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Selection of background electrolyte for CZE analysis by a chemometric approach Part I. Separation of a mixture of acidic non-steroidal anti-inflammatory drugs

Sandra Furlanetto [a,*], Silvia Lanteri [b], Serena Orlandini [a], Roberto Gotti [c], Iacopo Giannini [a], Sergio Pinzauti [a]

[a] *Department of Pharmaceutical Sciences, University of Florence, Via U. Schiff 6, 50019 Sesto Fiorentino, Florence, Italy*
[b] *Department of Chemistry and Technology of Drugs and Foods, University of Genoa, Via Brigata Salerno (s/n), 16147 Genoa, Italy*
[c] *Department of Pharmaceutical Sciences, University of Bologna, Via Belmeloro 6, 40126 Bologna, Italy*

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Abstract

This paper is the first part of the presentation of a chemometric approach for the rapid selection of a suitable background electrolyte (BGE) in CZE analysis of small drug molecules. The strategy is based on principal component analysis and experimental design. In this first section, the approach is applied to the analysis of a mixture of six arylpropionic anti-inflammatory drugs. Initially, 222 possible aqueous background electrolytes (objects) were characterized using as descriptors pH, conductivity, ionic strength and relative viscosity. In order to allow the dissociation of the acidic analytes, this original data set was reduced to 154 background electrolytes with pH values higher than or equal to 5. Principal component analysis made it possible to graphically represent the new set of objects, described by the four variables, in a two-dimensional space. Among these electrolytes, Kennard–Stone algorithm selected ten objects to be tested by CZE, covering homogeneously principal component space. CZE analyses were carried out with the selected electrolytes, and 0.1 M borax was identified as the most suitable one for the specified application. Finally, the characteristics of the analysis were finely tuned by means of a response surface study, which allowed the best conditions to be determined: borax concentration, 0.09 M; methanol, 6% (v/v); temperature, 24 °C, voltage, 20 kV. Applying these conditions, a baseline resolution among the six compounds was obtained in less than 10 min.

Keywords: Background electrolyte selection; CZE; Experimental design; Non-steroidal anti-inflammatory drugs; Principal component analysis

1. Introduction

In CZE, the background electrolyte (BGE) forms the chemical environment where separation takes place [1]. The electrophoretic mobility of an ion takes into account the environment where the ion exists during the separation process and also the electroosmotic flow (EOF) is influenced by the nature of the background electrolyte [2–6]. In fact, the electroosmotic flow depends on many parameters, such as zeta potential, dielectric constant and buffer viscosity, which in turn depend on the composition of the background electrolyte (pH, ionic strength, organic modifier) [4]. Modulation of EOF can be very useful and even necessary because EOF affects the amount of time a solute takes to migrate through the capillary, and therefore it can affect both efficiency and resolution indirectly [3,4]. Thus, the choice of BGE composition during method development gives the analyst a powerful tool to implement separation performances (*i.e.* selectivity, efficiency, analysis time) [1,7,8]. Also considering only aqueous medium, there are many possible BGEs, thus selection can be a time consuming step. Gaš et al. [9] proposed a mathematical and computational model to optimize background electrolytes for CZE of anions, but to our knowledge, no paper regarding a general approach for the selection of BGE has been reported in literature until now.

* Corresponding author. Tel.: +39 055 4573717; fax: +39 055 4573779.
E-mail address: sandra.furlanetto@unifi.it (S. Furlanetto).

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In general, knowledge of the structure, or more specifically the pK_a of the analytes, constitutes the starting point for the selection of an electrolyte with appropriate pH [2,7,10], but some other physico–chemical BGE characteristics can be relevant for an electrophoretic analysis: viscosity, ionic strength and conductivity) [2,3,7,8,11,12].

The aim of this work, which is divided into two parts, was to develop a rational strategy for the choice of the BGE, based on chemometric techniques. This general approach can be briefly summarized as follows: (i) characterization of a large set of possible BGEs by appropriate physico–chemical descriptors; (ii) reduction of the original data set considering the characteristics of the specific CZE analysis; (iii) visualization of the new data set by means of principal component analysis; (iv) selection of a limited number of BGEs to be tested in CZE capable of covering principal component space; (v) choice of a suitable BGE; (vi) response surface methodology for finding the global optimum conditions.

The first step in this study was the characterisation of a relevant and representative number of aqueous type BGEs considering the descriptors pH, ionic strength and two basic parameters of electroanalytical significance, conductivity and relative viscosity [13].

The pH of the BGE has a key role in capillary zone electrophoresis, since it contributes to the degree of dissociation of weak acids or bases, that each in turn, influences their effective mobility [2,7,14,15]. In addition, over the pH range 3–11 the electroosmotic flow can range ten-fold in untreated capillaries [5].

The conductivity (κ) of the BGE is also important for a CZE analysis [3]. In a capillary, the charge from electrode to electrode is conducted by the BGE and variations in its conductivity influence the EOF and electrophoretic mobility [16]. Conductivity variations can influence the migration time of analytes also under well controlled conditions [17]. Moreover, the generated current is determined by the BGE conductivity in addition to capillary dimension and applied voltage [18]. Finally, differences in sample zone and BGE conductivity can determine skewed peak shapes, solute concentration or defocusing [3,18]. Theoretically, the specific conductivity of a BGE, consisting of strong electrolytes, is given as $\sigma = F\sum_{i=1}^{n} c_i|z_i m_i|$, where F is the Faraday's constant and c_i, z_i and m_i are the concentrations, valences and mobilities of all ionic forms present in the BGE [6]. Thus, conductivity is related to the ionic strength of the BGE, but it also depends on the type of co-ions and counter-ions.

The effect of ionic strength (μ) [19] on selectivity and efficiency has been extensively described [8]. Ionic strength modulates the EOF and electrophoretic mobility of the analyte [2–4,7,10,18]. Additionally, buffer concentrations can modulate the effective charge at the capillary wall and thus the interactions between the wall and the solute [18].

The relative viscosity (η) of the BGE should also be considered since the translation movement of ions during CZE analysis is opposed by a retarding frictional force [2,3,8]. In particular, the electrophoretic and electroosmotic mobilities are inversely proportional to the viscosity of the surrounding medium [4,8,12].

The four selected variables allowed the electrolytes to be described in function of their own characteristics, independently from the conditions of analysis and the type of analyte. Other potential factors, such as ion association between analytes and buffer components, supramolecular interactions, stability constants of inclusion complexes either of chiral or achiral analytes, and many others that can influence the complex electrophoretic phenomena, were not considered as they are related to each analyte. This may help in understanding experimental findings and simplifies their interpretation, because the a priori identification for a non-bonding intermolecular interaction between a background electrolyte component and a particular analyte may be a difficult task.

In order to jointly consider all pertinent properties characterizing each BGE, multivariate methods [20] were used. The present approach involved principal component analysis (PCA) [21] and experimental design [22] and was set up with the aim of being of general utilization for CZE analysis of small drug molecules. Principal components can be associated with physico–chemical information, thus the characteristics of the BGEs can be described according to their principal properties [23]. Kennard–Stone algorithm [24] was used for the selection of a set of electrolytes to be screened by CZE, capable of homogeneously covering principal component space.

