REVIEW

REVIEW – RECENT ENANTIOMER SEPARATION STRATEGIES OF NONSTEROIDAL ANTI-INFLAMMATORY DRUGS (NSAIDs) BY CAPILLARY ELECTROPHORESIS

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Abstract

This report highlights the most recent enantiomer separation strategies applied in capillary electrophoresis (CE), using nonsteroidal anti-inflammatory drugs (NSAIDs) 2-arylpropionic acids as model compounds. It is mainly focused on method development and validation, along with pharmaceutical and biomedical application, where chiral selectors (CSs) are used. There is a large number of CSs that have, over the years, been synthesized and used in electrophoretic enantioseparations. In this review article, their advantages, their chiral recognition mechanisms and their performance are presented and discussed in particular.

Rezumat

Scopul acestei lucrări este prezentarea celor mai noi strategii aplicate în enantioseparareа prin electroforeză capilară (CE), utilizând ca model de compuşi chirali antiinflamatoarele nesteroidiene (AINS) din grupa acidului 2-arilpropionic. În principal se concentrează pe dezvoltarea şi validarea metodelor ce folosesc selector chiral (CSs), dar şi aplicabilitatea în domeniul farmaceutic şi medical. Există un număr mare de CSs sintetizaţi şi utilizaţi în separarea compuşilor chirali prin electroforeză capilară. În acest articol sunt prezentate şi discutate avantajele, mecanismele de recunoaştere şi eficienţa acestora.

Keywords: Capillary electrophoresis (CE), chiral selectors (CSs), enantioseparations, nonsteroidal anti-inflammatory drugs (NSAIDs)

Introduction

Current requirements for enantiospecific analysis in pharmaceuticals research, life sciences, food chemistry, toxicology and classical organic chemistry was the impulse for the rapid development of chiral separation methods in the last decade. Through those techniques there has been a significant increase in the level of knowledge on enantio-selective pharmacology, metabolism, catalysis and preparative scale synthesis, purification and production of chiral compounds.

Thus, the Food and Drug Administration and the European Medicines Agency require enantiomer drug purity of pharmaceuticals in pharmacokinetic and metabolic studies [45], therefore, a great variety of chromatographic methods have been developed and applied, through the years, for the enantioseparation of nonsteroidal anti-inflammatory drugs (NSAIDs), such as ibuprofen, ketoprofen, naproxen, pranoprofen, flurbiprofen, carprofen, indoprofen, fenoprofen and etodolac.

In this review, the first section provides an overview on the publication rates and time trends on the CE-enantiosepartion-NSAIDs topic in its most active period, 2000–2015 (not covered by previous more general CE-reviews). The rest of the paper covers contributions over the last five years period, including reviews and research articles dealing with NSAIDs–CSs interactions studies and their applications.

Overall publication rates and trends on the CE–NSAIDs–enantioseparation topic

Publication databases are a common way to access research information on target topics. SCOPUS database search in June 2015 using the key words "capillary electrophoresis" and "enantioseparation" into the 'Title-Abstract-keywords' search field, revealed a total of about 1940 hits with 80 - 160 papers published each year on this topic since 2000. As it can be observed, smaller values but similar time trends are found from the Web of Knowledge or WOK databases (merging terms related to "capillary electrophoresis" and "enantioseparation" into the 'Topic' search field), which provides extra confidence on the results. Although they offer just approximate results, due to non-matched or missing records, relative results can offer overall trends.

According to SCOPUS database, the most active journals publishing on the searched topic (% publications) are Journal of Chromatography A (21%), Electrophoresis (17%), Analytical Chemistry (13%), followed by a group of journals in the 2–7% range in both databases (Chirality, Biomedical Chomatography, Chromatographia and Analytica Chimica Acta).

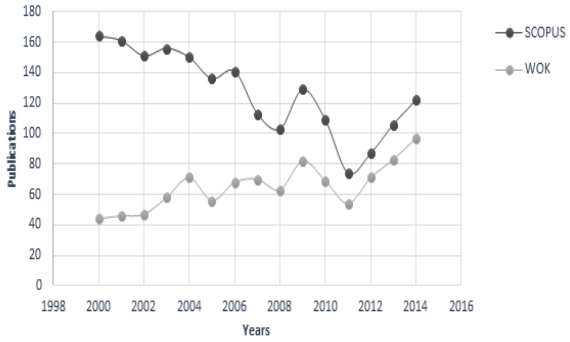

Figure 1.
Number of publications per year and trend including the CE-enantioseparation topics

After a well-known rapid increase of the number of publications during the 1990 decade, followed by a stabilization period (2000 - 2005), a small decrease was between 2006 and 2010, and a last period showing a slight increase (2011 - 2014). According to the last trend, a moderate increase for the next years should be expected.

A new search using the key words "capillary electrophoresis" and "enantioseparation" combined with "NSAIDs" gave about, 101 results on SCOPUS and 124 hits on WOK, this search certainly demonstrating the large interest for the NSAIDs as model chiral compounds.

