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Journal of Pharmaceutical and Biomedical Analysis
34 (2004) 861–870
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Investigating the potential of erythromycin and derivatives as chiral selector in capillary electrophoresis

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Received 16 October 2003; received in revised form 17 November 2003; accepted 18 November 2003

Abstract

Macrocyclic antibiotics are present in an increasing number of chiral separation applications. In this study, erythromycin and some derivatives, belonging to the group of macrolide antibiotics, were investigated for their potential as chiral selector. Erythromycin A itself and five related substances namely erythromycin A N-oxide, anhydroerythromycin A, anhydroerythromycin A N-oxide, erythralosamine and erythralosamine N-oxide were included. Twenty-one chiral compounds with a wide difference in physico-chemical properties were used to test the chiral activity of the erythromycins. The chiral compounds were analysed using capillary zone electrophoresis with the erythromycins dissolved in the running buffer at different concentrations ranging from 0.1 to 10 mM, with three different BGEs: sodium phosphate pHs 3.0 and 7.0 and sodium borate pH 9.2. The erythromycins showed more interactions with the acidic compounds (as observed with leucovorin, dopa, carbidopa, ketoprofen, indoprofen and warfarin) than with the neutral or weakly basic ones, especially in acidic medium. Unfortunately, no chiral separations were obtained for any of the 21 racemic mixtures. The complexation constants were calculated for the compounds showing interaction, based on the variation of electrophoretic mobility of the compounds in the presence of different concentrations of erythromycins. Finally, the size of erythromycin A was calculated using computational modelling. It was shown that the aglycone ring is only half as big as the β-cyclodextrin cavity, giving a possible explanation for the negative response of erythromycin in this study.

Keywords: Capillary electrophoresis; Chiral; Derivatives; Erythromycins; Selector

1. Introduction

In recent years, an increasing number of studies on chiral compounds have been observed, including studies dealing with the separation of enantiomers. Many new chiral selectors have been found so far, offering a better choice of selectors for all types of pharmaceutical compounds. Although cyclodextrins and their derivatives are present in the majority of chiral

Abbreviations: AEA, anhydroerythromycin A; AEANO, anhydroerythromycin A N-oxide; EA, erythromycin A; EANO, erythromycin A N-oxide; E, erythralosamine; EO, erythralosamine N-oxide

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doi:10.1016/j.jpba.2003.11.004

applications [1,2], the extensive use of macrocyclic antibiotics, in capillary electrophoresis in particular, can be observed more and more [2]. Thanks to the macrocyclic structure and the diversity in chemical groups, they exhibit a variety of interactions (inclusion, electrostatic, hydrogen-bond, hydrophilic-lipophilic, or other Van-der-Waals bond type) which enable them to achieve high chiral resolution with a wider range of analytes (acidic or basic, with large or small molecular sizes, etc.) [2]. A typical macrocyclic antibiotic selector is vancomycin. A major drawback of vancomycin, however, is its strong UV absorbance, which limits the detection of substrates when this selector is used.

The number of macrocyclic antibiotics being used as chiral selectors, as counted from 1994 with the introduction of vancomycin [2,3], has now exceeded the number of 10 [2] including four main groups: glycopeptides, polypeptides, ansamycins and aminoglycosides (although this group is not always considered as macrocyclic). However, there has not been much literature on the study of macrolide antibiotics as chiral selector, despite the fact that most of the macrolides consist of a macrocyclic lactone ring containing 14, 15 or 16 atoms, a structure which is rather promising for the formation of inclusion interactions. The use of erythromycin as impregnated reagent for the chiral separation of dansyl-amino acids by TLC was reported [4]. During the course of our work, a paper was published on the use of erythromycin as chiral selector [5]. The optimum conditions of this paper were applied to all chiral compounds used in our investigation, but no chiral separations were found. In our hands, the current was unstable, and dropped quite often.

In this study, erythromycin A and five other related compounds (see structures in Fig. 1) were used as an additive in capillary electrophoresis for a screening of their activity as chiral selector.

Erythromycin is a complex 14-membered macrolide antibiotic produced by the actinomycete, *Streptomyces erythreus* [6]. Erythromycin A (EA) is the main component. With the lack of aromatic rings in the structure (see Fig. 1), EA exhibits very weak UV absorption. This property, that has taken a lot of time and effort of researchers in improving analytical method sensitivity, in this case turns out to be an advantage.

Due to its instability in acidic condition, erythromycin is not suitable for oral administration in the sense that its decomposition results in low availability, unpredictable serum and tissue concentrations, and also several gastrointestinal adverse effects [6].

