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Determination of aminoglycoside antibiotics in pharmaceuticals by capillary zone electrophoresis with indirect UV detection coupled with micellar electrokinetic capillary chromatography

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ABSTRACT

Aminoglycoside antibiotics can be determined by capillary zone electrophoresis (CZE) with indirect UV detection in the anionic mode with a reversed electroosmotic flow (EOF) by addition of FC 135 to the background electrolyte. The effective mobilities of thirteen aminoglycoside antibiotics were determined as a function of pH. Applying CZE with indirect UV detection in the anionic mode and reversed EOF coupled with micellar electrokinetic capillary chromatography with the cationic surfactant cetyltrimethylammonium bromide, both neutral and charged antibiotics can be determined in combined pharmaceuticals. As an example, neomycin and hydrocortisone were determined in Otosporin eardorps.

INTRODUCTION

Capillary zone electrophoresis (CZE) has proved to be a highly efficient separation method, generally applicable for the determination of charged components. Using a UV detector, non-UV-absorbing components can also be detected in the indirect UV mode. Neutral components can be separated by micellar electrokinetic capillary chromatography (MECC), a hybrid technique, combining both chromatographic and electrophoretic separation principles. Since the introduction of MECC [1,2] and CZE [3,4], many components of pharmaceutical interest have been determined [5–10] using these techniques. No attention has been paid, however, to analyse for aminoglycoside antibiotics. So far, the non-UV-absorbing aminoglycoside antibiotics have been determined by, *e.g.*, ion-pair reversed-phase high-performance liquid chromatography using a refractive detector [11,12] and spectrometric methods using derivatization reagents [13].

Combined pharmaceuticals often contain both charged and neutral compounds, which may or may not be UV absorbing. In this work, we studied the possibilities of applying CZE with indirect UV detection for the determination of aminoglycoside antibiotics and CZE with indirect UV detection coupled with MECC for the determination of aminoglycoside antibiotics and neutral components in combined pharmaceuticals. In Fig. 1 the structural formulae of some representative aminoglycoside antibiotics are given.

EXPERIMENTAL

Instrumentation

For all experiments a P/ACE System 2000 HPCE instrument (Beckman, Palo Alto, CA, USA) was used. All experiments were carried out in a fused-

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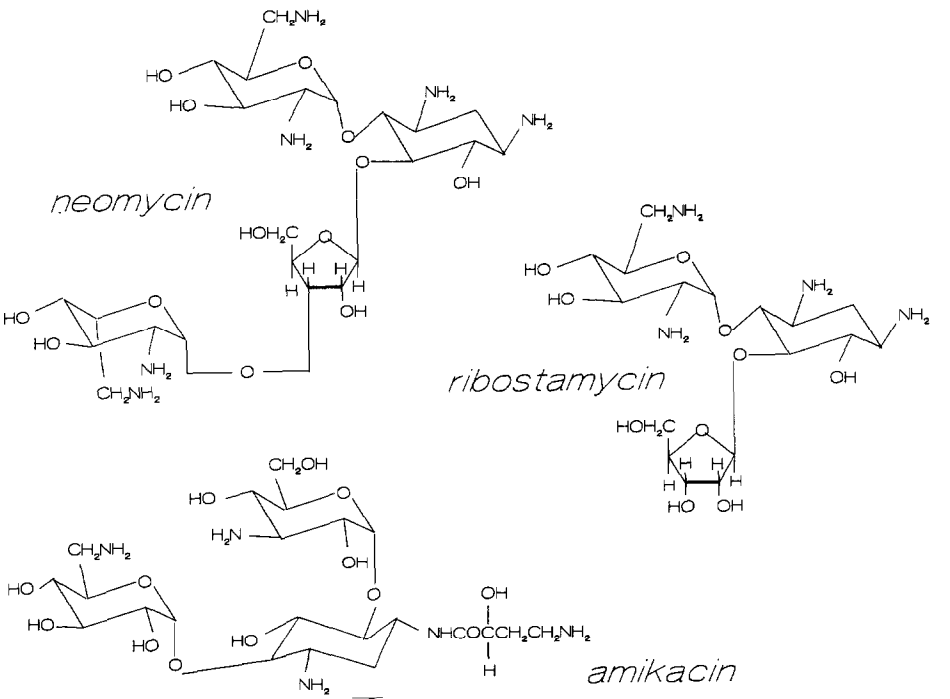