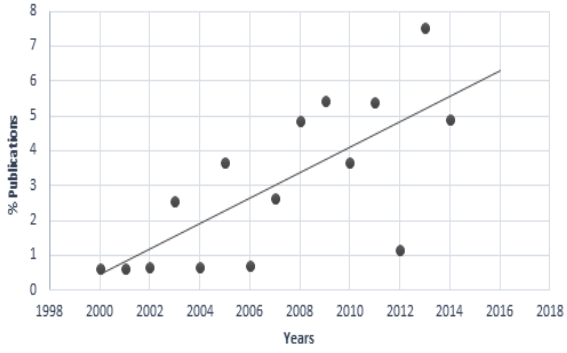

Figure 2.
Percentage of publications per year and trend, using NSAIDs as model chiral compounds in CE

Over the reviewed period, only 52.2% are research articles and 42.4% are reviews. Some of these reviews are summarized in Table I. As it can be observed none of the commented reviews is specific on enantiomer separation strategies of NSAIDs drugs by capillary electrophoresis.

Table I
Reviews containing CD – CE information

Year	Revised period	Main elements	Ref.
2012	Jan 2007 - Mar 2012	Recent development of cyclodextrin chiral stationary phases (CD-CSPs) and their applications in chromatography development and applications of novel CD-CSPs in liquid chromatography (LC), capillary electrochromatography (CEC), gas chromatography (GC) and supercritical fluid chromatography (SFC)	[56]
2013	2008 - 2011	Fundamentals and application of cyclodextrins (CDs) as chiral selector (native, neutral and charged, polymerized, dual systems) in all CE modes and CE–MS	[53]
2013	2010 - 2012	MEEKC applications. Microemulsion-CD synergy	[40]
2014	Aug 2007 - Feb 2013	Multivariate optimization in capillary zone electrophoresis (CZE), micellar electrokinetic chromatography (MEKC) and microemulsion electrokinetic chromatography (MEEKC). Experimental design; effect of cyclodextrin (CD) nature and concentration	[35]
2014	2008 - 2013	Application of cyclodextrins in chiral capillary electrophoresis (CE)	[38]
2014	2010 - Apr 2013	On-line preconcentration techniques in CE	[24]
2014	Up to May 2013	CE-applications in pharmaceutical analysis (small molecules and biomolecules)	[10]
2014	Jun 2011 - May 2013	Strategies to increase sensitivity in chiral CE	[44]
2014	Jan 2013 - Feb 2014	Cyclodextrins in capillary electrophoresis: Recent developments and new trends	[12]
2015	2012 - mid 2014	Chiral selectors (CSs) applied in CE; method developments and validations, along with pharmaceutical and biomedical applications	[47]

NSAIDs enantiomers and their biological importance

As most pharmaceutical substances currently used in drug therapy, NSAIDs have a centre of chirality in their molecule. Chiral drugs are usually synthesized as racemic mixtures. Enantiomers differ from one to another on the absorption, distribution, protein binding and receptor affinity. Often, it may be different even the metabolic pathways.

In case of NSAIDs there are three situations of enantiomers effects manifestation *in vivo*.

a) In first case, the pharmacological effect is restricted to one of the enantiomers, called eutomer. The pharmacologically inactive enantiomer, called distomer can produce unwanted side effects; in

some cases antagonistic effects are observed or even toxic [3], even so, currently less than 25% of chiral drugs are administered as a single enantiomer [20]. For example: naproxen is used as a single enantiomer (S-form) in the clinical field. This formulation containing a single enantiomer require optical purity testing of the active form and the determination of the very low concentrations of enantiomer impurity levels below 1% or sometimes even less than 1% of the nominal concentration of the main enantiomer. The quantification of these concentration levels are often a challenge for analysts, especially when there are required a good selectivity and a high sensitivity.

b) In the second case, enantiomers can have similar pharmacological properties, but may differ in terms of potential. Initially ketoprofen was introduced into medical practice as a racemic mixture, later demonstrating pharmacological differences of its enantiomers. As in other 2-arylpropionic acid derivatives, the S-isomer proved ketoprofen responsible for inhibiting the biosynthesis of PG, and currently exists due to formulation containing pure enantiomer: dexketoprofen [22].

c) In the third case, the example of ibuprofen, even though pharmacological properties are attributed exclusively to the S-enantiomer, recent studies have shown that R-ibuprofen is not totally devoid of pharmacodynamic activity, being in a proportion of 50-60% a precursor of the S-enantiomer by unidirectional conversion reaction. R-ibuprofen is metabolised as R-CoA thioester intermediate which is then epimerized to the S-CoA thioester, resulting S-ibuprofen conversion. This explains only a 25% difference between racemic mixture and a single enantiomer, considering pharmacological properties potential (Figure 3) [13].

