ELSEVIER

Available online at www.sciencedirect.com

SCIENCE DIRECT

Journal of Chromatography B, 789 (2003) 417–424

JOURNAL OF CHROMATOGRAPHY B

www.elsevier.com/locate/chromb

Short communication

Determination of dialysate creatinine by micellar electrokinetic chromatography

Ewa Poboży, Anna Radomska, Robert Koncki*, Stanisław Głąb

Department of Chemistry, University of Warsaw, Pasteura 1, 02-093 Warsaw, Poland

Received 1 November 2002; received in revised form 10 January 2003; accepted 10 January 2003

Abstract

Micellar electrokinetic chromatography with UV absorbance detection has been applied for fast and selective determination of creatinine in samples of postdialysate fluid. Optimization of the method was performed, with the best results being obtained using a 30 m*M* borate–100 m*M* sodium dodecyl sulphate background electrolyte, pH 9, with the detector set at 235 nm and an applied voltage of 17 kV across a fused-silica capillary of 67 cm/75 μm I.D. The linear range of the technique was over 2 orders of magnitude (5–1000 μ*M*). The developed analytical procedure is useful for the monitoring of clinical hemodialysis treatment, because creatinine levels in real undiluted samples of postdialysate range from 80 to 350 μ*M*. The separation system allows the analysis of about six to seven samples of spent dialysate per hour in almost real time. The determinations are not influenced by other components of dialysate fluid nor by other surrogates extracted from patient blood. The results of analysis using the developed procedure and the kinetic spectrophotometric Jaffe method conventionally used in clinical settings for creatinine determination are fully comparable. Successful clinical evaluation of the analytical system was performed. The developed system is useful for bloodless estimation of bioanalytical parameters of hemodialysis sessions such as creatinine–time profiles and total creatinine removal. Both these parameters are important in clinical models of hemodialysis therapy.
© 2003 Elsevier Science B.V. All rights reserved.

Keywords: Creatinine

1. Introduction

Owing to diminished renal functions, toxic products of protein metabolism are accumulated in the body and are dangerous for the health of man. In such a case blood purification by an artificial kidney is an effective life-saving therapy [1]. Moreover, replacement of renal functions by hemodialysis (HD) aims at restoring a relative control of fluids, electrolytes and acid–base balance. However, the main goal of this clinical treatment is the removal of toxic metabolites from a patient's body. Over 200 uremic toxins are reported in the medical literature. In a clinical setting it is impossible to identify and determine all of them. While the whole spectrum of these products is unknown, urea and creatinine have become the markers for these unidentified toxins [1,2], as nearly 95 and 5% of protein nitrogen is metabolized to urea nitrogen and creatinine nitrogen, respectively. In a patient on dialysis, urea is considered to be the molecule of reference for removing low-molecular mass toxic solutes. An estimation of changes in the creatinine level is useful in the

*Corresponding author. Fax: +48-22-8225-996.
E-mail address: rkoncki@chem.uw.edu.pl (R. Koncki).

1570-0232/03/$ – see front matter © 2003 Elsevier Science B.V. All rights reserved.
doi:10.1016/S1570-0232(03)00075-8

evaluation of HD effectiveness with regards to toxic molecules of intermediate size.

The nutritional status of dialysis patients is a major criterion for assessing treatment adequacy, because morbidity and mortality are strongly correlated with malnutrition in dialysis patients [3]. Urea generation reflects the protein catabolism rate of the patient, including rapid changes both in dietary protein intake and patient protein turnover. Conversely, creatinine produced from phosphocreatine and creatine is a marker of muscle mass and its generation reflects the slow turnover of muscle protein. Biomedical modelling of both urea and creatinine, far from being redundant, should provide complementary information about patient protein status. Urea kinetic modeling (UKM) [4,5] and creatinine kinetic modeling (CKM) [6–10] are complementary tools for clinicians. While UKM is useful for evaluation of HD dose and protein catabolism rate, CKM would provide information on muscle mass and nutritional status. The creatinine index may be regarded as an index of amino acid pool stored in the muscle mass (patients with muscular dystrophy have low creatinine output), and any reduction in the index over time means a proportional loss of nitrogen reserve and therefore these changes are a strong predictor of patient morbidity and mortality [7,8].