Two main acid degradation products of EA, anhydroerythromycin A and erythralosamine (structure in

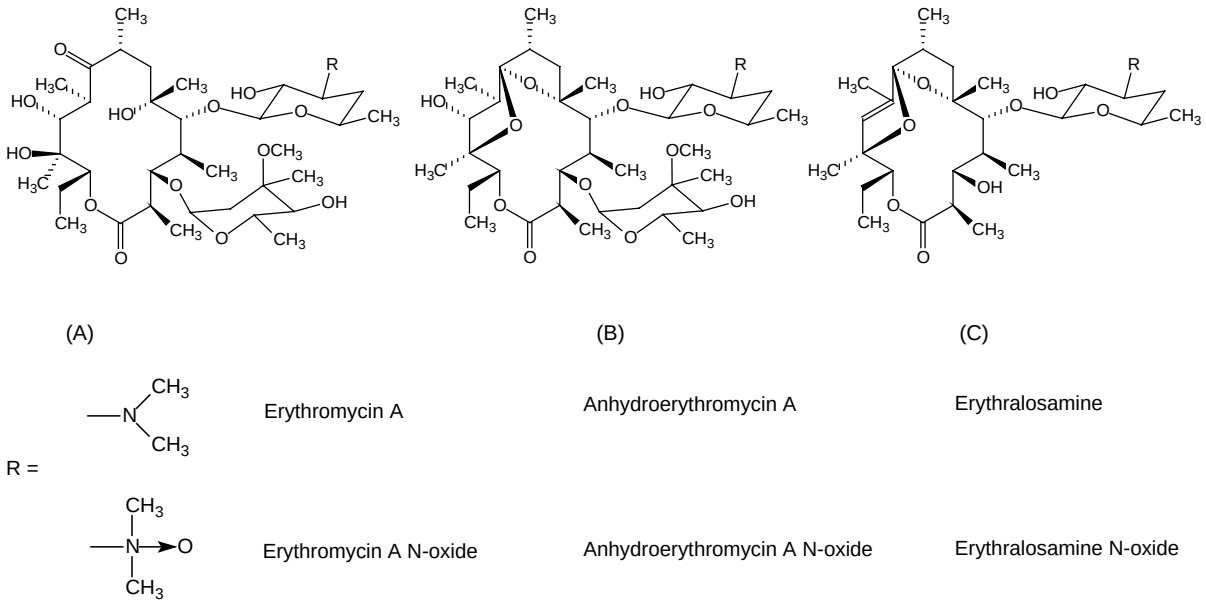

Fig. 1. Chemical structures of erythromycin A and related substances.

Fig. 1), which are much more stable than erythromycin A itself, are included in the investigation. With one dimethyl amino group in the desosamine sugar, with a pK_a of 8.8 [7], the three above compounds have a possibility of being positively charged in acidic medium while their N-oxide derivatives (the three remaining compounds investigated) are supposed to be neutral at any pH.

Twenty-one chiral compounds with different pK_a were chosen so that at each condition, different charge states exist. The compounds (in their racemic mixture) were analysed by capillary zone electrophoresis with the selectors dissolved in the running buffer at different concentrations. The influence of the selectors was followed by the change of mobility of the analytes.

2. Materials and methods

2.1. Chemicals

Erythromycin A (EA), erythromycin A N-oxide (EANO), anhydroerythromycin A (AEA), anhydroerythromycin A N-oxide (AEANO), erythralosamine (E), erythralosamine N-oxide (EO) were prepared as described before [8]. These substances are further called "erythromycins". Ten chiral compounds namely indoprofen, econazole nitrate, miconazole nitrate, sulconazole nitrate, metoprolol tartrate, oxprenolol hydrochloride, pindolol, fenfluramine hydrochloride, bupivacaine hydrochloride and isoprenaline hydrochloride were kindly provided by Profs. D. Massart and Y. Vander Heyden, Laboratory of Pharmaceutical and Biomedical Analysis, Free University of Brussels whereas eight other chiral compounds leucovorin, betaxolol hydrochloride, propranolol hydrochloride, ketoprofen, chlorpheniramine maleate, propiomazine maleate, verapamil hydrochloride and warfarin were a gift from Prof. J. Crommen, Department of Analytical Pharmaceutical Chemistry, University of Liège. Dopa and carbidopa were originated from Fluka Chemie (Buchs, Switzerland) and bamethane sulphate was from Federa (Brussels, Belgium). Mesityl oxide, sodium dihydrogen phosphate and sodium tetraborate decahydrate were purchased from Acros Organics (Geel, Belgium). Disodium hydrogen phosphate was from Federa (Brussels, Belgium). Sodium hydroxide and *ortho*-phosphoric acid 85% p.a. were obtained from Riedel-de Haën (Seelze, Germany) and hydrochloric acid 37% and methanol were purchased from Fisher Chemicals (Leicestershire, UK). All aqueous solutions were prepared with water purified by a Milli-Q 50 ultra purification system (Millipore, Bedford, MA, USA).

