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Thermosensitive and mucoadhesive in situ gel based on poloxamer as new carrier for rectal administration of nimesulide

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ABSTRACT

Poloxamer 407 has excellent thermo-sensitive gelling properties. Nevertheless, these gels possess inadequate poor bioadhesiveness and high permeability to water, which limited its' application as a thermoresponsive matrix. The main aim of the present investigation was to develop thermosensitive and mucoadhesive rectal in situ gel of nimesulide (NM) by using mucoadhesive polymers such as sodium alginate (Alg–Na) and HPMC. These gels were prepared by addition of mucoadhesive polymers (0.5%) to the formulations of thermosensitive gelling solution containing poloxamer 407 (18%) and nimesulide (2.0%). Polyethylene glycol (PEG) was used to modify gelation temperature and drug release properties. The gelation temperature and drug release rate of the prepared in situ gels were evaluated. Gelation temperature was significantly increased with incorporation of nimesulide (2.0%) in the poloxamer solution, while the addition of the mucoadhesive polymers played a reverse role on gelation temperature. The addition of PEG polymers increased the gelation temperature and the drug release rate. Among the formulations examined, the poloxamer 407/nimesulide/sodium alginate/PEG 4000 (18/2.0/0.5/1.2%) exhibited the appropriate gelation temperature, acceptable drug release rate and rectal retention at the administration site. Furthermore, the micrographic results showed that in situ gel, given at the dose of 20 mg/kg, was safe for no mucosa irritation. In addition, it resulted in significantly higher initial serum concentrations, C_{max} and AUC of NM compared to the solid suppository.

1. Introduction

Nimesulide (NM), an acyclic sulfonamide derivative that is significantly selective towards COX-2 inhibition, exerts potent pyretolysis, analgesic and anti-inflammatory effects when administered orally, rectally or topically (Anil et al., 2000; Suleyman et al., 2008). As a typical Class II drug according to the Biopharmaceutics Classification System (BCS) (Amidon et al., 1995; Meriani et al., 2003) the low aqueous solubility (virtually insoluble in aqueous systems, 5 μg/ml when pH <6.0) (Piel et al., 1997) and the poor bioavailability of this drug often hinders the full exploitation of their therapeutic properties. In particular, a rapid onset of action is required in the case of analgesics or pyretolysis, but the slow dissolution resulting from poor solubility retards the occurrence of the desired effects. Moreover, slow dissolution sometimes also exacerbates the local side effect of the drug, e.g., mucosal irritancy (Rainsford, 1999; Bolten, 1998). Therefore, aqueous solubility is always a challenging issue in both liquid and solid dosage form development. Several studies have been carried out in the effort to increase the aqueous solubility of NM (Neelam and Sonu, 2003) by complexing NM with β-cyclodextrin (Chowdary and Buchi, 2000; Pradeep and Nisharani, 1999; Buchi et al., 2007) or by incorporation of NM to form a NM-L-lysine-β-cyclodextrin complex (Piel et al., 1997). Most recently, investigations on the effects of pH combined with surfactant (Grbic et al., 2009) or water-soluble polymers (Alexanian et al., 2008) on the solubility and the drug dissolution of NM have been published.

In order to increase the water solubility of poorly soluble drugs, surfactants, especially block copolymers, can also be employed (Chiappetta and Alejandro, 2007; Zentner et al., 2001). Among these, Poloxamer 407 (P 407), also known as Pluronic F 127, is of particular interest. P 407 is of good tolerability for provoking no irritation and sensitivity on skin, and has thus far been found to be useful in topical, rectal and ocular formulations (Chiappetta and Alejandro, 2007; Dumortier et al., 2006; Bansal et al., 2007).

An oral administration of NM has generally been recommended. However, oral administration is not always feasible or desirable in all cases. As a result, the rectal drug administration by suppository,

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as an alternative to oral administration, has been effectively utilized to treat local diseases of the anorectal area as well as to deliver drugs systemically, especially on certain patient populations who are often difficult to receive treatment with oral tablets or capsules, such as young children, the elderly, and those with swallowing problems. Rectal suppository is also commonly used as an antipyretic and analgesic drug for infants and children. However, the conventional suppository is in a solid form which melts or softens in the rectum slowly. Moreover, such a solid suppository could be uncomfortable for patients and even lead to patient refusal. Therefore, various formulation strategies have been described to develop rectal dosage forms different from the suppository, such as thermosensitive poloxamer gel. This form is easy for administration and exact dose-volume control for the fact that it is a liquid form at room temperature and will turn into a gel instantly at physiological temperature, and also mucoadhesive to the rectal tissues without leakage after the dose.

