In vivo Assessment of Diltiazem HCl Immobilized Co-polymeric pH Sensitive Microgels

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Abstract

Novel stimuli responsive copolymeric 2-ethyl hexyl acrylate-co-itaconic acid diltiazem HCl (DLZ) loaded microgels were fabricated and characterized successfully to reduce the degradation of drugs in acidic pH of the stomach resulting in reduced bioavailability to minimize dosing frequency and enhance patient compliance. To evaluate the clinical significance, *in-vivo* studies of recently prepared novel pH sensitive co-polymeric microgels are performed and different pharmacokinetic parameters were investigated. Maximum plasma concentration (C_{max}), time to gain the highest plasma concentration (T_{max}), area below the plasma concentration-time bend (AUC $_{0-\infty}$), half-life ($t_{1/2\beta}$) and eradicate rate constant (K_{21}) are analyzed. Mean plasma concentration of assisted release diltiazem HCl loaded microgels exhibit higher T_{max} for prepared microgels (4.0758±0.22 hrs) than standard drug solution. The half-life $t_{1/2\beta}$ of test microgels is relatively higher (5.68±5.86 hrs) while the C_{max} is measured that is 41.06±2.02ng/mL for microgels with $AUC_{0-\infty}$ calculated as 460.35±39.99 ng.hr/mL. The eradicate rate constant K_{21} for diltiazem HCl microgels is 0.18 ± 0.07 hrs^{-1}, which showed the absorption phase is broadened and the drug is present for a extended spell of time in the body. This work proved that novel prepared pH-sensitive copolymeric p(EHA-co-IA) microgels released model drug in a sustained release manner in pH sensitive medium.

Keywords: Microgels, Diltiazem HCl, C_{max}, T_{max}, (AUC $_{0-\infty}$), K_{21}.

1. Introduction

Microgels are a subclass of polymer colloids that have attracted great attention in the research community in recent years due to their many applications. According to their unique properties, they can be defined as cross-linked polymer particles with hair-like morphology that respond to environmental stimuli by adjusting their size, density and other properties.[1] Stimulus-responsive hydrogel microspheres refer to hydrogel microspheres that counter to various exterior changes (temperature, pH, electric and magnetic fields, light,). Compared to hydrogels, microgels have features like high surface area, ease of synthesis and tiny particles.[1] Moreover, they can be utilized as a filler in any line, as well as internally and in vivo. Due to their excellent properties, functional microspheres are widely used in areas such as controlled release,[2] biomolecular separations,[3] sensors ,[4] catalysis[5] and enzyme immobilization.[6] Controlled drug delivery system hold clinical potential based on number of significances as (i) possible maintenance of optimal plasma drug levels within therapeutic range, (ii) reduced detrimental side effects by the local administration, (iii) improved drug effect (iv) enhanced half-life inside the body (v) steady small doses of drug with reduced pain as compared to intensive several large doses, (vi) cost effective products and reduced pharmaceutical waste.[7] Microgel based drug release mechanism can be modified by controlling the size, geometry and pH dependence of the drug carrier. The diffusion rate of the drug also relies on polymer mesh size and can be modified using different synthetic routes. These parameters are

tunable but can change mechanical resistance resulting in degradation of the hydrogel.[8] Diltiazem HCl act as calcium channel blocker and is primarily significant in treatment of coronary heart disease, hypertension, and angina pectoris.[9] Regular dosing is prescribed for optimal drug requirement as the oral bioavailability is only 40%. The result is a decrease in the concentration of the drug at the receptor site and a change in the plasma drug causing more side effects. For heart patients, it is even more important to maintain regular plasma levels. Therefore, higher doses of the drug should be administered to maintain a constant plasma concentration to achieve an effective therapeutic response and improve patient compliance.

In present work, previously reported novel pH sensitive DLZ loaded microgels[10] is validated by reverse phase HPLC in rabbit plasma. The best formulation is chosen for *in vivo* analysis after successful *in vitro* studies.

2. Experimental

2.1 Chemicals and Reagents

Diltiazem HCl was obtained from Highnoon laboratories Lahore, Citrated tubes and reagents such as acetonitrile (ACN), double distilled water and HPLC grade methanol were bought from Sigma Aldrich.