Both the models are important for clinicians because HD treatment should, just like any other therapy, be adequately administered and assessed. The models have been applied using pre- and post-dialysis concentrations of the markers in blood. However this approach is complex, time-consuming, requires blood sampling and is inaccurate. Evaluation of toxin removal by serum analysis is an indirect methodology and its popularity stems from the practice of measuring serum concentrations of a wide variety of analytes important for clinical diagnostic. Since the major effect of HD is solute removal, measurements of urea and creatinine in spent dialysate constitute direct methods for control of the effectiveness of the therapy. Moreover, direct dialysate methods eliminate inaccuracy problems caused by disequilibrium in the body of the patient on dialysis (rebound phenomena) and offer the ability of real-time control of the HD therapy process. Finally, from the analytical point of view, spent dialysate is more easily available, and a simpler, less hazardous sample than blood or serum. However, it should be noted that dialysate levels of all surrogates extracted from blood, including analytes and interfering species, are 1–2 orders of magnitude lower than in the biofluids.

Bloodless methodologies for UKM are well described in the literature [4,5]. For on-line analysis of dialysate urea, various conductometric [11], potentiometric [12,13] and spectrophotometric [14] bioanalytical systems were recently developed and some of them are now commercially available [15]. Determination of creatinine in spent dialysate and consequently bloodless CKM is much more difficult. The traditional colorimetric Jaffe method based on reaction of the analyte with picric acid is widely accepted by clinicians, although this method is not specific (serious interferences from glucose, keto-acids, amines and proteins) and is time- and work-consuming. Enzymatic assays that have enhanced selectivity for creatinine have also been developed. Only recently, a potentiometric bioanalytical system [12] and an amperometric biosensor [16] for dialysate creatinine determination have been reported. However, these analytical devices are not free of all interferences and are rather expensive in exploitation.

On the other hand, chromatographic and electrophoretic techniques are powerful analytical tools recommended for biomedical measurements because of the inherent ability to determine the analytes of interest with minimal interference from other species. The use of capillary electrophoresis (CE) for analysis of biological fluids is an attractive alternative to many other analytical schemes because of the speed of analysis, high selectivity, possibility of multicomponent analysis and minimal waste production. Only recently Kochansky and Strein [17] reviewed modern instrumental separation techniques for the determination of uremic toxins in biofluids. Electrophoretic methods have been successfully applied for determination of creatinine in urine [18–22] and serum [22–25]. In order to separate creatinine from other UV absorbing neutral species micellar electrokinetic capillary chromatography (MEKC) is employed. In MEKC, separation takes place through the equilibrium distribution of a solute between two phases that are moving at different velocities in opposite directions. The slow moving phase is

composed of molecular aggregates/micelles, which will interact with/retain neutral species [26]. Creatinine has pK_a values of 4.8 and 9.2 and its zwitterionic form, predominant between these pH values, does not interact hydrophobically with micelles. Therefore, MEKC is much better suited to separation of creatinine from various neutral solutes than standard CE. The most common pseudo-stationary phase in MEKC applied for biofluids analysis [20–26] is formed by adding sodium dodecyl sulphate (SDS) to the background electrolyte/buffer.

Although there are many cited papers devoted to electrophoretic analysis of serum and urine samples [17–25], in this work the utility of MEKC for simple and accurate determination of creatinine in spent dialysate is presented. The major aim of this contribution was to develop a rapid, reliable, accurate and efficient analytical procedure for real biomedical uses. Therefore clinical evaluation of the developed analytical system and its utility for control of HD session progress as well as for estimation of total creatinine removal useful for CKM of HD are also demonstrated.

2. Experimental

2.1. Instrumentation and measurements

All the CE experiments were performed with a Beckman P/ACE MDQ Capillary Electrophoresis System (USA) equipped with a multiwavelength UV detector. Separations were carried out in a fused-silica capillary (Beckman Instruments, Fullerton, USA) 67 cm total length/50 cm effective length, 75 μm I.D., 375 μm O.D, The temperature of the capillary cartridge was maintained at 25 °C. All injections were made hydrodynamically with a pressure of 0.5 p.s.i.. For conditioning, the capillary was pretreated with 0.1 *M* NaOH for 5 min. Next, it was rinsed with deionised water (from Milli-Q system, Millipore, USA) for 5 min, and with the electrolyte for 5 min. This procedure was repeated everyday prior to the measurements and prior to each change of the running electrolyte. Between measurements the capillary was rinsed with electrolyte.