Taking the above factors into consideration, we developed a thermosensible and mucoadhesive rectal in situ gel for NM, and evaluated its gelation temperature, in vitro release properties, in vivo retention, as well as rectal tissue irritation. The optimized in situ gel with favorable gelation temperature, release properties, favorable mucous membrane adhesion and remarkable safety was subsequently given to rabbits in order to investigate its bioavailability in comparison to that of a conventional solid suppository.

2. Materials and methods

2.1. Materials

Poloxamers (P 407, P 188) were purchased from BASF (Ludwigshafen, Germany), Hydroxypropylmethyl-cellulose (HPMC, Methocel K4M) was a gift from Colorcon. Polyethylene glycol (PEG, m.wt. 400, 4000) (Tiantai Chemical Reagent Co., Ltd., Tianjin, China), Sodium Alginate (Sinopharm Industry Reagent Co., Ltd., China), nimesulide were gifted from the Institute of Pharmaceutical Research (Tianjin, China). Methanol (HPLC grade, Concord Ltd., Tianjin, China) and all other reagents were of commercially analytical-grade. The water was distilled before use.

2.2. Preparation of in situ gels

The cold method as described previously (Choi et al., 1998a; Cafaggi et al., 2008) was adopted. Formulations containing NM were prepared by weight. A particular procedure was adopted in order to avoid both drug precipitation during mixing of the components and the difficulty of handling concentrated aqueous solutions of poloxamer. For this purpose, various components such as PEG and/or mucoadhesive polymers were added in the calculated amount of distilled water in room temperature. P 407 was added slowly into cold water to obtain an aqueous solution containing 30% poloxamer. Both solutions were stored in a refrigerator until use. An appropriate amount of these solutions was added to 2% NM salt solutions, which were prepared through mixing NM with the equivalent molar amount of NaOH.

2.3. Measurement of the sol–gel transition temperatures ($T_{sol-gel}$)

$T_{sol-gel}$ of the prepared formulations was determined as described previously (Kim et al., 1998). A 20-ml transparent vial containing a magnetic bar and 10 g of the gel was placed in a low-temperature thermostat water bath at 20 °C with a thermosensor immersed in the gel and heated at speed of 2–3 °C/min with constant stirring. When the magnetic bar stopped moving due to gelation, the temperature showed by the thermistor was determined as the gelation temperature. Measurements were performed in quadruplex and statistical tests of significance were performed using Student's t-test at the level of $\alpha = 0.05$.

2.4. Release test

Drug release was monitored using the USP paddle method (Zaki et al., 2007). The in situ gel formulations containing 50 mg of NM were inserted into the semipermeable dialysis bag. Both sides of the bag were sealed with a clamp to prevent leakage. The dialysis bag was then placed in a dissolution tester (RCZ-5A, Tianjin University Precision Instrument Factory, China). Release test was performed with 500 ml borate buffer (pH 9.1) as a release medium at 37 ± 0.5 °C and stirred at speed of 100 rpm. At predetermined interval, 5 ml of the medium was sampled and filtered. After sampling, an equivalent amount of borate buffer (pH 9.1) was added to the dissolution tester to maintain a constant volume. The filtrate was analyzed by a UV spectrophotometer (UV 1800 Shimadzu, Japan) at $\lambda = 393$ nm after appropriate dilution (Shoukria et al., 2009) with plain formulation as the blank. The concentration of drug was calculated from a previously constructed calibration curve. The release experiments were run in triplicates and the results were averaged.