2.2. Equipment

Uncoated fused-silica capillary was purchased from Polymicro Technologies (Phoenix, AZ, USA). The pH of the solutions was adjusted with a Metrohm 691 pH meter (Metrohm, Herisau, Switzerland). CE was performed on an Agilent CE system G1600A (Agilent Technologies, Waldbronn, Germany) equipped with a PDA (photodiode array) detector and controlled by Agilent 3D-CE Chemstation software (Revision A.08.04). Samples were injected by pressure (50 mbar, 5 s).

2.3. Sample preparation

The chiral compounds used as analytes were dissolved in different solvents depending on their solubility (see Table 1). Final sample solutions were all at a concentration of 0.2 mg/ml.

2.4. Capillary electrophoresis

A 50 μm i.d. uncoated fused-silica capillary was used with a total length of 50 cm (effective length 41.5 cm). Temperature of the capillary was maintained at 20 °C using an air-cooling system. Applied voltage was kept at 20 kV. The UV detection was set at different wavelengths depending on the absorbance properties of the compounds (see Table 1).

Three different buffer pHs were examined, using the following background electrolytes:

- Buffer pH 3.0 was obtained by mixing 20 mM sodium dihydrogen phosphate with 20 mM phosphoric acid.
- Buffer pH 7.0 was obtained by mixing 20 mM sodium dihydrogen phosphate with 20 mM disodium hydrogen phosphate.
- Buffer pH 9.2 was 20 mM sodium tetraborate decahydrate. The pH was not adjusted.

Each of the six erythromycins was tested at all pHs, except for erythromycin A and erythromycin A

Table 1
Racemates for screening enantioselectivity of erythromycins

Compound	Abbreviation[a]	pK_a	Solvent	λ (nm)	Compound	Abbreviation[a]	pK_a	Solvent	λ (nm)
Dopa	Do	2.3 8.7 9.8 13.1	HCl 0.1 M	198	Sulconazole Isoprenaline	Su Ip	NA 8.6 10.1 11.4	MeOH–H_2O[c] H_2O	198 198
Leucovorin	Le	3.1 4.8 10.4	H_2O	287	Bamethane Bupivacaine Verapamil	Ba Bu Ve	NA 8.1 8.6	H_2O H_2O H_2O	198 198 198
Carbidopa	Ca	NA[b]	HCl 0.1 M	198	Chlorpheniramine	Ch	9.0	H_2O	198
Warfarin	Wa	5.0	NaOH 0.1 M	205	Fenfluramine	Fe	9.1	H_2O	205
Ketoprofen	Ke	5.9	MeOH–H_2O[c]	266	Betaxolol	Be	NA	H_2O	198
Indoprofen	In	NA	MeOH–H_2O[c]	282	Metoprolol	Me	9.7	H_2O	198
Propiomazine	PM	6.6	HCl 0.1 M	245	Oxprenolol	Ox	9.5	MeOH–H_2O[c]	198
Econazole	Ec	NA	MeOH–H_2O[c]	198	Pindolol	Pi	9.7	MeOH–H_2O[c]	217
Miconazole	Mi	7.4	MeOH–H_2O[c]	205	Propranolol	PP	9.4	H_2O	217

[a] Compound name abbreviation.
[b] NA: not available.
[c] Mixture of methanol–water (1:1).

N-oxide which were tested only at pH 7.0 due to their instability in both acidic [8,9] and basic [10] conditions. They were dissolved in the buffer at different concentrations namely 0.1, 0.2, 0.5, 1.0, 2.5, 5.0, and 10 mM, which is considered the highest possible concentration and which corresponds to about three times the reported solubility for erythromycin in water [7,11]. Due to solubility problems of most erythromycins in basic aqueous medium, at pH 9.2 the highest concentration still feasible for investigation is only 5.0 mM. Table 2 shows more details on the concentration ranges of each erythromycin in each buffer. At each concentration of erythromycin, the solutions of racemic mixtures were analysed in duplicate.

The racemates were analysed both in the absence and presence of erythromycins. Even when no chiral separation was found, the formation constant of the complexation between each analyte and each erythromycin was calculated, assuming there was such a complex.

Table 2
Range of tested concentrations of erythromycins

	pH 3.0	pH 7.0	pH 9.2
EA	Not tested	0.2–10 mM	Not tested
EANO	Not tested	0.1–2.0 mM	Not tested
AEA	0.5–10 mM	0.2–10 mM	0.1–5.0 mM
AEANO	0.5–10 mM	0.2–10 mM	0.1–5.0 mM
E	0.5–10 mM	0.2–4.0 mM	0.1–1.0 mM
EO	0.5–10 mM	0.2–10 mM	0.1–5.0 mM

3. Results and discussion

Unfortunately, over all the 3000 runs performed to cover almost 70 experimental conditions, no chiral separation of any pair of enantiomers, with any erythromycin, at any concentration investigated was achieved.

