Polyelectrolyte multilayer assembly bearing ketoprofen for transdermal delivery

Gupta R., Yadav P. and Saraf S.A.*
Faculty of Pharmacy, BBDNITM, Lucknow, 226016. INDIA.
Email: guptrachna78@gmail.com

Introduction

A novel microencapsulation technology based on layer-by-layer assembly has been extensively studied and used for controlled delivery of drug microcrystal having poor aqueous solubility and low bioavailability [Qiu et al. 2001]. A non-steroidal anti-inflammatory drug was selected for encapsulation using biodegradable and biocompatible polyions and synergistically the suspension was embedded in gel matrix for topical application. Topical application of the drugs at the pathological sites offer potential advantages of delivering the drug directly to the site of action and thus producing high tissue concentrations of the drug [Jain et. al. 2005].

Material and methods

Sodium aliginate (SA), chitosan (CHI) and poloxamer F-68 (PF-68) procured from Sigma, USA, Ketoprofen (KF, MW: 254.29) gifted from SPARC, India, polyacrylamide and glycerol were purchased from Hi media, all were used without further purification. The water used throughout the experiment was purified with a Milli Q system from Millipore Co., USA.

Fabrication of multilayered assembly followed by embedding in gel

One hundred milligram drug as such obtained along with PF-68 was agate in mortar and suspended in acetate buffer (pH=4.0) and separated in different fraction by sedimentation technique. The multilayer assembly on fractioned and separated drug crystal (1%w/v taken for layering) was effectuated by alternate deposition of SA and CHI (0.2% of each dissolved in acetate buffer) using LBL technique [F-1, KF $(SA/CHI)_2$] as reported by Arida and Al-Tabakha [2007] with slight modification. The resultant preparation was stored at 4 ± 1°C. The matrix dispersion (20%) was finally added into the polyacrylamide gel (prepared by using 0.5% polyacrylamide, 10% glycerol, and 69.5% water) [KF $(SA/CHI)_2$ gel, F-2] and were transferred in a beaker and stirred.

In vitro-characterization

The developed systems were characterized for various physicochemical attributes. Particle size and polydispersity index of drug microcrystal was measured by Malvern mastersizer (UK) after suitable dilution. The shape and surface morphology of multilayered assembly were visualized under phase contrast microscopy (Olympus India, attached with Yoko CCD Camera, Taiwan). Layer-by-layer growth was determined by the ζ-potential of each adsorbing layer on the drug microcrystal dispersed in acetate buffer using Zetasizer Nano ZS (Malvern Zetasizer, 3000 HS). The ζ-potential value was the average of three successive measurements. To calculate its encapsulation rate, 20 mg of multilayered KF particles was gently stirred into 100 ml ethanol and left to settle freely for 10 min. Samples from the supernatant solution were withdrawn and analyzed spectrophotometrically at 256nm (Systronic 2201, UV-visible double beam spectrophotometer, Japan). The UV-method was calibrated and complied with generally accepted specifications for linearity and precision.

In-vitro drug release studies

In vitro drug release from different formulations was determined using Franz diffusion cell in which the donor compartment contained the formulation while the receptor compartment was filled with PBS (pH 4.5). The sink condition was maintained by using 40% v/v PEG-400 in PBS in the receptor compartment, and the temperature was maintained at 37±1°C with the help of a circulating water bath. Samples (1 ml) were withdrawn at appropriate time intervals and compensated with equal quantity of PBS (pH 4.5) containing 40% v/v PEG-400. Samples were filtered through Whatmann filter paper and drug content was determined spectrophotometrically using calibration curve of KF.

Results and Discussion

Ketoprofen is metabolized by first hepatic pass effect and having short biological half-life. So on the curative front it's needed to develop for improvement of therapeutic effectiveness and reduction in the severity of NSAID by altering the dosage forms and route of administration as well. Topical application of polyelectrolyte multilayered assembly bearing ketoprofen at the pathological sites offers potentials advantages of delivering the drug directly to the site of action and thus producing high tissue concentrations of the drug. Therefore, it is aimed to develop novel formulation bearing KF for transdermal delivery which would effectively manage the pain and inflammation in osteoarthritis and rheumatoid arthritis patients. This study reports the successful liquid dispersion of cohesive sub-micronized KF crystals by electrostatic coating with a charged polyelectrolyte and the subsequent self-assembling of multiple layers of polyions around the crystals to produce controlled release multilayered formulation along with gel for easy application at the dermal site. Agitation of KF with surfactant (2% w/w) reduced the size and of extent of aggregation, the powder is still cohesive. Fractionated drug crystal (Size and PI was shown in Table 1) was alternatively coated with natural PEs. A pH of 4 was used because the low solubility of KF at this pH [Hanson G.R. 2000] ensured that the particle shapes and sizes were not altered. SA was used as the first layer because the electric potential measurements showed that the surface of KF is positively charged at this pH. Size and size distribution of bared drug crystal (2.104 µm, PI: 0.061) and coated one (2.208 µm, PI: 0.151) was measured and shown in Fig. 1 and 2. Shape and surface morphology of bared and assembled structure was depicted in Fig. 3 and 4 as observed by Phase contrast microscopy.

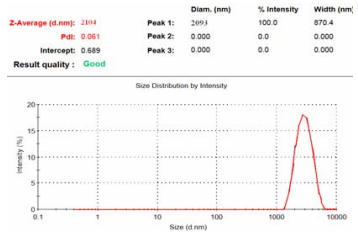

Fig. 1. Size and size distribution of bared KF selected for encapsulation.