The description of release data of NM rectal in situ gels with or without PEG by a model function was attempted by four mathematical models, namely zero, first order kinetics, the Higuchi square-root model and the Peppas model (Karen et al., 2008). Then the rate of release was described using the semi empirical model of Peppas model to understand the release mechanism of NM from the in situ gels:

$$\frac{M_t}{M} = kt^n$$

$$\log \frac{M_n}{M} = \log k + n \log t$$

where M_t/M is the fraction of released drug at time t, k is a characteristic constant of the liquid suppository and n is an indicator of release mechanism. As the k value becomes higher, the drug is released faster. The n value of 1 corresponds to zero-order release kinetics, $0.5 < n < 1$ means a non-Fickian release model, and $n = 0.5$ indicates Fickian diffusion (Higuchi model).

2.5. Retention in vivo

Six albino rabbits were divided into two groups randomly. Two types of optimal thermosensitive gels [Formulation A (P407-Alginate-PEG4000 (18:0.5:1.2)) and Formulation B (P407-HPMC-PEG400 (18:0.5:10))] were administered at a dose of 20 mg/kg into the rectum 4 cm above the anus using a plastic syringe (Choi et al., 1998a).

2.6. Rectal histopathological evaluation

Male albino rabbits weighing 2.5–3.0 kg were purchased from PLA Academy of Military Medical Laboratory Animal Center with an Animal Certificate of Conformity No SCXK2007-0001. Animals with free access to water were fasted for 36 h prior to each experiment. At 24 h after rectal administration of the gel at a dose of 20 mg/kg into the rectum 4 cm above the anus using a plastic syringe, the rectum was rejected. The segment was then rinsed with saline solution and immersed in 4% neutral phosphate-buffered formaldehyde, embedded in paraffin and cut into slices. The slices were stained with hematoxylin–eosine and examined by light microscopy for morphology.

2.7. Pharmacokinetic study

2.7.1. In vivo experiments

Six male albino rabbits weighing 2.5–3.0 kg were randomly assigned into two groups and housed individually in stainless steel cages, fed a commercial laboratory rabbit diet and allowed to tap water ad libitum. The rabbits were fasted for 36 h prior to and during the pharmacokinetic study. The animals were conscious throughout the duration of the experiments and were held in rabbit restrainers only during blood sampling. The Formulation A (20 mg/kg NM) was administered into the rectum 4 cm above the anus using a plastic syringe while the conventional solid suppository [NM/glyceryl monostearate (2.0:98.0%), prepared by fusion method] was administered into the rectum 4 cm above the anus (Choi et al., 1998a). After administration of the different formulations, blood samples (1.0 ml) were collected at time intervals of 10, 20, 30, 45, 60, 90, 120, 180, 240, 360, 480, 720, 1440 min from the marginal ear vein of the rabbits.

2.7.2. Blood sample analysis

Blood samples were allowed to clot and then centrifuged at 3000 rpm for 20 min. The obtained serum samples were deep-frozen at −20 °C prior to HPLC analysis. The protein precipitation procedure described previously was followed with some modification (Adriana et al., 2004). Briefly, a 200 μl aliquot of rabbit serum sample was pipetted into a 1.5 ml PE tube, and a 200 μl aliquot of acetonitrile was added in order to precipitate protein. The sample was mixed by vortexing vigorously for 60 s and centrifuged at 12,000 × *g* for 15 min (2–16 K, Sigma, USA). The supernatant was aspirated with a plastic syringe, and then filtered through a filter equipped with 0.22 μm nylon membrane before injected into the HPLC system. The mobile phase consisted of methanol:doubled distilled water:acetic acid (620:320:8, v/v/v). Prior to use, the mobile phase was filtered through a 0.22 μm nylon membrane filter. The mobile phase was run at a flow rate of 1.2 ml/min at 40 °C and the aliquots of 10 μl were injected into the column, and the UV detector was set at 299 nm.

2.7.3. Data analysis

All the parameters were calculated by the standard method. The maximum serum drug concentration (C_{max}) and the time to achieve this peak (T_{max}) were determined. The elimination rate constant (K_{el}) was determined by the linear regression analysis of the terminal linear part of the log serum concentration vs. time curve. The absorption rate constant (K_a) was estimated by the method of feathering, and mean absorption time (MAT) was calculated as $1/K_a$. The area under the plasma concentration–time curve (AUC_{0-24}) was calculated using the trapezoidal rule. The non-compartmental parameters area under first moment curve (AUMC) was calculated using the linear trapezoidal rule with extrapolation to infinite time; mean residence time (MRT) was calculated as AUMC/AUC. Results are expressed as mean ± standard error of the mean (S.E.M.) of six determinations.

