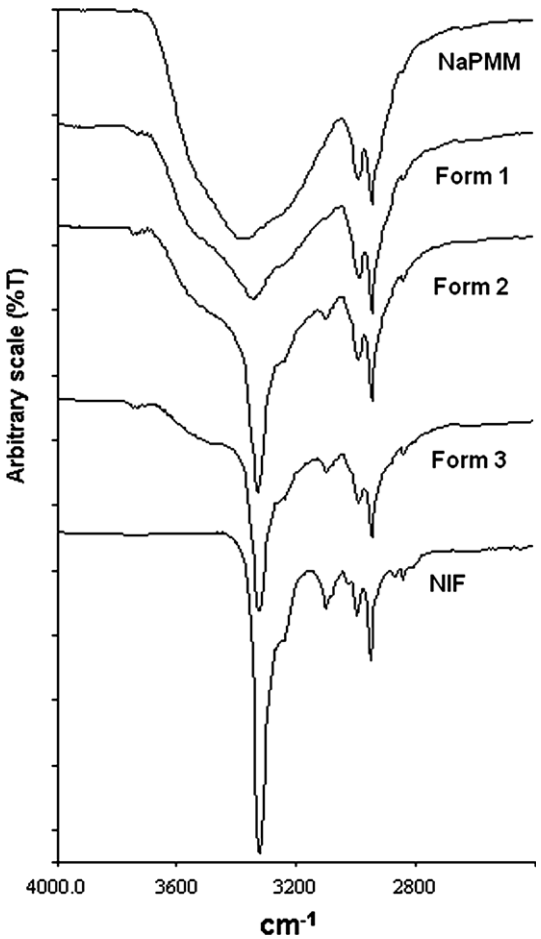


Fig. 3. ATR-FTIR spectra of NIF, NaPMM microparticles and raw NaPMM in the –NH region.

occurring between the NH function of the dihydropyridine ring and the C═O group of another NIF molecule [11,19]. In the microparticles loaded with the lowest drug amount (formulation No. 1), the ν-(NH) peak of NIF appeared as a broad band centered at about 3342 $cm^{-1}$ suggesting the absence of crystalline form of NIF.

For formulations Nos. 2 and 3 the ν-(NH) peak was centered at 3327–3330 $cm^{-1}$ and a shoulder arised at 3350–3353 $cm^{-1}$, respectively (Fig. 3). Furthermore, in all formulations the ν-(C═O) peak of the ester group of NaPMM at 1718 $cm^{-1}$ was detected at lower wavelength regions as a shoulder of ν-(C═O) peak of NIF esteric group. On the basis of these results, it can be assumed that NaPMM could interact with NIF by forming H-bonds between the amino group of NIF and the ester group of the copolymer.

These results suggested that NIF was either amorphous or crystalline in NaPMM microparticles. This hypothesis is supported also by the DSC data. Indeed, in freshly prepared microparticles the endothermic peak attributable to the NIF melting transition and $T_g$ [11] were detectable at about 171–172 °C and 45–50 °C, respectively. Additionally the peak areas for the melting events were lower than that of pure crystalline drug, indicating the co-existence of amorphous and crystalline NIF within the microparticles. Based upon the enthalpy of fusion of thermodynamically stable form of NIF ($\Delta H = 110.6 \pm 0.3$ J/g) and the enthalpy of fusion of NIF within the microparticles ($\Delta H_m$), the percentage of crystalline drug was calculated, and resulted in the range of 15–65%.

### 3.2.2. HPMC series

In the physical mixtures the main bands of NIF were clearly detectable over the broad pattern of the amorphous polymer according to the drug/HPMC ratio (data not shown). In the X-ray diffraction patterns of the microparticulate systems, the characteristic diffraction peaks of the drug crystalline form were not detected independently of the NIF/HPMC ratios (Fig. 4). The modification of NIF solid state was also supported by ATR-FTIR spectroscopy. Indeed, ν-(NH) of NIF within the microparticles shifted towards higher wavenumber region (3336–3337 $cm^{-1}$). Similar modifications were also recorded for the ν-(C═O) of NIF. These findings differed considerably from that of