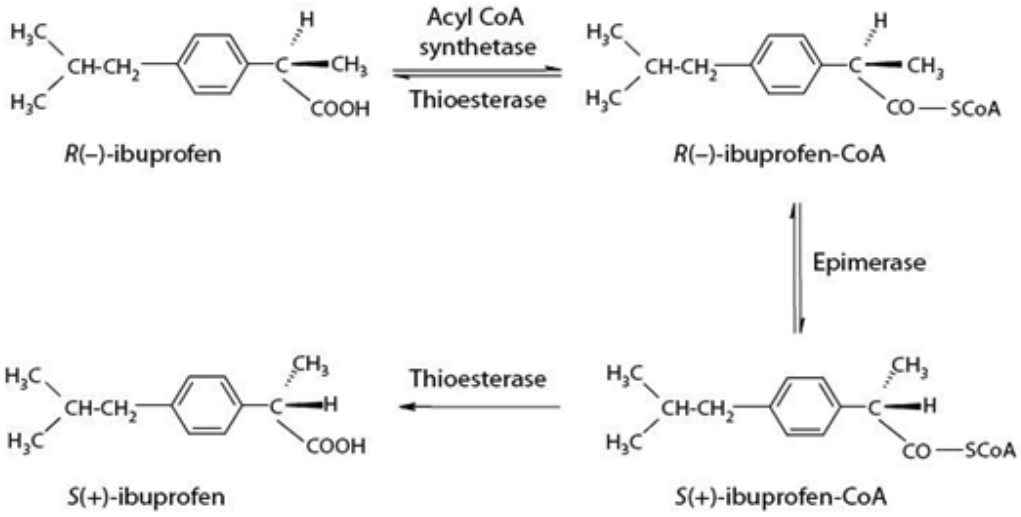

Figure 3.
Conversion of R-Ibuprofen to S-Ibuprofen

Due to a very poor efficiency, high costs and difficulties of quantification of HPLC methods (High-Performance Liquid Chromatography), capillary electrophoresis became the most applied chiral analysis. The major advantages of capillary electrophoresis (CE) are simplicity, high efficiency, versatility, rapid analysis, high resolution, small sample volume and low operating costs [31].

CE has various operation modes, depending on the composition of the background electrolyte (BGE) and the nature of the capillary. The most common CE modes are CZE (capillary zone electrophoresis), EKC (electrokinetic chromatography), MEKC (micellar electrokinetic chromatography), MEEKC (microemulsion electrokinetic chromatography) and NACE (non-aqueous capillary electrophoresis). In all cases, one or more chiral selectors (CSs) are added into the BGE in order to perform chiral discrimination.

Enantioseparations

The basic chiral separation principles are explained based on the different effective electrophoretic mobility of the enantiomers. Since two enantiomers possess similar physico-chemical properties, for a successful enantiomer resolution in CE, it is necessary to selectively modify the effective mobilities of the two enantiomers. Based on the different binding constants of the enantiomers with the chiral selector, in CE the separation of chiral compounds is achieved mainly by the direct separation method where the chiral selector is simply added to the BGE or bonded to either the capillary wall or to a stationary phase, forming stereo-selective complexes with different mobilities.

The enantioresolution can be influenced by several parameters such as the type and concentration of CS, pH and composition of the BGE, capillary temperature, applied voltage, additive to the BGE etc. [16].

Chiral selectors

Enantioseparation can be achieved in CE using chiral selectors, which discriminate between enantiomers by an enantioselective complexation, such as the formation of inclusion complexes (cyclodextins, polysaccharides, cyclic ethers or macrocyclic antibiotics); covalent bonds (ligand exchange) and electrostatic bonds (ion pairs). The complex formed during the electrophoretic process, in equilibrium with the free analyte, possesses a different mass responsible for the change of the effective mobility. Chiral recognition is the result of secundary interactions such as dipole-dipole, hydrogen bonding, π-interactions and hydrophobic interactions [7].

Numerous chiral selectors are currently available and can be used for enantioseparation, such as cyclodextrins (CDs), chiral crown ethers, proteins, chiral surfactants, macrocyclic antibiotics, ligand-exchange complexes and linear polysaccharides [20]. Among them, the cyclodextrins are the most widely used chiral selectors [16].

The most suitable chiral selector for each specific purpose is usually selected by trial, which is expensive and time-consuming. There are review articles which provide criteria for the choice of chiral selectors in terms of molecular size, charge, and the presence of specific functional groups or substructures in the analytes in order to minimize the number of trials needed [5].

The main properties of the material that can be used as chiral selector in CE are as follows: a) the material should be able to interact with chiral compounds stereoselectively through intermolecular forces; and b) the complexes formed should possess different mobility compared to the free analyte.

Cyclodextrins

Cyclodextrins (CDs) can often fulfil these requirements better than other chiral selectors available for CE enantioseparation. Hydrogen bonding, hydrophobic, dipole-dipole and van der Waals interactions are the main intermolecular forces responsible for the stereoselective reactions using CDs [5].

The first reference to dextrin was published in 1891 by Villiers, but the first detailed description of the preparation and isolation was made a few years later, in 1903 by Schardinger. In that time the structures of these molecules were not yet known. The research was continued by Freudenberg, Further and Borchert who first introduced in 1942, the concept of cyclodextrins, due to the cyclical structure. Cramer showed that the cyclodextrins are able to include foreign neutral molecules in their cavity. Since then, the interest in cyclodextrins has increased. In 1980 Saenger published an article, an analysis of cyclodextrins mentioning some industrial applications [42].

The First International Symposium on cyclodextrins was organized by Szejtli and held in Budapest in 1981, and the following year was published by the same Szejtli, the first book about cyclodextrins. Since then it has increased the interest in these compounds, there were found new industrial methods for producing cyclodextrins and at the same time decreasing the cost of production. So far in the literature have appeared numerous applications in pharmaceutical technology, analytical chemistry or cosmetic applications [6].

Cyclodextrins (CDs) can be obtained by enzymatic degradation of starch, using cyclodextrin-glucosyl-transferase (CGTase). CDs are cyclic oligosaccharides composed by 6 to 12 units of D (+) glucopyranose connected to each other through α-(1,4)-glucosidic bonds, thus creating a rigid structure. Only those CD with 6, 7 and 8 units, called α- , β- , and γ-CD, are currently used in analytical chemistry. They have a shape of a truncated cone, with the wider side ronded by a secondary hydroxyl groups and the narrow side with a primary hydroxyl groups. CDs presents an inner cavity lined with hydrogen atoms and glycosidic oxygen bridges, which favours hydrophobic interactions between a guest molecule and the CD host, while the outside is hydrophilic due to the presence of hydroxyl groups (positions 2, 3 and 6 of glucopyranose) (Figure 4) [2].