The lack of rigidity of the lactone ring, and lack of strong interaction groups as carboxylic or primary amine groups could be unfavourable properties of erythromycin compared to the common chiral antibiotics, for example vancomycin. And although it contains a number of hydroxyl groups, their interaction possibility is lower due to the high hydrogen-bonding environment of water. It is interesting, however, to analyse more carefully the obtained experimental data in order to find a more specific explanation for the negative response of erythromycin in the screening.

It was observed that the presence of erythromycin in the running buffer had an influence on the mobility of certain compounds, showing an interaction between these compounds and erythromycin. If the change of the migration time of the analyte over different ‘selector’ concentrations was more than 10 times

Table 3
Interactions between erythromycins and analytes

Compounds	Type	AEA			AEANO			E			EO			EA	EANO
	pH	3.0	7.0	9.2	3.0	7.0	9.2	3.0	7.0	9.2	3.0	7.0	9.2	7.0	7.0
Dopa		+	+	+	−	+	+	+	+	+	+	+	−	+	+
Carbidopa		+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leucovorin		+	+	+	+	+	−	+	+	−	+	+	+	+	+
Warfarin		+	+	−	+	−	+	+	−	−	+	−	−	−	−
Indoprofen		+	+	+	+	+	−	+	+	−	+	+	−	+	+
Ketoprofen		+	+	+	+	+	−	+	+	−	−	+	−	+	+
Propiomazine		−	−	−	+	−	−	−	+	−	−	−	−	−	−
Econazole		−	−	−	−	−	−	−	−	−	−	−	−	−	−
Miconazole		−	−	−	−	−	−	−	−	−	−	−	−	−	−
Sulconazole		−	−	−	−	−	−	−	−	−	−	−	−	−	−
Isoprenaline		−	−	−	−	−	−	−	−	−	−	−	−	−	−
Bamethane		−	−	−	+	−	−	+	−	−	−	−	−	−	−
Bupivacaine		−	−	−	−	−	−	−	−	−	−	−	−	−	−
Verapamil		+	−	−	−	−	−	+	+	−	−	−	−	+	−
Chlorpheniramine		−	−	−	+	−	−	−	−	−	−	−	−	−	−
Fenfluramine		+	−	−	−	−	−	−	−	−	−	−	−	−	−
Betaxolol		−	−	−	−	−	−	−	−	−	−	+	−	−	−
Metoprolol		−	−	−	−	−	−	−	−	−	−	+	−	−	−
Oxprenolol		−	−	−	−	−	−	−	+	−	−	+	−	−	−
Pindolol		−	−	−	−	−	−	−	+	−	−	+	−	−	+
Propranolol		−	−	−	+	−	−	+	−	−	−	−	−	−	−

(+) and (−), respectively, indicates there is/is not interaction between the analyte and the erythromycin.

the average deviation of migration times, it was considered that there was interaction between them.

Table 3 gives a brief description of the behaviour of erythromycins towards the chiral compounds in all investigated conditions. A (+) indicates a situation where an interaction was present, and (−) indicates a situation where no interactions were considered to be present. Fig. 2 gives an example of an interaction indicated as (+), with electropherograms of indoprofen in the absence and presence of EO at different concentrations. There is clearly an influence of EO on the migration behaviour of indoprofen, which is partly due to a change in EOF, and partly due to a change in electrophoretic mobility of indoprofen. The calculated electrophoretic mobilities are given in Fig. 2 for each condition.

3.1. Influence of parameters

3.1.1. Type of erythromycin

The main difference among the six erythromycins is not assumed to be the size of the aglycone ring, but the charging property. At pHs 7.0 and 3.0, the three N-oxides are neutral while the other three are positively charged at the N atom, which, theoretically, would enable them to interact more with the negatively charged compounds. However, results obtained did not show such a tendency. All erythromycins did interact mainly with the same group of compounds.

3.1.2. Type of chiral compounds

Interactions occurred most with the following compounds: Dopa, carbidopa, leucovorin, warfarin, indoprofen and ketoprofen. These compounds vary in size, and show no particular selectivity towards inclusion.

On the other hand, it can easily be recognised that all of them are acidic compounds. The interaction might be due to the presence of electrostatic interactions between the basic groups of erythromycins and the negative charges of the carboxylic groups. However, this finding does not go in line with the previous comment on the similarity in behaviour of the erythromycins, despite their difference in charging properties.

