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PHARMACOKINETIC EVALUATION OF PH-SENSITIVE P(BA-CO-IA) HYDROGEL MICROSPHERES OF NIFEDIPINE IN RABBITS

Original Research

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ABSTRACT

Background: pH-sensitive hydrogel microspheres have gained increasing attention as advanced drug delivery systems because of their ability to modulate drug release in response to physiological conditions. Nifedipine, a widely prescribed calcium channel blocker for hypertension and angina pectoris, suffers from low oral bioavailability, rapid first-pass metabolism, and short systemic residence time. These limitations often necessitate frequent dosing and contribute to variable therapeutic response, underscoring the need for a sustained-release delivery approach.

Objective: The objective of this study was to develop and evaluate pH-sensitive butyl acrylate-co-itaconic acid (p(BA-co-IA)) hydrogel microspheres for sustained oral delivery of nifedipine and to assess their in vivo pharmacokinetic performance.

Methods: Nifedipine-loaded p(BA-co-IA) hydrogel microspheres were synthesized using a modified suspension polymerization method. In vivo pharmacokinetic evaluation was conducted in healthy male albino rabbits following oral administration of nifedipine standard solution and hydrogel microspheres at a dose of 10 mg/kg. Plasma nifedipine concentrations were quantified using a validated reverse-phase high-performance liquid chromatography method, and pharmacokinetic parameters were calculated using compartmental analysis.

Results: The hydrogel microspheres demonstrated a delayed absorption profile with a higher Tmax (4.98 ± 0.43 h) compared with the standard solution (1.216 ± 0.02 h). Peak plasma concentration was lower for the microspheres (1.43 ± 0.08 µg/mL) than for the standard formulation (2.24 ± 0.01 µg/mL). The elimination half-life was prolonged for the microspheres (4.42 ± 0.96 h versus 2.24 ± 0.5 h), and systemic exposure was markedly enhanced, as reflected by a higher $AUC_{0-\infty}$ (19.6 ± 0.9 µg·h/mL compared with 10.92 ± 0.16 µg·h/mL). Drug levels remained detectable for up to 24 hours following administration of the sustained-release formulation.

Conclusion: The findings confirmed that pH-sensitive p(BA-co-IA) hydrogel microspheres provided sustained release and improved oral bioavailability of nifedipine, supporting their potential as a promising delivery system for long-term antihypertensive therapy.

Keywords: Bioavailability, Drug Delivery Systems, Hydrogels, Nifedipine, Pharmacokinetics, Rabbits, Sustained-Release Preparations.

INTRODUCTION

Hydrogel microspheres are crosslinked polymeric systems capable of absorbing large quantities of water while maintaining their three-dimensional structure, a property that has positioned them as highly attractive platforms for controlled drug delivery applications (1). Owing to their tunable size range from the nanometer to micrometer scale, these carriers offer precise control over drug loading, release kinetics, and biodistribution. Their highly hydrated polymeric networks provide an extensive surface area that facilitates multivalent bioconjugation, while also allowing therapeutic agents and biomolecules to be entrapped within the interior matrix of the microspheres (2). These features collectively make hydrogel microspheres particularly relevant in the context of modern pharmaceutical development, where sustained drug release and improved therapeutic efficacy are persistent clinical needs. For successful translation into biomedical use, hydrogel microspheres must meet several critical criteria, including chemical and mechanical stability during systemic circulation, protection of the encapsulated drug from premature degradation, surface modifiability for ligand attachment, biodegradability, and an optimal particle size that ensures safety and efficient clearance following drug release (3). Recent advances have led to the development of stimuli-responsive hydrogel microspheres that undergo reversible swelling or deswelling in response to environmental triggers such as pH, temperature, light, and electromagnetic fields (4). Compared with conventional bulk hydrogels, these microgels offer practical advantages including simpler preparation methods, higher surface-to-volume ratios, and improved responsiveness due to their small particle size (5). As a result, functional microspheres have been extensively investigated for applications extending beyond drug delivery, including biomolecular separations, biosensing, catalysis, and enzyme immobilization (6).