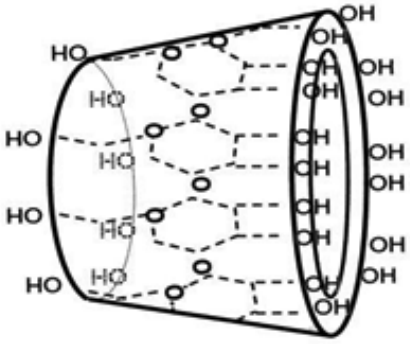

Figure 4.
Cyclodextrins structure

The physical properties of the three native CDs are different, e.g. width of the cavity, solubility, molecular mass etc., however, they possess the same cavity depth. Cyclodextrins contain 18 (α-CD), 21 (β-CD) and 24 (γ-CD) hydroxyl groups which can be easily modified by chemical reactions in order to obtain alkyl [23], hydroxyalkyl [46], carboxyalkyl [8], aminoalkyl and sulfated [14]

cyclodextrins, with different degrees of substitution. The composition of the modified CD depends on several parameters such as reaction conditions, reagent type and ratio (Table II) [16].

Table II

Native cyclodextrins and derivatives

Cyclodextrins type	Abbreviation	Substituents	Ref.
Native cyclodextrins			
α-Cyclodextrin	α-CD	H	[20]
β-Cyclodextrin	β-CD	H	
γ-Cyclodextrin	γ-CD	H	
Neutral cyclodextrins derivatives			
Methyl-α-cyclodextrin	M-α-CD	CH3, randomly substituted	[23]
Methyl-β-cyclodextrin	M-β-CD	CH3, randomly substituted	
Heptakis-2,6-dimethyl-β-cyclodextrin	DM-β-CD	CH3 in positions 2,6	[32]
Heptakis-2,3,6-trimethyl-α-cyclodextrin	TM-α-CD	CH3 in positions 2,3,6	[43]
Heptakis-2,3,6-trimethyl-β-cyclodextrin	TM-β-CD	CH3 in positions 2,3,6	[36]
Heptakis-2,3,6-trimethyl-γ-cyclodextrin	TM-γ-CD	CH3 in positions 2,3,6	
Hydroxypropyl-α-cyclodextrin	HP-α-CD	CH2-CH2-CH2-OH, randomly substituted	[48]
Hydroxypropyl-β-cyclodextrin	HP-β-CD	CH2-CH2-CH2-OH, randomly substituted	
Hydroxypropyl-γ-cyclodextrin	HP-γ-CD	CH2-CH2-CH2-OH, randomly substituted	
Glucose-β-cyclodextrin	Glu-β-CD	Glucosyl- , randomly substituted	[57]
Heptakis(2,3-di-O-acetyl)-β-CD	HDA-β-CD	(2,3-di-O-acetyl)- randomly substituted	[8]
Acetylated β-CD	Ac-β-CD	CH_3CO randomly substituted	[8]
Negatively charged cyclodextrin derivatives			
Carboxymethyl-β-cyclodextrin	CM-β-CD	CH_2-COONa, randomly substituted	[8]
Sulfated α-cyclodextrin	S-α-CD	SO_3Na, randomly substituted	
Sulfated β-cyclodextrin	S-β-CD	SO_3Na, randomly substituted	[36]
Sulfated γ-cyclodextrin	S-γ-CD	SO_3Na, randomly substituted	
Sulfobutyl-β-cyclodextrin	SB-β-CD	CH_2-CH2-CH2-CH2-SO_3Na randomly substituted	[16]
Heptakis-6-sulfo-β-cyclodextrin	HS-β-CD	SO_3Na in position 6	[41]
Hexakis(2,3-di-O-methyl-6-O-sulfo)-α-cyclodextrin	HDMS-α-CD	(2,3-di-O-methyl-6-O-sulfo)-randomly substituted	[26]
Heptakis-(2,3-diacetyl-6-sulfo)-β-cyclodextrin	HDAS-β-CD	CH_3CO in positions 2, 3 and SO_3Na in position 6	[28]
Positively charged cyclodextrin derivatives			
Heptamethylamino-β-cyclodextrin	[MeNH]7-β-CD	6A-methylamino	[15]
6-monodeoxy-6-mono(2-hydroxy)propylamino-β-cyclodextrin	IPA-β-CD	6-monodeoxy-6-mono(2-hydroxy) propylamino-single-isomer	[39]
4-dimethylamino-1,8-naphthalimid-β-cyclodextrin	DMAN-β-CD	4-dimethylamino-1,8-naphthalimide	[51]
2-hydroxy-3-trimethylammoniopropyl-β-cyclodextrin	TMA-β-CD	CH_2-CH(OH)-CH_2-N$(CH_3)_3$Cl, randomly substituted	[27]

Native cyclodextrins

Native CDs are cyclic oligosaccharides consisting of six (α-CD), seven (β-CD) or eight (γ-CD) glucopyranose units. β-CD is used more often in chiral CE applications than the other native CDs due to its cavity size, which enables tight inclusion complex formation with most of the analytes. Although native cyclodextrins demonstrated good enantioseparation abilities of numerous chiral compounds (β-blockers, amino acids), their applicability in CE for nonsteroidal anti-inflammatory drugs (NSAIDs) has been limited.