3.1.3. Influence of buffer pH

The change in migration time of the analyte was more significant at low pH. This again is not consistent with a mechanism of electrostatic interaction

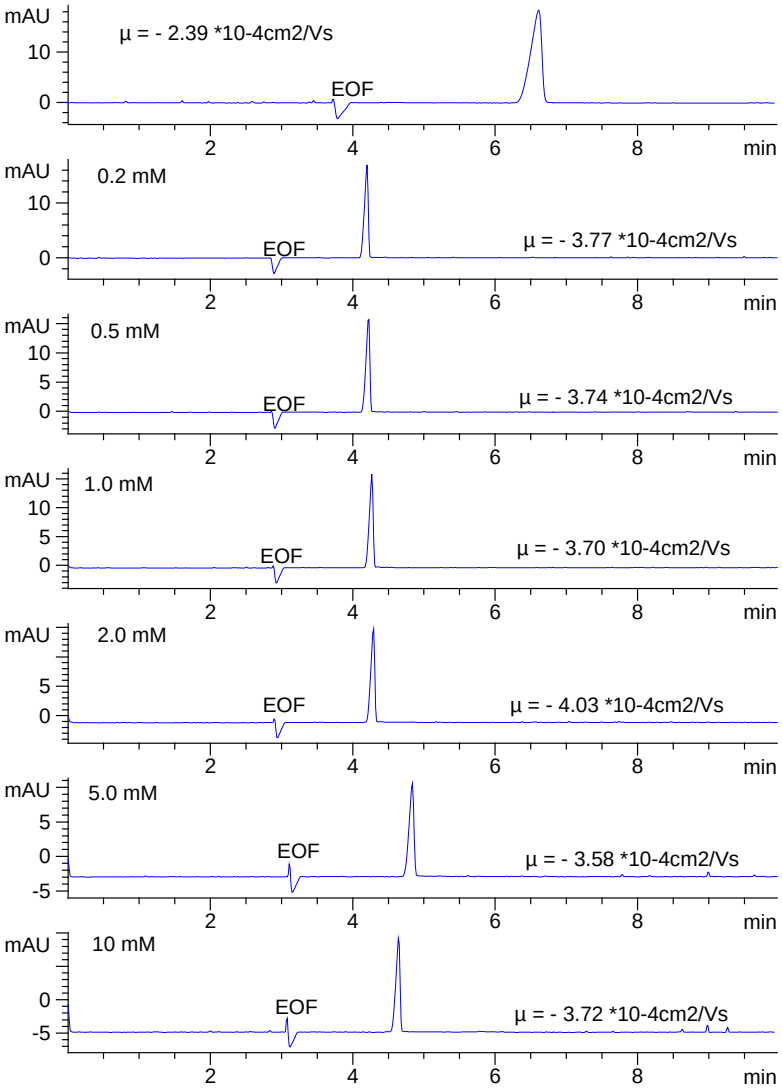

Fig. 2. Electropherograms of indoprofen in pH 7.0 buffer without and with 0.2, 0.5, 1.0, 2.0, 5.0 and 10 mM EO, from top to bottom consecutively.

between "selectors" and selectands, since at pH 7, they should be both well charged (except N-oxide derivatives), which means more possibilities for electrostatic interactions. On the contrary, in practice the influence was less at this pH than at pH 3, for all erythromycins. An example with EO is shown in Fig. 3.

To exclude the influence of changes in EOF, electrophoretic mobilities were calculated from the migration times. This will allow evaluating purely the influence of the charge status of the compounds on the interactions with the erythromycin "selectors". Fig. 4 presents the change in electrophoretic mobilities of ketoprofen and warfarin with different concentrations of EO. The changes were actually more significant at acidic pH, and these results thus show the same tendency as observed with migration time.

As a result of this, one can expect that there is actually formation of complexes between erythromycins and the indicated compounds. Based on

