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Use of cyclodextrins in the capillary electrophoretic separation of *erythro*- and *threo*-mefloquine enantiomers

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Abstract

Capillary zone electrophoresis has been used for the enantiomer separation of mefloquine diastereoisomers and enantiomers using a background electrolyte at acidic pH supplemented with β -cyclodextrin derivatives as chiral selectors. The cyclodextrin type and concentration strongly influenced the chiral resolution and among the cyclodextrins used (β -cyclodextrin, dimethylated-, trimethylated- and carboxymethylated- β -cyclodextrin), 2,6,-di-O-methyl- β -cyclodextrin allowed to obtain very good resolution for all the studied optical isomers even at very low concentration. The optimized electrophoretic method resulted to be very reproducible for both migration time and peak areas (STD%1%) with a good detection limit (1·10⁻⁶ M gave a signal to noise ratio=3 for each enantiomer). The analysis of a pharmaceutical preparation did not reveal the presence of the *threo* isomers but only the racemic *erythro*-mefloquine. Method recovery values, performed on a pharmaceutical preparation, were found in the range 99–102%.

Keywords: Enantiomer separation; Diastereomer separation; Buffer composition; Pharmaceutical analysis; Mefloquine; Cyclodextrins

1. Introduction

Capillary electrophoresis (CE) has been shown to be a powerful tool for the separation of enantiomers mainly in the field of pharmaceutical analysis where high-resolution analytical methods are requested for, e.g., chiral purity control of drugs, pharmacokinetic studies etc.

In CE enantioselective separations are performed using a chiral environment interacting with the two enantiomers either before or during the electrophoretic run (indirect and direct separation method) forming diastereoisomers that can be separated due to their differences in physico-chemical properties [1].

In the direct separation method the chiral selector is simply added to the background electrolyte (BGE) or bound to the capillary wall as well as included/bound to polyacrylamide gel [2–5]. Different separation mechanisms have been used including ligand exchange [6,7], complexation with chiral micelles [8–10], with crown-ether derivative [11,12], interaction with protein [13–15] or antibiotics [16] or cyclodextrins [1,5,17–22].

Cyclodextrins (CDs) are chiral oligosaccharides, widely used in CE for enantiomer separations, with a shape similar to a truncated cone; their cavity is relatively hydrophobic while the exterior is hydrophillic due to the presence of hydroxyl groups responsible for stereoselective bonds with analytes. Due to the easy chemical reaction of the hydroxyl groups, a wide number of CD derivatives are commercially available with different properties in com-

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parison to the native α -, β - and γ -CD, e.g., solubility, depth, charge etc. [23].

For the optimization of the enantiomer separation method, the appropriate CD have to be selected in accord to the published data and the own experience. In this study, capillary electrophoresis was used for the direct chiral separation of optical isomers of mefloquine (MF) employing β -cyclodextrin or some of its derivatives, namely heptakis-2,6-di-O-methyl- β -cyclodextrin, heptakis-2,3,6-tri-O-methyl- β -cyclodextrin and carboxy methylated- β -cyclodextrin.

Mefloquine is a relatively new antimalarial agent against drug-resistant strains of *Plasmodium falciparum* [24]. In its chemical structure two asymmetric centres at position 11 and 2' (see Fig. 1) are responsible for the existence of four optical isomers, namely (11R,2'S)-, (11S,2'R)-erythro-mefloquine (EMF) and (11S,2'S)-, (11R,2'R)-threo-mefloquine (TMF). The drug is usually administered as racemic *erythro*-form. In a recent pharmacokinetic study by

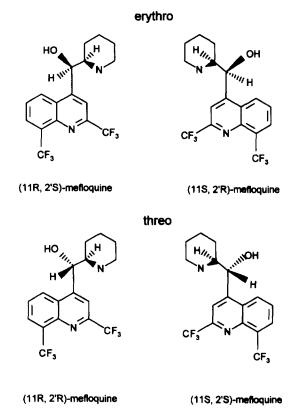


Fig. 1. Chemical structure of mefloquine optical isomers.

high-performance liquid chromatography (HPLC) [25] it has been shown that in a healthy male human patients the plasma concentration of (SR)-erythromefloquine was 2–3 times higher than that of its RS-enantiomer.

2. Experimental

2.1. Apparatus

Capillary electrophoresis experiments were carried out with HP3D Electrophoresis System (Hewlett-Packard, Waldbronn, Germany) equipped with a diode-array detector (190–600 nm); the signal was recorded at 223 nm. The data were collected on the HP ChemStation.