Among these systems, pH-sensitive hydrogel microspheres have gained particular attention for oral and targeted drug delivery. Their swelling behavior is governed by ionizable functional groups present on the polymer chains, which undergo protonation or deprotonation in response to changes in the surrounding pH. This process alters electrostatic repulsion within the polymer network, leading to controlled expansion or contraction of the microspheres and, consequently, modulation of drug release (7). Such behavior is especially advantageous for drugs that exhibit poor stability or bioavailability in the gastrointestinal tract, as pH-responsive carriers can protect the drug in unfavorable environments and release it at desired absorption sites. Nifedipine, a dihydropyridine calcium channel blocker widely used as a first-line therapy for hypertension and angina pectoris, represents a clinically important candidate for controlled delivery strategies (8). Despite being almost completely absorbed from the gastrointestinal tract, nifedipine exhibits low systemic bioavailability due to its high lipophilicity, extensive first-pass metabolism in the intestinal wall and liver, and rapid conversion to more polar metabolites that are eliminated renally. Furthermore, its pronounced photosensitivity and susceptibility to oxidative biotransformation pose additional challenges to maintaining therapeutic plasma concentrations (9). These limitations underscore a critical gap in current dosage forms and highlight the need for innovative carrier systems capable of enhancing drug stability, controlling release, and improving bioavailability. In this context, the objective of the present research is to develop and evaluate pH-sensitive hydrogel microspheres as a rational drug delivery system for nifedipine, with the aim of improving its stability, modulating its release profile, and ultimately enhancing its therapeutic effectiveness.

METHODS

This experimental study was designed to develop and evaluate a pH-sensitive copolymeric hydrogel microsphere system for the controlled delivery of nifedipine, followed by in vivo pharmacokinetic assessment in an animal model. Nifedipine was kindly provided by Highnoon Laboratories, Lahore, while all analytical-grade reagents and solvents, including acetonitrile, methanol (HPLC grade), ethanol, and double-distilled water, were procured from Sigma-Aldrich. All chemicals were used as received without further purification. Citrated tubes containing EDTA disodium salt were used for blood sample collection to prevent coagulation. Novel pH-sensitive butyl acrylate–co–itaconic acid [p(BA-co-IA)] hydrogel microspheres loaded with nifedipine were synthesized using a modified suspension polymerization technique. Double-distilled water served as the dispersion medium, containing polyvinyl alcohol (PVA, 0.4 g) dissolved in 100 mL of distilled water to stabilize the emulsion. The dispersed organic phase consisted of varying ratios of monomers dissolved in 24 mL of toluene, along with ethylene glycol dimethacrylate (EGDMA, 5%) as a cross-linking agent and benzoyl peroxide (BP, 1%) as the polymerization initiator. Polymerization was carried out under controlled conditions to obtain uniform microspheres, which were subsequently washed, dried, and characterized in earlier optimization studies to select the most suitable formulation for in vivo

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evaluation. Quantitative determination of nifedipine in rabbit plasma was performed using a validated reverse-phase high-performance liquid chromatography (RP-HPLC) method. Chromatographic separation was achieved using an Agilent 5 TCC18 column (250 mm × 4.6 mm). The mobile phase consisted of methanol and water in a 70:30 (v/v) ratio, with the pH adjusted to 3.0 using orthophosphoric acid. Detection was carried out at a wavelength of 238 nm with a flow rate of 1 mL/min. The method demonstrated a retention time of 3.38 minutes for nifedipine and showed excellent linearity over the tested concentration range, with a correlation coefficient (R^2) of 0.9995. Intra- and inter-day precision and accuracy were evaluated at three quality-control concentrations (12.5, 25, and 50 µg/mL), yielding acceptable percent relative standard deviation and high recovery. The limit of detection and limit of quantification were determined as 0.001754 mg/mL and 0.005315 mg/mL, respectively (10).

