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Capillary electrophoresis as a clinical tool Determination of organic anions in normal and uremic serum using photodiode-array detection

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Abstract

We report the use of free solution capillary electrophoresis to identify and quantify low-molecular-mass compounds found in normal and uremic serum as well as in hemodialysate fluid. The method reported provides a multicomponent analysis, allowing a single-step screening for more than 19 metabolites in less than 16 min. Serum samples from healthy individuals and from patients who have been diagnosed with chronic renal failure are analyzed using a borate buffer system at pH 9.0, and an extended light path capillary. Several ionic sample constituents are identified by electrophoretic mobility, UV spectra, and spiking with authentic standards. An analysis of the relative concentration of several metabolites, including hypoxanthine, pseudouridine, hippuric acid, and uric acid is presented. Each of these four metabolites is found in both normal and uremic serum samples (limits of detection 1 to 6 μM). Moreover, each of these metabolites is present at significantly elevated levels in uremic patients. The method reported is shown to have promising clinical utility for profiling serum sample constituents, and for quantitative determination of a few important metabolites.

1. Introduction

Capillary electrophoresis (CE) is a highly efficient separation technique that affords the separation of small volumes of complex samples in a short period of time. In the recent literature, one can find numerous reviews of CE [1–3] and reports describing the application of CE to determine many important biological moieties including amino acids [4], peptides [5], proteins [6,7], and pharmaceutical agents [8]. Recently, the application of CE methodology to the analysis of biological fluids [9–16] has provided great promise for its use as a clinical tool. To illustrate the utility of CE for the determination of low-molecular-mass metabolites in human biological fluids, we report here the use of CE to examine normal and uremic blood serum samples as well as hemodialysate fluid from patients with chronic renal failure.

Uremia ("urine in the blood") has been used to describe the re-adsorption of hundreds of nitrogenous compounds into the tissues and blood that would normally be excreted in the urine [17]. Efforts to analyze hemodialysate, serum, and urine samples using immunoassay, GC, and HPLC analyses of biological fluids have revealed the presence of many important metab-

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olites [18–31]. Some organic metabolic end products known to be present at elevated levels in uremic sera include urea, creatinine, peptide fragments, organic acids, guanidines, phenols, and indoles [17]. Immunoassay experiments can be very specific, but can often only determine an individual substance. Conversely, HPLC separations are capable of multicomponent determination; however, most separations are either too time-consuming or many compounds are incompletely resolved. CE, a relatively new chemical separation technique, may be capable of multicomponent determinations in substantially less time.

Recently, a few studies employing CE for the rapid analysis of biological fluids have been reported [11–16]. Schoots et al. [11] used CE to identify hippuric acid, 2-hydroxyhippuric acid, and uric acid in the blood serum of chronic renal-failure patients, and used a complementary HPLC analysis to verify the CE data and to determine creatinine, pseudouridine, and hypoxanthine. Miyake et al. [12] used MEKC to quantify the levels of creatinine and uric acid in human plasma and urine. Another study, using free solution CE, quantified creatinine concentrations in the urine of normal and pathological individuals [14]. Lee et al. [15] used free solution CE to quantify creatinine in serum. Finally, a study by Buchberger et al. [16] used free solution CE with indirect detection for the determination of low-molecular-mass metal cations in biological fluids.

In this paper, we present a free solution CE method that may be clinically useful for the analysis of biological fluids for low-molecular-mass species that are anionic at pH 9.0. The novel aspect of this work is that we include a large list of target compounds expected to be found in uremic (and normal) serum samples, and can accomplish a multicomponent determination of these chemical moieties in a relatively short amount of time (ca. 16 min). Using the method reported herein, we have been able to quantitatively determine levels of hypoxanthine, pseudouridine, hippuric acid, uric acid, and 2-hydroxy-hippuric acid, as well as a few other minor components in biological fluids. More in general, we report the differences in composition and concentration of metabolites found in samples obtained from "healthy" individuals and from those affected by chronic renal failure.

2. Experimental

2.1. Reagents

Indican, 2-hydroxyhippuric acid, indole-3-acetic acid, kynurenic acid, pseudouridine, theobromine, and theophylline were purchased from Sigma (St. Louis, MO, USA). Phenyltriethylammonium iodide and 1-methyl-nicotinamide iodide were purchased from Lancaster Laboratories (Windham, NH, USA). Allopurinol, hypoxanthine, 4-hydroxyphenylacetic acid, mesityl oxide, and xanthine were purchased from Aldrich Chemical Company (Milwaukee, WI, USA). Ferulic acid and nicotinic acid were purchased from ICN Biochemicals (Cleveland, OH, USA). Boric acid, m-hydroxybenzoic acid, and hippuric acid were purchased from J.T. Baker Chemical Company (Phillipsburg, NJ, USA). Uracil, L-(−)-tyrosine, and L-(−)-tryptophan were purchased from Eastman Kodak Company (Rochester, NY, USA). Uric acid was purchased from Mallinckrodt (Saint Louis, MO, USA). All reagents were used as received without further purification.