Fig. 1. Structural formulae of aminoglycoside antibiotics.

silica capillary from Siemens (Karlsruhe, Germany) of 50 μm I.D., total length 27 cm, distance between injection and detection 20 cm, or total length 67 cm, distance between injection and detection 60 cm. The wavelength of the UV detector was set at 214 nm. Data analysis was performed using the laboratory-written data analysis program CAESAR.

Chemicals

Amikacin dihydrate, gentamycin sulphate, streptomycin sulphate and tobramycin were obtained from Fluka (Buchs, Switzerland), butirosin disulphate salt, dibekacin sulphate salt, dihydrostreptomycin sesquisulphate salt, kanamycin B sulphate salt, lividomycin sulphate salt, neomycin sulphate, paromomycin sulphate, ribostamycin sulphate salt and sisomycin sulphate salt from Sigma (St. Louis, MO, USA), paracetamol from Merck-Schuchardt (Hohenbrunn, Germany), dexamethasone No. 85G30-50971 from De Onderlinge Pharmaceutische Groothandel (Utrecht, Netherlands), dapsone was donated by the State Institute for Quality Control of Agricultural Products (Wageningen, Netherlands), the fluorochemical surfactant FC 135 was obtained from Fluorad/3M (Leiden, Netherlands) and hydrocortisone from Aldrich (Brussels, Belgium).

Standard solutions

Standard solutions of the aminoglycoside antibiotics were prepared by weighing accurately 50.0 mg of the standards and dissolving them in 50.0 ml of a 100 m*M* cetyltrimethylammonium bromide (CTAB) solution (the stock solution of CTAB was stored at 30°C). For calibration, dilutions of this stock solution were used at concentrations of 1.0, 0.8, 0.6, 0.4, 0.2 and 0.1 mg/ml. For the determination of neomycin and hydrocortisone in Otosporin eardrops, a stock solution of 0.2 mg/ml of hydrocortisone and 0.1 mg/ml neomycin was prepared (in 100 m*M* CTAB) and six dilutions were prepared spread between one- and tenfold dilution, so that the concentration of the sample is near the middle of the linear range of the calibration graph.

Sample preparation

Otosporin eardrops (Wellcome Foundation, London, UK), labelled to contain 10 mg/ml of hydrocortisone and 5 mg/ml of neomycin, were diluted 100-fold with distilled water. This dilution was used for the injection without further pretreatment.

RESULTS AND DISCUSSION

Determination of aminoglycosides by CZE with indirect UV

Aminoglycoside antibiotics are non-UV-absorbing components, positively charged in their protonated form at pH 3–8. Isotachophoretic (ITP) experiments showed that they migrate at intermediate pH with effective mobilities of $20 \cdot 10^{-5}$–$50 \cdot 10^{-5}$ $cm^2/V \cdot s$ with positive charges of 2^+ to 5^+, as could be concluded from their response factors [14]. In the first instance the aminoglycoside antibiotics were determined using CZE in the cationic mode (cathode at the detection side) with the indirect UV mode. Very bad peak shapes, due to strong attractive forces between the highly positively charged components and the negatively charged capillary wall, and a low resolution were the result. Because higher separation numbers [15] can be obtained at low apparent mobilities and to suppress the attractive forces between the analytes and the capillary wall, experiments were carried out in the anionic mode (anode at the detection side) with a reversed electroosmotic flow (EOF) by the addition of FC 135 to the background electrolyte [16]. All aminoglycoside antibiotics now migrated in the upstream mode [17]. With the addition of FC 135, values for the mobility of the electroosmotic flow (m_{EOF}) can easily be obtained down to $-90 \cdot 10^{-5}$ $cm^2/V \cdot s$.