In vivo pharmacokinetic studies were conducted using twelve healthy male albino rabbits weighing 2.0 ± 0.3 kg. The study protocol was reviewed and approved by the Ethical Committee of Bahauddin Zakariya University (BZU), Multan, Punjab, Pakistan. All experimental procedures were carried out in accordance with institutional guidelines for the care and use of laboratory animals. Rabbits were housed under standard laboratory conditions and were acclimatized prior to experimentation. Animals were fasted for twelve hours before drug administration and up to 24 hours post-dosing, while free access to water was maintained throughout the study period. The best-performing formulation was selected for in vivo testing based on prior in vitro characterization. Nifedipine standard solution and nifedipine-loaded hydrogel microspheres, equivalent to a dose of 10 mg/kg, were administered orally using a stomach tube with distilled water as the vehicle. Blood samples (2 mL) were collected from the marginal ear vein at baseline (0 hour) and at predetermined intervals of 0.5, 1, 2, 4, 6, 8-, 10-, 12-, and 24-hours following administration. Samples were immediately transferred into citrated tubes containing EDTA and centrifuged at 5000 rpm for 10 minutes. Plasma was separated and stored at −20 °C until analysis. For sample preparation, plasma was alkalinized with 100 µL of 1 N sodium hydroxide and vortex-mixed, followed by liquid–liquid extraction using 5 mL diethyl ether. After centrifugation at 2400 g for 10 minutes, the organic layer was collected and evaporated to dryness under a gentle stream of nitrogen at room temperature. The residue was reconstituted in 250 µL of mobile phase, vortex-mixed, and centrifuged at 8500 g for 5 minutes to remove particulate matter. A 20 µL aliquot was injected into the HPLC system for analysis. Pharmacokinetic parameters were calculated using the Scientific Application Package Kinetica® version 4.1.1 (Thermo Electron Corporation), employing standard non-compartmental analysis.

RESULTS

The comparative in vivo evaluation demonstrated clear differences in the pharmacokinetic behavior of nifedipine administered as a standard oral solution and as sustained-release pH-sensitive hydrogel microspheres in healthy rabbits. Following oral administration, the standard solution produced a rapid rise in plasma drug concentration, reaching an early peak and declining quickly thereafter, whereas the hydrogel microspheres maintained relatively stable plasma concentrations over an extended period. This prolonged systemic exposure indicated effective sustained-release behavior of the pH-responsive microgels. Pharmacokinetic analysis showed that the time to reach maximum plasma concentration was markedly delayed for the hydrogel microspheres (4.98 ± 0.43 h) compared with the standard solution (1.216 ± 0.02 h), reflecting a slower and more controlled absorption process. The maximum plasma concentration was higher for the standard solution (2.24 ± 0.01 µg/mL) than for the microsphere formulation (1.43 ± 0.08 µg/mL), indicating attenuation of the initial peak with the sustained-release system. In contrast, the overall systemic exposure to nifedipine, as reflected by the area under the plasma concentration–time curve from zero to infinity, was substantially greater for the hydrogel microspheres (19.6 ± 0.9 µg·h/mL) than for the standard solution (10.92 ± 0.16 µg·h/mL), demonstrating improved bioavailability in plasma.

Elimination-related parameters further supported the sustained-release profile of the test formulation. The terminal half-life was prolonged for the hydrogel microspheres (4.42 ± 0.96 h) compared with the standard solution (2.24 ± 0.5 h), indicating extended drug persistence in systemic circulation. The elimination rate constant differed between formulations, with the microsphere system showing a lower elimination rate (0.16 ± 0.03 h^{-1}) relative to the standard solution (0.81 ± 0.09 h^{-1}), consistent with slower drug clearance. Mean residence time was also prolonged for the hydrogel microspheres, confirming sustained availability of nifedipine in plasma and reduced fluctuation in drug levels (11). The plasma concentration–time profile revealed that the standard solution achieved peak levels within the first hour and declined rapidly, with near-complete disappearance of measurable drug concentrations by 12 h. In contrast, the hydrogel microspheres exhibited a gradual increase in plasma concentration, reaching maximum levels between 4 and 6 h and maintaining detectable concentrations up to 24 h. This pattern confirmed prolonged absorption and sustained systemic exposure following administration of the pH-sensitive microspheres.