A fused-silica capillary tube 48.5(39.5 effective length) cm \times 50 μ m I.D. (Hewlett-Packard) was used for the electrophoretic runs. Injection was done by pressure at 20 mbar \times 10 s at the anodic end of the capillary. For fast enantiomers separations the polarity was reversed and the chiral selector rinsed only part of the capillary; the shortest part of the capillary was used for the run and injection or chiral selector rinsing was done by vacuum.

A constant voltage of 30 kV was applied and the capillary temperature was kept at 25°C. The new fused-silica capillaries were conditioned for 30 min with 1 M sodium hydroxide and then flushed with water. The following rinsing procedure was used before each run: (1); water for 1 min (2); 0.1 M sodium hydroxide for 1 min (3); water for 1 min and (4); background electrolyte for 1 min.

2.2. Chemicals

Phosphoric acid (85%, w/w) and sodium hydroxide were purchased from Carlo Erba, Milan, Italy. β -Cyclodextrin (β -CD), Heptakis-2,6-di-O-methyl- β -cyclodextrin (di-OMe- β -CD), heptakis-2,3,6-tri-O-methyl- β -cyclodextrin (tri-OMe- β -CD) and carboxymethyl- β -cyclodextrin (CM- β -CD) were from Cyclolab, Budapest, Hungary. *erythro*- and *threo*-mefloquine racemic compounds were supplied by Hoffmann-La Roche, Basle, Switzerland. Tablets declared to contain 274.09 mg of mefloquine·HCl, Lariam were purchased from the market. Double

distilled water was used for the preparation of all solutions and every solution was filtered using 0.45 μ m Millipore filters (Molsheim, France).

Background electrolyte (BGE) was 100 mM of phosphoric acid titrated at pH 2.5 with concentrated sodium hydroxide; the appropriate amounts of CD were daily dissolved in the BGE.

Three tablets of the drug were ground and 74.65 mg (containing 41.477 mg of *erythro*-mefloquine· HCl) were dissolved in water, stirred at room temperature for 30 min, the final volume was 100 ml. The prepared sample solution was diluted 5 times and injected for analysis. The recovery was verified by spiking the sample (74.65 mg) with standard racemic mefloquine·HCl (20.74 or 41.48 mg). The two spiked solutions were prepared with the same procedure used for the sample and injected for the electrophoresis.

3. Results and discussion

Based on our experience a phosphate buffer at pH 2.5 was selected for the electrophoretic experiments, the mefloquine isomers moved as positively charged compounds with a relatively high mobility and thus in a relatively short time. Using the phosphate buffer in absence of cyclodextrin we could be able to only partly separate the *erythro* and *threo* isomers. These results confirm our previous findings [26,27] that two diastereoisomers can be separated in CE in absence of a chiral selector because the optical isomers possess different physico-chemical properties.

Considering the chemical structures of the studied compounds (see Fig. 1) and based on our experience, β -CD was selected and added to the BGE in the concentration range of 1–16 mM and the experiments performed injecting separately the two standard racemic mixtures. No enantiomers resolution was achieved for *threo*-mefloquine while the two enantiomers of *erythro*-mefloquine were resolved (R=0.6) at 16 mM of β -CD. Clearly, at the operating experimental conditions, the enantiomers resolution of at least one of the studied diastereoisomers was strongly influenced by the concentration of the chiral selector, however we could not further increase its concentration because the solubility is relatively low (1.85%, w/w). Therefore modified

 β -CDs were selected as buffer additives for the study of enantiomers -separation including dimethylated- β -CD, trimethylated- β -CD and carboxymethylated- β -CD.

The addition of tri-OMe- β -CD to the BGE at pH 2.5 in the concentration range 1–50 mM caused an increase of migration time of both *erythro*- and *threo*-mefloquine probably due to both increase of the BGE's viscosity and complexation. The two diastereoisomers were not separated in their enantiomers.

Recently it has been shown that mefloquine can be easily separated in its enantiomers by CE using sulfobutylated- β -CD [28]. In this case the modified CD is negatively charged even at pH 2.5 and was moving in the opposite direction of analytes improving the enantiomers separation.

For the enantiomers resolution of TMF and EMF we used CM- β -CD, a modified β -CD with carboxylic groups in its structure, that can be negatively charged at pH \geq 4-5 [29]. However in this study, being the pH of the BGE 2.5, the chiral selector behaves as a quasi stationary phase where the stereoselectivity can be ascribed to hydrophobic interaction with the CD cavity and hydrogen bonds between the hydroxyl or carboxyl groups on the CD rim and the substituent groups on the asymmetric center of the analytes.

