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ORIGINAL ARTICLE

Pharmacokinetics of a novel nifedipine and pH-sensitive *N*-succinyl chitosan/alginate hydrogel bead in rabbits

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Abstract

Context: A novel N-succinyl chitosan/alginate hydrogel bead was prepared by the ionic gelation method for controlled delivery of nifedipine (NF). *Objective*: The delivery behavior of NF from the hydrogel bead was studied in rabbit body. *Materials and methods*: Nitrendipine was used as the internal standard and the concentration of NF in serum was determined by reversed-phase high-performance liquid chromatography. *Results*: The assay was linear from 5 to 755 ng/mL. The limit of quantitation for NF was 5 ng/mL in serum, and the recovery was greater than 90%. The method was used to determine the concentration–time profiles of NF in the serum. The pharmacokinetic parameters were calculated by Drug and Statistics (ver 1.0) program. The mean Cmax was 320.2 ± 71.3 μg/L, the mean Tmax was 3.2 ± 0.5 hours, the mean t1/2 was 6.60 ± 2.17 hours, the mean AUC0-24 was 2.03 ± 0.25 mg h/L, the mean AUC0-∞ was 2.50 ± 0.36 mg h/L, the mean MRT0-24 was 8.57 ± 0.19 hours, and the mean MRT0-∞ was 15.2 ± 1.8 hours. *Discussion and conclusion*: The pharmacokinetic characteristics were found by a two-compartment model following the oral administration of NF-loaded N-succinyl chitosan/alginate hydrogel beads in rabbits.

Key words: *HPLC–UV; hydrogel; nifedipine;* N-*succinyl chitosan; pharmacokinetics; pH sensitivity; rabbit serum*

Introduction

Nifedipine (NF) is a strong calcium ion antagonist with actions of expanding peripheral blood vessel and coronary artery, and it has less side effects. It is widely used in the treatment of arterial hypertension, angina pectoris, and other cardiovascular diseases. Although NF is absorbed almost completely, it displays a low bioavailability because of its short biological half-life with significant fluctuations in plasma concentrations[1] and its extensive first-pass metabolism[2]. It is easily decomposed in light. Therefore, it is essential to prepare a controlled release formulation of NF, and the pH-sensitive succinyl chitosan/alginate hydrogels were developed.

A variety of synthetic or natural polymers with acidic or basic pendant groups have been employed to fabricate the pH-sensitive hydrogels to obtain the desired controlled release of drugs[3]. The use of natural polymers in the design of pH-sensitive hydrogels has received much attention because of their excellent biocompatibility and biodegradability[4]. Among them, alginate and chitosan are very promising and have been widely exploited in pharmaceutical industry for controlling drug release[5,6]. The pH-sensitive hydrogels have attracted increasing attention because of their unique properties. Chitosan with amino groups is soluble at low pH and insoluble at high pH, whereas alginate with carboxyl groups has the property of shrinking at low pH and being dissolved at high pH. Swelling of such hydrogels in the stomach is minimal and thus the drug release is also minimal. Because of increase in pH, the swelling degree increases as the hydrogels pass down the intestinal tract[7].

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The *N*-succinyl chitosan/alginate hydrogel (Suc-Chi/alginate) hydrogel beads were prepared; the beads were used as a pH-sensitive controlled release system for the delivery of NF[8]. The release properties of NF from the hydrogel beads were studied in simulated gastric and intestinal fluid. The result indicates that the drug release properties from NF-loaded hydrogel beads are pH-dependent[9]. Therefore, the Suc-Chi/alginate hydrogel beads are good candidates as a drug delivery system in the intestinal tract.

To date, there has been more attention on the release properties of the hydrogel[10–13]. Many of them study the swelling behaviors and release kinetics in vitro[14,15]. Only a few release of the Suc-Chi/alginate hydrogel in vivo have been reported previously[16]. The studies focused on their biocompatibility and biodegradability, in vivo degradation properties and so on[7,17]. Therefore, on the basis of our previous work on *N*-succinyl chitosan/alginate hydrogel bead[8], the hydrogel beads were used as a pH-sensitive controlled release system for the delivery of NF. We studied the pharmacokinetic characteristics of NF following oral administration of the pH-sensitive succinyl chitosan/alginate hydrogel beads in vivo.