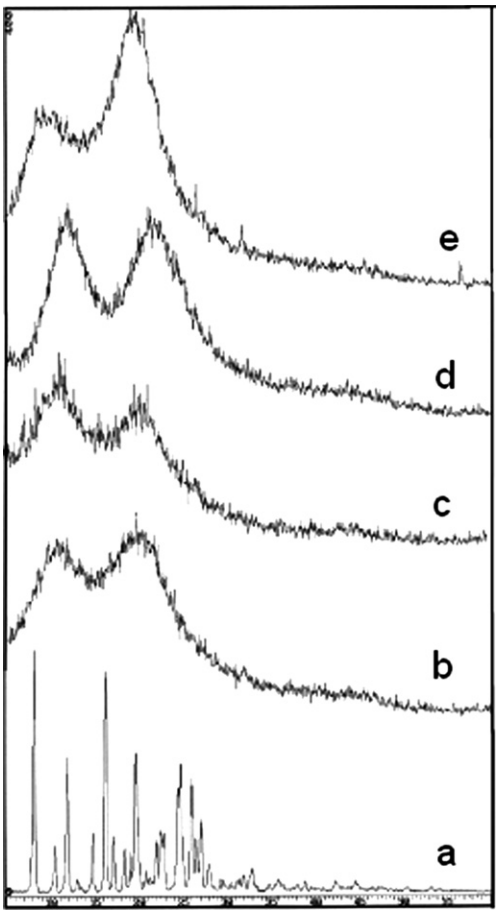


Fig. 4. X-ray diffraction patterns of (a) NIF, HPMC microparticles containing (b) 75, (c) 50 and (d) 25%, w/w NIF (time 0) and (e) raw HPMC.

microparticles made of Methocel® E50, a hydroxylpropylmethylcellulose with higher nominal viscosity [11]. In the latter case, on increasing NIF loading up to 50% w/w, the spectra of the microparticles exhibited clear diffraction peaks attributed to the drug crystalline form.

DSC data of formulation No. 4 indicated the presence of totally amorphous NIF. In the thermograms of formulations Nos. 5 and 6 the glass transition of amorphous NIF around 43 °C was followed by an exothermic event detected at about 110 °C and an endothermic peak with the onset temperature of about 170 °C which were attributed to NIF recrystallization and melting of the thermodynamically stable form of NIF, respectively. Only in the case of formulation No. 6 the presence of an additional small and sharp endotherm peak at about 151 °C ($\Delta H = 2.4 \pm 0.2$ J/g), which corresponds to NIF modification II [20], suggests that the recrystallization induced by temperature led to a mixture of polymorphs (Fig. 5). The solid–solid transition occurring during heating prevented the proper quantification of the amount of crystalline NIF into the microparticles at room temperature. These findings underline the influences of molecular weight and, consequently, glass transition temperature of the selected hydroxypropylmethylcellulose on NIF molecular mobility, which could affect microparticulate systems stability leading to drug crystallization. As a matter of fact, the $T_g$ of a high-viscosity Methocel® E50 ($T_g = 162$ °C [11]) was about 30 °C higher than that of a low-viscosity Methocel® E5 ($T_g = 129$ °C [1]); and NIF/Methocel® E50 solid dispersions did not show such exothermic peak independently of the drug/polymer ratio [11]. It might be assumed therefore that physical mechanism of protective effect on NIF crystallization involves the polymer anti-plasticizing effect on the amorphous drug, or specific interactions between the drug and hydroxypropylmethylcellulose, or a combination of both. In particular, the anti-plasticizing effect may contribute significantly by increasing the temperature at which the molecular mobility becomes significant with