The versatility of this approach was verified considering two different applications in the field of drug analysis, where capillary electrophoresis is now recognized as an alternative and complement to HPLC: the separation of acidic drugs, presented in this first part of the study, and the separation of basic drugs, which is the subject of the second part.

2. Materials and methods

2.1. Chemicals

All chemicals and reagents used were of analytical-reagent grade with no further purification. All the anti-inflammatory drugs (fenoprofen (FEN), flurbiprofen (FLU), indoprofen (IND), ketoprofen (KET), naproxen (NAP), suprofen (SUP)) were purchased from Sigma–Aldrich (St. Louis, MO, USA). All the substances used to prepare background electrolytes, reported in Table 1, were from Sigma–Aldrich. Methanol was obtained from Riedel-de-Haën (Seelze, Germany). Ultrapure water was used throughout the study and was obtained with a Milli-Q system (Millipore/Waters, Milford, MA, USA).

2.2. Solutions

Standard stock solutions of the anti-inflammatory drugs (about 5.0×10^{-4} M) were prepared in methanol. Working standard solutions were prepared by diluting 1:10 with methanol (up to a concentration of about 5.0×10^{-5} M for all the analytes). All the standard stock solutions were stored at 4 °C and used within one week, while all the working standard solutions were prepared daily. Compositions of all considered BGEs (pH range 2–12) are reported in Table 1. The standard run buffer used for

Table 1
Composition of the 222 considered background electrolytes

	Background electrolyte,acronym when appropriate	Composition	x (mL)	y (mL)
1	W09A	Walpole: 20 mL 0.1 M sodium acetate + x mL 0.1 M HCl made up to 100 mL	20.4	
2	W14A		18.0	
3	W16A		16.0	
4	W20A		8.0	
5	W22A		4.0	
6	W01B	Walpole: mixtures of x mL 0.2 M acetic acid and y mL 0.2 M sodium acetate	90	10
7	W02B		80	20
8	W03B		70	30
9	W04B		60	40
10	W05B		50	50
11	CL01B	Clark and Lubs: 50 mL 0.1 M KH_2PO_4 + x mL 0.1 M NaOH made up to 100 mL	46.85	
12	CL02B		42.74	
13	CL04B		34.90	
14	CL06B		23.60	
15	CL08B		12.60	
16	CL09B		8.55	
17	CL11B		3.66	
18	CL01C	Clark and Lubs: 50 mL 0.01 M boric acid (0.01 M KCl) + x mL 0.01 M NaOH made up to 100 mL	1.30	
19	CL04C		4.25	
20	CL07C		10.65	
21	CL10C		20.40	
22	CL01D	Clark and Lubs: 50 mL 0.1 M boric acid (0.1 M KCl) + x mL 0.1 M NaOH	2.61	
23	CL02D		3.97	
24	CL04D		8.50	
25	CL06D		16.30	
26	CL08D		26.70	
27	CL10D		36.85	
28	CL12D		43.90	
29	BW01A	Britton and Welford: 50 mL 0.2 N citric acid neutralised with x mL 0.2 M NaOH	0.0	
30	BW03A		5.0	
31	BW04A		7.5	
32	BW05A		10.0	
33	BW07A		15.0	
34	BW09A		20.0	
35	BW11A		25.0	
36	BW13A		30.0	
37	BW15A		35.0	
38	BW17A		40.0	
39	BW19A		45.0	
40	BW20A		47.5	
41	BW01B	Britton and Welford: 50 mL 0.1 M KH_2PO_4 neutralised with x mL 0.2 M NaOH	0.0	
42	BW02B		2.5	
43	BW05B		10.0	
44	BW08B		17.5	
45	BW09B		20.0	
46	BW10B		22.5	
47	BW11B		25.0	
48	BW12B		27.5	
49	MI01A	McIlvaine: mixtures of x mL 0.1 M citric acid and y mL of 0.2 M Na_2HPO_4	19.60	0.40
50	MI02A		18.76	1.24
51	MI03A		17.82	2.18
52	MI05A		15.89	4.11
53	MI07A		14.30	5.70
54	MI08A		13.56	6.44
55	MI10A		11.72	8.28

Table 1 (*Continued*)

	Background electrolyte,acronym when appropriate	Composition	*x* (mL)	*y* (mL)
56	BR01A	Britton and Robinson: titration of 50 mL of modified universal buffer mixture with *x* mL 0.2 M NaOH	0.00	
57	BR02A		1.25	
58	BR05A	Universal buffer mixture: 50 mL of a solution of mixed acids, being 0.04 M H_3PO_4, 0.04 M acetic acid and 0.04 M boric acid	5.00	
59	BR06A		6.25	
60	BR08A		8.75	
61	BR09A		10.00	
62	BR11A		12.50	
63	BR13A		15.00	
64	BR15A		17.50	
65	BR17A		20.00	
66	BR18A		21.25	
67	BR19A		22.50	
68	BR20A		23.75	
69	BR21A		25.00	
70	BR24A		28.75	
71	BR26A		31.25	
72	BR28A		33.75	
73	BR30A		36.25	
74	BR32A		38.75	
75	BR36A		43.75	
76	BR41A		50.00	
77	K01A	Kolthoff: mixtures of *x* mL 0.1 M KH_2PO_4 and *y* mL 0.1 M borax	55.26	4.74
78	K03A		49.80	10.20
79	K05A		43.32	16.68
80	K06A		40.02	19.98
81	K07A		37.38	22.62
82	K08A		33.00	27.00
83	K13A		23.22	36.78
84	K17A		3.00	57.00
85	Succinic ac. A	0.05 M succinic acid		
86	Succinic ac. B	0.01 M succinic acid		
87	K01B	Kolthoff: mixtures of *x* mL 0.05 M succinic acid and *y* mL 0.05 M borax	96.5	3.5
88	K02B		86.3	13.7
89	K03B		73.8	26.2
90	K04B		63.2	36.8
91	K05B		55.7	44.3
92	Na_2CO_3 A	0.05 M Na_2CO_3		
93	Na_2CO_3 B	0.01 M Na_2CO_3		
94	K01C	Kolthoff: 50 mL 0.1 M Na_2CO_3 + *x* mL 0.1 M HCl made up to 100 mL	20	
95	K02C		10	
96	K03C		3	
97	S01A	Sørensen: mixtures of *x* mL 0.05 M borax and *y* mL 0.05 M HCl	31.5	28.5
98	S02A		33.0	27.0
99	S03A		34.5	25.5
100	S07A		45.0	15.0
101	S11A		57.0	3.0
102	S01B	Sørensen: mixtures of *x* mL 1/15 M KH_2PO_4 and *y* mL 1/15 M Na_2HPO_4	54	6
103	S05B		30	30
104	S06B		24	36
105	S09B		6	54
106	S04C	Sørensen: mixtures of *x* mL of 0.1 M glycine (0.1 M NaCl) and *y* mL of 0.1 M HCl	50	50
107	S05C		60	40
108	S06C		80	20
109	S07C		95	5
110	S01D	Sørensen: mixtures of *x* mL of 0.1 M glycine (0.1 M NaCl) and *y* mL of 0.1 M NaOH	97.5	2.5
111	S02D		95.0	5.0