Several methods for the determination of NF in vitro and in plasma have been described in the literature. This includes a UV spectrophotometer[14] and high-performance liquid chromatography (HPLC) with UV[18–22]. To obtain the available pharmacokinetic parameters of NF sample, sensitive HPLC method was developed for the determination of NF in rabbit serum after oral administration of the pH-sensitive *N*-Suc-Chi/alginate hydrogel bead. The pharmacokinetics following oral administration of the NF-loaded Suc-Chi/alginate hydrogel beads to rabbits were studied.

Materials and methods

Materials

NF and nitrendipine reference substance were purchased from National Institute for the Control Pharmaceutical and Biological Products (Beijing, China). Acetonitrile and methanol were of chromatographic grade obtained from Shandong Yuwang Chemical Reagent Co. Ltd. (Shandong, China). Ethyl and disodium hydrogen phosphates were of analysis grade obtained from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China).

The NF-loaded *N*-Suc-Chi/alginate hydrogel beads were provided by the Department of Pharmacy, the Second Hospital of Lanzhou University (Gansu, China). *N*-Suc-Chi/alginate hydrogel beads were prepared by the ionic gelation method; NF was chosen as a model drug. The *N*-Suc-Chi/alginate hydrogel beads were prepared by dropping aqueous succinyl chitosan/alginate into a calcium chloride solution[8]. The NF pH-sensitive *N*-Suc-Chi/alginate hydrogel bead in drug loading was 9.8% (g/g).

Instrumentation and chromatographic conditions

The analysis was performed by a Waters 515 pump, a Waters 2487 UV–Vis detector, a Waters autosampler (Waters, Milford, MA, USA). A LUNA C_{18} packed column, 4.6 × 250 mm, 5 μm (Phenomenex Co. Ltd., Torrance, CA, USA) was used. The mobile phase was acetonitrile–water (60:40, v/v), filtered through a 0.45 μm millipore filter and degassed prior to use. The flow rate was 1.0 mL/min. The detection was performed at 235 nm at room temperature.

Animal, drug administration, and blood sampling

Rabbits were obtained from the Laboratory Animal Center at the Lanzhou (Gansu, China), and rabbits weighing 2.5–2.6 kg were housed individually over 2 weeks in a temperature-controlled environment (20–25°C). The relative humidity varied between 50% and 60%. Unless otherwise noted, the rabbits had free access to a standard diet and water 1 week prior to the experiments. They were fasted for 12 hours with free access to water prior to the experiments. The NF-loaded *N*-Suc-Chi/alginate hydrogel beads were then orally administered to rabbits at a dose of 12 mg/kg. Blood samples (1.5 mL) were collected through the femoral artery at times 1, 2, 3, 4, 6, 8, 10, 12, and 24 hours after oral administration. After taking a blood specimen, the homeostasis of the rabbits was maintained by injecting the same volume of physiological saline through the ear vein.

Sample preparation

Each blood sample was collected from the femoral artery at specific times and placed in centrifugal tubes. The tubes were centrifuged at 1290 × *g* for 10 minutes to separate precipitated proteins. The upper layer of serum was transferred to an appropriately labeled tube and stored at −20°C until subsequent assays. 0.5 mL, of serum was pipetted into a 10-mL centrifuge tube, and 0.5 mL of 50 mmol/L disodium hydrogen phosphate solution (pH = 12) and 0.2 mL of methanol containing nitrendipine (500 ng/mL) as internal standard, then vortex-mixed 2 mL ethyl for 2 minutes. After centrifugation for 10 minutes at 1290 × *g*, the organic solvent phase was transferred to another tube; the serum was extracted by ethyl twice (2, 2 mL) and evaporated to dryness under a stream of nitrogen gas at 40°C. The residue was dissolved in 0.5 mL methanol by vortex mixing for 3 minutes, after centrifuging at 1750 × *g* for 5 minutes, and 50 μL of the solution was filtrated by 0.45 μm millipore filter, then injected into the HPLC.

During all the operations, samples were protected from light. The same sample-handling process was used for recovery and precision determinations in serum. All the courses of sample preparation were kept from light until assayed.

Pharmacokinetic study

Twelve rabbits were divided into four groups, each group having three rabbits. The blood samples were collected from each rabbit for each time point in four groups. The serum concentrations of NF are the average of three rabbits in the study group and the pharmacokinetic results are the average of four groups. This method was used to determine the serum concentrations of NF in rabbit serum after oral administration at a dose of 12 mg/kg NF. All data and the pharmacokinetic parameters were calculated by the computer program Drug and Statistics (DAS) ver 1.0.

