Journal of Pharmaceutical Analysis 2015;5(1):70–73

HOSTED BY

Contents lists available at ScienceDirect

Journal of Pharmaceutical Analysis

www.elsevier.com/locate/jpa
www.sciencedirect.com

SHORT COMMUNICATION

Capillary electrophoresis to determine entrapment efficiency of a nanostructured lipid carrier loaded with piroxicam

Jessica Otarola, Adriana Guillermina Lista, Beatriz Fernández Band, Mariano Garrido*

Analytical Chemistry Section, INQUISUR (UNS-CONICET), Av. Alem 1253, Bahía Blanca, Argentina

Received 20 February 2014; revised 16 April 2014; accepted 19 May 2014
Available online 27 May 2014

KEYWORDS

Capillary electrophoresis;
Drug delivery system;
Nanostructured lipid carrier;
Piroxicam

Abstract A simple and fast capillary electrophoresis method has been developed to determine the amount of piroxicam loaded in a drug delivery system based on nanostructured lipid carriers (NLCs). The entrapment efficiency of the nanostructured lipid carrier was estimated by measuring the concentration of drug not entrapped in a suspension of NLC. The influence of different parameters on migration times, peak symmetry, efficiency and resolution was studied; these parameters included the pH of the electrophoretic buffer solution and the applied voltage. The piroxicam peak was obtained with a satisfactory resolution. The separation was carried out using a running buffer composed of 50 mM ammonium acetate and 13.75 mM ammonia at pH 9. The optimal voltage was 20 kV and the cartridge temperature was 20 °C. The corresponding calibration curve was linear over the range of 2.7–5.4 μg/mL of NLC suspension. The reproducibility of migration time and peak area were investigated, and the obtained RSD% values ($n = 5$) were 0.99 and 2.13, respectively.

1. Introduction

A drug delivery system is defined as a formulation or device that allows the introduction of a therapeutic substance in the body and improves the efficacy and safety of this substance, controlling the speed, time and place of release of the drug in the body [1,2]. From the pharmaceutical point of view, these systems show several advantages such as stability and potential for carrying drugs of different polarity, for increasing the drug bioavailability and improving the absorption efficiency [2]. Among these delivery systems, the solid lipid nanoparticles (SLNs), which emerged in the early 1990s, are of great interest. Basically, the SLNs are defined as lipid arrays at nanometric scale, solid at physiological temperatures and stabilized by surfactants [3]. In addition, in recent years, a second generation of these lipid nanoparticles have arisen as nanostructured lipid carriers (NLCs). These carrier systems have improved the properties and

*Corresponding author. Tel.: +54 291 4595100; fax: +54 291 4595160.
E-mail address: mgarrido@uns.edu.ar (M. Garrido).

Peer review under responsibility of Xi'an Jiaotong University.

2095-1779 © 2014 Xi'an Jiaotong University. Production and hosting by Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jpha.2014.05.003

stability of the SLNs [1]. In the last years, owing to the great economic and health interest in this topic, the research in this area has significantly increased.

The development of delivery systems involves a multidisciplinary approach. Analytical chemistry provides the fundamental tools to characterize the systems, quantify the drugs loaded in the carrier systems, perform drug release profiles and study the pharmacokinetics in living organisms.

Nowadays, liquid chromatography is the principal analytical technique used to carry out the determination of the amount of drug loaded in this kind of systems. Normally, using this technique requires a previous step consisting of the centrifugation of NLC suspensions in a membrane concentrator [4–6] with the consequent increase of potential errors arising from the sample handling.

In the last years, capillary electrophoresis (CE) has been increasingly used as a separation analytical technique, especially in some fields like pharmaceutical analysis, biology and modern medicine [7–9]. This separation technique shows some advantages such as higher speed, lower cost of analysis, and lower residues generation [10]. Therefore, CE appears as an alternative technique to quantify the concentration of drugs in different delivery systems.

On the other hand, non-steroidal anti-inflammatory drugs (NSAIDs) are widely used in therapeutics because of their anti-inflammatory and analgesic properties.