2.2. Preparation of standard solutions

CE running buffer solutions were prepared by dissolving boric acid in HPLC-grade water; adjustment of pH to 9.0 was accomplished by the addition of 1.0 *M* sodium hydroxide. Standard solutions were prepared by dissolving appropriate amounts of the reagent(s) in 20.0 m*M* borate buffer (pH 10.0) containing 1.0 m*M* mesityl oxide as a neutral marker. Phenyltriethylammonium iodide was added as an internal standard (I.S.) to each standard solution such that the final concentration was 2.0 m*M*. Linearity studies were performed by preparing standard analysis solutions of each reagent between 1.00 m*M* and 1.00 μM. The running

buffer (150.0 m*M* borate, pH 9.0) was vacuum filtered through a 0.2-μm Nylon 66 membrane purchased from Supelco (Bellefonte, PA, USA).

2.3. Preparation of serum samples

Serum samples from healthy and uremic individuals were obtained from Dr. Jay Jones (Geisinger Medical Center, Danville, PA, USA). A 20.0-μl aliquot of 100.0 m*M* I.S. was diluted to 1.00 ml with serum such that the resulting concentration of IS was 2.0 m*M*. This mixture was ultrafiltered by centrifugation at 2000 rpm (311 *g*) for 60 min at room temperature using a Centrifree Micropartition System (M_r cut-off 30 000), purchased from Amicon (Beverly, MA, USA), in order to remove unwanted serum proteins. The ultrafiltrate was directly used for CE analysis.

2.4. Preparation of hemodialysate

Spent hemodialysate fluid was obtained from Dr. Joe Bisordi (Geisinger Medical Center, Danville, PA, USA). Solutions of hemodialysate for each patient were prepared by diluting 1.00 ml of 20.0 m*M* I.S. to 10.00 ml with hemodialysate.

2.5. Equipment

A Hewlett-Packard ^{3D}Capillary Electrophoresis System (Hewlett-Packard, Wilmington, DE, USA) with a diode-array UV detector was used to carry out all CE separations. Data were collected on an HP Vectra 486 personal computer using the Hewlett-Packard ^{3D}CE Chemstation Software.

2.6. Separation conditions

A Hewlett-Packard extended light path capillary (50 μm I.D., 64.0 cm total length, 55.8 cm injection-to-detection) was used for all CE separations. The running buffer for all analyses was 150 m*M* borate, pH 9.0. Injection was accomplished by an application of 50.0 mbar pressure to the inlet vial for 5.0 s. Separations were performed at 22 kV, 25°C, using diode-array UV detection. The current generated across the capillary was typically 32.5 to 34.5 μA, which is within the operable range for the experimental conditions.

2.7. Peak identification and quantification

Peak identification was accomplished by comparing both electrophoretic mobilities and UV spectra of suspect peaks with those of authentic standards. In addition, the identity of peaks corresponding to pseudouridine, hypoxanthine, hippuric acid, and uric acid were further supported by spiking an ultrafiltrate sample with each component. Quantification was accomplished using a ratio of metabolite peak area to that of the internal standard, phenyltriethylammonium cation.

3. Results and discussion

3.1. CE of standard mixture

A CE separation of a standard mixture of "target" compounds that have been reported to be present in the blood serum of patients with chronic renal failure is shown in Fig. 1. A list of these target compounds along with their electrophoretic mobilities and corresponding peak numbers in Fig. 1 is given in Table 1. This electrophoretic separation allows the simultaneous determination of a fairly large list of compounds in relatively short time. Hypoxanthine (peak 10), hippuric acid (peak 12), uric acid (peak 16), and 2-hydroxyhippuric acid (peak 21) are important metabolites found in biofluids obtained from chronic renal-failure patients. Allopurinol (peak 8) and theophylline (peak 11) are medicinal agents commonly found in serum samples. The neutral flow marker, mesityl oxide (peak 3), which is not found in physiological fluids, was intentionally added to indicate the electroosmotic flow-rate. Creatinine, which is often used as a clinical marker for renal failure, is not separable from other neutral compounds using free solution CE, and thus co-elutes with other neutral