Result

HPLC chromatograms

Under the condition described above, the HPLC chromatograms of blank, serum spiked with NF at concentration of 236 ng/mL, and the serum obtained 3 hours after the oral administration of the NF-loaded *N*-Suc-Chi/alginate hydrogel beads are shown in Figure 1. The retention times for NF and nitrendipine (internal standard) were approximately 6.1 and 7.3 minutes, respectively. The peaks were sharp and symmetrical with good baseline resolution and minimal tailings, thus facilitating accurate measurements of the peak-area ratios. No endogenous serum components elute at the retention time of NF or internal standard.

Linearity and limits of quantification

There was good linearity over the range of 5.0–755 ng/mL in the serums and the calibration curve was $y = 0.0205 + 1.55 \times 10^{-3}x$ with a correlation coefficient of 0.9998. The limit of quantification for the assay was 5.0 ng/mL.

Precision and recovery

The reproducibility of the method was defined by examining both intra- and interday variance. Analytical accuracy and precision data are shown in Tables 1 and 2 and expressed as mean detected concentration and relative standard deviation (RSD). The recovery was assessed by comparing the peak-area (analyte:internal standard) obtained from spiked serum samples of different analyte concentrations to the peak-area ratios for the samples containing the equivalent amounts of the analyte and internal standard directly dissolved in methanol. The recoveries of NF from rabbit serum are shown in Table 3. The intra- and interday RSDs were less than 2%. The recovery ranged from 92.56% to 93.64%.

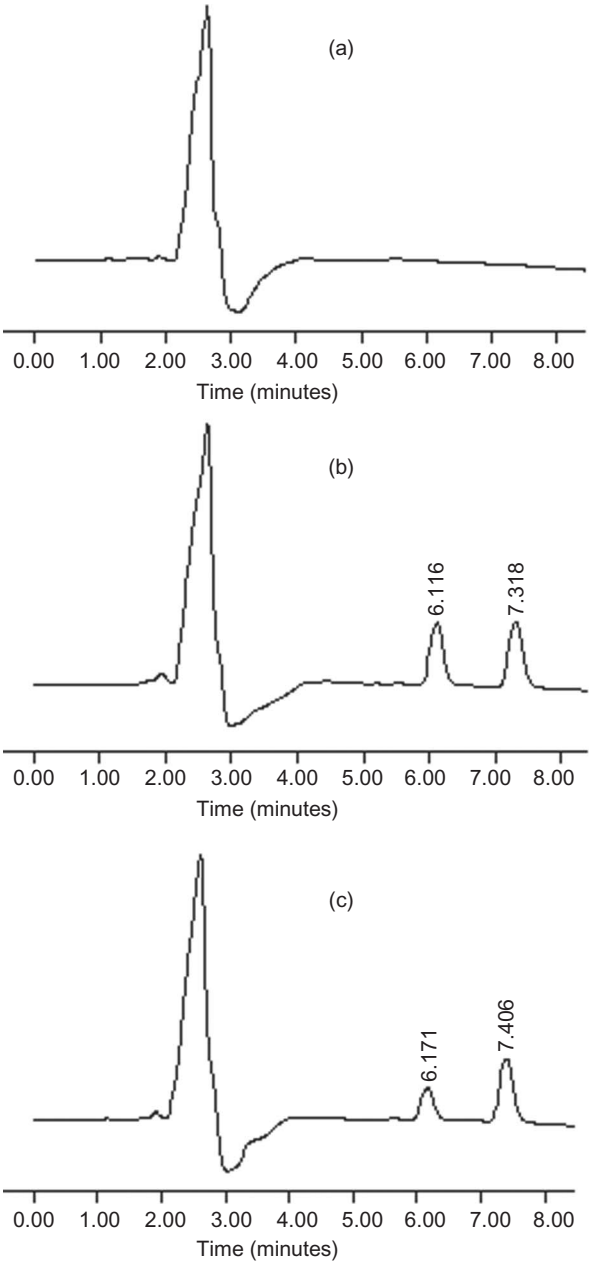

Figure 1. Chromatograms of nifedipine (NF) in rabbit serum: (a) blank serum; (b) serum-spiked NF (503.96 ng/mL) and nitrendipine (internal standard); (c) serum sample 3 hours after oral administration of NF-loaded succinyl chitosan/alginate hydrogel beads at a dose of 12 mg/kg. Retention times of NF and nitrendipine (internal standard) were approximately 6.1 and 7.4 minutes, respectively.