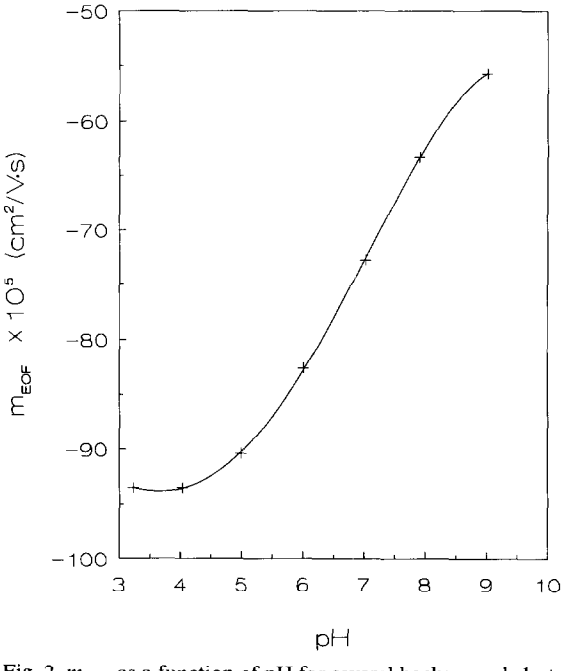

Fig. 2. m_{EOF} as a function of pH for several background electrolytes with 50 μg/ml of FC 135 added.

In Fig. 2, the m_{EOF} values as a function of the pH of the background electrolyte are shown for the background electrolytes with FC 135. In Table I, the compositions of all the background electrolytes are given. As can be seen from Fig. 2, the absolute values of m_{EOF} increase with decreasing pH, in contrast to the m_{EOF} values in background electrolytes without FC 135, which increase with increasing pH. This can be easily understood as follows. In fused silica the negative charge of the capillary wall increases with increasing pH (higher ζ-potential, higher m_{EOF}). An adsorbing layer of FC 135 molecules shields this negative charge. At high pH this shielding is less effective, resulting in a lower $|m_{EOF}|$.

Owing to this effect, the peak shape of the aminoglycosides in the reversed mode will also be the best at low pH, and will deteriorate at higher pH values. In Fig. 3 an example of the separation of a mixture of amikacin, dihydrostreptomycin, kana-

TABLE I

COMPOSITIONS OF BACKGROUND ELECTROLYTES AT DIFFERENT pH VALUES

All buffers were prepared by adding the buffering counter ion to the cations until the desired pH was reached. To all buffers, FC 135 was added at a concentration of 50 μg/ml.

Cation	Buffering counter species	pH
0.01 *M* imidazole	Formic acid	3.3
0.01 *M* imidazole	Formic acid	4.0
0.01 *M* imidazole	Acetic acid	5.0
0.01 *M* imidazole	MES[a]	6.0
0.02 *M* imidazole	Acetic acid	7.0
0.02 *M* imidazole	Acetic acid	7.9
0.02 *M* benzylamine	Acetic acid	9.0

[a] 2-(N-Morpholino)ethane sulphonic acid.