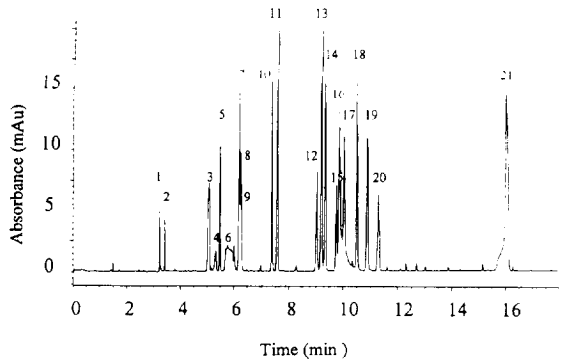

Fig. 1. Electropherogram of a standard mixture of suspect metabolites. CE operating conditions: 22 kV separation (34.5 μA current), 150 m*M* borate, pH 9.0, 50 μm I.D. extended light path capillary, 25°C, 5.0 s pressure injection (50 mbar), UV detection at 210 nm. 1 = 1-methylnicotinamide; 2 = I.S. (phenyltriethylammonium iodide); 3 = mesityl oxide (neutral marker); 4 = unknown impurity; 5 = theobromine; 6 = tryptophan; 7 = allopurinol; 8 = uracil; 9 = tyrosine; 10 = hypoxanthine; 11 = theophylline; 12 = hippuric acid; 13 = kynurenic acid; 14 = indole-3-acetic acid; 15 = 4-hydroxyphenyl-acetic acid; 16 = uric acid; 17 = xanthine; 18 = ferulic acid; 19 = indican; 20 = nicotinic acid; 21 = 2-hydroxyhippuric acid. Concentration of analytes as given in Table 1.

compounds in peak 3. Peak 4 is an unidentified anionic chemical impurity.

Almost all of the target compounds in the standard mixture are baseline resolved, and those which are not resolved can be quantitatively determined by employing detection at multiple wavelengths. Quantitation has been accomplished using phenyltriethylammonium iodide as an internal standard. The choice of a cationic internal standard allows correction for variation in injection volume while insuring no interference with the elution and quantification of any of the target components. Calibration plots and regression data for each metabolite in Fig. 1 were obtained and are summarized in Table 2. Calibration plots for all compounds were linear ($r^2 > 0.997$) between 1.00 m*M* and 0.0100 m*M* using UV detection at 210 nm. In addition, linearity for uric acid was determined at 291 nm to account for compounds interfering with uric acid quantification at 210 nm for some serum samples. Detection limits were set at a signal-to-noise ratio of 3.

3.2. CE of blood serum and hemodialysate

Clinical samples of blood serum from healthy individuals and from renal-failure patients as well as hemodialysate samples were injected according to the conditions adopted for the separation shown in Fig. 1. Representative electropherograms of normal serum, uremic serum, and hemodialysate fluid samples taken from different patients are shown in Fig. 2. The peak profiles obtained for all three samples are very similar suggesting that there are many common metabolites in these biofluids. Peak heights for the neutral peak (N), as well as for the peaks corresponding to hypoxanthine (HX), pseudouridine (PS), hippuric acid (HA) and uric acid (UA) are much larger in the separation of uremic serum (Fig. 2B) than in normal serum (Fig. 2A), as can be seen upon comparing the peak heights and absorbance axes in Fig. 2A and B. Hippuric acid and uric acid were uniformly present at high levels in the uremic serum samples. The hemodialysate fluid sample, obtained from another uremic individual (Fig. 2C), was shown to contain neutral components (N), hippuric acid (HA), uric acid (UA), and other minor components. As expected, the peak heights and areas in the hemodialysate electropherogram are much smaller than those in Fig. 2B since they are diluted in the artificial kidney with hemodialysate carrier fluid.

3.3. Peak identification and quantitation

Tentative peak identities in serum and hemodialysate separations were assigned by matching electrophoretic mobilities with those obtained for standard compounds. Since the pK_a values of hypoxanthine (8.9) and pseudouridine (9.2) [32] are very close to the running buffer pH (9.0), small deviations in pH (< 0.1) can result in significant changes ($> 3\%$) in the electrophoretic mobility for these two analytes. Photodiode-array detection (with UV spectra acquisition) and spiking with authentic standards have provided powerful secondary means of identification. Normalized UV spectra for authentic standards and identified peaks from Fig. 2B have