Table 1. Validation of the intraday assay.

Spiked concentration (ng/mL)	Measured concentration (ng/mL)	Accuracy (%)	RSD (%)
5.03	5.06 ± 0.06	100.56	1.18
100.79	100.42 ± 1.34	99.63	1.33
251.98	251.51 ± 1.00	99.81	0.40
503.96	501.59 ± 3.02	99.53	0.56
755.94	755.66 ± 1.31	99.96	0.17

Each value is represented as mean ± SD (n = 5).

Table 2. Validation of the interday assay.

Spiked concentration (ng/mL)	Measured concentration (ng/mL)	Accuracy (%)	RSD (%)
5.03	5.02 ± 0.06	99.67	1.19
100.79	100.30 ± 1.10	99.51	1.09
251.98	251.43 ± 1.41	99.78	0.56
503.96	502.85 ± 1.18	99.78	0.23
755.94	755.10 ± 4.74	99.89	0.63

Each value is represented as mean ± SD (n = 3).

Table 3. Recovery of the nifedipine assay.

Spiked concentration (ng/mL)	Peak-area ratio		Recovery (%)	RSD (%)
	Untreated	Treated		
5.03	0.031 ± 0.000	0.029 ± 0.002	92.58 ± 1.86	2.0
251.98	0.413 ± 0.004	0.387 ± 0.070	93.64 ± 1.98	2.1
503.96	0.822 ± 0.005	0.761 ± 0.020	92.56 ± 2.16	2.3

Each value is represented as mean ± SD (n = 5).

Pharmacokinetic analysis statistics

To estimate the absorption rate of NF in gastrointestinal tract, the serum concentration of NF was analyzed in rabbit serum, following oral administration of NF. The mean serum concentration of NF was determined at 0, 1, 2, 3, 4, 6, 8, 10, 12, and 24 hours after oral dosing. Figure 2 shows the mean ± SD serum concentration–time profile of NF after oral administration.

Pharmacokinetic analyses of NF were performed using DAS ver 1.0 program. The peak serum concentration and the time to reach C_{max} were obtained directly from the raw date. The area under the serum concentration–time profile (AUC) of NF was calculated by linear trapezoidal summation with extrapolation to infinity. The elimination rate constant (β) was calculated from the slope of the terminal phase of log concentration–time profile, and the elimination half-life ($t_{1/2(\beta)}$) was calculated by $0.693/\beta$.

The serum NF levels were calculated by NF concentration of the subjects at different time. The C_{max} (maximum plasma concentration), the t_{max} (time to reach C_{max}), the AUC_{0-24} (the area under concentration–time curve from 0 to 24 hours), the $AUC_{0-\infty}$ (the area under concentration–time curve from infinity), the $t_{1/2}$ (elimination half-time), and other parameters were automatically calculated by DAS ver 1.0 program. The pharmacokinetic parameters of NF are summarized in Table 4.

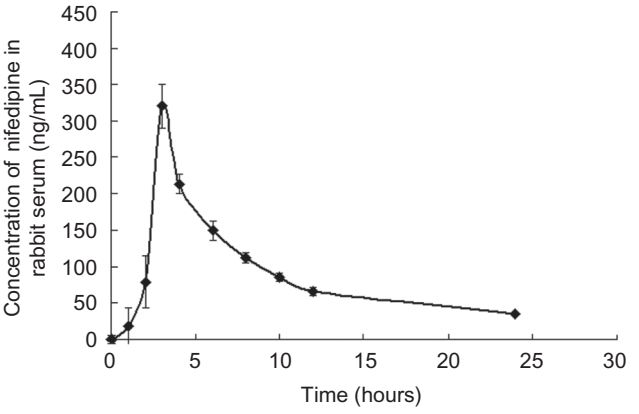

Figure 2. Serum concentration–time curve of nifedipine (NF) in rabbits after oral administration of NF-loaded succinyl chitosan/alginate hydrogel beads. Data are shown as mean ± SD of four experiments.

Table 4. Pharmacokinetic parameters of single dose of nifedipine-loaded Suc-Chi/alginate hydrogel beads in rabbits.

Parameters	Results
AUC_{0-24} (μg·h/L)	2026.7 ± 250.0
$AUC_{0-\infty}$ (μg·h/L)	2503.7 ± 363.0
T_{max} (hours)	3.2 ± 0.5
C_{max} (μg/L)	320.2 ± 71.3

Each value is represented as mean ± SD (n = 4).