2.2 HPLC Method Development

A specific, robust and reproducible reverse phase liquid chromatographic (RP-HPLC) mode has been establisheded using plasma obtained from rabbit treated with Diltiazem hydrochloride (DTZ). Standard chromatographic treatment was adopted employing TC- RP column (2) with an Agilent 5 with dimensions of 250 mm x 4.6 mm and detection through UV identification at 237 nm and optimized flow rate of 1 mL min^{-1}. A mixture of water and methanol in ratio of 90:10, v/v was utilized as mobile phase. The retention time R_t of 4.2 min was observed for DTZ. By using disperser solvent ACN, drug extraction from spiked rabbit plasma was performed for protein precipitation. The obtained supernatant was treated an extraction solvent (100mL of dichloromethane) followed by centrifugation. The extracted phase with drug was separated and dried by evaporating solvent. The collected drug leftover was dropped in 50 µL of mobile phase and characterized via HPLC analysis.

2.3 *In vivo* Studies

In vivo studies were investigated with a set of dozen control male albino rabbits with weigh around 2 ± 0.3 kg with consent of the Ethical Committee of BZU University, Multan (Punjab) Pakistan in association with The Women University Multan. The optimized formulation was chosen for *in vivo* analysis on behalf of results obtained from *in vitro* analysis. The rabbits were not given any food 12 hrs before drug administration with continued starvation for twenty-four hours post-dosing. Drinking water was provided to rabbits during the whole drug administration period.

The dose of standard amount of drug, Diltiazem hydrochloride and synthesized microgels equal to 10mg/kg was combined with deionized water by carrying stomach tube into stomach. Blood plasma (2 mL) were collected from the vein of ear at series of time segments starting from zero time (before dosing) and then after operation of a standard dose of the drug and synthesized microgels respectively at 0.5, 1, 2, 4, 6, 8, 10, 12 and 24 hrs. Whole blood trials were stored in citrated tubing with EDTA disodium salt already present as an anticoagulant followed by centrifugation at 5000 rpm for 10 min. The plasma samples were collected, frozen and stored at −20 °C prior to analysis.

Protein precipitation was done by using 1320 µL ACN to 660 µL of plasma samples, vortexed for 1min and centrifugation at 8000 rpm for 5min. A colorless supernatant (1800 µL) was collected and further added to 100 µL dichloromethane as selected extraction solvent. The solvent was evaporated in an oven at 35ºC and resultant drug residual was again taken in 50 µL of mobile phase, methanol: water (90:10: v/v). 20 µL of prepared material was introduced for HPLC scrutiny. Scientific application package Kinetica®

version 4.1.1 (Thermo Electron Corporation) was used to investigate pharmacokinetic parameters: maximum plasma concentration (Cmax), time to attain the highest plasma concentration (Tmax), area beneath the plasma concentration-time bend ($AUC_{0-\infty}$), elimination rate constant (K21) and half-life ($t_{1/2\beta}$).

3. Results and Discussion

3.1 *In vitro* Studies

Previously different pH dependent copolymeric 2-ethyl hexyl acrylate-co-itaconic acid p(EHA-co-IA) microgels were synthesized through optimized suspension polymerization. 2-ethyl hexyl acrylate and itaconic acid were polymerized in an aqueous phase with benzoyl peroxide (BPO) as an initiator and ethylene glycol dimethacrylate (EGDMA) as a cross-linker. The fabricated microgels were successfully loaded with selected an antihypertensive drug diltiazem HCl (DLZ) through equilibrium swelling method. The chemical stability of drug loaded on microgels was also investigated. Thermogravimetric analysis represented that loaded microgels reveal much improved thermal stability than standard drug DLZ. The spherical surface morphology of fabricated microgels is shown by SEM micrographs. The particle size distribution for microgels is calculated approximately 4.145 to 20 µm by Malvern nanosizer ZS instrument. About 96 % highest percentage drug loading was observed with yield near to 76 %. *In vitro* analysis showed the sustainable release of DLZ and its pH dependence under applied phosphate buffer at pH 1.2, 5.5, and 7.4.