Table 1 (*Continued*)

	Background electrolyte,acronym when appropriate	Composition	*x* (mL)	*y* (mL)
112	S03D		90.0	10.0
113	S04D		80.0	20.0
114	S05D		70.0	30.0
115	S06D		60.0	40.0
116	Na_2Hcitrate A	0.05 M disodium hydrogen citrate		
117	Na_2Hcitrate B	0.01 M disodium hydrogen citrate		
118	GV01A	German and Vogel: *x* mL 0.01 M sodium hydrogen succinate (0.005 M succinic acid + 0.005 M sodium succinate) and *y* mL 0.01 M sodium succinate	97.6	2.4
119	GV02A		80.8	19.2
120	GV03A		49.4	50.6
121	GV04A		24.0	76.0
122	GV05A		11.2	88.0
123	P01A	Palitzsch: mixtures of *x* mL 0.2 M boric acid and *y* mL 0.05 M borax	94	6
124	P02A		80	20
125	P03A		65	35
126	P04A		40	60
127	P05A		10	90
128	NT01B	Naegeli and Tyabji: 100 mL 0.05 M borax + *x* mL 0.1 M NaOH	66.6	
129	NT02B		99.6	
130	NT03B		119.6	
131	NT04B		150.3	
132	BW01C	Britton and Welford: 100 mL 0.2 N boric acid neutralised with *x* mL NaOH 0.2 M	10	
133	BW02C		30	
134	BW03C		50	
135	H_3PO_4 A	0.05 M H_3PO_4		
136	H_3PO_4 B	0.01 M H_3PO_4		
137	KH_2PO_4A	0.05 M KH_2PO_4		
138	KH_2PO_4B	0.01 M KH_2PO_4		
139	Na_2HPO_4 A	0.05 M Na_2HPO_4		
140	Na_2HPO_4 B	0.01 M Na_2HPO_4		
141	$NH_4H_2PO_4$ A	0.05 M $NH_4H_2PO_4$		
142	$NH_4H_2PO_4$ B	0.01 M $NH_4H_2PO_4$		
143	$(NH_4)_2HPO_4$ A	0.05 M $(NH_4)_2HPO_4$		
144	$(NH_4)_2HPO_4$ B	0.01 M $(NH_4)_2HPO_4$		
145	KH_2PO_4/H_3PO_4A	0.1 M KH_2PO_4/0.01 M H_3PO_4		
146	KH_2PO_4/H_3PO_4B	0.05 M KH_2PO_4/0.005 M H_3PO_4		
147	H_3PO_4/KH_2PO_4 A	0.04 M H_3PO_4/0.01 M KH_2PO_4		
148	H_3PO_4/KH_2PO_4 B	0.025 M H_3PO_4/0.025 M KH_2PO_4		
149	H_3PO_4/KH_2PO_4 C	0.01 M H_3PO_4/0.04 M KH_2PO_4		
150	KH_2PO_4/H_3PO_4 C	0.05 M KH_2PO_4/0.05M H_3PO_4		
151	KH_2PO_4/H_3PO_4 D	0.01 M KH_2PO_4/0.01M H_3PO_4		
152	CH_3COOH A	0.05 M CH_3COOH		
153	CH_3COOH B	0.01 M CH_3COOH		
154	CH_3COONa A	0.05 M CH_3COONa		
155	CH_3COONa B	0.01 M CH_3COONa		
156	CH_3COONH_4 A	0.05 M CH_3COONH_4		
157	CH_3COONH_4 B	0.01 M CH_3COONH_4		
158	CH_3COONH_4/CH_3COOH A	0.05 M CH_3COONH_4/0.005 M CH_3COOH		
159	CH_3COONH_4/CH_3COOH B	0.01 M CH_3COONH_4/0.001M CH_3COOH		
160	CH_3COONa/H_2SO_4 A	0.1 M CH_3COONa/0.01 M H_2SO_4		
161	CH_3COONa/H_2SO_4 B	0.05 M CH_3COONa/0.005 M H_2SO_4		
162	CH_3COONa/H_3PO_4 A	0.1 M CH_3COONa/0.01 M H_3PO_4		
163	CH_3COONa/H_3PO_4 B	0.05 M CH_3COONa/0.005 M H_3PO_4		
164	CH_3COONa/H_3PO_4 C	0.1 M CH_3COONa/0.05 M H_3PO_4		
165	KCl A	0.05 M KCl		
166	KCl B	0.01 M KCl		
167	LiCl A	0.05 M LiCl		
168	LiCl B	0.01 M LiCl		
169	NaCl A	0.05 M NaCl		
170	NaCl B	0.01 M NaCl		
171	NH_4Cl A	0.05 M NH_4Cl		

Table 1 (*Continued*)

	Background electrolyte,acronym when appropriate	Composition	*x* (mL)	*y* (mL)
172	NH_4Cl B	0.01 M NH_4Cl		
173	NH_3/NH_4Cl B	0.1 M NH_3/0.1 M NH_4Cl		
174	NaOH/KCl B	0.01 M NaOH/0.01 M KCl		
175	K_2CO_3 A	0.05 M K_2CO_3		
176	K_2CO_3 B	0.01 M K_2CO_3		
177	$NaHCO_3$ A	0.05 M $NaHCO_3$		
178	$NaHCO_3$ B	0.01 M $NaHCO_3$		
179	KNO_3 A	0.05 M KNO_3		
180	KNO_3 B	0.01 M KNO_3		
181	HCOOH A	0.05 M HCOOH		
182	HCOOH B	0.01 M HCOOH		
183	Citric ac. A	0.05 M Citric acid		
184	Citric ac. B	0.01 M Citric acid		
185	Na citrate A	0.05 M Sodium citrate		
186	Na citrate B	0.01 M Sodium citrate		
187	H_3BO_3 A	0.05 M Boric acid		
188	H_3BO_3 B	0.01 M Boric acid		
189	H_3BO_3/borax A	0.1 M Boric acid, 0.03 M borax		
190	H_3BO_3/borax B	0.1 M Boric acid, 0.01 M borax		
191	Borax A	0.05 M Borax		
192	Borax B	0.01 M Borax		
193	Borax C	0.1 M Borax		
194	Borax/NaCl A	0.05 M Borax/0.05 M NaCl		
195	Borax/NaCl B	0.05 M Borax/0.01 M NaCl		
196	TRIZMA HCl A	0.05 M TRIS HCl		
197	T01A	0.0445 M TRIS HCl/0.0055 M TRIS		
198	T02A	0.0363 M TRIS HCl/0.0137 M TRIS		
199	T03A	0.0255 M TRIS HCl/0.0245 M TRIS		
200	T04A	0.0140 M TRIS HCl/0.0360 M TRIS		
201	T05A	0.0048 M TRIS HCl/0.0452 M TRIS		
202	TRIZMA A	0.05 M TRIS		
203	TRIZMA HCl B	0.01 M TRIS HCl		
204	T01B	0.0089 M TRIS HCl/0.0011 M TRIS		
205	T02B	0.00726 M TRIS HCl/0.00274 M TRIS		
206	T03B	0.0051 M TRIS HCl/0.0049 M TRIS		
207	T04B	0.0028 M TRIS HCl/0.0072 M TRIS		
208	T05B	0.00096 M TRIS HCl/0.00904 M TRIS		
209	TRIZMA B	0.01 M TRIS		
210	TRIZMA HCl 01A	0.05 M TRIS HCl/0.01 M NaOH		
211	TRIZMA HCl 03A	0.05 M TRIS HCl/0.04 M NaOH		
212	TRIZMA HCl 01B	0.01 M TRIS HCl/0.002 M NaOH		
213	TRIZMA HCl 03B	0.01 M TRIS HCl/0.008 M NaOH		
214	TRIZMA 01A	0.05 M TRIS/0.01 M HCl		
215	TRIZMA 03A	0.05 M TRIS/0.04 M HCl		
216	TRIZMA 01B	0.01 M TRIS/0.002 M HCl		
217	TRIZMA 03B	0.01 M TRIS/0.008 M HCl		
218	TRIS BORATE A	0.05 M TRIS/0.05 M boric acid		
219	TRIS BORATE B	0.01 M TRIS/0.01 M boric acid		
220	TRIS PHOSPHATE A	0.05 M TRIS/0.05 M KH_2PO_4		
221	TRIS PHOSPHATE B	0.01 M TRIS/0.01 M KH_2PO_4		
222	TRIS CITRATE	0.01 M TRIS/(0.01/3) M citric acid		