3. Results and discussion

3.1. Preparation of in situ gel

A sufficient drug-loading is needed in a liquid formulation to compensate the leakage after rectal administration resulting from the max volume limitation. NM is practically insoluble in water; therefore, NM was suspended directly in the gels in the preliminary tests (for instance 1%, which is actually insufficient for the dose needed in clinical). However, an unstable suspension was obtained, which was hard to resuspend due to its poor solubility. This problem was solved by changing NM into its soluble salt form through addition of an equivalent molar amount of NaOH, at the same time, considering the optimized pH range of the rectal mucosal. As the result, a 2% NM sodium salt solution, showing good compatibility with P 407, was prepared, which could form a stable and homogeneous gel at a suitable temperature after mixture with the other two solutions.

Table 1
Sol–gel transition temperatures of NM 18% P 407 rectal in situ gels with different additives.

Mucoadhesive polymer	Concentration (%)	Mean $T_{sol-gel}$ ± S.D. (°C, n = 4)
Control (no mucoadhesive)		35.3 ± 0.36
NaCl	1.0	33.6 ± 0.61
	2.0	29.6 ± 0.81
	3.0	23.8 ± 0.45
P188	5.0	34.3 ± 0.79
	10.0	37.2 ± 0.47
	15.0	30.3 ± 0.47
	20.0	26.4 ± 0.39
HPMC	0.5	32.4 ± 0.31
	1.0	31.2 ± 0.78
	2.0	29.7 ± 0.96
Alg–Na	0.5	32.7 ± 0.54
	0.8	30.8 ± 0.16
	1.0	30.1 ± 0.62
CS	0.5	–[a]

[a] CS was discarded because of precipitation under alkaline environment.

3.2. Sol–gel transition temperature

Preliminary studies showed that the incorporation of (2.0%) NM into P 407 solutions markedly increased the $T_{sol-gel}$ (data not shown), while that medicated solutions containing 18, 20 and 22% P 407 had $T_{sol-gel}$ of 35.3, 30.2 and 25.2 °C individually. With the basement of in situ rectal gel, only 18% P 407 solutions were selected for further studies. However, it was reported that bioadhesive polymers must be added to reinforce the gel viscosity and bioadhesive force, both of which were significantly reduced by the drugs added (Choi et al., 1998a; Yong et al., 2004). The mucoadhesives used were selected as the neutral polymer hydroxypropylmethylcellulose (HPMC), the very water-soluble material sodium chloride (NaCl), poloxamer analogue poloxamer 188 (P 188), the anionic polymer sodium alginate (Alg–Na) as well as the cationic polymer chitosan (CS). The impact of bioadhesive polymers on the gelation temperatures depended on the nature of bioadhesive additives (polymers) and the concentration in the formulations. As shown in Table 1, the mucoadhesive polymers, except P 188, had a concentration dependent $T_{sol-gel}$ lowering effect. The most pronounced was with the addition of Alg–Na at 1.0%, with which the $T_{sol-gel}$ was lowered by 5.2 °C. Meanwhile, the same parameter was decreased by 4.1 °C with HPMC. The $T_{sol-gel}$-lowering effect of mucoadhesive polymers could be explained by their ability to bind to polyethylene oxide (PEO) chains present in the poloxamer molecules promoting dehydration and causing an increase in entanglement of adjacent molecules with more extensive intermolecular hydrogen bonding (Choi et al., 1998a; Ryu et al., 1999; Zaki et al., 2007). By means of differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA), the influence of the addition of mucoadhesive addictives, such as hyaluronic acid (HA), was recently investigated. The results indicated that the addition of mucoadhesive addictives into poloxamer gels hindered the interactions between water and poloxamer molecules; whereas the water was strongly bound to the gel network. As the temperature increasing, the gelation process driven by poloxamer micelle assembly and packing was facilitated, by which a lower $T_{sol-gel}$ was achieved (Mayol et al., 2011).