The use of CM- β -CD in the concentration range (1-10 mM) allowed to achieve enantiomers resolution for only EMF and the resolution increased by increasing the concentration of the chiral selector (see Fig. 2).

The addition of 2.5 mM of di-OMe- β -CD to the BGE allowed to obtain good enantiomers resolution for both diastereoisomers in less than 6 min as can be seen in Fig. 3. The migration order of the *threo*-and *erythro*-mefloquine enantiomers was not verified due to the difficulty to get the separated analytes.

The effect of di-OMe- β -CD concentration was studied in the range 2.5–10 mM in order to find the optimum experimental conditions for the quantitation of *erythro*-mefloquine and its impurity (*threo*) in pharmaceutical preparations.

Table 1 shows the effect of di-OMe- β -CD added to the phosphate buffer at pH 2.5 on migration time and resolution of *threo*- and *erythro*-mefloquine.

Increasing the CD concentration a general increase

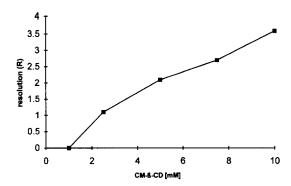


Fig. 2. Effect of the concentration of carboxymethylated- β -cyclodextrin (CM- β -CD) added to the background electrolyte on the resolution (R) of *erythro*-mefloquine enantiomers. For experimental conditions see Table 1.

of migration time for all the studied compounds was recorded. The resolution increased by increasing the concentration of CD for *threo* while for the *erythro* stereoisomers a maximum of resolution was recorded at 5 mM of CD, however very high resolution was achieved at 7.5 and 10 mM of chiral selector.

3.1. Fast enantiomers separation

Recently it has been shown that fast enantiomers separations can be performed by CE; Aumatell and Guttman reported the enantiomers separation of metaproterenol and isoproterenol in less than 40 s [30].

The separation of the four optical isomers of mefloquine was performed reversing the polarity and injecting the sample at the end of the capillary

Table 1 Effect of the concentration of dimethylated- β -cyclodextrin on the migration time and resolution (R) of threo- and erythro-mefloquine

CD (mM)	Threo			Erythro		
	$\overline{t_1}$	t ₂	R	$\overline{t_1}$	t_2	R
2.5	5.16	5.28	1.6	5.44	5.99	6.5
5.0	5.41	5.58	2.5	5.77	6.41	7.9
7.5	5.87	6.08	2.8	6.27	6.96	7.6
10	6.30	6.52	2.8	6.74	7.43	7.3

Capillary, 48.5 (39.5 effective length) cm \times 50 μ m I.D.; background electrolyte 100 mM phosphate buffer pH 2.5; applied voltage 30 kV; injection by pressure 20 mbar 10 s of racemic *threo*- and *erythro*-mefloquine (0.01 and 0.02 mg/ml, respectively); temperature at 25°C. t (min).

closest to the detector. The capillary was rinsed with the BGE in absence of cyclodextrin and then the CD-buffer at 50 mbar for 10–120 s filling only part of the capillary. Resolution and migration time were strongly influenced by the zone length of cyclodextrin and increased by increasing the chiral filling time; the optimum experimental conditions (base line resolution of both optical isomers) were found to be at 90 s.

Fig. 4 shows the enantiomers separation of *threo*-and *erythro*-mefloquine obtained in less than 110 s.

3.2. Reproducibility

When the fast separation method was used we obtained a very good reproducibility values for

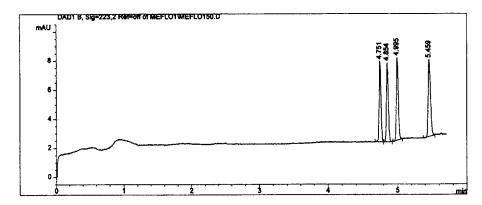


Fig. 3. Electropherogram of the enantiomers separation of the four optical isomers of mefloquine. The peaks at 4.751 and 4.854 represents the *threo*-mefloquine while at 4.995 and 5.459 the *erythro*-isomer. Experimental conditions as in Table 2.