the analysis of anti-inflammatory drugs consisted of an aqueous solution of 0.09 M borax containing 6% (v/v) methanol.

2.3. Equipment

A Metrohm 691 pH Meter (Metrohm, Herisau, Switzerland) was used to measure pH. A Model 120 Microprocessor Conductivity Meter (Analytical Control, Milan, Italy) was used to measure conductivity. Viscosity measurements were carried out with a glass Ubbelohde viscometer with a capillary diameter of 1 mm. The viscometer was arranged in a double-wall glass cylinder filled with water. The temperature was kept constant at 25.0 °C using a circulating thermal bath, model Haake D1 Fisons (Haake, Berlin, Germany). The relative viscosity of the electrolyte solutions was calculated by the ratio of the outflow time for the solution to the outflow time for water [25]. The val-

ues reported for pH, conductivity and relative viscosity of the electrolytes are the mean of three replicate measurements. The order of magnitude for the observed R.S.D. values were: pH, 0.1–0.5%; relative viscosity, 0.1–0.5%; conductivity, 0.1–1.0%. However, for this latter descriptor, R.S.D. values can reach 5–10% for BGE with low conductivity values.

A Spectra PHORESIS 1000 (Thermo Separation Products, Fremont, CA, USA) equipped with an UV-VIS DAD and driven by CE software (version 3.01) operating under IBM os/2™ (version 1.2) was used to carry out CZE experiments.

2.4. Capillary electrophoretic conditions

Fused silica capillaries (50 μm i.d., 375 μm o.d.) were purchased from Composite Metal Services (Ilkley, UK) and had a total length of 44 cm (36 cm to detector). Before use, a new capillary was flushed with 1 M NaOH and water for 5 min each. At the beginning of each day, the capillary was rinsed with 0.1 M NaOH for 5 min and then with water for 5 min. Between two runs, the capillary was flushed with water (1 min), 0.1 M NaOH solution (2 min), water (2 min) and run buffer (4 min).

Detection wavelength was 195 nm. The detection was towards the cathodic end and the detection window was built-in by burning off the polyimide coating on the capillary. Samples were injected hydrodynamically and the vacuum system of the instrument applied a constant negative pressure of 5.17 kPa for the injection (5 s). Initial screening of the electrolytes was carried out setting capillary temperature at 25 °C and voltage at 18 kV.

The final optimized conditions for the analysis of anti-inflammatory drugs were the following: BGE, 0.09 M borax containing 6% (v/v) methanol; voltage, 20 kV; temperature, 24 °C (generated current about 100 μA).

2.5. Calculations and software

Ionic strength μ was calculated by:

$$\mu = \frac{1}{2}\sum_{i=1}^{n} c_i z_i^2$$

where c_i is the molarity concentration of ion i, z_i is the charge of that ion, and the sum is taken over all ions in the solution.

Resolution values R were calculated according to the formula:

$$R = 1.18\left(\frac{t_{RB} - t_{RA}}{w_{1/2A} + w_{1/2B}}\right)$$

where t_{RA} and t_{RB} are the migration times and $w_{1/2A}$ and $w_{1/2B}$ the peak widths at half height of adjacent peak pairs [26].

The PARVUS software package [27] was used to perform principal component analysis. The NEMROD-W software package [28] was employed to select BGEs by means of Kennard–Stone algorithm, to generate experimental designs and to perform statistical analysis of the data.

3. Results and discussion

3.1. Characterisation of BGEs by physico–chemical descriptors

The first step of the work was the selection of variables able to describe an aqueous electrolyte solution in order to characterize the considered electrolytes. Four variables were chosen as BGE descriptors: pH, conductivity, ionic strength and relative viscosity.

A total of 222 electrolytes of different type, able to cover the pH range useful in a CZE analysis (2–12), were considered as BGEs (Table 1). For each electrolyte, the descriptors pH, conductivity and relative viscosity were experimentally measured, while ionic strength was calculated.

3.2. Reduction of the original data set for the analysis of anti-inflammatory drugs

After characterization of the electrolytes, a reduction of the original data set according to an appropriate pH range was made for the considered application, taking into account the pK_a of the considered analytes. This reduction was due to the fact that CZE requires charged compounds, and analytes that behave as weak electrolytes are ionized at different pH values depending on their pK_a [8,12,14,15].

The proposed approach was applied to the separation of six anti-inflammatory drugs, derivatives of arylpropionic acid: fenoprofen, pK_a 4.5 [29]; flurbiprofen, pK_a 4.35 [30]; indoprofen, pK_a 5.8 [29]; ketoprofen, pK_a 4.5 [29]; naproxen, pK_a 4.2 [29]; suprofen, pK_a 3.9 [29].

In order to allow the dissociation of the analytes, the original set of 222 buffers was reduced selecting BGEs with pH values higher than or equal to 5, thus constituting a new data set of 154 objects. The corresponding data matrix, composed of 154 rows (electrolytes) and four columns (descriptors), is reported in Table 2.

3.3. Visualisation of the data set by means of principal component analysis

Principal component analysis [21,31] was applied to the new set of selected BGEs, making it possible to describe the characteristics of the objects according to their principal properties [23]. A set of new mutually orthogonal variables (principal component, PC) was defined. The first two PCs of autoscaled data, PC1 and PC2, explained most of the variation of the data (80.4% of explained variance) and were significant according to the K correlation index [32], while PC3 retained 16.3% of the explained variance. The first two PCs contain the useful information without noise present in the data set, thus it was possible to use PC1 and PC2 for describing the objects.