Neutral CD derivatives

The application range increased when different derivatives of the three native CDs (α-, β-, γ-CD) were synthesized. A broad spectrum of neutral CD derivatives is commercially available. Substituents increase hydrophobic cavity depth and increase the diameter of CDs, or may enter another stereogenic centre positioned just above the mouth of cyclodextrin, e.g. hydroxypropyl cyclodextrin proved to be useful in analysis of compounds where the chemical group involved in the inclusion complex or hydrogen bonds is not directly adjacent to chiral centres [48].

The separation of ketoprofen enantiomers was achieved by using of three TM-CDs derivatives, namely trimethyl-α-CD (TM-α-CD), TM-β-CD, and trimethyl-γ-CD (TM- γ -CD) in CE [43]. It was observed that the elution order of the enantiomers depended on the CD's cavity size. Therefore, with TM-α-CD and TM-β-CD, the R enantiomer eluted first, while in the case of TM-γ-CD the enantiomer migration order was reversed. The change in the structure of the complexes between the analyte enantiomers and the CSs was confirmed by nuclear magnetic resonance (NMR) experiments. In particular, ketoprofen entered the cavity from the

wider secondary side of TM-α-CD with the benzoyl moiety located deep inside the cavity, while in the second case, the phenyl and alkyl moieties of ketoprofen entered the cavity from the narrower, primary side of TM-β-CD. In the third and last case, the analyte entered the TM-γ-CD cavity from the narrower side with the benzyl ring located outside (Figure 5).

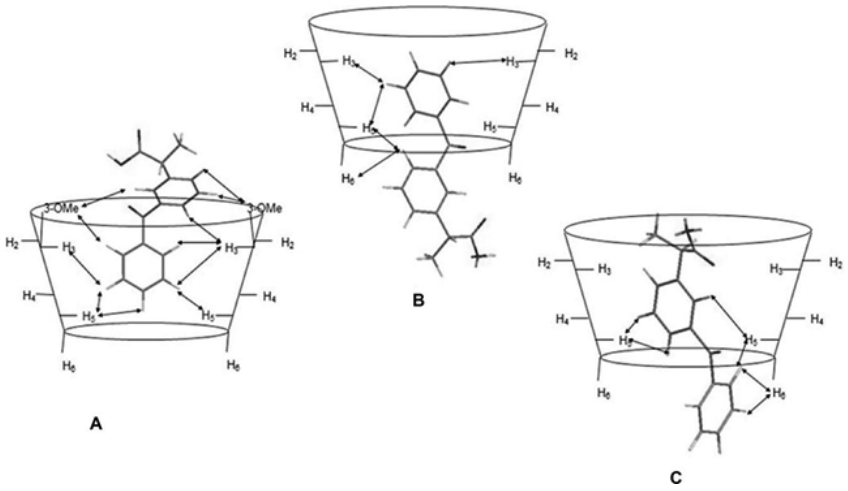

Figure 5.
Structure of the ketoprofen /TM-α-CD (A), ketoprofen/TM-β-CD (B) and ketoprofen/TM-γ-CD (C) [43]

In order to optimize the enantioseparation it may be necessary to incorporate an additive in the mobile phase. Chiral ionic liquids (CILs) have successfully been applied in CE enantioseparation of 2-arylpropionic acids as model analytes.

François *et al.* studied the influence of two CILs (ethyl- and phenylcholine of bis(trifluoromethylsulfonyl)-imide) as BGE additives, in the presence of classical chiral selectors: DM-β-CD or TM-β-CD. Although no general trend could be established, an increase in separation selectivity and resolution was observed, suggesting synergistic effects [18].

Wang *et al.* reported the combined use of the CIL-type surfactant N-undecenoxy-carbonyl-L-leucinol bromide (L-UCLB) and TM-β-CD as a dual chiral selector for the simultaneous enantioseparation of profens. It was proved that a low concentration of L-UCLB (1.5 - 2.0 mM) provided enantioseparations with resolution values in the range of 2.0-2.4 [54]. In another study by the same authors, the interactions among TM-β-CD, L-UCLB and profens were investigated by the use of affinity capillary electrophoresis (ACE). The different binding constant values of R- and S-fenoprofen suggested that enantioseparation is achieved due to the synergistic effect of the two CSs [55].

Zhang *et al.* used methyl-β-CD (M-β-CD), HP-β-CD and glucose-β-CD as CSs for the enantioseparation of six anionic racemic drugs (naproxen, pranoprofen, warfarin, carprofen, ibuprofen and ketoprofen). Since the resolution values were lower in the case the CD derivative was used as the sole CS, it was investigated the synergistic effect of CDs and amino acid-derived chiral ionic liquids (CILs): L-alanine tertbutyl ester bis (trifluoromethane) sulfonamide, L-alanine tert butylesterbis(trifluoro-methane) sulfonimide (L-AlaC_4NTf_2), and L-valine tert butyl ester bis (trifluoromethane) sulfonimide, alanine L-valine tert butylesterbis(trifluoro-methane)-sulfonimide (L-ValC_4NTf_2). The synergistic effect was significant for the first three analytes. It is worth though mentioning that, when the amino acid-derived CILs were used alone, no enantio-separations were observed [57].

Negatively charged cyclodextrin derivatives

Several negatively charged CDs have been synthesized, over the years, and have been widely used as CSs in CE. Due to their ionisable character, negatively charged CD derivatives interact strongly with basic compounds, and high migration differences between the enantiomers are observed. They can also be used for the separation of neutral compounds in their ionized state. This review reveals that sulphated CD derivatives, and particularly HS-β-CDs, have received the most attention and appear to be the most effective [41].

