Analytica Chimica Acta 676 (2010) 68–74

Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

Nanoengineered optical urea biosensor for estimating hemodialysis parameters in spent dialysate

M. Swati [a], N.K. Hase [b], Rohit Srivastava [a,*]

[a] Department of Biosciences and Bioengineering, Centre for Research in Nanotechnology & Science (CRNTS), IIT Bombay, Powai, Mumbai 400076, Maharashtra, India
[b] Department of Nephrology, King Edward Memorial Hospital, Parel, Mumbai 400012, India

ARTICLE INFO

Article history:
Received 8 April 2010
Received in revised form 16 June 2010
Accepted 20 July 2010
Available online 27 July 2010

Keywords:
Hemodialysis monitoring
Optical urea biosensor
Urea kinetic modeling
Calcium alginate microspheres
Cresol red
Layer-by-layer

ABSTRACT

An optical biosensing scheme based on urease encapsulated calcium alginate microspheres which are coated with polyelectrolyte nanofilms predominantly composed of cresol red (CR) dye is demonstrated in this paper. The dye molecules within the nanofilms are deposited via the layer-by-layer (LbL) self-assembly technique on the microspheres and used as the optical transducer. A flow through cell constructed using a cuvette attached to a fiber optic spectrometer was used to determine the response of the biosensor to standard urea solutions of different concentrations. The change in pH and the absorbance ratio was monitored with time and these results were used for measurements of urea concentrations in the spent dialysate fluid. The biological parameters controlling hemodialysis such as dialyzer clearance or Kt/V and percent removed urea (PRU) have also been reported. The results demonstrate that the urea biosensor is pH reversible with a sensitivity of 0.09 pH units/min and is able to detect a change of 0.005 ratio units in urea concentration ranging 0.1–60 mg dL^{-1}. The response time of the sensor was calculated as 8 min while the detection range of urea covered the levels that are present in the spent dialysate fluid. The results obtained in the analysis of biological samples were in good agreement with those obtained by a reference method, showing no significant differences at a confidence level of 95%.

1. Introduction

Dialysis is necessary to remove waste products from the blood, when kidneys fail to function. Nearly all uremic toxins are products of protein metabolism and clinically it is difficult to identify and determine all of them. While whole spectrum of these products is unknown, urea (having mild toxicity) has become the marker for these unidentified toxins because it is the end product of protein metabolism and is present in millimolar (mM) concentration which is easy and relatively inexpensive for determination [1]. Dialysis efficiency is monitored periodically by testing a patient's blood which is sampled at the start and at the end of dialysis. The levels of urea in the two blood samples are then compared. Urea kinetic modeling [2–7] is increasingly recognized as the most efficient way for quantifying and monitoring hemodialysis treatments. Mathematical models that have been applied for urea kinetic modeling are complex, time-consuming, require blood sampling and potentially inaccurate. Direct dialysate methods bypass any solute disequilibrium in the patient's body. This simplifies the mathematics and allows accurate predictions to equilibrium concentrations in the body, even before all solute gradients have dissipated [8].

Measurement of urea in the spent dialysate makes it possible to calculate the amount of urea removed during one session directly and is termed as true removed urea (TRU). This value is highest at the beginning of the week and lowest at the end of the week. The bioanalytical systems dedicated for dialysate urea nitrogen (DUN) determination predominantly are based on enzymatic reactors containing urease. The enzyme converts urea into ionic products (ammonium and hydrocarbonate/carbonate ions). Usually, for detection of the products, conductimetric [9,10] or potentiometric [11,12] techniques are adopted. For example, disposable conductimetric biosensors for measurements in spent dialysate have been developed using screen-printing technology [13,14]. These biosensors for measurement of urea in spent dialysate have one of the major disadvantages that their fabrication takes long time with the requirement of expensive reagents at several steps. Even though they display faster response time, the range of urea levels detected by such sensors is usually narrow which might not be helpful in dealing with biological samples. Two methods are generally used to assess dialysis adequacy, percent removed urea (PRU) and Kt/V where K stands for the dialyzer clearance (mL min^{-1}) and t stands for time. Kt/V is the fraction that represents the volume of fluid completely cleared of urea during a single treatment (www.kidney.niddk.nih.gov accessed on 14.07.09).