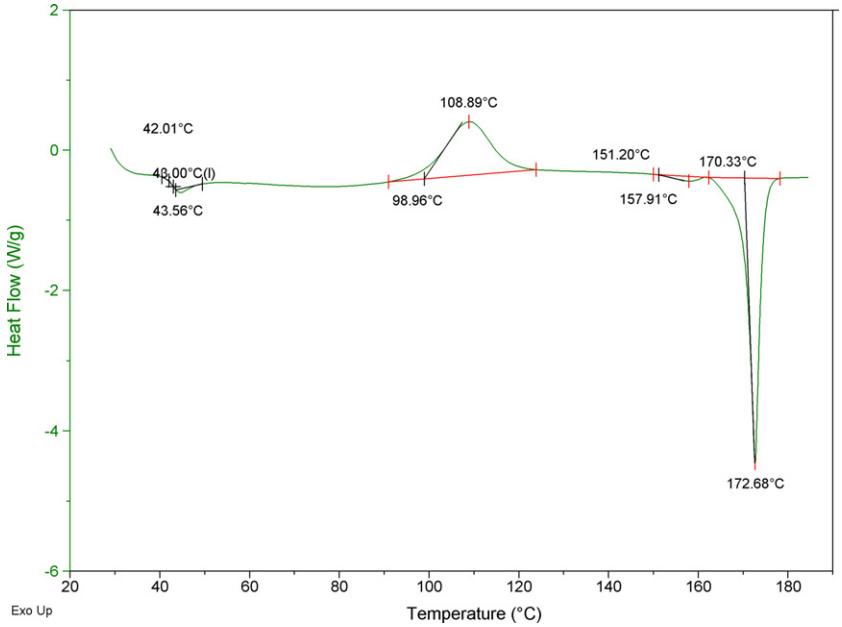


Fig. 5. DSC data of NIF within HPMC microparticles (formulation No. 6) upon heating at 10 K/min.

respect to recrystallization. In other words, the polymer might inhibit or slow down the crystallization process increasing the viscosity of the binary systems and decreasing the diffusion of drug molecules necessary to form a lattice. These observations are also in agreement with the stabilization mechanism of amorphous drugs in solid dispersions with polyvinylpyrrolidone [21–24].

### 3.3. Technological characterization of NIF loaded microparticles

NIF contents reported in Table 1 showed that the selected preparation conditions guaranteed good encapsulation. The residual moisture content within HPMC microparticles was ranging 1.0–1.7%, while in NaPMM microparticles the values were deeply influenced by the amount of loaded NIF (Table 1).

The results of texture analyses are reported in Table 2. The maximum detachment force (MDF) and the detachment work (WoA) values of the formulations Nos. 1–3 were significantly higher than those of the negative control ($p < 0.007$). The presence of NIF decreased MDF value of NaPMM even if the difference was not statistically significant ($p > 0.081$). The analysis of the force versus elongation graphs evidenced some differences in the debonding process (Fig. 6). As described in previous works, during the detachment process of NaPMM, the deformation force homogeneously increased before the maximum due to nucleation. This step was followed by the rapid growth of micro-cavities and the force values dropped down when the detachment process was completed [1,3]. In our set of experiments, during the nucleation phase, the detachment force in presence of NIF increased much slower with respect to the raw polymer. In the case of formulation No. 1 the highest value in detachment work (Table 2) can be justified considering the maximum detachment force maintained its value because of a progressive nucleation of a second population of cavities at the polymer-mucin interface, resulting in their coalescence and fibril debonding. This modification in the detachment pattern was drug loading dependent. Indeed, in the case of formulation No. 2, a shoulder in the first portion of the curve was detected, while the plateau was not recorded at highest NIF content, namely formulation No. 3. These features could be justified considering that the NIF/NaPMM interactions, evidenced by means of ATR-FTIR spectroscopy, can change the viscous modulus of the gel layer at NaPMM/mucin interface according to the NIF content.

Table 2
Maximum detachment force (MDF) and work of adhesione (WoA) of NIF microparticulate systems

| Formulation No. | Mucoadhesion | |
|---|---|---|
| | MDF (N) | WoA (mJ) |
| 1 | 3.73 ± 0.18 | 5.21 ± 1.04 |
| 2 | 3.30 ± 0.49 | 2.48 ± 0.30 |
| 3 | 3.59 ± 0.42 | 2.29 ± 0.79 |
| 4 | 2.19 ± 0.44 | 2.40 ± 0.29 |
| 5 | 2.15 ± 0.21 | 2.17 ± 0.22 |
| 6 | 1.80 ± 0.18 | 1.89 ± 0.07 |
| NaPMM | 5.06 ± 1.35 | 4.88 ± 1.10 |
| HPMC | 2.07 ± 0.36 | 2.28 ± 0.25 |
| Steel punch[a] | 1.62 ± 0.17 | 0.74 ± 0.24 |

[a] Negative control.