Table 2
Sol–gel transition temperatures of NM 18% P407 mucoadhesive rectal in situ gels.

Mucoadhesive polymer	PEG (%)	Mean $T_{sol-gel}$ ± S.D. (°C, n = 4)
Control (no mucoadhesive/no PEG)		**35.3 ± 0.36**
Alg–Na (0.5%)	0% PEG 4000	32.7 ± 0.54*
	0.5% PEG 4000	32.5 ± 0.59*
	0.8% PEG 4000	32.3 ± 0.48*
	1.2% PEG 4000[a]	**34.9 ± 0.69**
	1.5% PEG 4000	37.7 ± 0.41*
HPMC (0.5%)	0% PEG 400	32.4 ± 0.31*
	5% PEG 400	32.5 ± 0.59*
	8% PEG 400	32.2 ± 0.55*
	10% PEG 400[b]	**35.1 ± 0.72**
	15% PEG 400	30.4 ± 0.48*

* Significantly different from control formulation at $P < 0.001$, $n = 4$.
[a] Formulation A.
[b] Formulation B.

The impact of NaCl on the gelation temperatures depended on the concentration. For example, the addition of 1.0% NaCl, the $T_{sol-gel}$ decreased 1.7 °C. This was consistent with previous reports, in which there was a decrease in poloxamer solution in the presence of neutral salts like NaCl (Yong et al., 2001; Su et al., 2003). Conversely, when the concentration of P 188 increased to 10%, the $T_{sol-gel}$ upgraded from 35.3 to 37.2 °C. According to Chiappetta and Alejandro (2007), the temperature-dependent gelation of poloxamer solutions was explained by the micellization of PEO–PPO–PEO. The higher the content of PEO (and lower EO/PO ratio) and the lower the molecular weight of the polymer, the higher the $T_{sol-gel}$ observed. An increase in the content of PPO results in lower $T_{sol-gel}$ values. This parabola phenomenon might be explained by the EO/PO ratio in the mixed poloxamer solutions of P 407/P 188. However, extensive studies performed on the P 188 as an additive to Pluronic solution had reported that although the $T_{sol-gel}$ of the gels could be appropriately regulated, it was not a considerable mucoadhesive polymer (Choi et al., 1998a; Lin et al., 2004; Koffi et al., 2006; Zaki et al., 2007). On the other hand, NaCl containing formulations crystallize within only a few months, while CS was found to precipitate resulting from poor solubility in the alkaline environment of the gel. Therefore, only the formulations in Table 1 containing HPMC and Alg–Na at concentration of 0.5% were used for further studies with consideration of the $T_{sol-gel}$ value.

As a rectal in situ gel, the $T_{sol-gel}$ range should be 33–37 °C to avoid any difficulties in manufacture procedure, storage and leakage from the anus. In addition, it should be kept in mind that the added mucoadhesive additives, which augmented bioadhesive force and gel viscosity, may retard the drug release. Therefore, in order to develop gels with appropriate $T_{sol-gel}$ and release properties, a release enhancer such as PEG polymers must be added. Such a dosage form would be of therapeutic interest in avoiding the issues in the emergency treatment such as serious infantile serious fever.The effect of PEG incorporation showed that PEG increased the $T_{sol-gel}$ of the corresponding formulations in a concentration-dependent manner (Table 2), which was in accordance with that obtained by Zaki et al. (2007). This increasing effect could be attributed to the interference of PEG with the process of micellar association of poloxamer chains (Edsman et al., 1998). As the different mucoadhesive formulations had different $T_{sol-gel}$, a variety amount of PEG was required to get the $T_{sol-gel}$ of 35 °C, and it was 1.2% for PEG 4000 in Alg–Na containing formulations. In case of HPMC, macroscopic phase separation between the two polymers was observed upon addition of PEG 4000; hence PEG 400 was applied instead at concentration of 10%. The higher concentrations of PEG 400 needed could be explained by sol–gel transition temperature of poloxamer on PEG chain length (Chiappetta and Alejandro, 2007).