Discussion

The pharmacokinetic parameters were calculated by the Drug and Statistics (ver 1.0) program. The main pharmacokinetic parameters of NF pH-sensitive hygrogel were listed as follows: AUC_{0-24}: 2.03 ± 0.25 mg·h/L; $AUC_{0-\infty}$: 2.50 ± 0.36 mg·h/L; MRT_{0-24}: 8.57 ± 0.19 hours; $MRT_{0-\infty}$: 15.2 ± 1.8 hours; T_{max}: 3.2 ± 0.5 hours; C_{max}: 320.2 ± 71.3 μg/L; and $t_{1/2}$: 6.60 ± 2.17 hours. The pharmacokinetics characteristics were fitted to a two-compartment model after oral administration NF pH-sensitive hydrogel in rabbits. Table 5 lists the pharmacokinetic parameters.

The release properties of NF from the hydrogel beads were studied in simulated gastric and intestinal fluid. The release from *N*-Suc-Chi/alginate beads was about

Table 5. Pharmacokinetic parameters for nifedipine after oral administration obtained from the two-compartment model.

Parameters	Results
K_a	0.75 ± 0.20
$t_{1/2}$, $t_{1/2(\alpha)}$ (hours)	6.60 ± 2.17
$t_{1/2(\beta)}$ (hours)	46.7 ± 16.3
$t_{1/2(ka)}$ (hours)	0.98 ± 0.27
k_{10} (hour^{-1})	0.05 ± 0.03
k_{12} (hour^{-1})	0.04 ± 0.04
k_{21} (hour^{-1})	0.05 ± 0.04
MRT_{0-24} (hours)	8.57 ± 0.19
$MRT_{0-\infty}$ (hours)	15.2 ± 1.8
CL/F (L/kg)	0.0038 ± 0.0009

Each value is represented as mean ± SD ($n = 4$).

8% in 3 hours at pH 1.5. The amount of NF released increased significantly at pH 7.4 (~68%). The results clearly suggested that the drug release properties of NF-loaded hydrogel beads are pH-dependent.

The pharmacokinetic characteristics were fitted to a two-compartment model after oral administration of NF-loaded *N*-Suc-Chi/alginate hydrogel beads to rabbits. The NF serum concentration–time curves showed that the mean maximum concentration of NF in serum was 320.2 μg/L at 3.2 hours after oral dosing, the mean concentration of NF in serum was 17.9 μg/L at 1 hour, and the mean concentration of NF in serum was 79 μg/L at 2 hours. The quantity of drug release in the stomach was minimal. Because of increase in pH, the quantity of drug released increased as the NF-loaded *N*-Suc-Chi/alginate hydrogel beads pass down the intestinal tract. The results suggested that the drug release properties from NF-loaded Suc-Chi/alginate hydrogel beads are also pH-dependent in vivo. This showed that the in vivo and in vitro NF release properties from hydrogel beads are consistent.

The features of NF pharmacokinetics after oral administration of Suc-Chi/alginate hydrogel bead could be applied as a reference for its clinical application. This release system of hydrogel bead may have some interesting features: The release results noted that NF-loaded *N*-Suc-Chi/alginate hydrogel beads have pH-responsive release pattern, which can not only protect drug loss in acid environment but also control drug release in intestinal tract. Based on in vitro and in vivo drug release in rabbit, a new dosage form can be designed. If it is administered in patients before sleep, through predetermined lag time, the drug will be released before seizure disorders, which can efficiently prevent/cure cardiovascular diseases and decrease the side effect caused by the drug itself.

In conclusion, this article describes a simple, rapid, sensitive, accurate, and precise procedure for the determination of NF, suitable for the analysis of large numbers of serum samples. The assay was validated to meet the requirements of pharmacokinetic studies. The pharmacokinetic parameters and the results of oral administration of NF-loaded *N*-succinyl chitosan/alginate hydrogel beads can be used as a suitable reference in clinical application.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