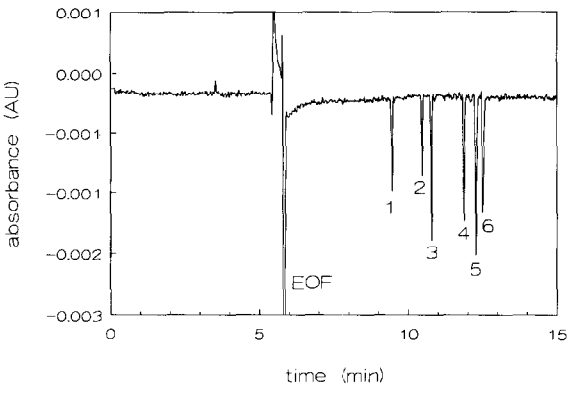

Fig. 3. Electropherogram for the separation of (1) dihydrostreptomycin, (2) lividomycin, (3) amikacin, (4) kanamycin, (5) tobramycin and (6) sisomycin (all 0.1 mg/ml) in the anionic mode with reversed EOF applying a background electrolyte of 0.01 *M* imidazole acetate at pH 5.0 containing the additive FC 135 (50 μl/ml). Capillary length, 67 cm; applied voltage, 12.5 kV; pressure injection time 2 s; UV detection wavelength, 214 nm.

mycin, lividomycin, sisomycin and tobramycin (all at a concentration of 0.1 mg/ml), obtained by applying a background electrolyte of 0.01 *M* imidazole at pH 5.0 adjusted by adding acetic acid with the additive FC 135 (50 μg/ml), is given [16]. The effective mobilities of the aminoglycoside antibiotics were determined as a function of pH. In Table II all the determined effective mobilities of the aminoglycoside antibiotics are given (see Table I for the background electrolyte composition). From Table II and Fig. 3, it can be concluded that aminoglycosides can easily be determined in the anionic CZE mode with reversed EOF by the addition of FC 135 with indirect UV detection at an optimum pH of about 5, although not all components can be separated at this pH.

Coupled capillary zone electrophoresis and micellar electrokinetic capillary chromatography

Pharmaceuticals often contain both neutral and charged components. In order to determine simultaneously both charged and neutral components, a micelle-forming surfactant has to be added to the background electrolyte.

Applying a coupled CZE and MECC system, negative, positive and neutral components can migrate in any order depending on their effective mobilities and capacity factors. As an illustration, a schematic representation of the different migration possibilities is given in Fig. 4. In Fig. 4a the original situation is shown, where the capillary is filled with background electrolyte containing a cationic surfactant. The cathode is placed at the injection side (i). In Fig. 4b the situation after some time is shown and Fig. 4c shows the corresponding electrophero-

TABLE II

CALCULATED EFFECTIVE MOBILITIES, $m \cdot 10^5$ ($cm^2/V \cdot s$), OF AMINOGLYCOSIDE ANTIBIOTICS AT DIFFERENT pH VALUES

For the composition of the background electrolytes, see Table I.

Component	pH					
	3.23	4.03	4.99	6.01	7.02	7.90
Amikacin	42.76	42.31	41.68	40.01	34.15	30.11
Butirosin	43.08	42.74	41.05	37.85	34.27	32.07
Dibekacin	52.35	51.46	48.45	46.34	40.26	34.33
Dihydrostreptomycin	35.31	35.00	34.58	35.26	32.51	31.47
Gentamycin	50.39	49.03	46.39	44.81	40.34	35.93
Kanamycin	50.31	49.31	46.18	43.30	35.37	27.90
Lividomycin	42.73	42.05	40.03	36.34	28.91	23.78
Neomycin	51.28	50.22	47.99	46.39	39.98	32.53
Paromomycin	47.52	46.94	44.51	41.50	34.68	28.00
Ribostamycin	46.05	44.85	41.10	39.14	34.52	28.54
Sisomycin	51.41	50.71	48.15	45.53	39.94	33.86
Streptomycin	34.99	39.94	34.70	34.92	33.22	32.36
Tobramycin	51.34	50.67	47.51	45.04	38.21	30.73