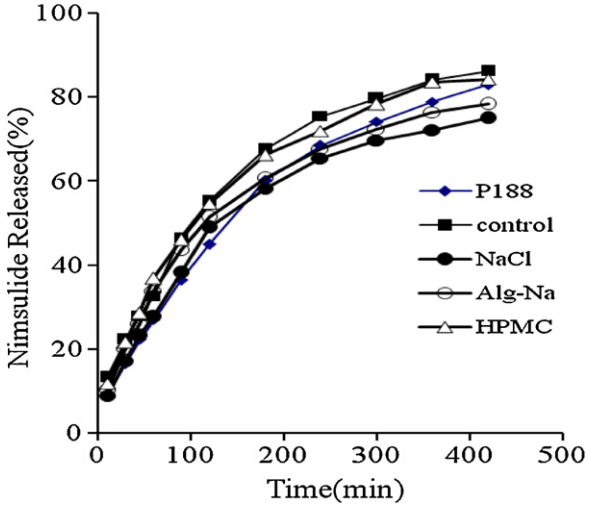

Fig. 1. Release profiles of NM from 18% P407 rectal in situ gels containing different mucoadhesive polymers in borate buffer using the paddle method (values are mean of three measurements; error bars were omitted for clarity).

3.3. In vitro drug release study

Cumulative amount of NM released versus time profiles for various polymer solutions without PEG are presented (Fig. 1). Compared to the control gel (P 407, 18%), the release was slowed down by addition of the additives (Alg–Na, HPMC, NaCl, P 188). However, it must be pointed out that release profiles of NM from the different formulations do not show significantly differences from the control gel, except Alg–Na and NaCl. These in vitro release results could be reasonably ascribed to that the release tests were performed in sink conditions, under which the strong dilution imposed when setting up in vitro release experiments. The volume ratio between release medium and gel was high; the gel underwent a strong dilution that caused the rapid formation and release of a sol phase (Mayol et al., 2011). As a result, a weak effect of mucoadhesives addition within poloxamer-based gels was observed in vitro release.

Ryu et al. (1999) also observed that association of mucoadhesive substance (sodium alginate, polycarbophil, carbopol, poly(vinyl pyrrolidone) and hydroxypropylcellulose) to a mixture of Poloxamers 407 and 188 slowed down the in vitro release of propanolol. The correlation coefficients calculated for each of these (Table 3) indicated that NM release was better described by the Peppas model for all the in situ gels.

The release data of NM rectal in situ gels with or without PEG are presented in Table 4. From the k value it could be concluded that the drug release was generally retarded by the addition of mucoadhesive additives as previously reported (Kim et al., 1998), while the addition of PEG enhanced the drug release (Zaki et al., 2007). The retardation effects of these mucoadhesives could be explained as the increase of the overall gel microviscosity or gel strength (Choi et al., 1998a; Kim et al., 1998; Choi et al., 1998b).

Table 3
Calculated correlation coefficients of NM release.

Polymer	PEG (%)	Curve fitting constant (R^2)			
		Zero	First	Higuchi	Peppas
Control	No	0.9142	0.9894	0.9819	0.9902
HPMC	No	0.9111	0.9911	0.9849	0.9899
	10% PEG 400	0.8545	0.9584	0.9565	0.9690
Alg–Na	No	0.8998	0.9767	0.9802	0.9875
	1.2% PEG 4000	0.9109	0.9823	0.9803	0.9921

Table 4
Release data of NM mucoadhesive rectal in situ gels at 37 °C ($n = 3$).

Polymer	PEG (%)	n	k	R^2	Mechanism
Control	No	0.5584	34.7776	0.9902	Non-Fickian
HPMC	No	0.5724	32.0184	0.9899	Non-Fickian
	10% PEG 400	0.6421	34.1822	0.9690	Non-Fickian
Alg–Na	No	0.5892	30.8461	0.9875	Non-Fickian
	1.2% PEG 4000	0.6365	31.3401	0.9921	Non-Fickian

n, k ($\%/h^n$) and R^2: release exponent, release constant and correlation coefficient respectively obtained from Eq.