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Table 1: Comparative Pharmacokinetic Parameters of Nifedipine After Oral Administration of Standard Solution and pH-Sensitive Hydrogel Microspheres in Rabbits (Mean ± SD)

Parameter	Standard solution (Mean ± SD)	Nifedipine hydrogel microspheres (Mean ± SD)
AUC 0-∞ (h.ug/ml)	10.92±0.16	19.6±0.9
Tmax (h)	1.216±0.02	4.98±0.43
Cmax (ug/ml)	2.24±0.01	1.43±0.08
t 1/2 β (h)	2.24±0.5	4.42±0.96
K 21(1/h)	0.81±0.09	0.16±0.03

Table 2: Plasma Concentration–Time Profile of Nifedipine Following Oral Administration of Standard Solution and pH-Sensitive Hydrogel Microspheres in Rabbits (Mean ± SD)

Time(hrs)	Standard solution (Mean ± SD)	Nifedipine hydrogel microspheres (Mean ± SD)
0	0±0	0±0
0.5	1.74±0.02	0.29±0.086
1	2.22±0.013	0.52±0.171
2	1.96±0.011	0.81±0.43
3	1.6±0.024	1.27±0.66
4	1.2±0.013	1.62±0.95
5	0.93±0.03	1.68±1.27
6	0.71±0.032	1.61±1.66
8	0.32±0.01	1.34±2.39
10	0.112±0.01	1.01±3.16
12	0	0.74±4.02
24	0	0.24±8.4

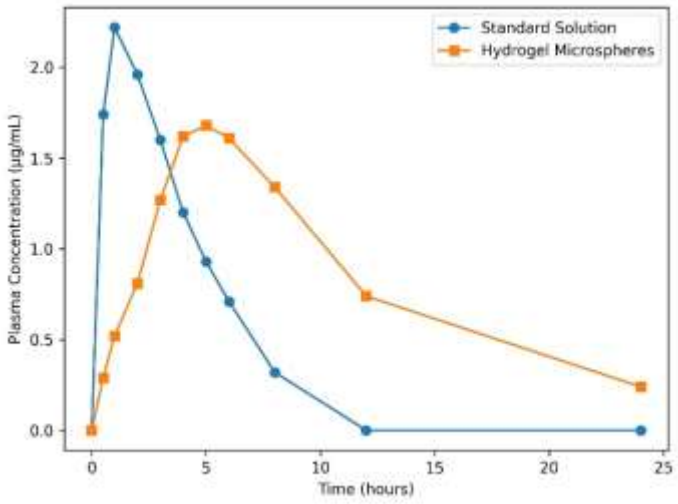

Figure 2 Plasma Concentration (ug/ml)