Clinical dialysis treatments were performed on Fresenius HD machines (Germany). The rate of blood flow ranged from 0.20 to 0.25 l/min. The flow-rate on the dialysate side was 0.5 l/min.

For reference spectrophotometric determinations of creatinine in spent dialysate, a Shimadzu 2401/PC spectrophotometer (Japan) and disposable cuvettes (Sarstedt, Germany) were used.

2.2. Reagents and materials

All chemicals were of analytical reagent grade. Carrier electrolyte was prepared using sodium tetraborate, (POCh, Poland) and sodium dodecyl sulphate (Fluka, Switzerland). Creatinine was purchased from Sigma (St. Louis, MO, USA).

The dialysis liquids were obtained in the form of two separate concentrates from Karima (Poland). The concentrates were mixed with deionised water in the HD machine. The final composition of the liquid for HD was: 140 m*M* sodium, 2 m*M* potassium, 1 m*M* magnesium, 3 m*M* calcium, 29 m*M* total acetate, 35 m*M* total carbonate and 111 m*M* chloride.

For reference creatinine determination a Sigma diagnostic test kit (spectrophotometric kinetic procedure No. 555) was used.

All standard, test and sample solutions were prepared immediately prior to use, and if necessary filtered using disposable syringe filters (Millipore, USA).

3. Results and discussion

3.1. Optimization of the analytical procedure

Preliminary experiments were performed to establish instrumental parameters. Increasing the injection time in the range 3–10 s did not change the migration time of creatinine, and only slightly increased the sensitivity, as determined using peak height. However, changes in the shape of peaks resulting from 10-s injections suggested that an 8-s injection was optimal. After numerous test runs, we chose a run voltage of 17 kV as a compromise between stability of measurements, migration time and effectiveness of separation. The maximum current generated was 95 mA.

Spectrophotometric UV detection of creatinine

could be carried out at a wide variety of different wavelengths ranging from 210 to 254 nm [17–25]. Higher sensitivities were obtained at shorter wavelengths (usually 210 or 214 nm), but then other metabolites/toxins could be also detected and strongly interfered in the creatinine determination. In this study we used 235 nm, because at this wavelength there is a local absorbance maximum specific for creatinine [19] and absorbance from the matrix (ionic dialysate fluid components) is not observed. Moreover at this wavelength, some blood surrogates that extracted into dialysate (for example urea), did not absorb the light.

Optimization of the background buffer composition is discussed below. Our particular interest was to find a CE procedure for dialysate creatinine determination, which would be selective and fast and not require any sample pretreatment or dilution. To achieve this goal we tried to implement methodology, earlier reported by Tran et al. [24] and Shirao et al. [20], developed for the analysis of serum and urine, respectively. Postdialysate samples contain creatinine and other metabolites at lower levels than in these biofluids, but the content of electrolytes is much higher (see Section 2). In the cited papers, phosphate buffer, pH 7 [20], and borate buffer, pH 9 [24], both with added SDS, were used as the electrolytes. In our application the use of phosphates is risky because of the possibility of slow precipitation of calcium and magnesium phosphates and blocking of the capillary. Due to the high content of carbonate in the samples, buffers of pH over 7, that prevent gas (CO_2) bubbles forming in the capillary, are necessary. For both these reasons borates rather than phosphates are recommended as the buffering agent in the background electrolyte. Preliminary CE experiments showed that addition of SDS to the buffer is necessary. In the course of pure dialysate electrophoresis without SDS a negative peak originating from electrolytes and water from the fluid was obtained (Fig. 1). The migration times for both creatinine and dialysate matrix were practically identical.

Separation of the peaks was possible using MEKC with SDS as the micelle-forming agent. The zwitterionic form of creatinine only interacts electrostatically with the micelles. However, these weak interactions are sufficiently effective because micelles carried the analyte against electroosmotic flow. As can be seen from Fig. 1, the addition of SDS caused both inversion of the peak from the dialysate matrix and its separation from the creatinine peak. An increase of the SDS amount in the buffer only slightly increased the migration time for creatinine. On the other hand, with higher concentrations of SDS worse reproducibility of the measurements was observed, mainly due to a large increase in the conductivity of the background electrolyte. The SDS concentration did not influence the sensitivity of the analyte determination.