Moreover, some researchers attributed the release retarding effect of mucoadhesives to their possible squeezing effect on the aqueous channels of poloxamer micelles, through which the drug diffuses (Choi et al., 1998b; Ryu et al., 1999). The release-enhancing effect of the PEG polymer could be attributed to its water solubility enhancement and viscosity-lowering effect (Zaki et al., 2007). Results also revealed that the in situ gels had values of n between 0.5584 and 0.6421, indicating non-Fickian (anomalous) release. These might indicate that the release of NM followed coupled erosion-diffusion mechanism (Zaki et al., 2007). In other words, both drug diffusion and polymer swelling were important processes during drug release. What's more, it was worthy of noting that the kinetics of drug release was unaffected by the addition of mucoadhesive polymers and/or PEG polymers. A similar result was already reported for ondansetron (Jadhav et al., 2009) and metoclopramide HCl (Zaki et al., 2007). Thus, the optimum formulation for a thermosensitive and mucoadhesive rectal in situ gel was determined to be NM 2% with 0.5% Alg–Na and 1.2% PEG 4000 in P 407 gels (18%) providing continuous and faster release of active material.

3.4. In vivo retention in the rectum

One of the important design criteria for the drug delivery platforms is their ability to exhibit mucoadhesion. Only Formulation A was retained in the rabbits' rectum without any leakage. This indicated that the bioadhesive force of rectal in situ gel was strong enough to ensure a good contact between the hydrogel and the mucosa of rabbits. The mechanism of the increase cannot be well explained, based on the data collected. One of the possible reasons for these increasing bioadhesive force effects might be related to hydrogen bonding between the hydrogel and the oligosaccharide chains of rectal mucous lining via carboxyl groups in the mucoadhesive polymers (Choi et al., 1998a; Ryu et al., 1999). Similar results were also reported with Carbopol (Lin and Sung, 2000) and polycarbophil (Choi et al., 1998b) as mucoadhesive additives, both of which have carboxyl groups.

3.5. Assessment of rectal mucosal integrity

It was important to investigate the safety of the optimized in situ gel. The morphological study of the administration effect on rectal tissues did not show signs of irritation such as epithelial necrosis, sloughing of epithelial cells and hemorrhage in any of the rabbit's rectal tissues (Fig. 2). Ryu et al. (1999) reported previously that alginate sodium and poloxamer caused no damage to mucous membranes. No irritation could also be partly explained by that the content of NaOH which was lower than the tissue-damaging threshold level.

3.6. Pharmacokinetic study

The bioavailability of NM for the optimized in situ gel was determined and compared to that of the conventional solid suppository. The mean serum drug concentration–time profiles were illustrated

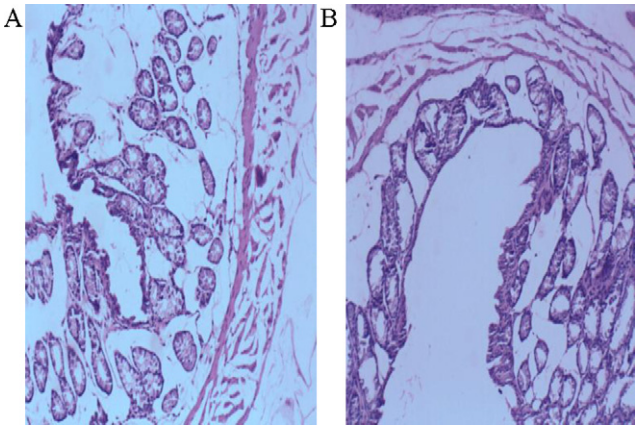

Fig. 2. Morphology of rectal mucosa of rabbits after rectal administration of NM in situ gel (A) before the dose and (B) after the dose.