Table 1
List of metabolites in mixture separated by CE in Fig. 1

Compound	Peak No. in Fig. 1	μ_{ep} ($\times 10^4$)[a] (cm^2/Vs)	Concentration (mM)
1-Methylnicotinamide	1	+3.09 ± 0.01	0.553
Phenyltriethylammonium (internal standard)	2	+2.62 ± 0.01	0.518
Mesityl oxide (flow marker)	3	(μ_{eo} = 5.32 ± 0.01)	8.64
Unknown impurity	4	−0.25 ± 0.01	
Theobromine	5	−0.39 ± 0.01	0.533
Tryptophan	6	−0.82 ± 0.01	0.524
Allopurinol	7	−0.97 ± 0.01	0.551
Uracil	8	−1.00 ± 0.01	0.500
Tyrosine	9	−1.03 ± 0.01	0.524
Hypoxanthine	10	−1.67 ± 0.01	0.558
Theophylline	11	−1.77 ± 0.01	0.500
Hippuric acid	12	−2.33 ± 0.01	0.508
Kynurenic acid	13	−2.39 ± 0.01	0.497
Indole-3-acetic acid	14	−2.43 ± 0.01	0.519
4-Hydroxyphenylacetic acid	15	−2.55 ± 0.01	0.513
Uric acid	16	−2.58 ± 0.01	0.523
Xanthine	17	−2.63 ± 0.01	0.506
Ferulic acid	18	−2.76 ± 0.01	0.520
Indican	19	−2.84 ± 0.01	0.430
Nicotinic acid	20	−2.93 ± 0.01	0.487
2-Hydroxyhippuric acid	21	−3.64 ± 0.01	0.656

[a] Error values represent standard deviation (n = 2).

been overlaid and are shown in Fig. 3. All spectra have been corrected for background absorbance and normalized to the maximum absorbance between 190 and 300 nm. Using this method of spectral analysis, a clear distinction between the suspect analyte and neighboring or co-eluting peaks can often be made. In addition, spectral data can also be used to speculate on the contents of peaks caused by co-eluting components (see Fig. 5 below).

3.4. Data analysis

To demonstrate the utility of this CE method, we have analyzed ten uremic and ten "normal" serum samples, each obtained from a different patient. As expected, numerous peaks were observed and many remain unidentified. Serum concentrations of uric acid, hippuric acid, hypoxanthine, pseudouridine, 2-hydroxyphenylacetic acid, indican and other minor components were determined and are given in Table 3 (dashes in this table indicate that the compound in question was not found in that particular sample). The four compounds uric acid, hippuric acid, pseudouridine, and hypoxanthine were found in a large number of both uremic and "normal" serum samples. The identity of these four peaks was further supported by "spiking" experiments. The average peak areas obtained for these four compounds in both populations indicate that these metabolites occur at elevated levels in many uremic individuals. The range of uric acid, hippuric acid, hypoxanthine, and pseudouridine concentrations, as well as the mean of the values for samples with detectable amounts of these four metabolites is illustrated in Fig. 4. The plateau of each bar represents the mean concentration for the samples found to contain the

Table 2
Linear regression data for calibration plots of concentration (mM) versus peak area (relative to internal standard) for the compounds separated in Fig. 1

Compound	Slope	Intercept	r^2	Detection limit (mM)
1-Methylnicotinamide	0.978	−0.00180	0.9998	0.0063
Theobromine	3.39	−0.00471	0.99996	0.0022
Allopurinol	3.66	0.00364	0.99997	0.0012
Uracil	2.13	0.00151	1.00000	0.0020
Tyrosine	1.85	0.00266	0.9993	0.0050
Hypoxanthine	4.44	0.00259	0.99999	0.0013
Theophylline	5.71	0.0123	0.99987	0.0018
Hippuric acid	3.37	0.00540	1.00000	0.0019
Kynurenic acid	8.81	0.00546	1.00000	0.00045
Indole-3-acetic acid	7.72	0.00027	0.99986	0.0010
4-Hydroxyphenylacetic acid	3.11	0.0153	0.99997	0.0020
Uric acid (210 nm)	7.63	−0.141	0.997	0.0011
Uric acid (291 nm)	4.89	−0.0382	0.99993	0.010
Xanthine	4.48	0.00957	0.99998	0.0020
Ferulic acid	6.72	−0.00937	0.99987	0.0015
Indican	7.73	−0.0255	0.99933	0.0015
Nicotinic acid	3.57	0.00407	0.99999	0.0024
2-Hydroxyhippuric acid	15.6	−0.180	0.9994	0.0023
Pseudouridine	4.77	0.00554	0.9998	0.0019

metabolite in question. As expected with clinical samples, the range of the data points for both sets of samples is quite large. Overall, the data fall within the expected range for "normal" and "elevated" concentrations [12,13,24]. It is clear, however, that the two populations are different with respect to concentration of these four analytes. The difference in the two populations is statistically significant ($p < 0.01$) for hypoxanthine ($t = 3.56$), pseudouridine ($t = 7.71$), hippuric acid ($t = 4.15$) and uric acid ($t = 3.61$). The range of the data and/or frequency of detectable peaks for other metabolites precludes the determination of statistically significant differences between the two populations.