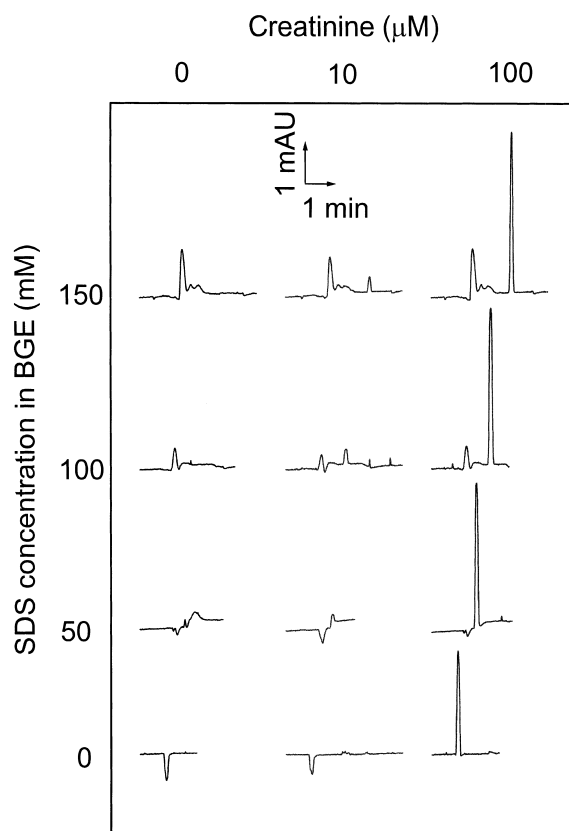

Fig. 1. Effect of SDS on electropherograms of dialysate fluid without creatinine, and dialysate fluid spiked with creatinine at levels of 10 and 100 µ*M*. Concentrations of SDS (in m*M*) are given in the figure. As background electrolyte 30 m*M* borate buffer, pH 9, was used.

In the next part of the study, the influence of the concentration and pH of the borate buffer on the determination of dialysate creatinine by MEKC was investigated, using 0.1 *M* SDS. With an increase of

the buffer concentration from 10 to 50 mM, the velocity of the electroosmotic flow decreased. Improved separation was observed with an increase in ionic strength of the buffer. On the other hand, migration time decreased and the electric current flowing through the capillary increased. As high electric current should be avoided in order to suppress the Joule heating effect, 30 mM borate was chosen for further investigations. These findings are in agreement with those reported by Shirao et al. [20] for phosphate buffer. The influence of buffer pH was investigated in the range of the effective buffer capacity of borate i.e., from pH 8 to 10. Migration times and sensitivities found in the pH range 8.5–10 were nearly the same. A pH of 9 was chosen as optimal.

We have found that the optimal composition of the background buffer is 30 mM sodium borate and 100 mM SDS. It is worth noting, that the solution has a pH of about 9.0, and therefore it could be easily prepared by simple mixing of the appropriate components, without need of pH adjustment and use of pH-meter. Moreover, this background electrolyte and the dialysate fluid have similar ionic strengths and therefore nonspecific effects from the dialysate matrix are minimized. The optimized electrolyte composition is similar to that recommended for MEKC analysis of deproteinized serum samples [24].

In this work, peak height was used as the analytical signal. The obtained calibration graph is linear from 10 to 1000 μM creatinine with a determination limit around 5 μM (figure not shown), and fully covers the range of the analyte concentrations founded in samples of postdialysate. It should be noted that height of peaks and migration times for creatinine standards prepared in the dialysate fluid and background electrolyte are the same. The use of the developed procedure gave good resolution of the dialysate matrix peak, creatinine and other blood surrogates extracted into dialysate. The zwitterionic form of creatinine does not interact with the hydrophobic interior of micelles and therefore eluted early in the separation. In consequence, creatinine was the first dialysed blood toxin to elute. The other neutral and anionic toxins expected to be present in postdialysate fluid are eluted next. This observation is in agreement with an earlier report by Tran et al. [24] on the separation of creatinine from 20 other uremic toxins. We have found that creatinine peaks are not affected by a large excess of alkaline cations, urea and uric acid in the sample. An interference from creatine was observed in the case of an 100-fold excess of creatine:creatinine, but not in the case of a 10-fold excess. As the creatine concentration in real postdialysate samples is well below this, creatine does not pose a problem. We can conclude that the developed method can be used for effective separation and selective determination of creatinine in postdialysate fluid.