The biplot of PC1 and PC2 is reported in Fig. 1. The 154 electrolytes, characterised by the four variables, were visualised in the two-dimensional space described by PC1 and PC2, making it possible to obtain information about similarity among the objects. In fact, BGEs with similar properties are close to each

Table 2
pH, conductivity (κ), ionic strength (μ) and relative viscosity (η) values of the selected background electrolytes with pH ≥ 5

	Background electrolyte, acronym when appropriate	pH	κ (mS cm^{-1})	μ (M)	η
1	W22A	5.25	1.430	0.020	1.011
2	CL01B	7.89	6.99	0.144	1.030
3	CL02B	7.58	6.68	0.135	1.026
4	CL04B	7.18	6.22	0.120	1.022
5	CL06B	6.78	5.48	0.097	1.022
6	CL08B	6.35	4.71	0.075	1.020
7	CL09B	6.14	4.43	0.067	1.021
8	CL11B	5.74	4.05	0.057	1.017
9	CL01C	7.46	1.180	0.005	1.009
10	CL04C	7.96	1.210	0.006	1.005
11	CL07C	8.54	1.260	0.007	1.004
12	CL10C	8.76	1.320	0.009	1.013
13	CL01D	7.56	10.58	0.100	1.007
14	CL02D	7.76	10.42	0.100	1.007
15	CL04D	8.22	9.98	0.100	1.007
16	CL06D	8.66	9.46	0.100	1.007
17	CL08D	9.07	8.98	0.100	1.009
18	CL10D	9.45	8.60	0.100	1.012
19	CL12D	9.85	8.34	0.100	1.013
20	BW15A	5.26	5.07	0.129	1.036
21	BW17A	5.70	5.50	0.155	1.037
22	BW19A	6.20	5.88	0.180	1.039
23	BW20A	6.66	6.04	0.189	1.040
24	BW02B	5.90	7.48	0.114	1.020
25	BW05B	6.63	8.08	0.150	1.018
26	BW08B	7.18	8.84	0.178	1.022
27	BW09B	7.42	8.92	0.186	1.026
28	BW10B	7.82	9.09	0.193	1.026
29	BW11B	9.87	9.16	0.200	1.026
30	BW12B	10.80	9.28	0.214	1.026
31	BR15A	5.17	3.17	0.052	1.030
32	BR17A	5.83	3.53	0.057	1.030
33	BR18A	6.16	3.71	0.063	1.030
34	BR19A	6.47	3.89	0.069	1.029
35	BR20A	6.65	4.09	0.075	1.031
36	BR21A	6.85	4.24	0.080	1.031
37	BR24A	7.70	4.75	0.095	1.029
38	BR26A	8.42	4.94	0.102	1.028
39	BR28A	9.01	5.11	0.104	1.028
40	BR30A	9.47	5.31	0.107	1.029
41	BR32A	9.92	5.45	0.110	1.030
42	BR36A	11.30	5.89	0.138	1.036
43	BR41A	11.90	6.90	0.134	1.040
44	K01A	6.05	7.55	0.124	1.013
45	K03A	6.53	7.92	0.151	1.025
46	K05A	7.04	8.31	0.183	1.030
47	K06A	7.43	8.42	0.200	1.033
48	K07A	7.76	8.53	0.200	1.033
49	K08A	8.26	8.56	0.200	1.033
50	K13A	8.77	8.41	0.200	1.039
51	K17A	9.24	8.41	0.200	1.049
52	K05B	5.59	3.01	0.060	1.017
53	Na_2CO_3 A	11.27	7.24	0.150	1.015
54	Na_2CO_3 B	10.89	1.73	0.030	1.009
55	K01C	10.12	7.23	0.130	1.016
56	K02C	10.53	7.19	0.140	1.015
57	K03C	10.98	7.16	0.150	1.016
58	S01A	8.59	3.52	0.053	1.014
59	S02A	8.63	3.54	0.055	1.015
60	S03A	8.67	3.64	0.058	1.017
61	S07A	8.94	4.13	0.075	1.022
62	S11A	9.14	4.62	0.095	1.025
63	S01B	5.93	5.28	0.08	1.024

Table 2 (*Continued*)

	Background electrolyte, acronym when appropriate	pH	κ (mS cm^{-1})	μ (M)	η
64	S05B	6.81	6.42	0.133	1.024
65	S06B	6.98	6.71	0.147	1.029
66	S09B	7.73	7.53	0.187	1.033
67	S01D	8.03	8.54	0.290	1.018
68	S02D	8.37	8.44	0.280	1.019
69	S03D	8.74	8.37	0.260	1.023
70	S04D	9.20	7.96	0.220	1.016
71	S05D	9.55	7.63	0.180	1.013
72	S06D	9.97	7.38	0.140	1.012
73	Na_2Hcitrate A	5.17	6.02	0.150	1.040
74	Na_2Hcitrate B	5.31	1.430	0.030	1.004
75	GV02A	5.04	0.890	0.010	1.007
76	GV03A	5.41	1.153	0.020	1.007
77	GV04A	5.86	1.396	0.030	1.006
78	GV05A	6.22	1.440	0.030	1.006
79	P01A	6.92	0.371	0.010	1.019
80	P02A	7.71	1.168	0.020	1.022
81	P03A	8.15	1.93	0.040	1.023
82	P04A	8.67	3.25	0.060	1.030
83	P05A	9.07	4.59	0.090	1.031
84	NT01B	9.88	5.27	0.100	1.026
85	NT02B	10.62	5.36	0.100	1.025
86	NT03B	11.73	5.97	0.100	1.023
87	NT04B	12.10	7.48	0.100	1.021
88	BW01C	7.73	1.086	0.020	1.021
89	BW02C	8.63	2.50	0.050	1.021
90	BW03C	9.13	3.55	0.070	1.021
91	Na_2HPO_4 A	9.02	6.07	0.150	1.048
92	Na_2HPO_4 B	8.77	1.440	0.030	1.027
93	$(NH_4)_2HPO_4$ A	7.98	6.97	0.150	1.020
94	$(NH_4)_2HPO_4$ B	7.88	1.68	0.030	1.007
95	CH_3COONa A	7.53	3.29	0.050	1.022
96	CH_3COONa B	7.12	0.750	0.010	1.018
97	CH_3COONH_4 A	6.60	4.19	0.050	1.018
98	CH_3COONH_4B	6.48	0.936	0.010	1.012
99	CH_3COONH_4/CH_3COOH A	5.49	3.91	0.050	1.022
100	CH_3COONH_4/CH_3COOH B	5.50	0.891	0.010	1.016
101	CH_3COONa/H_2SO_4 A	5.33	6.20	0.110	1.029
102	CH_3COONa/H_2SO_4 B	5.31	3.36	0.055	1.018
103	CH_3COONa/H_3PO_4 A	5.68	5.90	0.100	1.029
104	CH_3COONa/H_3PO_4 B	5.66	3.15	0.050	1.016
105	KCl A	5.40	5.42	0.050	1.015
106	KCl B	5.45	1.200	0.010	1.018
107	LiCl A	5.57	4.15	0.049	1.015
108	LiCl B	5.71	0.943	0.010	1.011
109	NaCl A	5.36	4.73	0.050	1.026
110	NaCl B	5.47	1.070	0.010	1.022
111	NH_4Cl A	5.29	5.65	0.050	1.013
112	NH_4Cl B	5.52	1.250	0.010	1.008
113	NH_3/NH_4Cl B	9.46	10.62	0.100	1.007
114	NaOH/KCl B	11.78	3.08	0.020	1.006
115	K_2CO_3 A	11.26	8.44	0.150	1.019
116	K_2CO_3 B	10.80	1.97	0.030	1.012
117	$NaHCO_3$ A	8.37	3.27	0.050	1.029
118	$NaHCO_3$ B	8.33	0.745	0.010	1.021
119	Na citrate A	7.72	8.91	0.250	1.024
120	Na citrate B	7.61	2.25	0.050	1.017
121	H_3BO_3 B	5.43	0.004	0.000	1.017
122	H_3BO_3/borax A	8.53	3.08	0.060	1.030
123	H_3BO_3/borax B	8.03	1.187	0.020	1.024
124	borax A	9.15	5.08	0.100	1.028
125	borax B	8.95	1.248	0.020	1.020
126	borax C	9.22	8.72	0.200	1.067
127	borax/NaCl A	9.07	8.81	0.150	1.042