Although we were able to determine and quantify most of the major and some of the minor peaks obtained in these analyses, there are several frequently observed peaks that we have not been able to conclusively identify. These include the neutral peak, that typically elutes at about 5 min, and several other peaks that occur at characteristic elution times. These peaks occur more frequently in uremic serum than in normal serum, and do not appear to correspond with any of the metabolites that we have considered in this study. Creatinine is likely to be a major component of the neutral peak in any given analysis, and small peptide fragments might explain some of the other unidentified peaks.

Several HPLC [20–22,24–27] and CE [12,14,15] analyses have reported the presence of creatinine and/or indican in blood serum of patients with chronic renal failure, yet our method did not determine either of these two metabolites with any regularity.

Creatinine is neutral at pH 9.0 and is most likely a major component in the neutral peaks that we have obtained. The UV spectrum for a creatinine standard does not match the spectra obtained from the neutral peak in the separations in Fig. 2, but spectral similarities are apparent. Overlaid normalized UV spectra of a

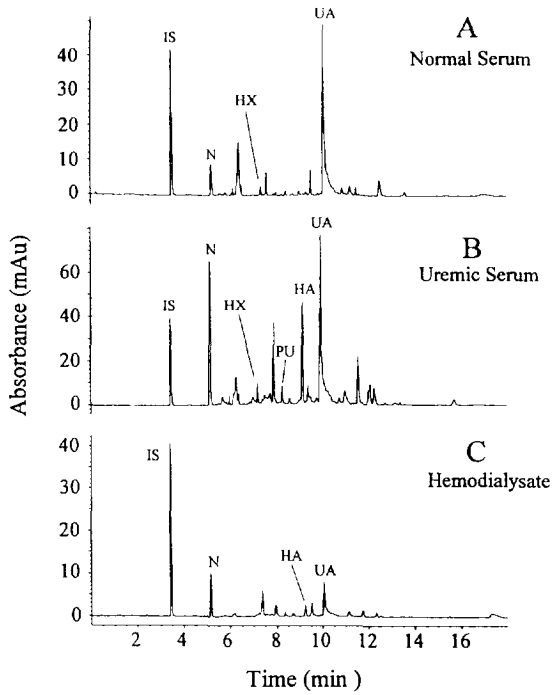

Fig. 2. Representative CE separations of clinical samples: (A) ultrafiltered normal serum; (B) ultrafiltered uremic serum; (C) hemodialysate. CE conditions are the same as in Fig. 1. Peak identities: IS = phenyltriethylammonium internal standard; N = neutral peak; HX = hypoxanthine; PU = pseudouridine; HA = hippuric acid; UA = uric acid.

creatinine standard and those of the neutral peaks in Fig. 2 are shown in Fig. 5. It is clear that creatinine is not the sole constituent in this group of neutral compounds. The magnitude of the absorbance at each of the spectral features (at 205 nm and 234 nm) might indicate that the uremic serum has the highest concentration of creatinine, followed by normal serum and hemodialysate, respectively. Because creatinine is uncharged over a wide pH range, determination of creatinine remains difficult with free zone capillary electrophoresis. Efforts to separate and identify the neutral components in sera using MEKC are currently being investigated.

The absence of indican in our CE analyses can be attributed to the removal of indican, which is known to be strongly serum protein-bound [24], in the ultrafiltration step. Indican was rarely detected (2 of 10 uremic serum samples) in any of the electropherograms for biological fluids that were subjected to Centrifree sample pretreatment.

Since some of the unidentified peaks had spectral features similar to those of tyrosine and/or tryptophan, we speculate that they could correspond to peptide fragments with different amino acid compositions and correspondingly different net charges and mobilities. One such peak characteristically elutes at about 6.3 min ($\mu_{ep} = -9.7 \cdot 10^{-5}$ cm^2/Vs) and was found in 40% of the normal sera and in 100% of the uremic sera. Two additional peaks, one at about 7.9 min ($\mu_{ep} = -1.8 \cdot 10^{-4}$ cm^2/Vs) and a second at about 8.6 min ($\mu_{ep} = -2.1 \cdot 10^{-4}$ cm^2/Vs) were observed in 10% and 0% of normal sera, respectively. These same two peaks were observed in 100% and 60% of uremic sera, respectively. Each of these three characteristic peaks can be seen in the separation of uremic serum shown in Fig. 2B. We found no correlation of the presence or absence of any of these peaks with other peaks in any individual sample for this limited population. It was evident, however, that the uremic samples had, on average, higher concentrations of each of these species.