In CE, contrary to other separation techniques (i.e. HPLC), components which migrate more slowly than the peaks of interest can be removed by capillary purging after the peaks of interest have passed the detector, thus considerably increasing sample throughput. In our system, the creatinine peak has a migration time of 7 min. One to 2 min capillary rinsing between consecutive samples injections only with background electrolyte (without caustic rinsing) is sufficient to give reproducible results for standards prepared in dialysate fluid (Table 1).

3.2. Biomedical application of the analytical method

The optimized procedure developed in this work has been applied for analysis of posthemodialysate effluent from artificial kidney in the course of HD session. In the inset of Fig. 2 a whole-time electropherogram of postdialysate sample is shown. As expected, creatinine had the shortest migration time, as confirmed by a CE run of the same sample spiked

Table 1
Reproducibility of the method tested on creatinine standards prepared in dialysate fluid (artificial samples without other extracting blood surrogates)

	Standard 1	Standard 2
Number of repetitions	24	18
Creatinine level	20 μM	100 μM
Average migration time	6.88 min	7.02 min
Range of migration time	6.78–6.96	6.88–7.08
SD of migration time	0.07 min	0.05 min
SEM of migration time	0.01 min	0.01 min
Average height of peak	0.75 mAU	3.46 mAU
SD of peak height	0.015 mAU	0.038 mAU
SEM of peak height	0.003 mAU	0.009 mAU

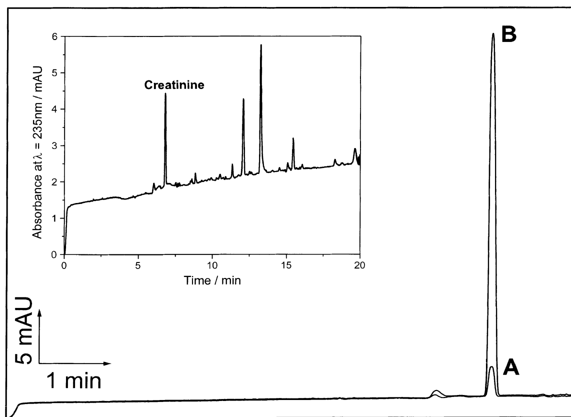

Fig. 2. Electropherogram of real sample of postdialysate fluid (A) and the same sample spiked with 100 μ*M* creatinine (B). Full electropherogram of the real sample is shown in the inset.

with creatinine (Fig. 2). Migration times for other uremic toxins were longer, as reported earlier [24]. A recovery test illustrated by Fig. 2 was repeated five times. The concentration of added standard was 100 μ*M* and the concentrations determined were: 97.7, 100.6, 98.0, 101.5 and 99.6 μ*M*. Respective migration times varied from 6.9 to 7.1 min. The reproducibility of real sample analysis was comparable with that found for artificial samples (Table 1). With 20 injections the migration times stayed nearly the same (7.00 min, SD=0.09, SEM=0.02) with average peak height of 3.45 mAU (SD=0.031, SEM=0.007).

In this work creatinine was analyzed directly from its peak height by reference to a standard calibration curve. The results of dialysate creatinine analyses using the developed MEKC procedure and kinetic colorimetric Jaffe method conventionally used in clinical settings are collected in Fig. 3. The correlation equation for analyses of 48 real postdialysate samples was: $Y=1.031(\pm 0.014)X-8.23(\pm 2.58)$ (μ*M*) with a regression coefficient of 0.997 and a standard deviation of 4.2 μ*M*. These results clearly confirm the excellent agreement between the developed analytical technique and the standard clinical method.