At present, there are some commercially available urea monitors for the automatic measurement of urea concentration in the

* Corresponding author. Tel.: +91 22 25767746; fax: +91 22 25723840.
E-mail address: rsrivasta@iitb.ac.in (R. Srivastava).

0003-2670/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.aca.2010.07.030

spent dialysate. These monitors are bulky and very expensive, due to which the cost per test may not be affordable to people of lower hierarchy. Their complicated construction and requirement of extra electrodes to compensate for electrical interferences is another drawback which could be overcome by usage of simple sensing systems like the one described in this work. The traditional once-per-month blood-based estimation of urea removal at the time of hemodialysis is done by using one of these commercial urea biosensors. In recent years such estimations have recognized limitations: the first is a consequence of the infrequent monitoring where under dialysis can persist for up to two months before the dialysis prescription is modified, by which time the patient's health can be detrimentally affected. The infrequency of blood-based measurements also puts substantial burden on the accuracy of the measurement. It is at this point where the requirement of a sensor is deliberated that would allow real-time, online monitoring of urea removal as a result of which systematic under dialysis of an individual can be identified and corrected on a much shorter time scale. These are some of the reasons that could justify the need for a urea biosensor which is both cost-effective and can give accurate results.

Urea biosensor developed in this work consists of cresol red as the pH sensitive dye which detects the pH changes produced during the enzyme–substrate reaction. It is an acid–base indicator where in alkaline conditions it is red or purple in color but after exposure to acid it turns light yellow in color. With increasing pH the ratio of basic to acidic form of the dye increases paving the way for ratiometric analysis. The advantages of such an analysis are that the instrumental errors, abnormalities of unequal dye loading and signal drifts that are usually associated with single wavelength measurements, are nullified. The absorption maxima of the protonated (HR) and the deprotonated (R^-) forms are 434 and 572 nm, respectively. The developed sensor was coupled to a cuvette based flow system connected to a fiber optic spectrophotometer for absorbance measurements. The USB4000 spectrometer is a miniature fiber optic UV/VIS/NIR detection system for low light level applications. It accepts light energy transmitted through single-strand optical fiber and disperses it via a fixed grating across the CCD array (www.oceanoptics.com accessed on 25-11-09). Fiber optic sensors based on immobilized enzymes are relatively new and offer some advantages over electrochemical sensors. These advantages include minimized electrical noises because the signal is optical, the lack of a reference cell or electrode, analysis of samples in an inaccessible location as well as the possibility of miniaturization [15,16]. They are immune to electromagnetic interference, because there are no electrical currents flowing at the sensing point.

2. Experimental

2.1. Materials

Sodium alginate (low viscosity; 250 cps,), Urease (EC3.5.1.5, activity of 50,000 U g^{-1}), phosphate buffered saline (PBS), sodium poly (styrene sulfonate) (PSS, MW ~ 70,000), poly (allylamine hydrochloride) (PAH, MW ~ 70,000) were obtained from Sigma (India). Agarose was obtained from SRL, Mumbai, India and Nessler's reagent for ammonium ion determination was purchased from Qualigens, Mumbai, India. Cresol red was purchased from Loba Chemei, Mumbai, India while, calcium chloride and urea were obtained from Merck Ltd., Mumbai, India. Quantichrom urea assay kit was purchased from Krishgen Biosciences, Mumbai, India. All the reagents were of analytical grade and were used without further purification. Experiments were carried out using solutions prepared with double distilled water.

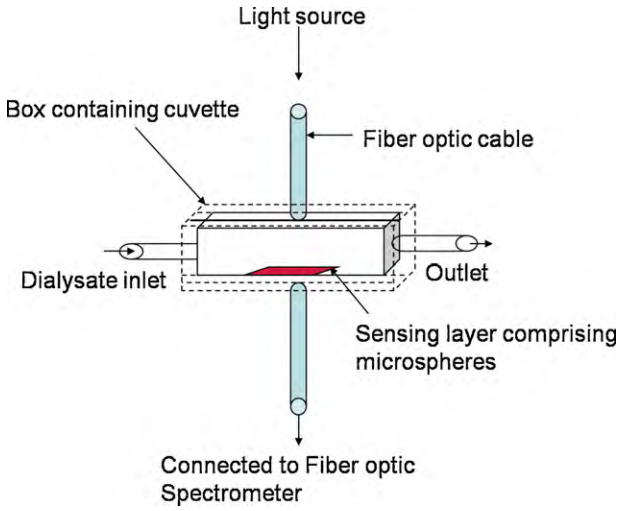

Fig. 1. Cuvette based flow cell.