Three additional unidentified peaks that had no spectral similarities to tyrosine or tryptophan were frequently observed in separations of uremic serum samples. These three peaks exhibited minimal spectral features as compared with the peaks discussed in the previous paragraph. First, a peak at about 7.3 min ($\mu_{ep} = -1.6 \cdot 10^{-4}$ cm^2/Vs) with a weak λ_{max} at about 240 nm was observed in 20% of the normal samples and 50% of the uremic samples. Second, a peak at 9.4 min ($\mu_{ep} = -2.4 \cdot 10^{-4}$ cm^2/Vs) was observed in 70% of the normal samples and 80% of the uremic samples. Finally, and most interestingly, a peak at about 12.1 min ($\mu_{ep} = -3.0 \cdot 10^{-4}$ cm^2/Vs) often with a characteristic hump in the UV spectra at about 280 nm was observed in 90% of the uremic serum samples, and was not present in any of the normal serum samples. The presence (or absence) of each of these peaks could provide interesting insight into the physio-

Table 3
Apparent metabolite concentration in m*M* for individual "normal" (N) and uremic (U) samples

Compound	N1	N2	N3	N4	N5	N6	N7	N8	N9	N10	Mean ± S.D.
Uric acid	0.143	0.259	0.138	0.54	0.405	0.392	0.29	0.33	0.15	0.348	0.30 + 0.13
Hippuric acid	0.007	0.0068	0.0096	0.007	0.004	0.0119	0.0041	–	0.0090	0.0128	0.008 ± 0.003
Hypoxanthine	0.026	0.0080	0.0082	0.0051	0.0059	0.0046	0.0106	0.0067	–	0.0172	0.009 ± 0.007
Pseudouridine	0.0054	0.0056	0.0067	0.0070	0.0006	0.0049	0.0057	0.021	0.0076	0.00894	0.007 ± 0.005
Theophylline	–	–	–		–	0.0065	0.0172	–	–		
4-Hydroxyphenyl-acetic acid	–	–	0.26	–	–	–			–	–	
Indican	0.023	–	–	–		–	–	–	0.0127	–	
2-Hydroxyhippuric acid						–				–	
	U1	U2	U3	U4	U5	U6	U7	U8	U9	U10	Mean ± S.D.
Uric acid	0.43	0.452	0.432	0.45	0.65	0.39	0.47	0.40	0.51	0.58	0.48 ± 0.08
Hippuric acid	0.049	0.334	0.080	0.56	0.261	0.066	0.209	0.213	0.36	0.091	0.22 ± 0.16
Hypoxanthine	0.0420	0.0302	0.0130	0.095	0.014	0.038	0.017	0.0216	0.0339	0.10	0.04 ± 0.03
Pseudouridine	0.029	0.040	0.0369	0.05	0.042	0.037	0.064	0.0364	0.066	0.0257	0.043 ± 0.014
Theophylline	–	–	–		–	–	–	–	–		
4-Hydroxyphenyl-acetic acid	–	–	–	–	–	–	0.060		–	–	
Indican	–	–	–	–	0.029	–	–	–	0.033	–	
2-Hydroxyhippuric acid	–	0.0226	–	0.0504	–	–	–	0.0193	–	0.0317	

Averages where calculated were done so by using the limit of detection for samples with analyte not detectable. Individual entries are averages of three injections (with exception of N1 which is only two). For individual entries, error in most precise digit is less than ± 5.