Positively charged cyclodextrin derivatives

Positively charged CD derivatives have limited number of applications in comparison to the negatively charged CDs, due to their absorbing character to the column wall and the more complicated synthesis procedure. However, they demonstrate an advantage over the anionic CDs, and this includes the lower migration times of the analytes. The majority of the positively charged CD derivatives are derived from β-CD (Table III).

Rousseau *et al.* performed enantiomeric purity determination studies of R-flurbiprofen using 6-monodeoxy-6-mono(2-hydroxy)propylamino-β-cyclodextrin (IPA-beta-CD) as chiral selector. The

nonaqueous BGE was made up of 20 mM IPA-beta-CD, 20 mM ammonium camphorsulfonate and 40 mM ammonium acetate in methanol. Flufenamic acid was selected as internal standard. The CE method was carefully optimized in order to prevent the adsorption of the cationic CD onto the capillary wall, and therefore, to avoid loss of peak efficiency and enantioresolution. To achieve this goal, the addition of ammonium camphorsulfonate was found to be necessary (Table III) [39].

Table III

Enantioseparation of nonsteroidal anti-inflammatory drugs (NSAIDs). Strategies applied in capillary electrophoresis (CE)

Selector chiral (concentration)	Analyte(s)	CE mode	BGE - buffer	pH	Ref.
Neutral CD Derivatives					
TM-β-CD 20 - 80mM	Ibuprofen, Ketoprofen	CZE	50 mM phosphate buffer	6.0	[50]
TM-β-CD 25mM	Ibuprofen, Ketoprofen	CZE	20 mM phosphate buffer / 20 mM triethanol-amina	5.0	[4]
TM-β-CD 8–15 mM	Ibuprofen, Ketoprofen	CZE	0.1 M phosphate buffer 0.1% hydroxipropilenmethyl cellulose (HPMC)	4.92	[59]
TM-α-, -β-, -γ-CD 50 mM	Ketoprofen	EKC	60 mM acetic acid	5.0	[43]
TM-β-CD 5-35 mM	Ibuprofen, Ketoprofen, Carprofen, Indoprofen, Flurbiprofen, Naproxen, Fenoprofen	EKC	5 mM sodium acetate/acetic acid + 5 - 20 mM L-$AlaC_4Lac$ (L-alanine tert butyl ester lactate), 20°C	5.0	[29]
TM-β-CD 35 mM	Ibuprofen, Fenoprofen, Indoprofen, Suprofen, Ketoprofen	CD-MECK	5 mM sodium acetate / L-UCLB 1.5 mM	5.0	[54]
TM-β-CD 35 mM	Fenoprofen	CD-MECK	5 mM sodium acetate / L-UCLB 1.5 mM	5.0	[55]
HP-β-CD 15 mM	Etodolac	CZE	0.1 M phosphate buffer	6.0	[11]
M-β-CD 20 mM , HP-β-, Glu-β-CD 30 mM	Naproxen, pranoprofen	EKC	30 mM sodium citrate / 20 % (v/v) ethanol or acetonitrile ± 15 mM L-$AlaC_4NTf_2$ or L-$ValC_4NTf_2$	5.0	[57]
Negatively charged CD derivatives					
HS-β-CD 5% w/v	Ketoprofen	EKC	25 mM phosphate buffer	2.5	[41]
DMAN-β-CD 5-30% w/v	Ibuprofen, Naproxen	NACE	ACN–MeOH (70:30, v/v) / 350 mM HAc and 5 mM TEA	-	[51]
Positively charged CD derivatives					
β-CD-EA 1 - 5 mM	Ibuprofen	CZE	50 mM phosphate buffer	4-7	[34]
[MeNH]7-β-CD 5 mM	Ketoprofen	CZE	75 mM Britton Robinson buffer	5.0	[15]
Et-NH-β-CD 2 - 20 mM	Ibuprofen, Ketoprofen	CZE	50 mM phosphate buffer	5-7	[21]
IPA-β-CD 20 mM	Flurbiprofen	NACE	40 mM ammonium acetate / 20 mM ammonium camphorsulfonate / methanol	6.8	[39]
Linear oligo- and polysaccharides					
Amycol 5-L maltodextrin (MD)	Naproxen, Ibuprofen, Flurbiprofen	CZE	20 mM phosphate buffer	7.0	[49]
Branched polysaccharides					
Glycogen (3% w/v)	Ibuprofen	CZE	90 mM Tris-borate (7.0)	7.0	[9]
Antibiotics					
Eremomycin 2.5 mM	Ibuprofen, ketoprofen	EKC	100 mM phosphate + 18% v/v ACN	6.5	[37]
Eremomycin 0.5 mM	Flurbiprofen, ketoprofen, Fenoprofen, Indoprofen	EKC	50 mM phosphate / methanol (40:60 vol%)	5.8	[25]
Vancomycin 2 mM	Naproxen, Ibuprofen, Carprofen, Ketoprofen, Pranoprofen	EKC	50 mM phosphate / 20% methanol + 15 mM L-$AlaC_4NTf_2$ or L-$ValC_4NTf_2$	7.0	[58]
Dual selector systems:					
4.0% (w/v) S-β-CD / 0.5% (w/v) TM-β-CD	Ketoprofen	CD-MEKC	50 mM phosphate / 20 mM SDS	2.5	[36]
DM-β-CD / β-CD DM-β-CD / 6-O-α-maltosyl-β-CD	Ibuprofen, Flurbiprofen (non-enantioselective separation)	CZE	50 mM TRIS 7 / 50 mM Tricine (N-[Tris(hydroxymethyl)methyl]glycine)	8.2	[32]

Oligo- and polysaccharides

Linear oligo- and polysaccharides

Maltodextrins (MDs) are the first CSs, in the family of linear oligo- and polysaccharides, which have been used for enantioseparations in CE. They consist of D-(+)-glucose units, which are connected through a Glu-(1–4)-α-D-Glu linkage. In addition, they are complex malto-oligo and polysaccharide mixtures, obtained from partial acid and/or enzymatic hydrolysis of starch. They are

characterized by their dextrose equivalent, which is the equivalent of the degree of polymerization of malto-oligosaccharides. MDs are neutral polysaccharides and have shown highly efficient chiral selectivity in CE for a broad range of acidic and basic compounds.