However, there are several secondary effects related to the use of these drugs, with gastric irritation being the most common [11,12]. Among these drugs, piroxicam is especially interesting because of its low solubility in both organic solvents and aqueous solutions, which could be a limitation on its passage to the systemic circulation. Therefore, this active principle was selected as a target analyte in the current study. The common structural feature of NSAIDs is an acidic group, characterized by pKa values in a range of 3–6. Thus, these compounds are mainly in the anionic form at pH ≥ 7. Therefore, this kind of analytes can be determined using capillary zone electrophoresis (CZE) [13,14] with a simple electrophoretic buffer.

The aim of this study was to evaluate the capability of capillary electrophoresis, as an analytical technique, to determine the concentration of piroxicam loaded in a NLC. The nanoparticles suspension was obtained from a warm transparent microemulsion and its entrapment efficiency was estimated indirectly on the base of the amount of drug not entrapped in the NLCs (i.e., the free drug). The influence of different electrophoretic parameters was investigated. The proposed method has several advantages such as low consumption of reagents, injection of small volumes of sample and short time of analysis. The principal advantage, in comparison with the most commonly used technique (liquid chromatography) is that the sample does not need previous treatment and can be directly injected in the capillary.

2. Materials and methods

2.1. Apparatus

CE experiments were carried out with a Beckman Coulter Capillary Electrophoresis Instrument MDQ (Fullerton, USA), equipped with a diode array detector. The capillary was also from Beckman System and before its first use it was sequentially rinsed with 1.0 M HCl (10 min), ultrapure water (5 min), 1.0 M NaOH (10 min), ultrapure water (5 min), 0.1 M NaOH (5 min), ultrapure water (3 min) and running buffer (10 min). Control and data processing were carried out with 32 Karat software.

A magnetic stirrer with temperature control (Autoscience AM-5250B, Tianjin, China) was used for NLCs preparation.

2.2. Chemicals and reagents

All solutions were prepared using ultrapure water (Millipore, Bedford, MA, USA).

Ethyl oleate (Sigma Aldrich, Buenos Aires, Argentina), soya lecithin (F.A.S. Córdoba, Argentina), polysorbate 80 (Tween 80) (Sigma-Aldrich) and n-butanol (Baker, Chemical Center S.R.L., Buenos Aires, Argentina) were used for preparation of the NLCs suspension. Piroxicam was obtained from local pharmacies.

Ammonium acetate and ammonia (Merck, Buenos Aires, Argentina) were used to prepare the electrophoretic buffer solution.

2.3. Preparation of nanoparticles

The process for the elaboration of the lipid nanoparticles suspension included the following steps: preparation of the lipid phase, preparation of the aqueous phase, formation of a microemulsion and the subsequent obtaining of the solid nanoparticles. The lipid phase was achieved by merging the solid (lecithin) and liquid (ethyl oleate) lipids at 62 °C, 25% and 75% w/v, respectively. The appropriate amount of the active principle (piroxicam) was weighed and dissolved in the lipid phase at the same temperature under continuous agitation. The aqueous phase was a 7.6% (m/v) surfactant (Tween 80) solution. The microemulsion was formed by mixing the lipidic and aqueous phases at 62 °C and with subsequent addition of n-butanol under continuous agitation until obtaining a unique transparent phase. Finally, nanoparticles were formed by dispersing the hot microemulsion in cold water (at approximately 4 °C). Also, control suspensions were prepared following the same procedure but without piroxicam.

All the analyses were carried out on the fifth day from the preparation of the NLCs suspension. After this interval of time, the system was stable and the turbidity was not significant, so the spectrometric measurements were not affected.

2.4. Electrophoretic method

Electrophoretic separation was carried out with a 65 cm × 75 μm i.d. fused-silica capillary. The piroxicam peak was satisfactory obtained working with a buffer solution consisting of 50 mM ammonium acetate and 13.75 mM ammonia at pH 9. This pH value was adjusted with 1.0 M NaOH. The applied voltage was 20 kV. Injections were made in hydrodynamic mode during 5 s at 0.5 psi. The capillary was thermostatized at 20 °C.

Before each injection, the capillary was rinsed with ultrapure water (1 min), 0.1 M NaOH (2 min), and running buffer (5 min). The electropherograms were recorded at 220 nm.

3. Results and discussion

3.1. Separation conditions

The optimization of the separation conditions was performed using a control suspension to which suitable amounts of piroxicam were added (i.e., control suspension of NLCs+free piroxicam).