Table 2 (*Continued*)

	Background electrolyte, acronym when appropriate	pH	κ (mS cm^{-1})	μ (M)	η
128	borax/NaCl B	9.13	5.85	0.110	1.043
129	T01A	7.07	3.44	0.040	1.020
130	T02A	7.57	2.81	0.040	1.017
131	T03A	8.01	2.00	0.030	1.017
132	T04A	8.45	1.180	0.010	1.020
133	T05A	9.01	0.430	0.005	1.022
134	TRIZMA A	10.19	0.048	0.000	1.017
135	TRIZMA HCl B	5.18	0.868	0.010	1.021
136	T01B	7.05	0.779	0.040	1.016
137	T02B	7.55	0.641	0.040	1.016
138	T03B	7.95	0.451	0.030	1.013
139	T04B	8.37	0.254	0.010	1.014
140	T05B	8.86	0.096	0.000	1.017
141	TRIZMA B	9.60	0.022	0.000	1.012
142	TRIZMA HCl 01A	7.39	3.95	0.050	1.015
143	TRIZMA HCl 03A	8.68	4.53	0.050	1.031
144	TRIZMA HCl 01B	7.43	0.900	0.010	1.013
145	TRIZMA HCl 03B	8.63	1.013	0.010	1.015
146	TRIZMA 01A	8.57	0.888	0.010	1.005
147	TRIZMA 03A	7.29	3.07	0.040	1.012
148	TRIZMA 01B	8.61	0.193	0.000	1.006
149	TRIZMA 03B	7.29	0.702	0.010	1.002
150	TRIS BORATE A	8.23	0.361	0.000	1.013
151	TRIS BORATE B	8.32	0.100	0.000	1.000
152	TRIS PHOSPHATE A	7.57	5.50	0.120	1.023
153	TRIS PHOSPHATE B	7.45	1.390	0.020	1.004
154	TRIS CITRATE	6.68	0.624	0.160	1.001

other in these plots, while BGEs whose properties are different are projected far from each other. Loadings for PC1 were: pH, 0.24673; κ, 0.60073; μ, 0.62188; η, 0.43762. Loadings for PC2 were: pH, 0.91102; κ, 0.01021; μ, −0.08788; η, −0.40277. From the graph it appears that the first principal component could be considered the direction of conductivity and ionic strength,

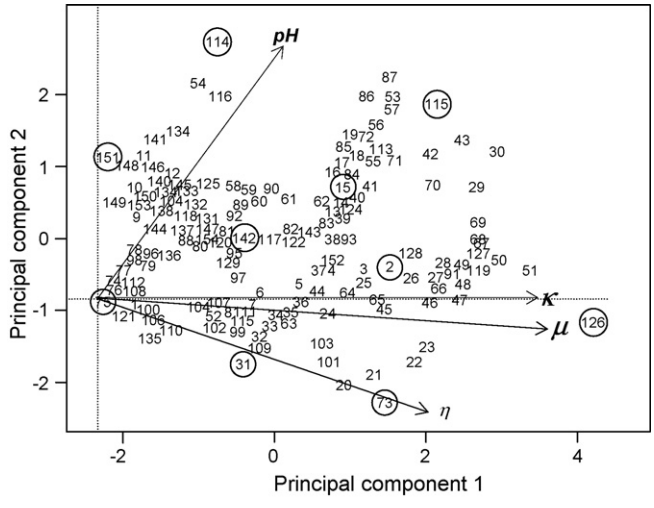

Fig. 1. Biplot on the first two principal components of 154 background electrolytes scores and loadings. Score axes are reported as abscissa and ordinate, loading axes are reported as dotted lines. Each index corresponds to a BGE (see Table 2). Variables are reported as: pH, κ (conductivity), μ (ionic strength), η (relative viscosity) and the arrows indicate the direction of the variables. The circles indicate the 10 BGEs to be tested in CZE, selected by means of Kennard–Stone algorithm.

the second one the pH direction, while relative viscosity could be associated with both directions. PCA points out that conductivity and ionic strength are highly correlated. This behaviour was expected, because, as already mentioned, the specific conductivity of the BGE is related to the concentration of the ions constituting the electrolyte. Anyway, it was considered useful to retain both the two variables, as chemical–physical considerations should be prevalent and the information is not the same. Finally, the plot reveals that objects are not homogenously distributed in the experimental space. This depends on the practical difficulty of preparing electrolytes with all possible combinations of the descriptors, thus the BGEs do not acquire all the possible values of the considered variables.

3.4. Selection of BGEs to be tested

In order to obtain general information about the suitable BGE properties for the considered analysis, a screening step involved the CZE testing of only some electrolytes, far from each other in the biplot since objects which are nearby have similar properties [23]. Therefore, it is fundamental to select a set of BGEs to be tested capable of covering homogeneously the experimental space, even if this latter has an irregular shape. This kind of problem can be solved by means of Kennard–Stone algorithm [24], which selects the points according to the Euclidean distance between them.