The analytical system reported in this paper can be used as an at-line monitor for control of HD treatment. Such a biomedical application is illustrated by Fig. 4. As can be seen from this figure, during HD

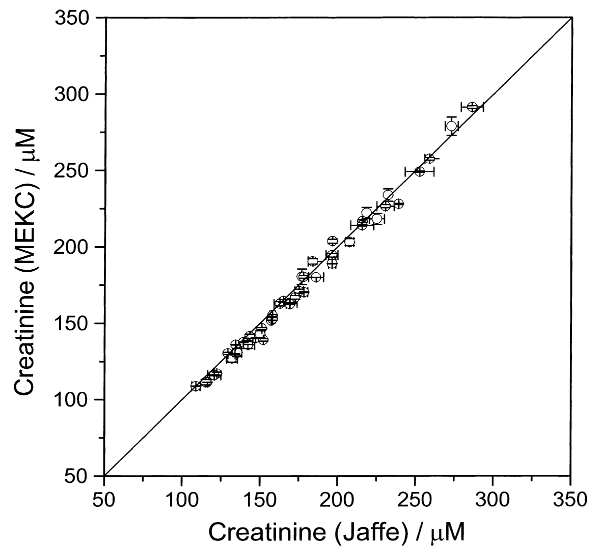

Fig. 3. Correlation of the results of dialysate creatinine determination in real clinical samples using the developed and reference method.

treatment dialysate creatinine levels fall exponentially and this is a common toxin–time profile. Any significant disturbance in this analytically controlled removal of the uremic toxin can be treated as a signal of malfunction of the artificial kidney. Creatinine removal (line B in Fig. 4) in the course of an HD session can be simply evaluated by multiply-

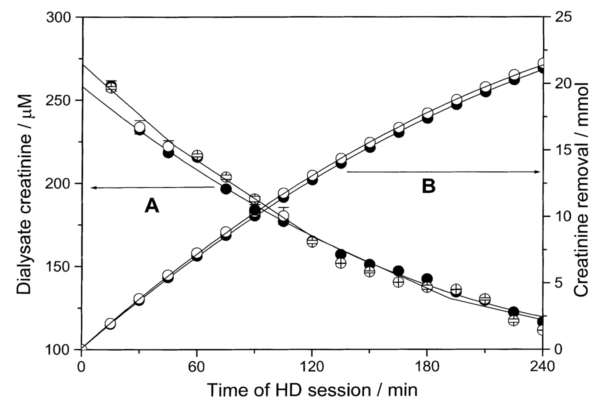

Fig. 4. HD progress controlled by monitoring of dialysate creatinine level using the developed (open points) and reference (solid points) method. Lines (A) represent exponential decay of dialysate creatinine level in the course of HD session. Lines (B) represent amount of creatinine removed from patient body by HD treatment.

Table 2
Biomedical CKM parameters of HD session monitored using both, the developed and reference method. The results correspond with experimental data showed in Fig. 4

CKM parameter	by MEKC	by Jaffe
Initial level of dialysate creatinine, C_0 (μM)	269	261
Final level of dialysate creatinine, C_T (μM)	111	112
Creatinine reduction ratio, calculated as $(1-C_T/C_0)\times 100$ (%)	58.8	56.7
KT/V, calculated as $\ln(C_0/C_T)$ (dimensionless)	0.89	0.84
K/V ($\times 10^{-3}$; 1/min)	3.70	3.50
Total creatinine removal (mmol), calculated as $F\times C_0/(K/V)\times[1-\exp(-KT/V)]$, where F is flow-rate of dialysate fluid	21.5	21.1

ing the area under the dialysate creatinine–time curve (line A in Fig. 4) by the flow-rate of the dialysate fluid. Taking into account that toxin removal by an artificial kidney is a first-order process it is possible to predict the final dialysate creatinine level as well as total creatinine removal before the end of HD session.

Estimation of pre-, post- and interdialytic creatinine levels as well as total creatinine removal is useful in kinetic modelling of HD therapy. Although conventional CKM is based on blood levels of the toxin, it is known that the relationship between concentrations of any surrogate at the start and the end of HD session is the same in blood and dialysate. Therefore, in many cases blood-side and dialysate-side measurements can lead to the same results. Kinetic modelling utilizing KT/V as a parameter (where K, V and T are, the dialysator clearance, body water volume and total time of HD, respectively) is a standard biomedical methodology of HD quantification. This dimensionless parameter is used by clinicians for quantitative description of the delivered HD dose. An alternative parameter is creatinine reduction ratio or percentage removal of creatinine. For calculation of each of these parameters the initial/final creatinine level ratio is useful (Table 2). Estimation of the total amount of creatinine removed by HD is necessary for calculation of creatinine index and body lean muscle [6–10]. As can been seen from Fig. 4 and Table 2, the pre-/post-HD creatinine level ratio as well as the total creatinine removal, both parameters necessary for CKM calculations, are easily determinable using the MEKC method reported in this paper.

