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SHORT COMMUNICATION

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



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## KEYWORDS

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**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.

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## 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

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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  $\text{pH} \geq 7$ . 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  $\times$  75  $\mu\text{m}$  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 thermostated 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).

A solution consisting of 50 mM ammonium acetate and 13.75 mM ammonia was selected as running buffer, taking into account the Fillet et al. publication [15]. The pH of this solution was tested over the range of 7.0–9.3. The peak of piroxicam was not appreciated using pH values between 7 and 8. At pH values around 9 the piroxicam signal was detected. Therefore, considering the separation efficiency, the symmetry of the peak shape and the stability of the baseline, the pH of buffer solution was kept at 9.0.

The effect of the applied voltage was also investigated (15–25 kV). The results indicated that higher voltage corresponded to shorter analysis time but to lower peak areas. So, 20 kV was selected as the optimum separation voltage.

### 3.2. Figures of merit

Fig. 1 shows the electropherogram of the control suspension, and the same suspension with the addition of 0.18 mg of piroxicam. The peak of free piroxicam appeared with a good resolution respect to one corresponding to the control suspension. Thus, the calibration curve was obtained by measuring the peak areas corresponding to different amounts of piroxicam added to the control suspension.

The relationship between the peak areas and the corresponding concentrations of piroxicam was linear in the range of 2.7–5.4  $\mu\text{g}/\text{mL}$ . The regression equation was  $A = 5904.5C - 10,361$ , where  $A$  is the peak area and  $C$  the concentration of piroxicam in  $\mu\text{g}/\text{mL}$  ( $R^2 = 0.997$ ). The reproducibility of migration time and peak area were investigated, and the obtained RSD% values ( $n = 5$ ) were 0.99% and 2.13%, respectively.

### 3.3. Entrapment efficiency

The study of the entrapment efficiency involved a set of four replicates of a suspension of piroxicam-loaded NLCs prepared using 8.10  $\mu\text{g}$  of piroxicam per millilitre of suspension. These suspensions were injected into the capillary and the peak areas of free piroxicam were measured. Thus, the entrapment efficiency was obtained by differences between the amount of piroxicam used to prepare the NLCs suspensions and the amount of piroxicam non-entrapped in the NLCs, which was quantified by CE. The average value of entrapment efficiency was 0.12 mg of loaded piroxicam per g of NLCs. The results were expressed as per

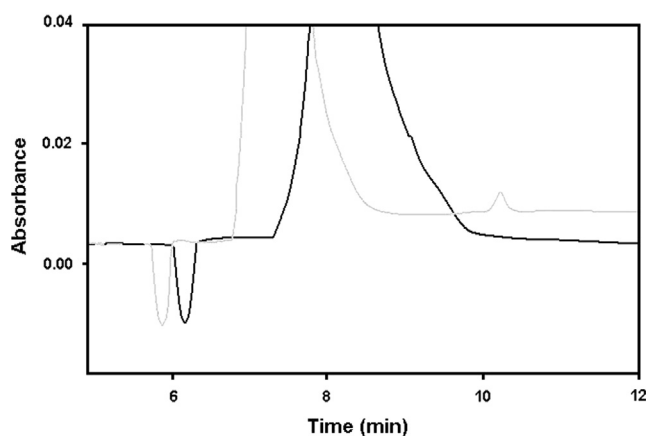


Fig. 1 Electropherograms of control solution (black line) and control solution with piroxicam (grey line).

**Table 1** Results for calculating the entrapment efficiency in different piroxicam-loaded NLCs.

Amount of piroxicam used to prepare the NLCs ( $\mu\text{g}$ ) <sup>a</sup>	Amount of free piroxicam ( $\mu\text{g}$ ) <sup>a</sup>	Difference ( $\mu\text{g}$ )	Amount of piroxicam loaded in NLCs (mg/g)
4.32	2.62	1.70	0.11
4.86	3.11	1.75	0.11
5.40	3.35	2.05	0.13
8.10	6.20	1.90	0.12
9.50	7.50	2.00	0.13
10.80	9.08	1.72	0.11
13.50	11.40	2.10	0.13
			$\bar{X} = 0.12$ ; RSD% = 8.3

<sup>a</sup>Corresponding to 1 mL of suspension.

gram of nanoparticles, taking into account that 1 mL of NLCs suspension corresponds to 15.8 mg of nanoparticles. The RSD% was 1.08 ( $n = 3$ ).

In order to investigate the performance of the proposed method, a set of different suspensions with increasing amounts of drug were prepared. Table 1 shows the obtained results.

In all cases the results were similar, even though the amounts of piroxicam used to prepare the NLCs were quite different. This fact would indicate that the proposed method provided reliable results.

## 4. Conclusion

A fast, effective and reliable CE method to determine the entrapment efficiency of an NLCs-based delivery system for piroxicam was developed. The proposed method used a simple electrophoretic buffer solution with a short time of analysis (about 10 min). It is important to note that the suspensions were injected into the capillary without any pre-treatment. Similar methods could be developed to determine the entrapment efficiency of others carriers system, based on the proposed method. Also, with the corresponding optimized electrophoretic conditions, other drugs could be analyzed.

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