2.2. Instrumentation and measurements

All optical measurements were performed at the characteristic wavelengths of cresol red dye at 434 and 572 nm with a Hitachi U2900 Spectrophotometer. Ocean Optics' Fiber optic spectrophotometer (USB4000), white LED light source, optical fibers and cuvette holder were used for flow cell construction. All spectrophotometric measurements were performed at room temperature without any special thermosetting. The pH values of all solutions were determined with SympHony SP70P pH meter (VWR). The fluorescent images of the microspheres were obtained from confocal laser scanning microscope (Olympus IX81).

2.3. Preparation of optical urea biosensor

Preparation of calcium alginate microspheres for the biosensor development was carried out according to the procedure previously described in detail [17]. Briefly, the composition of the alginate microspheres was: 2 wt.% of sodium alginate, 2 mg mL^{-1} concentration urease solution and 0.25 M calcium chloride solution. The microspheres were prepared by extrusion of the precursor solution (urease mixed with sodium alginate solution) from the nozzle of a commercial droplet generator into the calcium chloride solution for gelation. The size of the prepared microspheres was around 50–80 μm.

Further, the microspheres were coated with polyelectrolyte nanofilms with cresol red dye molecules encapsulated within them. The protocol briefly is as follows: a solution of 2 mg mL^{-1} each of poly (allylamine hydrochloride) or PAH (polycation) and cresol red-poly (styrene sulfonate) or CR-PSS (polyanion) was prepared in 0.5 M calcium chloride solution. To 200 μL microspheres, 1 mL of PAH was added and incubated for 20 min with intermittent shaking. After this step, washing was accomplished by centrifugation of the suspension at 1000 rpm for 1 min. The second layer of CR-PSS was deposited using a similar technique on the microspheres. This formed a bilayer of PAH/(CR-PSS) on the surface of the microspheres.

2.3.1. Flow cell arrangement

Approximately 100 μL of these microspheres was put inside a plastic cuvette placed horizontally as shown in Fig. 1. When partially dried, 0.1% agarose solution was poured on them. The temperature at which it was poured over urease encapsulated microspheres was approximately 40 °C which resulted in immediate solidification of the agarose solution forming the sensing layer.

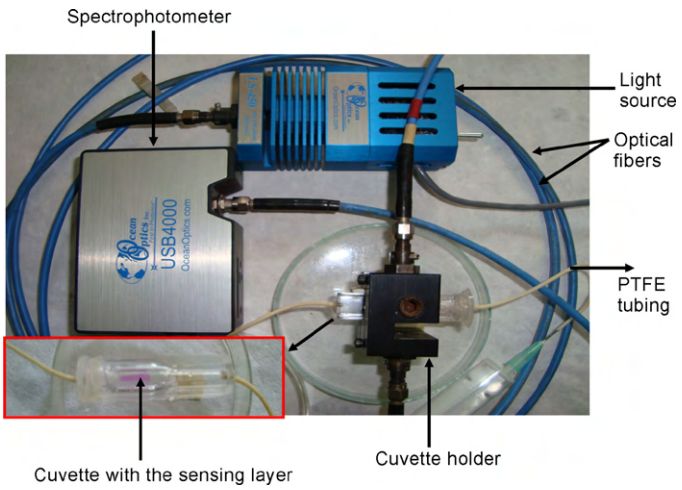

Fig. 2. Flow cell arrangement integrated with fiber optic spectrophotometer.

This arrangement of the sensing layer with the cuvette was connected to a syringe pump and tubing made of PTFE 0.3 mm. For absorbance measurements in the flow system, optical fibers (727-733-2447, Ocean Optics Ltd., India) connected to white light source (LED-White, Ocean Optics Ltd.), mini spectrometer (USB4000, Ocean Optics Ltd.) were used as shown in Fig. 2. Control of the photometer and data acquisition was accomplished with SpectraSuite software (Ocean Optics Ltd.) interfaced with a computer.