In the present study Kennard–Stone algorithm, included in experimental design software [28], was applied to the data matrix constituted by the 154 objects described by the scores on the first

Table 3
Ten BGEs selected by means of Kennard–Stone algorithm from the original data set of 154 electrolytes

	Background electrolyte	pH	κ (mS cm^{-1})	μ (M)	η
126	Borax C	9.22	8.72	0.200	1.067
151	TRIS BORATE B	8.32	0.100	0.000	1.000
115	K_2CO_3 A	11.26	8.44	0.147	1.019
31	BR15A	5.17	3.17	0.052	1.030
2	CL01B	7.89	6.99	0.144	1.030
114	NaOH/KCl B	11.78	3.08	0.020	1.006
142	TRIZMA HCl 01A	7.39	3.95	0.050	1.015
75	GV02A	5.04	0.89	0.014	1.007
73	Na_2Hcitrate A	5.17	6.02	0.150	1.040
15	CL04D	8.22	9.98	0.100	1.007

Electrolytes are coded with the acronym reported in Table 1 and the index reported in Table 2.

two principal components. In fact these principal components are significant according to K correlation index criterion [32], so it is better to use only the significant information (80.4% of the total variance) to select a set of BGEs excluding the noise and the useless information. Kennard–Stone algorithm selected a subset of objects to be tested as BGEs for the CZE analysis of the anti-inflammatory drugs. The subset was constituted by 10 objects in order to limit the number of electrophoretic analyses. The chosen points resulted to be as far as possible from each other, covering the space as uniformly as possible and thus containing different information. The composition and value of the descriptors of the 10 selected BGE is reported in Table 3 and their position in the biplot is indicated by circles (Fig. 1). The selected electrolytes are constituted by different buffering systems (borate, carbonate, phosphate, tris, succinate, citrate) and are highly different in the descriptors' values, covering all the range considered: pH, 5.04–11.78; k, 0.100–9.98 mS cm^{-1}; μ, 0.000–0.200 M; η, 1.000–1.067. Thus, as required, this set of BGE could represent a good starting point for a general screening effective for finding a suitable electrolyte for the analysis.

3.5. Choice of a suitable BGE

For each selected BGE, a CZE analysis was carried out applying the non-optimized experimental conditions 25 °C and 18 kV. These values were chosen taking into consideration the different nature of the electrolytes considered: higher values of temperature and voltage would lead to very high generated currents for the BGEs presenting high values of conductivity; on the other hand these values were high enough to assure a reasonable analysis time for BGEs with lower EOF. Peak identification was made using the spiking technique. Considering the resolution among the six analytes, the peak efficiency and analysis time, the "best" electropherogram was obtained using object 126, corresponding to 0.1 M borax (borax C, pH = 9.22, κ = 8.72 mS cm^{-1}, μ = 0.200 M, η = 1.067). Fig. 2a reports the obtained electropherogram, which reveals that it was possible to achieve a baseline resolution among the analytes with the exception of the pairs ketoprofen/suprofen (R = 1.34) and flunoxaprofen/naproxen (R = 1.38). Thus, it was decided to

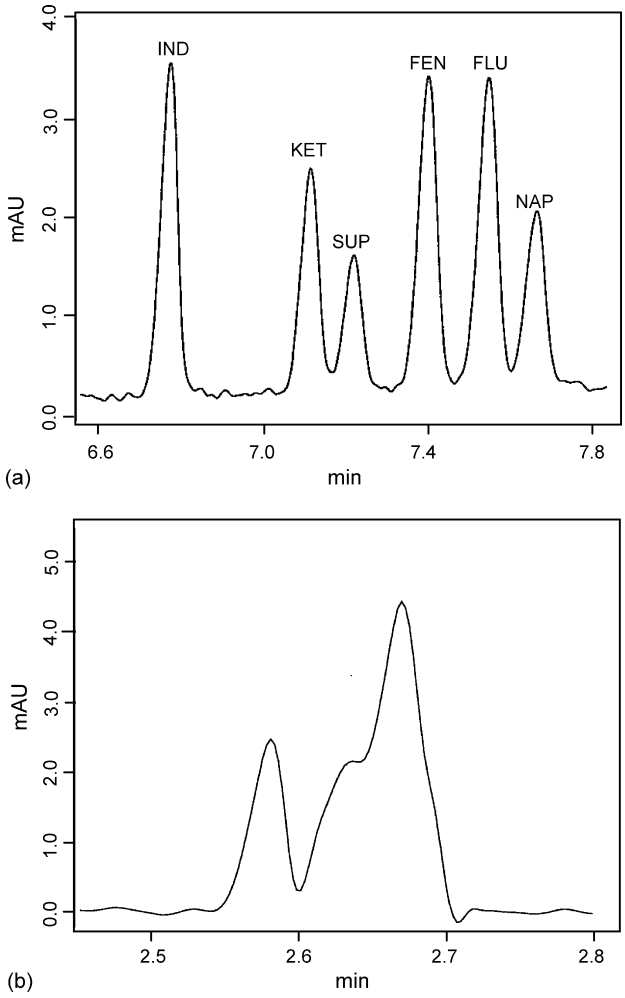

Fig. 2. Electropherograms of the anti-inflammatory drugs. Experimental conditions: temperature, 25 °C; voltage, 18 kV; background electrolyte: (a) object 126-Borax C (0.1 M borax), pH = 9.22, κ = 8.72 mS cm^{-1}, μ = 0.200 M, η = 1.067; (b) object 133-T05A (0.0048 M TRIS HCl/0.0452 M TRIS), pH = 9.01, κ = 0.430 mS cm^{-1}, μ = 0.005 M, η = 1.022.

choose this electrolyte as BGE to continue the study, also considering that this electrolyte was not near, and thus not similar, to any other object.

Moreover, it is possible to point out that other BGEs such as no. 128 (borax/NaCl B, pH = 9.13, κ = 5.85 mS cm^{-1}, μ = 0.110 M, η = 1.043) and no. 133 (T05A, pH = 9.01, κ = 0.430 mS cm^{-1}, μ = 0.005 M, η = 1.022), with similar pH to the chosen buffer but different values of the other descriptors, led to poorer responses. The electropherogram obtained using BGE 133, characterized by a low value of conductivity, is reported as example (Fig. 2b). This electropherogram shows an insufficient separation and confirms that electrolytes, apparently similar for pH value, can really give very different results. This bears out the importance of considering simultaneously all the considered factors for describing the characteristics of the BGE, and thus obtaining the resolution of a separation.

In addition, in order to verify that objects nearby in the plot have similar properties and show similar selectivity, object 151 was compared to the nearby object 133, and no. 23 to the nearby object 126. In the first case, as mentioned above, a poor electro-

Table 4
Doehlert design experimental plan and responses

No. exp.	BGE conc. (M)	MeOH conc. (%v/v)	V (kV)	T (°C)	R_2	R_5	t (min)
1	0.12	5	18	25	1.49	1.48	12.74
2	0.08	5	18	25	1.37	1.51	9.55
3	0.11	10	18	25	1.67	1.67	19.85
4	0.09	0	18	25	1.31	1.34	7.71
5	0.11	0	18	25	1.38	1.42	8.43
6	0.09	10	18	25	1.46	1.65	14.73
7	0.11	7	23	25	1.59	1.24	7.94
8	0.09	3	13	25	1.26	1.41	14.31
9	0.11	3	13	25	1.47	1.48	17.52
10	0.10	8	13	25	1.53	1.75	23.96
11	0.09	7	23	25	1.36	1.33	7.42
12	0.10	2	23	25	1.39	1.19	5.41
13	0.11	7	19	30	1.45	1.27	11.09
14	0.09	3	17	20	1.46	1.62	11.14
15	0.11	3	17	20	1.55	1.55	12.64
16	0.10	8	17	20	1.76	1.87	17.70
17	0.10	5	22	20	1.49	1.52	7.79
18	0.09	7	19	30	1.35	1.43	9.78
19	0.10	2	19	30	1.36	1.28	7.42
20	0.10	5	14	30	1.27	1.39	14.46
21	0.10	5	18	25	1.55	1.54	10.46
22	0.10	5	18	25	1.41	1.55	11.04
23	0.10	5	18	25	1.49	1.47	11.06
24	0.10	5	18	25	1.54	1.51	10.72

pherogram with very bad selectivity was obtained. On the other hand, object 23 gave an electropherogram with good selectivity, but the analysis time was longer than with object 126, strengthening the choice of this latter BGE. This bore out that nearby electrolytes also behave similarly from an electrophoretic point of view.