The calibration curve of DLZ demonstrated good linearity was calculated R2= 0.9999. The intra- and inter-days accuracy and precision of 25, 50, 100 ng/mL showed % relative standard deviation within range and outstanding percentage recovery. The limit of Quantification (LOQ) and limit of detection (LOD) values were 4.02ng mL^{-1} and 1.325ng mL^{-1}respectively.[11] As *in vitro* studies offered major improvement in drug plasma concentration, *in vivo* analysis will further help to estimate clinical significance of the developed optimized HPLC methodology for the examination of DLZ. Therefore, the developed HLC method is employed to perform pharmacokinetic studies using rabbit plasma.

3.2 *In vivo* Studies

The bioavailability of standard solution of diltiazem HCl and sustained release of drug loaded on pH sensitive microgels is investigated in rabbits to measure controlled release of drug through microgels with continuous maintenance of blood plasma level for about extended time interval. For the standard DLZ solution, peak plasma concentration is swift but reduced life time of drug affects its bioavailability adversely. By applying synthesized HPLC method, pharmacokinetic parameters (Mean ± SE) are studied and provided in Table 1.

To evaluate sustained release of drug loaded on microgels, the mean plasma concentration demonstrated T_{max} value of for 4.0758±0.22 hrs in as comparison to the T_{max} (1.8918±0.034 hrs) of reference standard DLZ solution. The half-life $t_{1/2\beta}$ of drug loaded microgels (5.68±5.86 hrs) is improved and extended as compared to standard DLZ solution ($t_{1/2\beta}$ = 1.48±0.28 hrs). The improved T_{max} and half-life $t_{1/2\beta}$ for microgels based formulations represent increased absorption phase and prolong presence of drug in the body. This effectively increased the bioavailability of the drug. The C_{max} for synthesized microgels is observed around 41.06±2.02ng/mL whereas C_{max} of standard DLZ solution (54.31±0.79 ng/mL) respectively. This ensured the controlled release of DLZ from fabricated microgels. $AUC_{0-\infty}$ is observed to have higher values for loaded microgels as compared to standard drug suspension. Elimination rate constant, K_{21} for drug loaded microgels is 0.18 ± 0.07 per hour and 0.48±0.06 per hour for the standard DLZ solution.

Table 1. Representation of pharmacokinetic parameters investigated as Mean ± SD for standard drug DLZ and drug loaded pH sensitive microgels.

Pharmacokinetic parameters	Standard Drug Solution (Mean ± SD)	pH sensitive Drug loaded Microgels (Mean ± SD)
t 1/2 β (h)	1.48±0.28	5.68±5.86
Tmax (h)	1.8918±0.034	4.0758±0.22
Cmax (ng/mL)	54.31±0.79	41.06±2.02
K 21(1/h)	0.48±0.06	0.18±0.07
AUC 0-∞ (h.ng/mL)	279.10±7.635	460.35±39.99

Figure 1 represented the mean plasma concentration plotted against time profile for orally administered standard drug solution and controlled release via drug loaded microgels. The profile shows a stable increase in bioavailability of DLZ drug over time interval of 24 hours for DLZ loaded microgels while the availability of standard drug suspension is reduced after 4 hours. The enhanced bioavailability showed the therapeutic effect of a DLZ loaded microgels for a precise dosage/formulation.

Table 2. Administration of rabbit plasma concentration given in ng/mL (Mean ± SD) for standard drug Diltiazem HCl (10 mg kg^{-1}) as orally and DLZ loaded microgels.

Time Intervals (hrs)	**Diltiazem HCl (10 mg kg^{-1}) (Mean ± SD)**	**DLZ Loaded Microgels $ngmL^{-1}$ (Mean ± SD)**
0	0±0	0±0
0.5	21.8±3.54	9.73±1.104
1	40.5±2.07	16.18±1.66
2	60.25±1.55	27±1.41
3	53.08±4.36	39.64±1.689
4	43.92±3.87	48.27±3.88
5	18.08±0.67	47±4.38
6	11.83±1.27	36.27±4.58
8	7.42±0.90	29.18±4.73
10	3.25±0.97	22.09±4.83
12	0.58±0.90	12.55±3.80
18	0	6.82±2.09
24	0	2.18±1.17

The performed kinetic parameters depict the significance of pH sensitive DLZ loaded microgels *in vivo* following exposure to several routes for example the level of distribution within the animal body, the amount of drug available for action and removal, role of certain organs in removal and the rate of elimination. This generated data can be useful to develop therapeutic effects of DLZ loaded microgels and the duration of exposure.