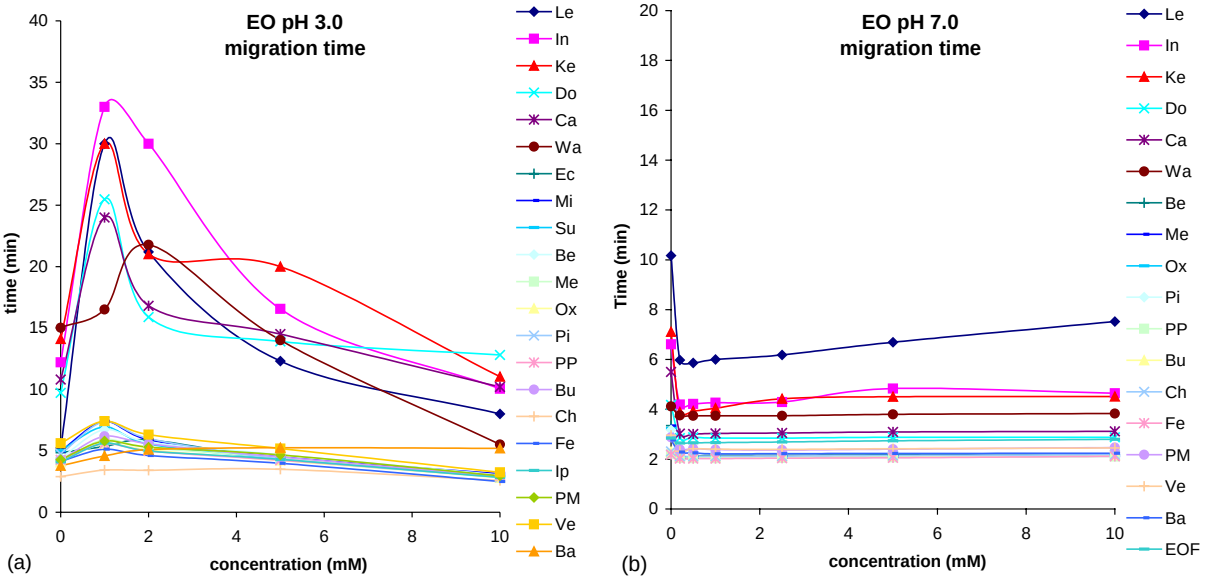

Fig. 3. Migration time of analytes in the presence of EO, at pH 3.0 (a) and pH 7.0 (b).

the experimental data, efforts were made to calculate the formation constants of these complexes.

3.2. Calculation of formation constant

In an ideal system of a one-to-one interaction scheme where a chiral compound (A) is reversibly bound to a selector (S),

$$A + S \overset{K_A}{\rightleftharpoons} AS$$

the experimentally measured electrophoretic mobility, μ is the weighted average of the mobilities of the analyte in the free and complexed forms [12]:

$$\mu = \alpha\mu_A + (1-\alpha)\mu_{AS} \quad (1)$$

where μ_A and μ_{AS} are the electrophoretic mobilities of the free analyte and the analyte–selector complex, respectively, and α is the ratio of free analyte in the mixture:

$$\alpha = \frac{[A]}{[A]+[AS]} \quad (2)$$

It can be assumed that the presence of selector does not influence significantly the electrophoretic mobility of

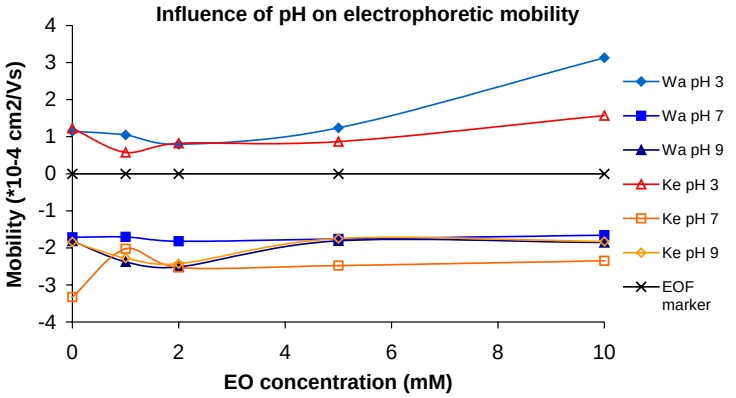

Fig. 4. Change in electrophoretic mobility of ketoprofen and warfarin with different concentrations of EO in the buffer, at different pHs.

the chiral compound in the free state. In other words, μ_A can be experimentally measured in the absence of chiral selector, whereas μ_{AS}, the mobility of the fully complexed form obtained in a condition where the concentration of chiral selector approaches infinity, can hardly be measured accurately in most cases [13,14].

On the other hand, the equilibrium constant of the analyte–selector complex can be defined in Eq. (3)

$$K_A = \frac{[AS]}{[A][S]} \tag{3}$$

The substitution of Eqs. (2) and (3) into Eq. (1), after rearrangement and consolidation gives the following relationship:

$$\frac{1}{\mu - \mu_A} = \frac{1}{K_A} \times \frac{1}{(\mu_{AS} - \mu_A)} \times \frac{1}{[S]} + \frac{1}{(\mu_{AS} - \mu_A)} \tag{4}$$

This equation, having been called double reciprocal [14,15], can be expressed in an easier way for a linear plot:

$$y = abx + b \tag{5}$$

where a and b can be determined from the intercept and slope of the linear curve by linear regression. For all the positive points (+) in Table 3, calculations were performed, but most of them did not fit the linear curve. Results in Table 4 include the best fitting data, from which the constant K_A was calculated.

The reason why a number of "selector"—selectand pairs could not be fitted by linear regression, could be that the assumption of 1:1 stoechiometry does not reflect reality, or in other words, a different complex ratio is present.