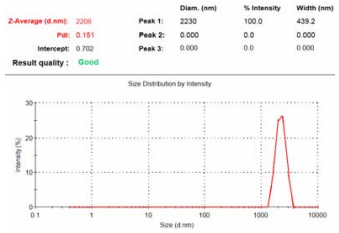

Fig. 2. Size and size distribution of polyelectrolyte encapsulated KF.

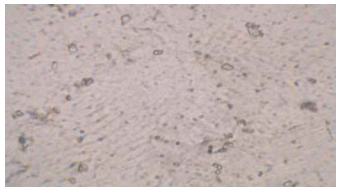

Fig. 3. Photomicrograph of bared KF at magnification (100x) showed mono-dispersity.

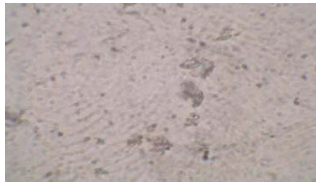

Fig. 4. Photomicrograph of encapsulated KF at magnification 100x.

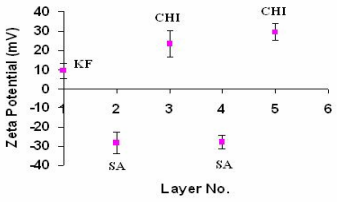

Fig. 5. Multilayer growth study by zeta potential measurement.

Fractions collected (Top to bottom)	Size (µm)	Polydispersity index
1	2.104	0.061
2	2.345	0.151
3	2.88	0.328
4	3.056	0.203
5	3.924	0.28

Table 1. Size and PI of fractions collected by centrifugation after agitation.

The effective adsorption of the PEs was evidenced by the zeta potential measurement of the reversal in charge and the enhanced dispersability of the KF particles because of the colloidal stabilization as shown in Fig 5. The shell thickness and diameter of the multilayered assembled around the microcrystal can be varied with the precision of a few nanometers by varying the combinations of PEs used in the assembling process. The shell formed around the microcrystal also preserved the integrity and the shape of the original crystals. The encapsulation efficiency of this method was very high. Typically the polymer: drug ratio of 10:1 is used in conventional polymer-based drug delivery systems. In this novel approach we studied here, that the shell thickness of the capsule come ~100 nm, which is much smaller than the core drug crystal dimensions and gives a polymer shell to drug core thickness ratio of about 1:50. In vitro drug release, determined using cellophane membrane, showed that PE encapsulated KF (F-1) exhibited higher drug release compared to gel formulations of encapsulated KF (F-2).

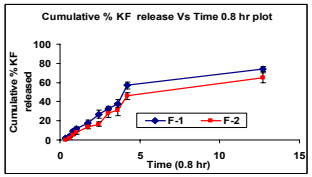

Fig. 6. In-vitro release profile of encapsulated formulations at pH=4.5.

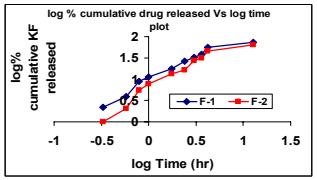

Fig. 7. Log% cumulative KF released Vs log time plot of KF released at pH=4.5.

A different release kinetic was observed for the both the formulations. Fick's law of diffusion seems not to be applicable in each case. An initial rapid drug release was noted for F-1, whereas a lag time (~15 min) was observed with F-2 formulations which, could result from the time taken by the drug to diffuse across the gel. The direct exposure of F-1 to diffusion media and quick release of drug may account for rapid initial release. Both formulations showed controlled drug release over 6 hr and an increase in release rate was observed after 24 hr, but this sustained effect was more pronounced with F-2. The log percent cumulative drug released was plotted as a function of log time (Fig. 7). The slope of the curves was determined as the values of diffusional release exponent (n). The values of diffusional release exponent (n) from the straight lines were noted to be > 0.45 for both the formulations, showed that the release of drug from formulations followed Non-Fickian pattern [Langer and Peppas, 1981]. From the percent cumulative drug released versus time$^{0.8}$ plot, the slope values were determined as release rate constants (Fig. 6). F-2 released slowly the drug as compared with F-1, accounted for by the time the drug takes to diffuse through gel. The slower release of drug from F-2 maintained the drug concentration for longer period of time. Burst releases as well as sustained release both are of interest for dermal application. Burst release can be useful to improve the penetration of drug. Sustained release supplied the drug over a prolonged period of time.In this way, it is concluded that the release of drug from formulations followed zero order kinetics. A lag time (15-30 minutes) was observed in every case but more pronounced in F-2. Because in these formulations the drug has to cross two diffusion barriers, one the gel entangled polymer matrix and the other is drug encapsulated in PEs. In case of the F-2 water evaporation is reduced because of water-binding properties of glycerol and the polymer polyacrylamide. Therefore, polymorphic transitions and successive drug expulsion should be reduced.

Conclusions

In the present investigation, we aimed to develop polyelectrolyte assembled multilayered KF mixed with gel for transdermal delivery, which effectively would manage the pain and inflammation in osteoarthritis and rheumatoid arthritis. Thus obtained data showed better effectiveness of the novel formulation. Further investigations are still underway to gather stability studies of developed formulation and for in-vivo performance by taking suitable model.

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