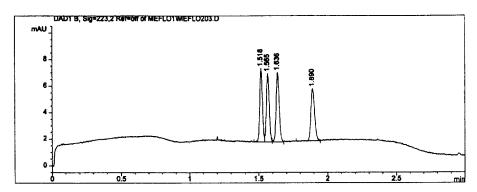


Fig. 4. Electropherogram of the fast enantiomers separation of threo- and erythro-mefloquine. The background electrolyte, 0.1~M phosphate buffer and 2.5~mM di-OMe- β -CD rinsed the capillary at 50 mbar for 90 s; injection at 20 mbar for 5 s. Threo-mefloquine (1.518, 1.565 min); erythro-mefloquine (1.636, 1.890 min). The distance injection end of the capillary-detector was 9 cm. For other experimental conditions as in Table 2.

migration time of the four optical isomers (STD 0.6–1%) while for peak area was not satisfactory 20–24%. The very poor reproducibility for the peak areas is not clear and will be further investigated. For the method optimization we turned back to the preliminary experiments, normal polarity and 39.5 cm effective length of the capillary A mixture containing racemic *erythro*- and *threo*-mefloquine was injected ten times for the electrophoretic separations using the phosphate buffer supplemented with 2.5 mM of di-OMe- β -CD in order to verify the reproducibility of the method for either migration time and corrected peak areas and resolution. As can be seen in Table 2 the method resulted to be very reproducible.

The sensitivity of the electrophoretic method was tested analyzing mixture of the four enantiomers at different concentrations and the detection limit found (signal to noise ratio 3:1) was $1.2 \cdot 10^{-6}$ M for each enantiomer.

The linearity was studied injecting ten racemic Table 2 Reproducibility (STD%) of migration time (t), area and resolution (R) for the enantiomers separation of *threo*- and *erythro*-mefloquine (n=10 measurements)

Analyte	t_1	<i>t</i> ₂	area,	area ₂	R
Threo	0.70	0.71	1.19	0.76	0.72
Erythro	0.73	0.78	0.77	1.69	0.60

Experimental conditions as in Table 1 except for the CD concentration (2.5 mM) and sample concentration that was 0.02 mg/ml for each diastereomer.

mixtures containing threo- and erythro-mefloquine at different concentrations (2–180 μM and 25–250 μM , respectively). The calibrations curves resulted to be linear in the studied concentrations ranges with a correlation coefficient of 0.9992 for both enantiomers of erythro- and threo-mefloquine.

Considering the good results obtained for reproducibility, linearity and detection limit we applied the optimized method to the analysis of mefloquine enantiomers in a commercial pharmaceutical preparation declared to contain mefloquine.

The analysis was performed injecting the extracted sample and revealed only the presence of the two enantiomers of *erythro*-mefloquine and the found amounts were in accord to the declared ones.

As an example Fig. 5 shows the electropherogram of the enantiomers separation of a tablet's extract with 100 mM phosphate buffer at pH 2.5. The presence of only *erythro* mefloquine was confirmed spiking the sample with standard compound.

Recovery of the method was performed spiking the sample with standard racemic *erythro*-mefloquine and was found to be for the lowest level 102 and 98% while for the highest level 99 and 98% (first and second peak, respectively).

4. Conclusions

Capillary zone electrophoresis is a valuable separation technique for the analysis of optical isomers of

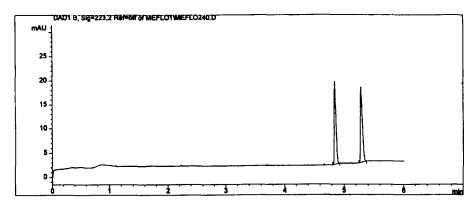


Fig. 5. Electropherogram of the enantiomers separation of tablet's extract containing mefloquine. For the experimental conditions see text and Table 1.

mefloquine. The *erythro*- and *threo*-enantiomers can be easily separated from each other in less than 6 min modifying the background electrolyte at pH 2.5 with 2,6-di-O-methyl- β -cyclodextrin. The effect of the concentration of the modified CD on resolution was studied; rising the CD concentration caused an increase of resolution with a maximum at 5 mM of chiral selector. A fast separation of the four optical isomers (less than 110 s) was achieved using the shortest effective length of the capillary (9 cm), reversing the polarity and the injection, filling only part of the capillary with the chiral selector.

Among the other cyclodextrins used (β -CD, tri-OMe- β -CD and CM- β -CD), CM- β -CD resulted to be a good stereoselective agent for only *erythro*-mefloquine ($R_{\text{max}} = 3.8$ at 10 mM of CD).

Reproducibility, linearity and detection limit were verified using 2.5 mM of di-OMe- β -CD as a chiral modifier of the BGE at pH 2.5 and resulted to be very good. The optimized method was applied for the assay of *erythro*-mefloquine enantiomers in a pharmaceutical preparation.

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