Table 4
Formation constant of erythromycin–compound complex

Complex	pH	K_A (mM^{-1})	R
Ca–AEA	7.0	0.292	0.989
Wa–AEA	7.0	0.037	0.991
Pi–E	7.0	0.782	0.994
Ca–E	7.0	2.88	0.995
Le–AEA	9.2	2.22	0.992
Do–AEA	9.2	22.6	0.999
Ca–AEA	9.2	0.175	0.998
Le–AEA	3.0	5.38	0.976
Do–E	3.0	17.6	0.948
Wa–E	3.0	97.1	0.927

3.3. Estimation of erythromycin ring size

Although the different three-dimensional conformations of EA and E have already been described in a study by Bertho et al., using two-dimensional transferred nuclear Overhauser effect NMR spectroscopy [16], we could not find in literature any documents on the size of erythromycin.

To have an idea of the size of the aglycone ring of erythromycin, the geometrical structure of erythromycin was built with the support of computational software, Gaussian 98 [17]. This program is used very frequently to predict the energies, molecular structures and vibrational frequencies of molecular systems. It provides calculations based on the basic laws of quantum mechanics. In quantum chemistry, computational chemical methods are used to solve complex equations as the Schrödinger equation, which describes the electronic and nuclear motion at the atomic or molecular level. Those methods can be divided into two categories namely the wave functional ab initio method, and Density Functional Theory [18,19]. In wave functional ab initio methods, the wave function is used as the basic source of information. The most common wave functional ab initio technique is the Hartree–Fock level that uses the one-electron functions (orbitals) to construct the multi-electron wave functions. At this level of theory, geometry of molecules can be accurately predicted. In this study, geometrical parameters of the structures considered were initially optimized and subsequently characterized by harmonic vibrational analyses using the Hartree–Fock theory with STO-3G basis set.

The stationary structure of erythromycin A is shown in Fig. 5. Since the ring of EA is asymmetric, a diameter of the aglycone ring can not really be specified. Various distances between different atoms on the aglycone rings were determined, as presented in Table 5. The ring of erythromycin was found to be narrower than the α-cyclodextrin cavity (5.7 Å), and just half of the size of β-cyclodextrin (7.8 Å) [20]. Since an aromatic ring has an outer diameter of around 5 Å (calculated with the same software), it would only fit in the aglycone ring in a particular direction. This might be considered as another reason of the low selector

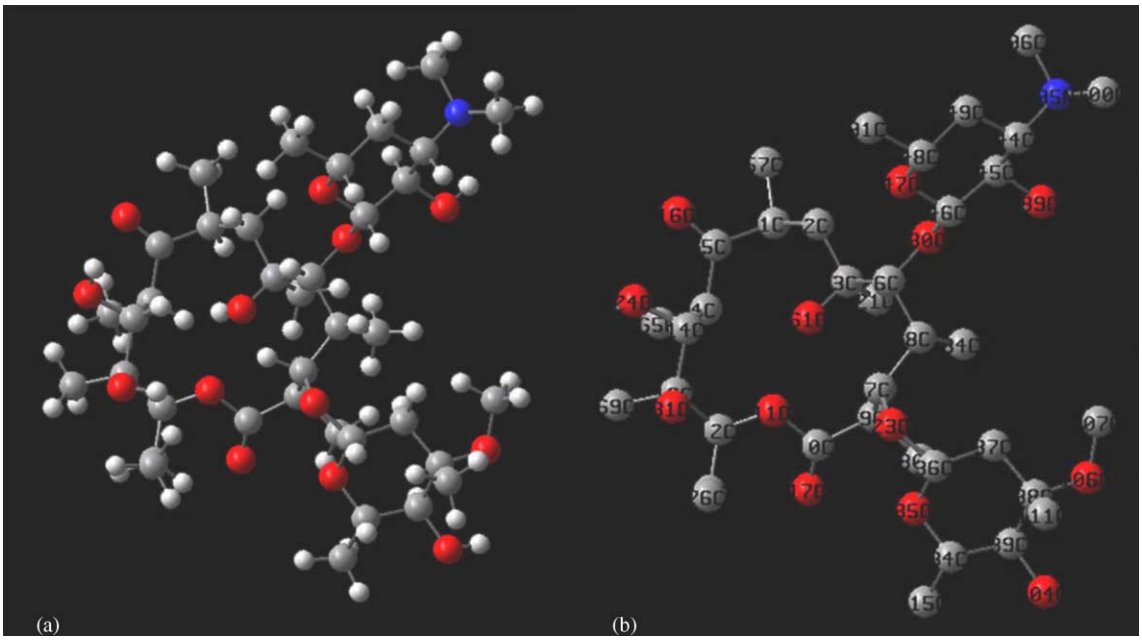

Fig. 5. Erythromycin A, complete structure (a) and simplified structure without hydrogen atoms (b). The numbering of atoms in (b) differs from the standard erythromycin numbering. This is due to the modelling approach in which atoms were gradually added to the basic aglycone ring.