3.6. Response surface methodology

After selecting BGE 126, with the aim of improving the poorer resolution values between the pairs KET/SUP and FLU/NAP while maintaining a low analysis time, an experimental design [33–36], in particular, a response surface study, was carried out. The response surfaces describe the response variation with respect to factor variation and were estimated by means of a Doehlert design [22]. This design offers the advantage of high efficiency because the points of the Doehlert matrix are equal to $k^2 + k + n$, where k is the number of factors and n the number of central points. Another advantage is that factors are studied at various levels: one at three and one at five, while the remaining $k - 2$ factors at seven levels [22]. The researcher can choose the number of levels at which to study a factor depending on the possibility of dividing the experimental domain and as a function of the desired information.

The considered responses were critical resolutions values R_2 (KET/SUP) and R_5 (FLU/NAP) and analysis time (t), calculated as migration time of the last peak.

In addition to the typical instrumental electrophoretic parameters such as voltage and temperature, the possible addition of an organic modifier (methanol) was taken into account to finely tune the characteristics of the selected BGE. For the same reason, the effect of a limited change of BGE concentration on the responses was also considered. The investigated experimental domain was the following: borax concentration (BGE conc.), 0.08–0.12 M; methanol concentration (MeOH conc.), 0–10% (v/v); voltage (V), 13–23 kV; temperature (T), 20–30° C. The range for BGE conc. and MeOH conc. was quite small, in order to avoid to affect deeply the characteristics of the chosen BGE. Instead, for the instrumental parameters it was possible and preferable to inspect a wide range of values. The experimental plan (24 experiments) and the measured responses are reported in Table 4.

Statistical treatment of the obtained responses, by means of the analysis of variance [21], indicated that the quadratic regression models assumed were valid and significant. R^2 and Q^2 for the different calculated models were: R_2, $R^2 = 0.905$, $Q^2 = 0.283$; R_5, $R^2 = 0.962$, $Q^2 = 0.632$; t, $R^2 = 0.995$, $Q^2 = 0.950$.

Examining the response surfaces obtained, a high level of buffer and methanol concentration and a low level of voltage and temperature were required to maximise R_2. High levels of methanol concentration and low levels of all the other considered factors maximised R_5, for which a negative quadratic effect was evidenced for voltage. Finally, in order to minimise analysis time, high voltage and BGE concentration values and low methanol concentration values were required, evidencing a positive interaction between these last two factors.

Partial desirability functions [22], varying from 0 to 1 according to the closeness of the response to its target value, were associated to each response. The first requirement was to achieve a baseline resolution for all the peaks, and for the critical resolutions R_2 and R_5 the target value was 1.5. The fully desired analysis time was set below 10 min, with partially accepted values between 12 and 10 min. The overall desirability function

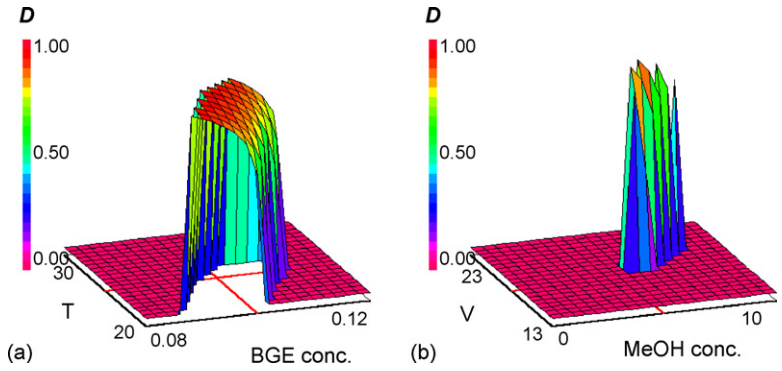

Fig. 3. Desirability function graphs obtained by plotting: (a) buffer concentration (BGE conc., M) *vs.* temperature (T, °C); (b) methanol concentration (MeOH conc., %v/v) *vs.* voltage (V, kV).

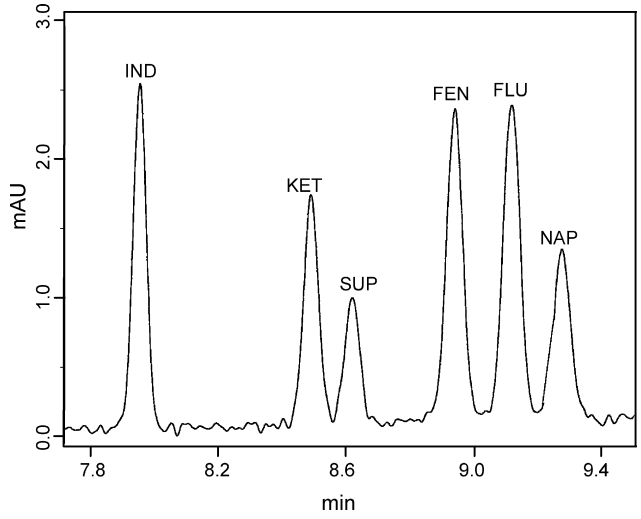

Fig. 4. Electropherogram of the anti-inflammatory drugs. Optimized experimental conditions: background electrolyte, 0.09 M borax; methanol, 6% (v/v); voltage, 20 kV; temperature, 24 °C.

(D) is calculated as the geometric mean of the partial desirability functions and its maximum corresponds to the experimental conditions able to give the best compromise among the responses. The graphical representation of D is depicted in Fig. 3, where D is shown for two factors at a time, setting the other two at their optimized values. The selected optimized conditions corresponded to borax concentration, 0.09 M; methanol, 6% (v/v); temperature, 24 °C; and voltage, 20 kV. Applying these conditions, a baseline resolution among the six compounds was obtained in less than 10 min (Fig. 4).

4. Conclusions

In this first part of the study, the selection of a suitable background electrolyte for the analysis of a set of acidic anti-inflammatory drugs has been efficiently performed by using a chemometric approach involving principal component analysis and experimental design. The results obtained can be used to draw some preliminary conclusions about the reliability of the presented approach. The four chosen descriptors were able to characterize the aqueous type electrolytes. The selection of ten representative objects described by the scores on the first two principal components was suitable to obtain information about the investigated experimental domain, allowing the best electrolyte for the real sample to be selected.

Further testing of the procedure is deemed necessary in order to verify its real versatility and usefulness. Thus, the second part of this study will continue the discussion about the proposed approach, presenting another application concerning the analysis of basic drugs.

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