3.3 Efficacy of Developed Microgels

Expanded controlled release of DLZ drug from pH sensitive microgels enhanced the therapeutic efficacy and generated a more consistent plasma drug profile in comparison compared to diltiazem oral tablets. The developed formulation demonstrated significant drug loading and bioavailability due to strong inter-molecular and hydrophilic binding between the microgel particles and selected drug. The pH sensitive microgels allowed a slow and more controlled release of the drug over time interval of 24 hrs. These results showed that DLZ loaded microgels could be adapted as treatment of several cardiac associated diseases as arrhythmia, angina etc. Initially the time profile showed drug release due to rapid dissolution from the surface of the microgels measured as the loading dose. Further steady drug release for a period of 24 hrs can be measured as the maintenance dose. The kinetics showed that the drug release from the formulation followed a matrix diffusion process. The microgels particle size, its distribution,

surface morphology, drug content loaded, *in vitro* release and kinetics added towards the *in vivo* investigation of derived formulation. The results of *in vivo* analysis of novel pH dependent microgels proved their safe and effective mode as controlled drug delivery system for certain drugs with low bioavailability and are affected by acidic pH.

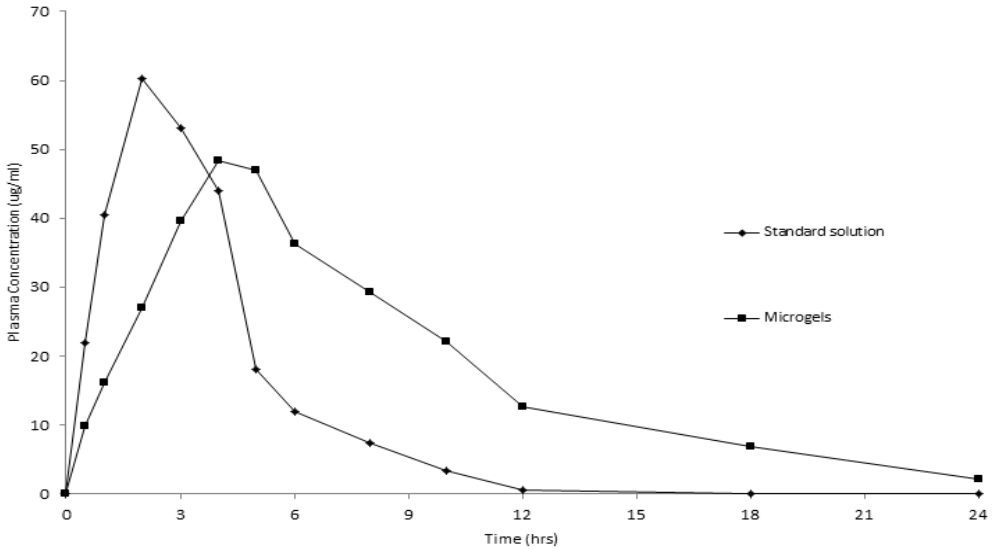

Figure 1. Profile for plasma concentration plotted against time curves of standard drug solution and drug loaded microgels equivalent to 10mg kg^{-1} in healthy rabbits (n = 12 concentrations considered as mean).

4. Conclusion

The current study investigated the *in vivo* application of pH sensitive DLZ loaded microgels by employing developed HPLC method. The pharmacokinetic studies showed improved mean plasma concentration T_{max} for controlled release of drug from microgels as compared to short lived standard drug solution. The half-life $t_{1/2\beta}$ of synthesized microgels is enhanced as compared to standard drug solution. The increased marks of T_{max} and half-life $t_{1/2}$ for controlled release formulation depict that absorption period is enhanced and drug remain available for extended spell of time in the body. DLZ loaded microgels offer controlled drug release pattern. Therefore, the pH sensitive microgels can be a therapeutic alternative for improved bioavailability of drugs in the body with superior efficacy and clinical significance.

Competing Interests

The authors declare that they have no competing interests.

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