Table 5
Dimensions of erythromycin ring

Atoms[a]	Distance (Å)	Atoms[a]	Distance (Å)
C13–C8	6.04	O61–C5	3.06
C6–C13	5.79	O61–C8	2.92
C1–C10	5.56	O61–C1	2.88
C10–C6	4.51	O61–C7	2.69
C3–C4	3.55	O61–O11	2.40

[a] Numbering is in accordance with Fig. 5.

ability of erythromycin on the chiral compounds investigated. However, an inclusion, if it exists, can also be formed with the aliphatic part of the chiral molecule.

4. Concluding remarks

The screening showed very low chance of using erythromycin as a chiral selector. Chiral separations were obtained at neither acidic, basic, nor neutral pHs. However, some interactions of erythromycins were observed with the acidic compounds: dopa, carbidopa, indoprofen, ketoprofen, warfarin and leucovorin, which can make erythromycins useful additives for the separation of related substances of these compounds. The size of the erythromycin A aglycone ring was also calculated on the optimized geometric structure, and proved to be quite narrow compared to the popular cyclodextrins. This fact might be one among the proposed reasons for the negative response of erythromycins in the screening.

Acknowledgements

Profs. J. Crommen and Y. Vander Heyden are acknowledged for fruitful discussions. Many thanks are sent to Pham Cam Nam for the assistance in computational calculations of molecular size.

References

[1] A. Amini, Electrophoresis 22 (2001) 3107–3130.
[2] T. Ward, A. Farris III, J. Chomatogr. A 906 (2001) 73–89.
[3] G. Gübitz, M. Schmid, Electrophoresis 21 (2000) 4112–4135.

[4] R. Bushan, V. Parshad, J. Chromatogr. A 736 (1996) 235–238.
[5] J.G. Hou, T.X. He, X.Q. Han, X.Z. Du, X.F. Mao, H.L. Deng, J.Z. Gao, Chinese Chem. Lett. 14 (2003) 280–282.
[6] S. Omura, Macrolide Antibiotics, Chemistry, Biology and Practice, Academic Press, San Diego, 2002, pp. 328–330.
[7] The Merck Index, 13th ed., Merck & Co Inc., Whitehouse Station, 2001, pp. 654–655.
[8] Th. Cachet, G. Van den Mooter, R. Hauchecorne, C. Vinckier, J. Hoogmartens, Int. J. Pharm. 55 (1989) 59–65.
[9] C. Vinckier, R. Hauchecorne, Th. Cachet, G. Van den Mooter, J. Hoogmartens, Int. J. Pharm. 55 (1989) 67–76.
[10] J. Paesen, K. Khan, E. Roets, J. Hoogmartens, Int. J. Pharm. 113 (1994) 215–222.
[11] W.L. Koch, in: K. Florey (Ed.), Analytical Profiles of Drug Substances, vol. 8, Academic Press, London, 1979, pp. 159–177.
[12] R. Kuhn, S. Hoffstetter-Kuhn, Capillary Electrophoresis: Principles and Practice, Springer-Verlag, Berlin, 1993, pp. 91–101.
[13] R. Vespalec, P. Boček, J. Chromatogr. A 875 (2000) 431–445.
[14] K. Rundlett, D. Armstrong, J. Chromatogr. A 721 (1996) 173–186.
[15] K. Rundlett, D. Armstrong, Electrophoresis 18 (1997) 2194–2202.
[16] G. Bertho, J. Gharbi-Benarous, M. Delaforge, J. Girault, Bioorg. Med. Chem. 6 (1998) 209–221.
[17] M. Frisch, G. Trucks, H. Schlegel, B. Johnson, M. Robb, J. Cheeseman, T. Keith, G. Petersson, J. Montgomery, K. Raghavachari, M. Al-Laham, V. Zakrzewski, J. Ortiz, J. Foresman, C. Peng, P. Ayala, M. Wong, J. Andres, E. Replogle, R. Gomperts, R. Martin, D. Fox, J. Binkley, D. DeFrees, J. Baker, J. Stewart, M. Head-Gordon, C. Gonzalez, J. Pople, Gaussian 98, Rev. C3, Gaussian Inc., Pittsburgh, 1998.
[18] F. Jensen, Introduction to Computational Chemistry, Wiley, Chichester, 1999.
[19] A. Szabo, N.S. Oslund, Modern Quantum Chemistry, Dover, New York, 1989.
[20] S. Fanali, in: N.A. Guzman (Ed.), Capillary Electrophoresis Technology, Chromatographic Science Series, vol. 64, Marcel Dekker, New York, 1993, pp. 731–734.