Tanaka *et al.* investigated the CE enantiomer separation of NSAIDs by using dextrin as chiral selector. Successful CE separation has been reported on naptroxen, ibuprofen and flurbiprofen enatiomeres [49].

Branched polysaccharides

If linear polysaccharides contain only α-1,4 bonds, there are polysaccharides which are branched by virtue of certain molecules being linked to a molecule *via* α-1,4 and another *via* α-1,6 glycosidic bonds. The rate at which these bonds appear may vary. The plant based amylopectin contains a branch every 30 units while the animal based glycogen contains a branch approximately every 10 units.

Chen *et al.* reported for the first time the use of branched polysaccharides as chiral selectors in CE. In his study glycogen belonging to the class of branched polysaccharides, was used as a novel chiral selector for the enantiomeric separations. Since glycogen is electrically neutral, the method is applicable to ionic compounds. Glycogen possesses not only high solubility, but also low viscosity in the water. Moreover, with the lack of aromatic rings in the structure, it exhibits very weak UV absorption. The potential of this chiral selector has been tested and demonstrated on 18 chiral compounds including 12 basic drugs and 6 acidic drugs. Among the tested compounds, the enantiomers of ibuprofen, which is an acidic drug, were successfully recognized by 3.0% w/v glycogen with 90mM Tris-H_3PO_4 buffer (pH 7.0) [9].

Antibiotics

Antibiotics are considered an important class of CSs, and their use in CE has been reported extensively. Glycopeptide antibiotics, is the most commonly used class of antibiotics. Vancomycin and eremomycin have been reported that were used as CS for the enantioseparation of some NSAIDs.

Eremomycin was added into the background electrolyte (BGE) and used as a CS for the enantioseparation of some NSAIDs (indoprofen, flurbiprofen, ketoprofen and fenoprofen) [25]. In this study, two different BGEs (aqueous BGE and water-methanol BGE) were used and their chiral separation performance was evaluated and compared. Eremomycin has amino groups; so, at low values of pH, it is adsorbed onto the capillary wall. On the other hand, the use of an organic-aqueous BGE reduces its adsorption onto the walls, when reversed polarity CE mode is applied, and the analysis time is shorter. After method optimization and validation, Lebedeva *et al.* determined ketoprofen in a sample of Bystrumgel®. It was found that a racemic mixture of ketoprofen was contained in the sample.

Zhang *et al.* used vancomycin in combination with amino acid ester-based chiral ionic liquid (AAIL) as additives (L-AlaC4NTf2, and L-ValC4NTf2) for the enantioseparation of non-steroidal anti-inflammatory drugs (NSAIDs). They demonstrated that the addition of L-AlaC4NTf2 and LValC4NTF2 into the BGE improved the chiral separation of NSAIDs drugs since the resolution was better than that obtained when vancomycin was used as the sole CS. In this report, pH was considered an important factor for the enantioseparation, because it affected the dissociation of the carboxyl groups of the NSAIDs, the carboxyl and amino groups in vancomycin and the amino group in the chiral ionic ligands (CILs). The concentrations of CIL and vancomycin in the BGE were also evaluated. As the first concentration increased from 0 to 15 mM, the RS increased, while in the case of a CIL concentration of above 15 mM, the RS decreased due to the gradually saturated complexation. As far as the vancomycin concentration is concerned, a concentration of 2 - 3 mM proved to be the optimum, since a higher concentration resulted in longer migration times due to the adsorption of vancomycin onto the capillary wall [58].

Other CSs

A great interest has been drawn these last years towards ionic liquids in analytical chemistry, especially in separation technology, and particularly in capillary electrophoresis.

François *et al.* studied the interactions in non-aqueous capillary electrophoresis (NACE), between an achiral IL (1-butyl-3-methylimidazolium bis-(trifluoromethylsulfonyl)imide) and a series of 2-arylpropionic acids. The results indicated a quadratic effect of the concentration of the achiral IL in the BGE on profen electrophoretic mobilities due to antagonistic interactions between anionic analytes and imidazolium cations either adsorbed to the capillary wall or free in the BGE electrolyte [18]. With a view to evaluate a new family of chiral selectors, another study by the same authors focused on the evaluation of two CILs (ethyl- and phenylcholine of bis(trifluoromethylsulfonyl)imide; $EtCholNTf_2$,$PhCholNTf_2$) by CE. No direct enantioselectivity was observed with regard to anti-inflammatory drugs 2-arylpropionic acids as model analytes. Neutral CDs were used as chiral selectors and CILs as BGE additives. Results showed an increase in separation selectivity and resolution, suggesting synergistic effects [19].

There is a large number of chiral ionic liquids (CILs) that have been synthesized, however, only a few have successfully been applied in separation technology, mainly used as background electrolyte

additives, and secondary as chiral ligands and chiral selectors.