MDF of HPMC microparticles became lower than those of NaPMM microparticles. The values were close to those of the negative control, namely the steel punch (Table 2). By increasing NIF content in the microparticles, mucoadhesive properties decreased until negligible in formulation No. 6. No variations in the texture profiles of formulations Nos. 4–6 were observed in comparison to that of raw HPMC (data not shown).

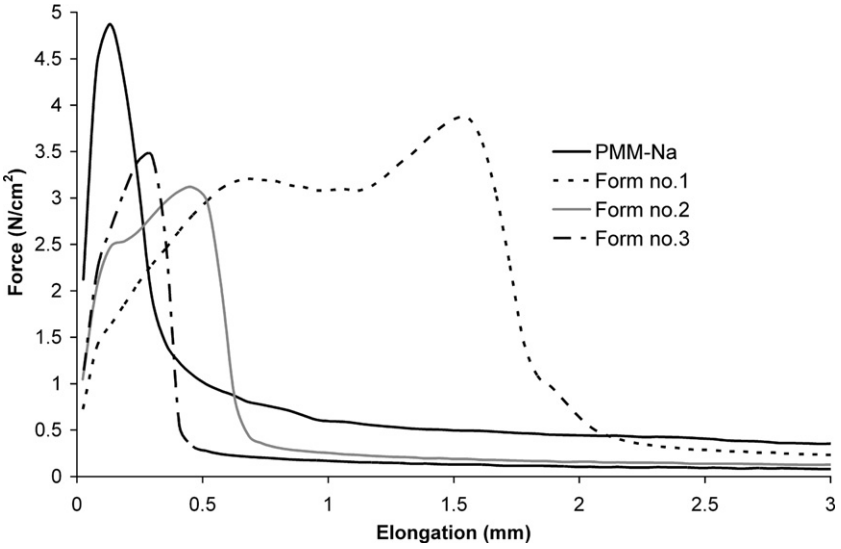


Fig. 6. Force versus elongation graphs of NaPMM and microparticles containing 25% (formulation No. 1), 50% (formulation No. 2) and 75% (formulation No. 3) w/w NIF.

The dissolution profiles of microparticle samples showed that NIF encapsulation in both polymers improved the drug dissolution rate with respect to micronized NIF (Fig. 7a and b). In the case of HPMC microparticles, the supersaturation degree (SD) of NIF was independent of the drug content (Table 3). NaPMM microparticles exhibited lower SD than the corresponding HPMC microparticles, which was influenced by the amount of loaded drug. In all cases the levels of supersaturation in the dissolution medium are maintained over time. The explanation for the differences between the dissolution profiles can be found in the different physicochemical properties of the microparticles, as evidenced in the solid state. Based upon this observation, it may be assumed that the interactions between NIF and HPMC or NaPMM were strong enough to maintain the drug solubilized in a supersaturated solution. The main differences were detected when NIF/polymer ratio was fixed at 25/75% w/w (formulations Nos. 1 and 4). Because of the high dissolution rate [1] and low viscosity of NaPMM, NIF could only dissolve along with the polymer and the dissolution process from NaPMM microparticles was faster with respect to that of HPMC microparticles (Fig. 7a and b). On the other hand, HPMC microparticles permitted to obtain the highest levels of supersaturation (Table 3) because it was more effective to stabilize a larger amount of amouphous NIF within the microparticles.