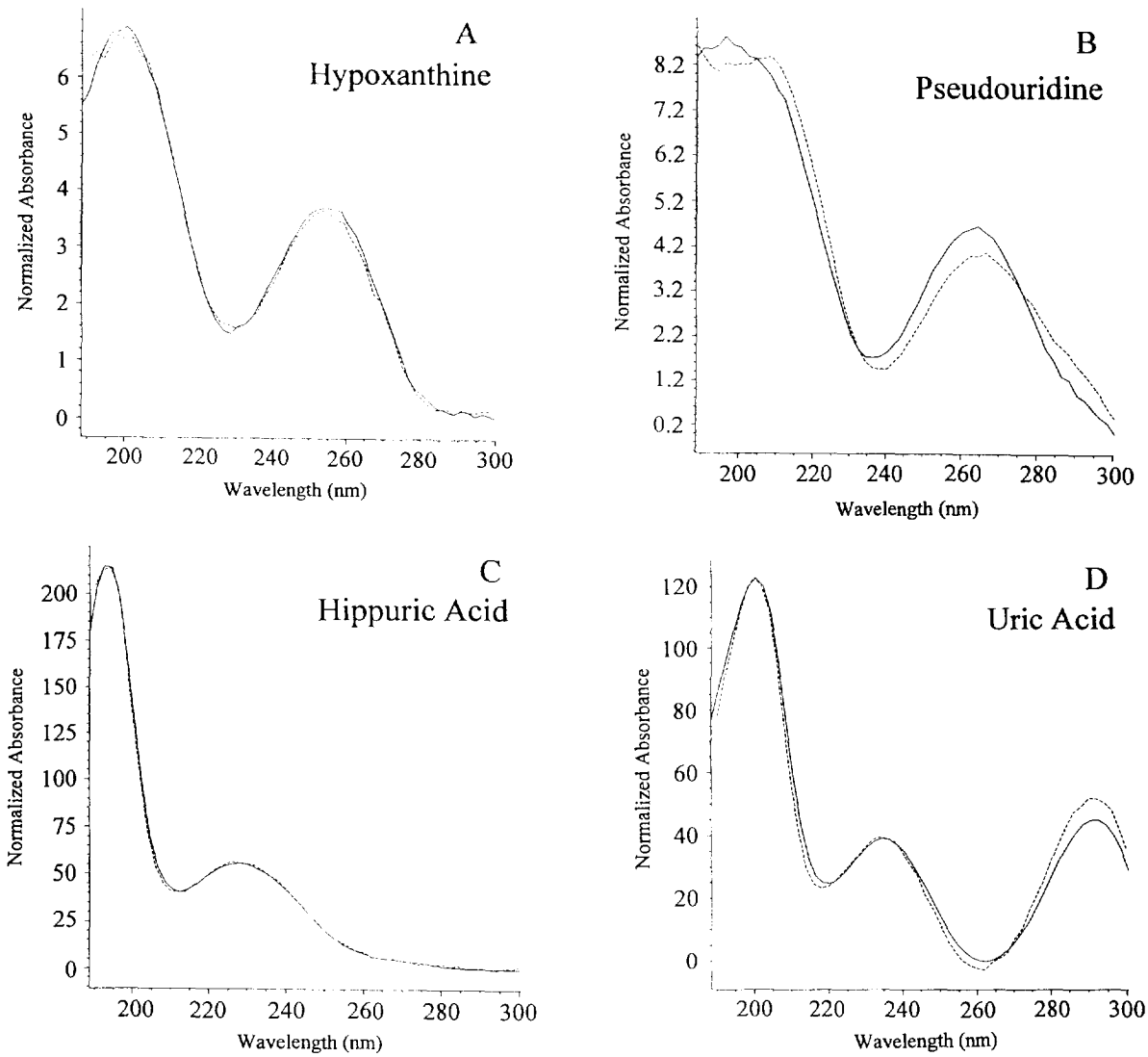

Fig. 3. Normalized UV spectral match for: (A) hypoxanthine; (B) pseudouridine; (C) hippuric acid; and (D) uric acid. In each case the solid line represents the spectrum of an authentic standard as collected upon elution, and the dashed line represents the spectrum obtained at the apex of the suspect peak in the electropherogram of the uremic serum sample shown in Fig. 2B.

logical and pathological factors associated with chronic renal failure and, thus, is worthy of further study.

4. Conclusions

Clearly, the use of CE to analyze biological fluids represents a powerful new technology for the study of the physiology and pathology of medical disorders including renal failure. This method allows for efficient profiling of serum contents including low-molecular-mass metabolites and peptide fragments. Quantitative determination of pseudouridine, hypoxanthine, hippuric acid, and uric acid in biological fluids has been accomplished in a single multicomponent screening of each of twenty serum samples.