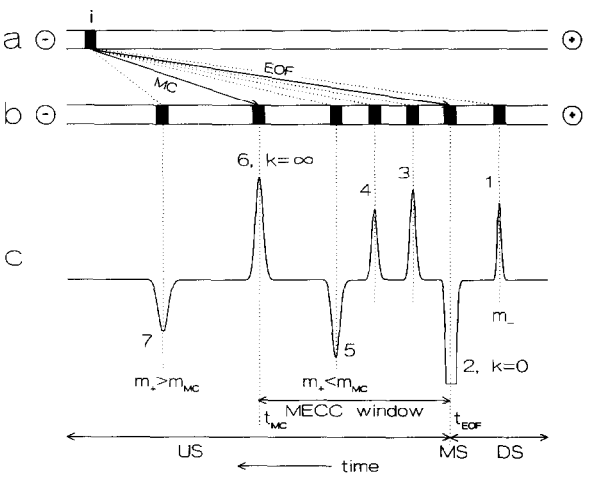

Fig. 4. Schematic representation of several migration modes in CZE with indirect UV detection in the anionic mode with reversed EOF coupled with MECC with a cationic surfactant. (a) Original situation; (b) separation after some time; (c) electropherogram of components migrating in different modes. Components 1, 3, 4, 6 are UV absorbing, components 5, 7 are non-UV-absorbing. For further explanation, see text.

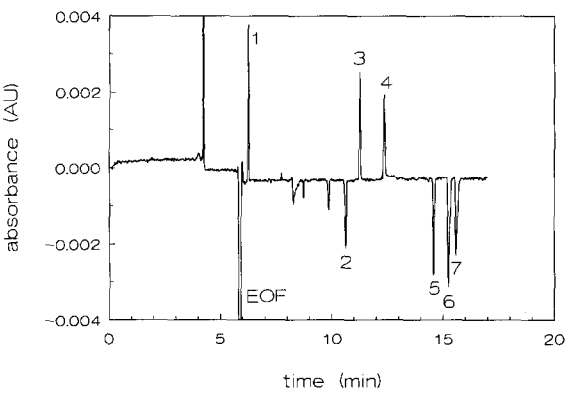

Fig. 5. Electropherogram for the separation of (1) paracetamol, (2) dihydrostreptomycin, (3) dapsone, (4) dexamethasone, (5) kanamycin, (6) tobramycin and (7) sisomycin obtained by applying a coupled CZE–MECC system consisting of 0.01 *M* imidazole acetate at pH 5.0 containing FC 135 (50 μl/ml) and 100 m*M* CTAB. Capillary length, 67 cm; applied voltage, 15 kV; pressure injection time, 5 s; UV detection wavelength 214 nm.

gram. In this electropherogram, component 1 is negatively charged and migrates in the downstream mode (DS) in front of a water dip (midstream mode, MS), that can act as an EOF marker [17]. A non-solubilized neutral component can also act as an EOF marker (with a capacity factor $k = 0$) if the component absorbs UV radiation. The completely solubilized component 6 acts as a micelle (MC) marker ($k = \infty$). The time window for neutral components migrating in the MECC mode is demarcated by t_{MC} and t_{EOF} and, *e.g.*, a neutral component (4) migrates in the MECC mode. Component 3, negatively charged but partially solubilized, migrates behind the EOF marker. Component 5, without UV absorption, is a positive component with a mobility smaller than that of the micelles, whereas the positive component 7, without UV absorption, with a mobility larger than that of the MC marker migrates behind the MC marker.

For the determination of neutral components simultaneously with aminoglycoside antibiotics in the anionic mode with reversed EOF, in first instance sodium dodecyl sulphate (SDS) was used. As the additive FC 135 probably solubilized in the SDS micelles, the mobility of the reversed EOF strongly decreased, as a result of which the aminoglycoside antibiotics could no longer be detected in the anionic mode. For this reason the cationic surfactant CTAB was tried as a micelle-forming surfactant causing, moreover, a reversed EOF. Good results in the separation of aminoglycoside antibiotics and several neutral components could be obtained with the addition of CTAB (100 m*M*) and FC 135 (50 μg/ml).