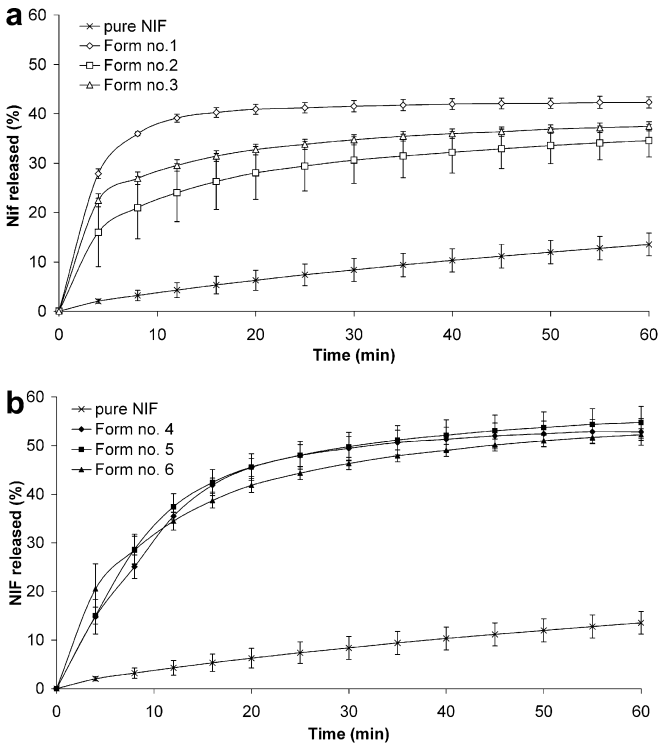


Fig. 7. Dissolution profiles of NIF from (a) NaPMM microparticles and (b) HPMC microparticles (time 0).

Table 3
Supersaturation degree of NIF in the dissolution medium after preparation of microparticles (time 0) and 1 month of storage in long-term and accelerated conditions

| Formulation No. | Time 0 | Time = 1 month | |
|---|---|---|---|
| | | (25 °C/60%RH) | (40 °C/75%RH) |
| 1 | 2.3 ± 0.1 | 2.2 ± 0.1 | 1.6 ± 0.0 |
| 2 | 2.1 ± 0.1 | 2.2 ± 0.1 | 1.9 ± 0.0 |
| 3 | 1.9 ± 0.2 | 1.8 ± 0.2 | 1.7 ± 0.4 |
| 4 | 3.1 ± 0.2 | 1.6 ± 0.1 | 1.8 ± 0.3 |
| 5 | 2.8 ± 0.2 | 1.8 ± 0.2 | 1.6 ± 0.0 |
| 6 | 2.8 ± 0.1 | 1.6 ± 0.1 | 1.5 ± 0.0 |

### 3.4. Evaluation of physical stability of NIF loaded microparticles

The microparticles kept the original shape during the stability study (Fig. 1c and d). In all the formulations, no significant variation of drug content was detected in both the storage conditions.

Conversion from the amorphous to the crystalline drug form occurred in HPMC microparticles. Indeed, a significant increase of the NIF crystalline fraction in the formulations Nos. 5 and 6 stored in accelerated conditions was verified by X-ray diffraction after a 1-month period (data not shown). At the lowest NIF content, no peaks were observed in the diffraction pattern, indicating that no significant phase change occurred with respect to time 0. The X-ray diffraction patterns of HPMC microparticles registered after 3 months of storage did not show any other significant modifications.

In the case of NaPMM microparticles, NIF was susceptible to crystallization when the microparticles were stored upon accelerated conditions. The temperature gap between storage temperature and $T_g$ is critical with respect to the physical stability of amorphous state because it can act on molecular mobility of an amorphous drug [24]. Hence, in the case of samples stored at 40 °C stability issues would arise, since the $T_g$ of amorphous NIF was around 45–50 °C. Indeed, the X-ray spectrum of formulation No. 1 clearly exhibited the typical pattern of an amorphous powder after storage at 25 °C for 1 month, while the intensity of main peaks of crystalline NIF increased after storage at 40 °C, revealing that the temperature enhanced the solid/solid transition. DSC data evidenced that the percentage of crystallized drug in formulation No. 2 significantly increased (crystallized NIF = 67 ± 2%) after 1 month storage at 40 °C with respect to 25 °C (crystallized NIF = 56 ± 0.7%). On the contrary, no significant variation in the solid state of formulation No. 3 was detected independently of storage conditions.

The lack of crystallinity in NaPMM microparticles can be ascribed to interactions, namely H-bonds, between NIF and NaPMM or caused by the fast drying of the feed. In the freshly prepared formulation No. 1, two $T_g$ values clearly detectable at about 45 and 59 °C