2.3.2. Confocal imaging

The fluorescent images of calcium alginate microspheres incorporated as the sensing layer in the cuvette were obtained by confocal laser scanning microscope by using Alexa Red filter with an excitation wavelength (WL) of 568 nm using the laser source (He-Ne-G) with an excitation WL of 485 and an emission WL of 596 nm.

2.3.3. Flow cell procedure

The performance of the cuvette based flow cell was evaluated under dynamic conditions with an internal volume of 1 mL. Phosphate buffered saline (or PBS) at pH 7.0 ± 0.15 having a molarity of 10 mM (for baseline measurement) and urea solution were introduced alternately through the syringe pump into the flow cell at a flow rate of 0.5 mL min^{-1} ensuring that the sensing layer does not shift from its place. The buffer was withdrawn after recording the dark and bright spectrum of the light source used and subsequently urea solutions of different concentrations were pumped through the flow cell for further analysis. The effect of buffers of varying pH on the absorbance of cresol red dye at its two wavelengths has been previously studied [18]. Enzymatic catalysis of urea produced ammonium ions in the surrounding medium which led to a pH increase that was sensed by the immobilized pH indicator, cresol red; its color varied from orange to red leading to an increase of absorbance at the corresponding wavelengths.

2.4. Characterization of the biosensor

2.4.1. pH change with time

The experiment was performed to demonstrate the response of the sensor and determine the maximum pH attained during the course of enzyme–substrate reaction; it was performed with urease in solution phase as well as in the immobilized phase. The pH was measured at the start of the reaction and then at intervals of 2, 4, 6, 8 and 10 min. The assay solution which was used for reaction of urease in solution phase consisted of urea and cresol red (in solution phase).

2.4.2. Absorbance ratio change with time

The experiment was performed in conjugation with the pH change with time experiment as described previously. At each point where pH was determined, the corresponding absorbance ratio values were also measured.

2.4.3. Analytical response

The results obtained with the experiment on determining the change in pH and absorbance ratio were further evaluated under flow conditions. A solution of 60 mg dL^{-1} urea concentration was passed through the sensing layer where its response was studied until a constant value was achieved. The reproducibility of the absorbance measurements with fresh urea solution in alternation with the buffer was carried out at 434 and 572 nm. Inter-device repeatability was evaluated with twelve different urea biosensors. Each biosensor was used once and the reproducibility of absorbance ratio and pH was studied for 60 mg dL^{-1} urea. The average and relative standard deviation of the values were calculated.

2.5. Clinical evaluation of biosensor

To evaluate the clinical efficacy of the biosensor, spent dialysate samples were used. These samples were obtained from 3 regular subjects undergoing dialysis sessions at the outpatient dialysis unit of the Department of Nephrology, KEM Hospital, Parel, Mumbai, India. The liquid was sampled at 10, 15, 30, 60, 120, 180 and 240 min of hemodialysis session and immediately refrigerated for later measurement of urea in our lab.

Quantichrom urea assay kit was used as the reference method for determining urea concentrations in the dialysate samples. A chromogenic reagent formed a colored complex specifically with urea and the intensity of the color, measured at 520 nm, was directly proportional to the urea concentration in the sample. This method has a linear detection range 0.08–100 mg dL^{-1} and no pretreatments are needed to be performed since assays can be directly performed on raw biological samples. The protocol for the experiment is as follows: equal volumes of Reagent A and Reagent B, present in the kit, were mixed shortly prior to the assay. In the tubes 20 μL water, 20 μL standard (50 mg dL^{-1}) and 20 μL samples were taken and added 1 mL of the working reagent, respectively. After incubation of 20 min the absorbance was noted at 520 nm. Urea concentration was calculated from Eq. (1):

$$[\text{Urea}] = \frac{A_{\text{sample}} - A_{\text{blank}}}{A_{\text{standard}} - A_{\text{blank}}} \times n \times [\text{STD}] \quad (1)$$

A_{sample}, absorbance of the sample; A_{blank}, absorbance of the blank; $A_{standard}$, absorbance of the standard; n, dilution factor (if any); [STD] = 50 mg dL^{-1} standard (provided with the kit). Conversions: blood urea nitrogen (BUN) in mg dL^{-1} = [Urea]/2.14.