Wang *et al.* reported two studies in 2009 about the combined use of 2,3,6-tri-O-methyl-β-cyclodextrin (TM-β-CD) and the CIL-type surfactant N-undecenoxy-carbonyl-L-leucinol bromide (L-UCLB), which formed micelles in aqueous BGEs, for the enantio-separation of anionic profens. In the first study different parameters were altered, such as the CIL concentration and chain length, in order to optimize the simultaneous enantioseparations of fenoprofen, ibuprofen, ketoprofen, suprofen, and indoprofen. The use of L-UCLB as a single chiral selector did not result in any enantioseparation, while the association between the CIL and the CD resulted in a baseline enatioseparation of almost all analytes (except from ibuprofen), and in an improvement in peak efficiency [54]. Their second study, was an investigation of the interactions among TM-β-CD, L-UCLB and profens in affinity CE. Fenoprofen was used as a model. The analyte enantiomers have different binding constant values due to the synergistic effect of the chiral selectors, which, in turn, results in an effective enantioseparation [55].

A similar study was performed by Zhang *et al.* using the amino acid ester-based chiral ionic liquids (AAILs) L-alanine tert butyl ester bis (trifluoro-methane) sulfonamide, L-AlaC_4NTf_2, and L-valine tert butyl ester bis (trifluoromethane) sulfonimide, L-ValC_4NTf_2 as BGE additives and β-CD derivatives (methyl-β-CD, hydropropyl-β-CD, glucose-β-CD) as chiral selectors in CE enantio-separations of six anionic racemic drugs. The synergistic effect was significant for half of the analytes examined, and particularly for naproxen, pranoprofen and warfarin. Another important observation, in this study, was the improvement of both resolution and effective selectivity factor with the addition of an organic component (ethanol or acetonitrile), possibly due to a decrease in electro-osmotic mobility, which, in turn, increases the interactions between the AAIL, CDs and analyte [57].

In a more recent study Zhang *et al.* used the vancomycin-based synergistic system with AAIL (L-AlaC_4NTf_2, and L-ValC_4NTf_2) as additives for the enantioseparation of five anti-inflammatory drugs by using CE. All enantioseparations were again significantly improved when the binary systems were used, and the resolution values were much higher than in the case where vancomycin was used as the sole chiral selector [58].

Even though application of chiral ionic liquids is still in its early stages, the scientific interest is increasing dramatically. The use of CILs as single chiral selector represents a new trend.

An attempt was made by Tran *et al.* to use a chiral ionic liquid (CIL), S-[3-(chloro-2-hydroxypropyl)-trimethylammonium][bis((trifluoromethyl)sulfonyl) amide], (S-[CHTA]+[Tf_2N]-) as chiral selector for CE. The results obtained seem to suggest that additional chiral selector(s) are needed to provide the three-point interactions needed for chiral separations. In the case of ibuprofen, a second chiral selector, namely a chiral anion (sodium cholate), is needed, and for furbiprofen, in addition to S-[CHTA]+[Tf_2N]− and sodium cholate, a third and neutral chiral selector, 1-S-octyl-β-d-thio-glucopyranoside (OTG), is also needed for the chiral separation. It is interesting to mention that no separation was obtained when sodium cholate was used as the sole chiral selector [52].

Recently Mavroudi *et al.* investigated the separation of five 2-arylpropionic acids, non-steroidal anti-inflammatory drugs (indoprofen, carprofen, ketoprofen, ibuprofen and flurbiprofen). It is important to mention that non-enatioselective separation was obtained by supporting the BGE either with sodium dodecyl sulphate (SDS) or an AAIL-(L-AlaC_4Lac). The performance of these additives was evaluated by comparing migration times, efficiencies and %RSD values. Finally, in an attempt to study the synergistic effect of SDS and AAIL, the results were similar to the ones obtained when SDS was used as the sole additive [30].

Dual selector systems

In some cases, the use of a single CS in the BGE cannot achieve partial or baseline separation. Therefore, the combination of two CSs may improve the enantioseparations. The most common dual selector systems include different CDs, usually a combination of a ionic with neutral CD derivatives [17] or with native CDs, more rarely two charged CDs [1] or two neutral CD derivatives [33]. Such a combination can lead to higher resolution due to the differences in the complexation mechanisms of the two CDs with the enantiomers, regarding the stability of complexation, the chiral recognition pattern, and the effect on analyte mobility.

In the study by Petr *et al.*, a dual selector system (S-β-CD and TM-β-CD), in combination with sodium dodecyl sulphate (SDS), was used for the chiral separation of ketoprofen enantiomers. The method was also applied for the determination of ketoprofen enantiomers in waste water samples by using simple filtration as a clean-up step. It was clearly demonstrated from this study that the addition of an extra modifier in a dual selector system is some-times crucial for successful enantioseparations [36].

Concluding remarks

Numerous chiral selectors have been used over the years, in CE for the chiral separation of NSAIDs. As demonstrated, in this review, even though CD derivatives are the most widely applied CSs in

electrophoretic separations, the search for new CSs still continues and the studies presented show a glimpse of a promising future. The successful use of linear oligo- and polysaccharides, branched polysaccharides, antibiotics, different dual selector systems as CSs and chiral surfactants and amino acid ester-based chiral ionic liquid as BGE additives in CE chiral separation of NSAIDs are also presented in this article. Overall, considering the large number of applications reported, we can conclude that CE enantioseparations have great potential for routine industrial separations.

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