In Fig. 5 an example is given of the separation of a mixture of the UV-absorbing neutral components paracetamol and dapsone (0.02 mg/ml) and dexamethasone and the aminoglycoside antibiotics dihydrostreptomycin, kanamycin, tobramycin and sisomycin (all 0.1 mg/ml). The background electrolyte consisted of 0.01 *M* imidazole at pH 5.0 adjusted by adding acetic acid with the additives 50 μg/ml FC 135 and 100 m*M* CTAB. In order to

TABLE III

REGRESSION COEFFICIENTS, *r*, AND LIMITS OF DETECTION, LOD, FOR THE CALIBRATION GRAPHS OF DIHYDROSTREPTOMYCIN, SISOMYCIN, PARACETAMOL AND DAPSONE

Component	*r*	LOD (μg/ml)
Dihydrostreptomycin	0.9998	23.38
Sisomycin	0.9995	35.89
Paracetamol	1.0000	9.87
Dapsone	0.9997	29.84

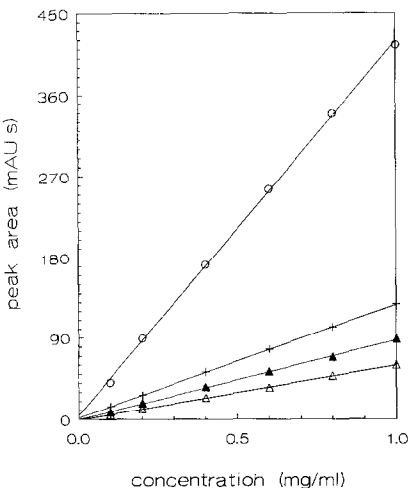

Fig. 6. Calibration graphs for peak area (mAUs) *versus* injected concentration (mg/ml) for (△) dihydrostreptomycin, (▲) sisomycin, (+) paracetamol and (○) dapsone. For separation conditions, see Fig. 5.

check quantitative aspects of separations using a coupled CZE–MECC system, calibration graphs of peak area *versus* injected concentration (5-s pressure injection) were set up for the aminoglycoside antibiotics dihydrostreptomycin and sisomycin and the neutral components paracetamol and dapsone in the same background electrolyte. The qualitative abilities of this separation were checked by measuring the effective and pseudo-effective mobilities of the separate components [15], and comparing them with the effective and pseudo-effective mobilities in the mixture. In Fig. 6 the calibration graphs are presented and in Table III all regression parameters are given, showing a linear relationship between peak area and injected concentration for both charged and neutral components.

As an application, we determined neomycin and hydrocortisone in Otosporin eardrops. The sample was measured four times. In Table IV the regression coefficients of the calibration graphs of the two components and the labelled and determined concentrations of the components in the sample are given. As can be seen, the determined and the labelled values agree well.

TABLE IV

REGRESSION COEFFICIENTS, r, FOR THE CALIBRATION GRAPHS OF NEOMYCIN AND HYDROCORTISONE, AND THE LABELLED AND MEASURED CONCENTRATIONS OF THESE COMPONENTS IN OTOSPORIN EARDROPS

Component	r	Concentration (mg/ml)	
		Labelled	Measured
Neomycin	0.9997	5.00	5.42
Hydrocortisone	0.9990	10.00	10.56

CONCLUSIONS

The determination of aminoglycoside antibiotics in the cationic mode is difficult owing to attractive forces between the positively charged aminoglycoside antibiotics and the negative charged capillary wall. By addition of FC 135 to the background electrolyte, resulting in a reversed wall charge, the aminoglycoside antibiotics could easily be determined in the anionic mode with reversed EOF. The effective mobilities of thirteen aminoglycoside antibiotics were measured as a function of pH. Charged and neutral components can be determined simultaneously by applying coupled CZE and MECC. By the application of an electrolyte consisting of 0.01 *M* imidazole adjusted to pH of 5.0 by adding acetic acid and the additives FC 135 (50 μg/ml, for reversed EOF) and CTAB (100 m*M*, as micelle-forming surfactant), the aminoglycoside antibiotics (in the anionic mode with reversed EOF with indirect UV detection) and neutral components (reversed MECC mode) could be simultaneously determined. The values obtained for neomycin and hydrocortisone in eardrops agreed with the labelled values.

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