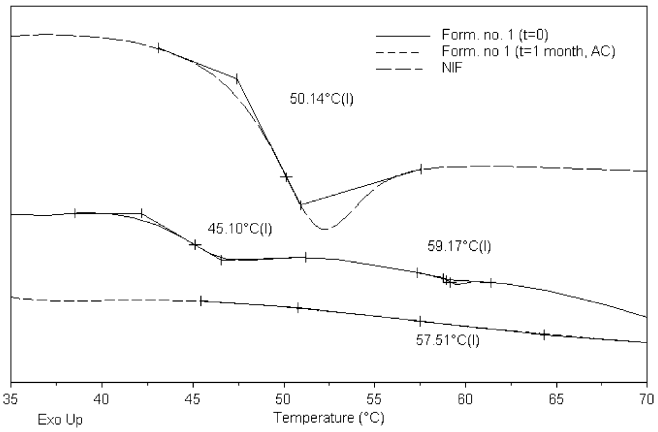


Fig. 8. DSC data of amorphous NIF (long dash line), freshly prepared formulation No. 1 (solid line) and formulation No. 1 upon 1-month storage in accelerated condition (dash line).

(Fig. 8) can be attributed to amorphous NIF and NIF/NaPMMM solid solution, respectively. Upon storage in accelerated condition, $T_g$ at 45 °C was not detectable indicating that the drug molecules, which were not physically stabilized by interacting with NaPMM, underwent the solid/solid transition from the amorphous to the stable polymorphic modification I. In all NaPMM formulations stored in accelerated condition, the amount of amorphous NIF became proportional to the polymer content in the microparticles ($r^2 = 0.9995$). This correlation leads to the possibility to conclude that NaPMM acted as a crystallization inhibitor by means of a saturable mechanism. After 3 months of storage in long-term and accelerated conditions no further physical modification was detected.

The modification of NIF solid state affected the biopharmaceutical performances of the microparticles. After 1 month storage at 25 or 40 °C, a significant decrease in the supersaturation degrees and dissolution rate was evidenced in the HPMC set of microparticles. As exemplified in Fig. 9, the percentage of NIF released within the first hour decreased from about 50% to 30%. The variation of the NIF supersaturation degree was less remarkable in the microparticles prepared by using NaPMM. Indeed, the supersaturation degrees were statistically different from those calculated at time 0 only in the case of formulations Nos. 1 and 2 stored at 40 °C (Table 3).

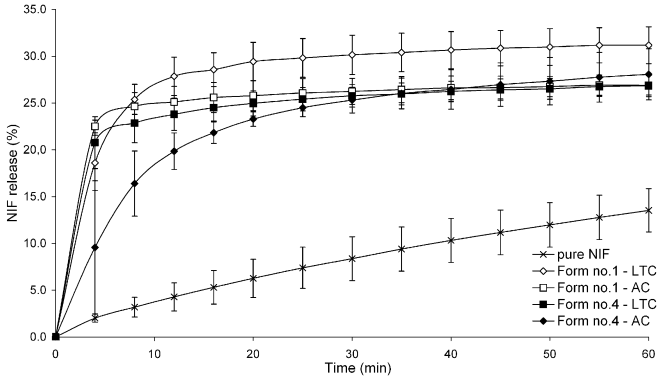


Fig. 9. Dissolution profiles of NIF from NaPMM (formulation No. 1) and HPMC microparticles (formulation No. 4) upon 1-month storage period in long-term condition (LTC) and accelerated condition (AC).

After 3 months of storage, the NIF release profiles overlapped with those determined after 1 month of storage. As formulations Nos. 1 and 2 stored in long-term and accelerated conditions exhibited different dissolution profiles due to NIF physical instability, a final cure of NaPMM microparticles could be required to prevent solid/solid transition by aging.

## 4. Conclusion

In this work, the suitability of a poly(methacrylic acid, methyl methacrylate) sodium salt (NaPMM), a novel mucoadhesive material, to prepare fast-dissolving microparticles containing nifedipine (NIF) was verified.

The performances of NaPMM were compared to those of HPMC. NaPMM could be preferable to HPMC because of its ability to confer satisfactory mucoadhesive properties and increase drug dissolution rate. Indeed, this investigation confirmed that the microencapsulation in NaPMM permitted to overcome solubility issue of poorly water-soluble active ingredients due to drug/polymer interactions. As a matter of fact, when NIF or piroxicam [2] was loaded, the formulations constituted of NaPMM promptly released the drug. This feature could be favorably exploited in the design of a sublingual solid dosage form since the microparticulate system should quickly dissolve in the limited volume of saliva under the tongue.

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