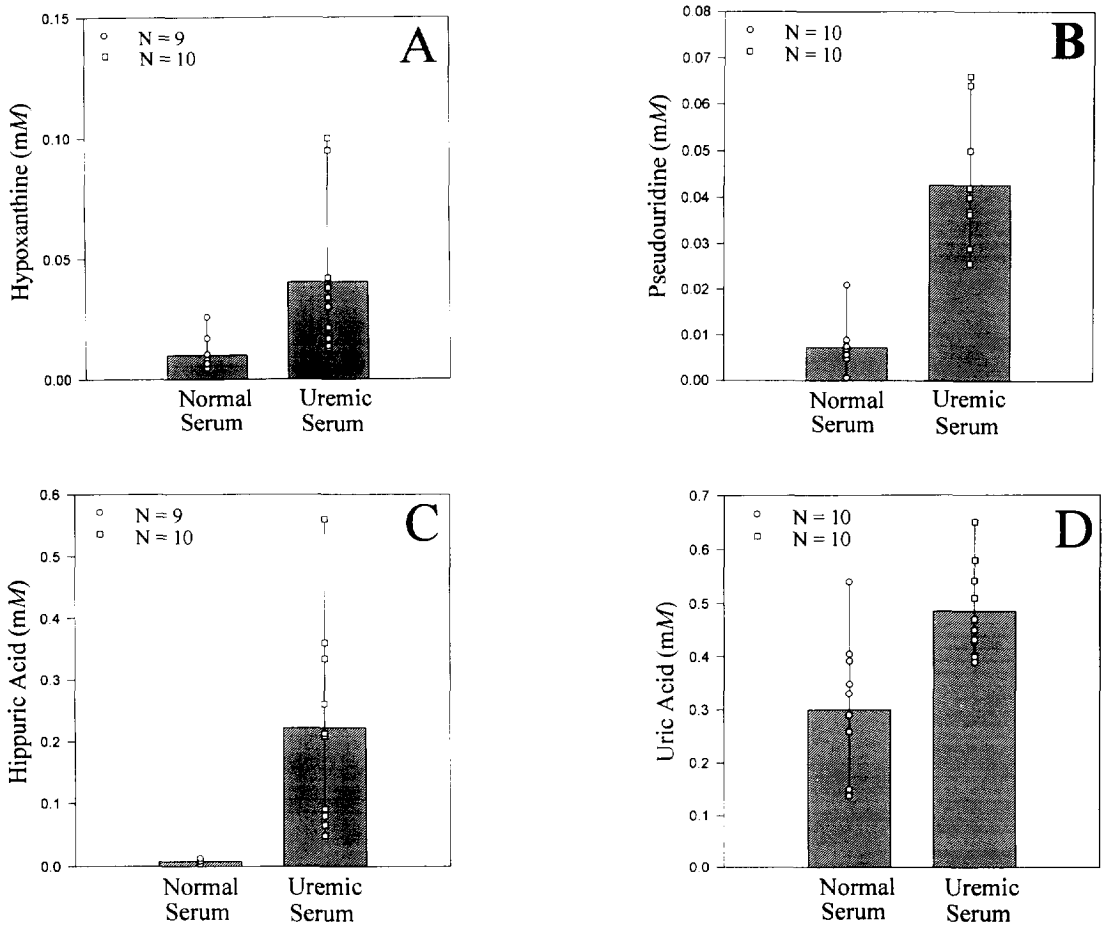

Fig. 4. Comparison of the range of concentrations of four particular metabolites found in ten "normal" and ten uremic serum samples. The value at the plateau of each bar represents the average (mean) concentration for those samples found to contain detectable amounts of the metabolite in normal (○) and uremic (□) serum: (A) hypoxanthine; (B) pseudouridine; (C) hippuric acid; (D) uric acid. N indicates the number of samples (out of ten) that contained a detectable amount of the metabolite.

Uremic sera were found to have significantly higher concentrations of these four metabolites. The other metabolites considered here were detected too infrequently to determine any difference in the two populations.

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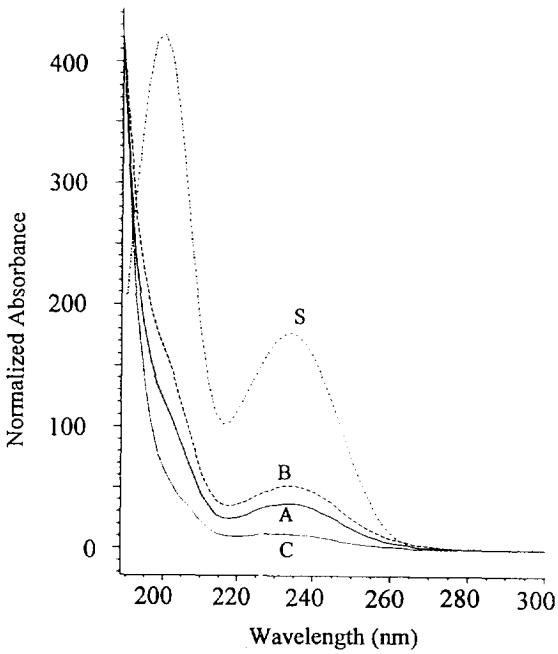

Fig. 5. Normalized UV spectral comparison of creatinine with the neutral peaks in Fig. 2. All spectra were obtained at the peak apex. Key: S = from authentic creatinine standard peak; A = from neutral peak in Fig. 2A (normal serum); B = from neutral peak in Fig. 2B (uremic serum); C = from neutral peak in Fig. 2C (hemodialysate).

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