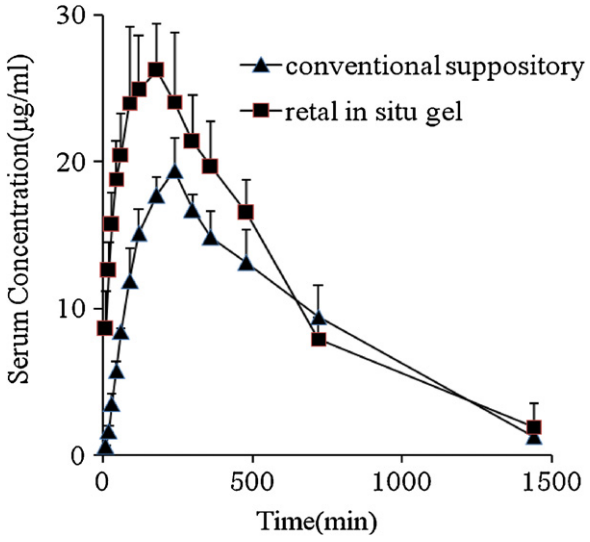

Fig. 3. Mean serum concentration–time profiles following rectal administration of in situ gel or conventional solid suppository. In situ gel was composed of [2/18/0.5/1.2/0.05% (w/w) NM/P407/Alg–Na/PEG 4000]. Conventional solid suppository was composed of [NM/glyceryl monostearate (2.0/98.0%)].

in Fig. 3. The profiles demonstrated that significantly higher serum drug levels were achieved in case of the rectal in situ gel when compared to that of the conventional solid suppository. Table 5 shows the pharmacokinetic and bioavailability data of the two formulations. Results revealed that faster absorption and higher serum NM levels were achieved in case of the rectal in situ gel as indicated by the C_{max} and AUC values. The mean residence time (MRT), elimination half-life $t_{1/2(\beta)}$ of NM from the rectal in situ gel (425.2 min and 262.35 min, respectively) were not significantly

Table 5
Pharmacokinetic parameters of NM delivered by the conventional solid suppository or rectal in situ gel ($n = 3$).

Parameters	Conventional suppository	Rectal in situ gel
T_{max} (min)	274.18 ± 44.1	131.33 ± 21.5*
C_{max} (μg/ml)	16.21 ± 3.66	26.69 ± 2.38*
$t_{1/2(\beta)}$ (min)	231.05 ± 87.5	262.35 ± 60.7
$t_{1/2(\alpha)}$ (min)	135.40 ± 13.3	31.42 ± 8.8*
$AUC_{0\to t}$ (μg min/ml)	9976.1 ± 1616.4	13,359.8 ± 1926.3
$AUC_{0\to\infty}$ (μg min/ml)	10,295.7 ± 1930.7	13,526.3 ± 2626.2
MRT (min)	484.82 ± 44.2	425.2 ± 41.8

Each value represents the mean ± S.D. ($n = 3$).
* Significantly different at $P < 0.05$ by Student's t-test.

different from those of the conventional suppository (484.82 min and 231.05 min, respectively). Although the $AUC_{0\rightarrow t}$ for the gel increased about 33.92% compared with that of the solid suppository group (13,359.8 μg min/ml and 9976.1 μg min/ml, respectively), the differences was not statistically significant ($P = 0.14$). That was attributed to the high inter-individual variability. In contrast, the rectal in situ gel significantly shortened the time to reach the maximum serum concentration (T_{max}) and increased the maximum serum concentrations of drug (C_{max}) of NM (131.33 min, 26.69 μg/ml) in comparison with that of the conventional suppository (274.18 min, 16.21 μg/ml). These indicated that NM from the former could be absorbed faster initially than that from the latter. The reason for this difference might be the dispersability (fluidity) and bioadhesive force. As a virtually insoluble in aqueous systems, NM in the conventional solid suppository was slowly dissolved and then dispersed into the rectum. In contrast, NM in the rectal in situ gel rapidly dispersed into the rectum (since NM was virtually in soluble salt form), gelled and attached to the rectal mucous membranes (Kim et al., 1998). Based on these results, the rectal in situ gels in our research would be useful to deliver NM in a pattern that allows fast absorption in the initial phase.

4. Conclusion

It is concluded that modulation of the adhesive properties of poloxamer 407 solutions by Alg–Na in the presence of PEG 4000 allowed a faster in vitro release of NM, as well as a quicker absorption of this drug in rabbits than the conventional suppository. What's more, it is likely to ensure a prolonged adhesion of the hydrogel at the mucosal surface, following its rectal administration. Therefore, these results revealed the potential usefulness of the NM rectal in situ gel, which could alleviate the feeling of alien, discomfort and refusal during application of the patients, is likely a more convenient and effective rectal antipyretic and analgesic dosage form specifically for infants and children.

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