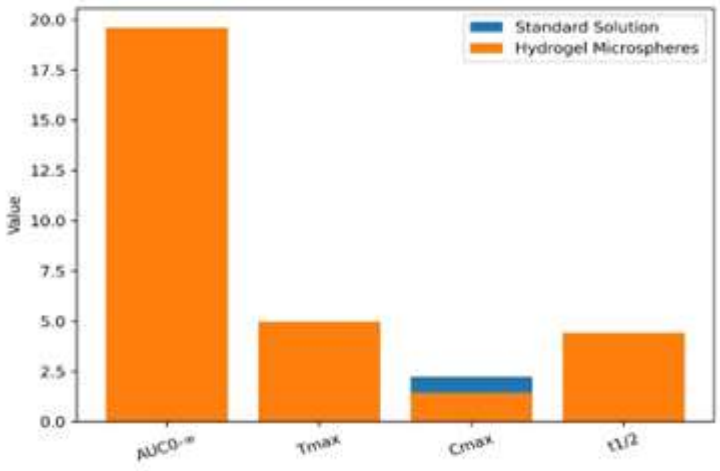

Figure 2 Values

DISCUSSION

The present investigation demonstrated that pH-sensitive hydrogel microspheres functioned as an effective sustained-release carrier for nifedipine, offering pharmacokinetic behavior that aligned well with the intended objective of prolonged systemic exposure and reduced plasma concentration fluctuations. The observed pattern of delayed absorption and extended presence of drug in circulation was consistent with the fundamental design of polymeric microgel systems, in which drug diffusion is governed by polymer swelling, ionization behavior, and matrix relaxation rather than rapid dissolution. This behavior is clinically relevant for nifedipine, a drug whose therapeutic utility is limited by rapid metabolism, short residence time, and pronounced peak–trough variations following conventional oral dosing. When interpreted in the context of existing literature, the findings were in agreement with prior in vivo evaluations of sustained-release nifedipine carrier systems, which reported delayed absorption, prolonged half-life, and enhanced systemic exposure compared with immediate-release formulations (12-14). Such consistency across different polymeric platforms supports the concept that controlled-release micro- and particulate systems can successfully mitigate the pharmacokinetic limitations of nifedipine by moderating the rate of drug input into systemic circulation (15). The comparatively lower peak exposure associated with sustained-release formulations is particularly advantageous in reducing dose-related adverse effects, while the extended exposure profile favors improved therapeutic coverage over time. The pH-responsive nature of the hydrogel microspheres represented a notable strength of the study, as it provided a mechanistic basis for controlled drug release in physiological environments. The ionizable functional groups within the polymeric network allowed the system to respond dynamically to surrounding pH conditions, facilitating gradual drug release and enhancing apparent bioavailability (16-18). Additionally, the use of a validated analytical method and standardized pharmacokinetic modeling strengthened the reliability of the interpretation. The in vivo evaluation further enhanced translational relevance by demonstrating formulation performance under biological conditions rather than relying solely on in vitro release data (19).

Despite these strengths, several limitations warranted consideration. The study was conducted in a single animal species with a relatively small sample size, which may limit extrapolation of the findings to humans. Inter-species differences in gastrointestinal physiology and drug metabolism could influence the magnitude of sustained-release effects. Furthermore, the investigation focused primarily on pharmacokinetic behavior, without assessment of pharmacodynamic outcomes or long-term safety of the polymeric carrier. The absence of statistical comparison between formulations and lack of relative bioavailability calculations also restricted the depth of comparative interpretation. Future research could address these limitations by incorporating larger cohorts, multiple animal models, and extended dosing regimens to evaluate chronic exposure and safety. Integration of pharmacodynamic endpoints, such as blood pressure control, would provide clinically meaningful correlations between sustained plasma levels and therapeutic response (20,21). Additionally, surface modification of the hydrogel microspheres or optimization of polymer composition may further refine release kinetics and improve targeting efficiency. Collectively, these considerations highlight the potential of pH-sensitive hydrogel microspheres as a promising delivery platform for nifedipine while underscoring the need for further systematic investigation to support clinical translation.

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CONCLUSION

The present study successfully demonstrated that the developed pH-sensitive copolymeric p(BA-co-IA) hydrogel microspheres functioned as an effective sustained-release delivery system for nifedipine. The in vivo evaluation confirmed a slower absorption profile, prolonged systemic availability, and improved overall exposure of the drug when compared with the conventional standard formulation, supporting the primary objective of enhancing bioavailability through controlled release. By maintaining therapeutic drug levels for an extended duration, the hydrogel microspheres addressed key pharmacokinetic limitations associated with nifedipine, including rapid clearance and short residence time. These findings highlight the practical potential of pH-responsive hydrogel microspheres as a promising oral drug delivery platform and provide a strong foundation for further optimization and translational research aimed at improving patient adherence and therapeutic outcomes.

AUTHOR CONTRIBUTIONS

Author	Contribution
Rabia Razzaq*	Substantial Contribution to study design, analysis, acquisition of Data Manuscript Writing Has given Final Approval of the version to be published
Nazar Mohammad Ranjha	Substantial Contribution to study design, acquisition and interpretation of Data Critical Review and Manuscript Writing Has given Final Approval of the version to be published
Asma Umer Khayam	Substantial Contribution to acquisition and interpretation of Data Has given Final Approval of the version to be published
Aasma Akram	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published
Mubashir Ali Khalique	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published
Nayla Javed	Substantial Contribution to study design and Data Analysis Has given Final Approval of the version to be published
Nabiha Iqbal	Contributed to study concept and Data collection Has given Final Approval of the version to be published
Hafiz Muhammad Usman Abid*	Writing - Review & Editing, Assistance with Data Curation

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