References

1. Varon J. (2008). Treatment of acute severe hypertension: Current and newer agents. Drugs, 68:283–97.
2. Hayashi K, Wakino S, Sugano N, Ozawa Y. (2007). Ca^{2+} channel subtypes and pharmacology in the Kidney. Circ Res, 100:342–53.
3. Kimura Y. (1993). In: Tsuruta T, Hayashi T, Katsoka K, Ishihara K, Kimura Y, eds. Biomedical applications of polymeric materials. Boca Raton, FL: CRC Press.
4. Kaplan DL, Wiley BJ, Mayer JM, Arcidiacono S, Keith J, Lombardi SJ, et al. (1994). In: Shalaby SW, ed. Biomedical polymers. New York: Hanser Publishers.
5. Murata Y, Jinno D, Liu D, Isobe T. (2007). The drug release profile from calcium-induced alginate gel beads coated with an alginate hydrolysate. Molecules, 12:2559–66.
6. Shi J, Alves NM, Mano JF. (2008). Chitosan coated alginate beads containing poly (*N*-isopropylacrylamide) for dual-stimuli-responsive drug release. J Biomed Mater Res B Appl Biomater, 84:595–603.
7. Mi FL, Tan YC, Liang HF. (2002). In vivo biocompatibility and degradability of a novel injectable-chitosan-based implant. Biomaterials, 23:181–91.
8. Dai YN, Li P, Zhang JP, Wang AQ, Wei Q. (2008). A novel pH sensitive *N*-succinyl chitosan/alginate hydrogel bead for nifedipine delivery. Biopharm Drug Dispos, 29:173–84.
9. Dai YN, Li P, Zhang JP, Wang AQ, Wei Q. (2008). Swelling characteristics and drug delivery properties of nifedipine pH sensitive alginate-chitosan hydrogel beads. J Biomed Mater Res B Appl Biomater, 86B:493–500.
10. Nochos A, Douroumis D, Bouropoulos N. (2008). In vitro release of bovine serum albumin from alginate/HPMC hydrogel beads. Carbohydr Polymers, 74:451–7.
11. Wang Q, Zhang JP, Wang AQ. (2009). Preparation and characterization of a novel pH-sensitive chitosan-g-poly (acrylic acid)/attapulgite/sodium alginate composite hydrogel bead for controlled release of diclofenac sodium. Carbohydr Polymers, 78:731–7.
12. Zhang JP, Li A, Wang AQ. (2006). Study on superabsorbent composite. VI. Preparation, characterization and swelling behaviors of starch phosphate-graft-acrylamide/attapulgite superabsorbent composite. Carbohydr Polymers, 65:150–8.
13. Zhang JP, Chen H, Wang AQ. (2005). Study on superabsorbent composite. III. Swelling behaviors of polyacrylamide/attapulgite

composite based on acidified attapulgite and organo-attapulgite. Eur Polym J, 41:2434–42.
14. Chandy T, Sharma CP. (1992). Chitosan beads and granules for oral sustained delivery of nifedipine: In vitro studies. Biomaterials, 13:949–52.
15. Wang FQ, Li P, Zhang JP, Wang AQ, Wei Q. (2010). A novel pH sensitive magnetic alginate-chitosan beads for albendazole delivery. Drug Dev Ind Pharm, in press.
16. Sandra G, César T, Enriqueta M, José TM, Dolores BM. (2010). Characterization and *in vivo* evaluation of ketotifen-loaded chitosan microspheres. Carbohydr Polymers, 79: 1006–13.
17. Chiu YL, Chen SC, Su CJ, Hsiao CW, Chen YM, Chen HL, et al. (2009). pH-triggered injectable hydrogels prepared from aqueous *N*-palmitoyl chitosan: *In vitro* characteristics and *in vivo* biocompatibility. Biomaterials, 28:4877–88.
18. Ioannis N, Daftsios AC. (2003). Determination of nifedipine in human plasma by solid-phase extraction and high-performance liquid chromatography: Validation and application to pharmacokinetic studies. J Pharm Biomed Anal, 32:1213–18.
19. Keinbloesem CH, Vanharten J. (1984). Liquid chromatographic determination of nifedipine in plasma and of its main metabolite in urine. J Chromatogr, 308:209–16.
20. Nakamura I, Takahashi M, Izumi H. (1999). Sensitive high-performance liquid chromatographic determination of nifedipine in cat plasma following improved sample treatment. J Chromatogr B, 729:265–70.
21. Nitsche V, Schutz H, Eichinger A. (1987). Rapid high-performance liquid chromatographic determination of nifedipine in plasma with online precolumn solid phase extraction. J Chromatogr B, 420:207–11.
22. Vertzoni MV, Reppas C, Archontaki HA. (2006). Sensitive and simple liquid chromatographic method with ultraviolet detection for the determination of nifedipine in canine plasma. Anal Chim Acta, 573–574:298–304.