4. Conclusions

To the best of our knowledge this is the first report on the application of CE for control of HD treatment. The developed procedure is easy to operate (no sample pretreatment) and enables fast, sensitive and selective determination of creatinine in real clinical samples of spent dialysate. The results clearly show that MEKC is useful as an at-line HD monitor working in bloodless (dialysate-side) methodology allowing estimation of biomedical parameters important for CKM and in consequence for quantitative administration and assessment of HD therapy.

Acknowledgements

The authors thank the staff and patients at the Dialysis Unit of the St. Anna Hospital in Warsaw. The University of Warsaw supported this work (grant BW-1562/09/2002).

References

[1] W.L. Heinrich, in: The Principles and Practice of Dialysis, Williams & Wilkins, Baltimore, MD, 1994.
[2] S. Ringoir, A. Schoots, R. Vanholder, Kidney Int. 33 (1988) S4.
[3] E.G. Lowrie, N.L. Lew, Am. J. Kidney Dis. 15 (1990) 458.
[4] R.M. Hakim, T.A. Depner, T.F. Parker, Am. J. Kindey Dis. 20 (1992) 107.
[5] T.A. Depner, Am. J. Nephrol. 16 (1996) 17.
[6] P.R. Keshaviah, K.D. Nolph, H.L. Moore, B. Prowant, P.F. Emerson, M. Meyer, Z.J. Twardowski, R. Khanna, L. Ponferrada, A. Collins, J. Am. Soc. Nephrol. 4 (1994) 1475.

[7] B. Canaud, L.J. Garred, A. Argiles, J.F. Flavier, C. Bouloux, C. Mion, Nephrol. Dial. Transplant. 10 (1995) 1405.
[8] T. Shinzato, S. Nakai, M. Miwa, N. Iwayama, I. Takai, Y. Matsumoto, H. Morita, K. Maeda, Artif. Organs 21 (1997) 864.
[9] A.C. Johansson, P.O. Attman, B. Haraldsson, Kidney Int. 51 (1997) 855.
[10] W.R. Clark, B.A. Mueller, M.A. Kraus, W.L. Macias, Kidney Int. 54 (1998) 554.
[11] L. Chiari, A. Cappello, R. Tartarini, F. Paolini, P. Calzavara, Int. J. Artif. Organs 21 (1998) 526.
[12] M. Jurkiewicz, S. Alegret, J. Almirall, M. Garcia, E. Fabregas, Analyst 123 (1998) 1321.
[13] R. Koncki, A. Radomska, S. Glab, Anal. Chim. Acta 418 (2000) 213.
[14] A. Radomska, S. Glab, R. Koncki, Analyst 126 (2001) 1564.
[15] J. Sternby, Adv. Renal Replace Ther. 6 (1999) 265.
[16] B. Tombach, J. Schneider, F. Matzkies, R.M. Schaefer, G.C. Chemnitius, Clin. Chim. Acta 312 (2001) 129.
[17] C.J. Kochansky, T.G. Strein, J. Chromatogr. B 747 (2000) 217.
[18] N.A. Guzman, C.M. Berck, L. Hernandez, J.P. Advis, J. Liq. Chromatogr. 13 (1990) 3833.
[19] H. Shi, Y. Ma, Y. Ma, Anal. Chim. Acta 312 (1995) 79.
[20] M.K. Shirao, S. Suzuki, J. Kobayashi, H. Nakazawa, E. Mochizuki, J. Chromatogr. B 693 (1997) 463.
[21] D.G. Burke, P.C. MacLean, R.A. Walker, P.J. Dewar, T. Smith-Palmer, J. Chromatogr. B. 732 (1999) 479.
[22] M. Miyake, A. Shibukawa, T. Nakagawa, J. High Resolut. Chromatogr. 181 (1991) 14.
[23] A. Schmutz, W. Thormann, Electrophoresis 15 (1994) 51.
[24] T.C. Tran, T.A. Huq, H.L. Kantes, J.N. Crane, T.G. Strein, J. Chromatogr. B 690 (1997) 35.
[25] D.A. Walsh, E. Dempsey, Anal. Chim. Acta 459 (2002) 187.
[26] R. Weinberger, in: Practical Capillary Electrophoresis, Academic Press, London, 1993.