The standard curve of urea prepared from the kit was used to calculate urea concentrations present in the dialysate samples.

3. Results and discussion

3.1. Performance of the optical urea biosensor

The optical urea biosensor used in this work consists of calcium alginate microspheres coated with cresol red incorporated within polyelectrolyte nanofilms. The particles immobilized in agarose gel on the inner surface of the cuvette formed the sensing layer as displayed in the confocal image (Fig. 3). The sensing layer of agarose gel does not affect the flow of the fluid because urea is a very small molecule (60.06 g molecular weight) and it can easily pass through the porous matrix of agarose. The microspheres on the other hand are of size range 50–80 μm which will not easily come out of the gel making them further stable in the gel. Urease enzyme catalyses the

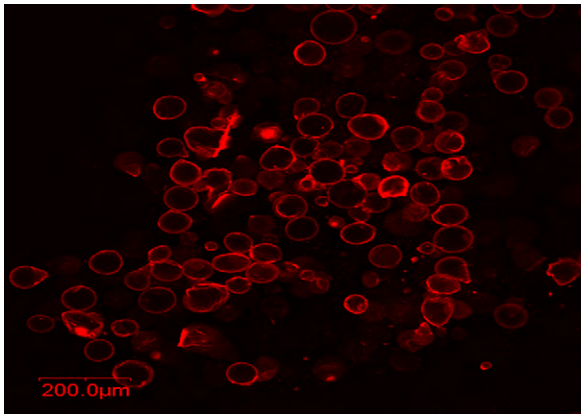

Fig. 3. Confocal image of microspheres embedded in agarose gel (red fluorescence due to the presence of CR dye). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

hydrolysis of urea and the ammonium ions formed in the course of the reaction increase the pH of the medium further causing a change in the absorbance of the dye molecules encapsulated within the nanofilms. Changes in the intensity of its color from orange to yellow to purple color were treated as analytical signals.

3.1.1. Response time of the biosensor

Fig. 4A represents the response time of urease in solution phase. The pH of the buffer (PBS) was 7.0 ± 0.15 and when urea solution was added, the production of ammonium ions caused increase in the pH which was recorded at intervals of 2 min for 10 min. Approximately, the highest pH recorded was 8.0 ± 0.15 and the time taken to reach this value was 6 min. Beyond this time there were no significant changes in the pH, whereas significant changes in the corresponding absorbance ratio values were observed till 8 min. Thus the response time of urease in solution phase was established as 8 min.

In Fig. 4B, the response time of urease in immobilized form in the sensor has been studied. Both the pH and absorbance ratio displayed a constant value with buffer solution (without urea) which is clearly indicated in the figure. When urea solution was added, the increase in pH (from 7.0 to 7.85 ± 0.03) was monitored until a constant value was achieved. The complete response of the sensor was achieved in 8 min beyond which there were no significant differences in the pH. Agarose did not affect urease activity as can be observed by the increasing pH due to continuous production of ammonium ions. Therefore the response time of the biosensor was established as 8 min.

3.1.2. Analytical response of the biosensor

The analytical characteristics of the biosensor were studied by evaluating the reversibility and repeatability of the biosensor. A solution of 60 mg dL^{-1} urea was passed through the sensing layer (flow rate = 0.5 mL min^{-1}) where its response was studied until a constant value was achieved. PBS buffer at pH 7.0 ± 0.15 was initially pumped through the flow cell and its corresponding absorbance ratio value was noted. After withdrawing the buffer, urea solution was pumped through the flow cell and allowed to stay in contact with the sensing layer of the cuvette for 8 min (response time of the sensor). The changes in the pH as well as absorbance ratio values were noted at intervals of 2 min within the time period of 8 min. These set of values corresponded to the response of the biosensor when urea solution was pumped for the first time through the flow cell. Urea solution was then replaced by the buffer in the flow cell. A second set of pH and absorbance ratio values were taken similarly when urea solution of the same

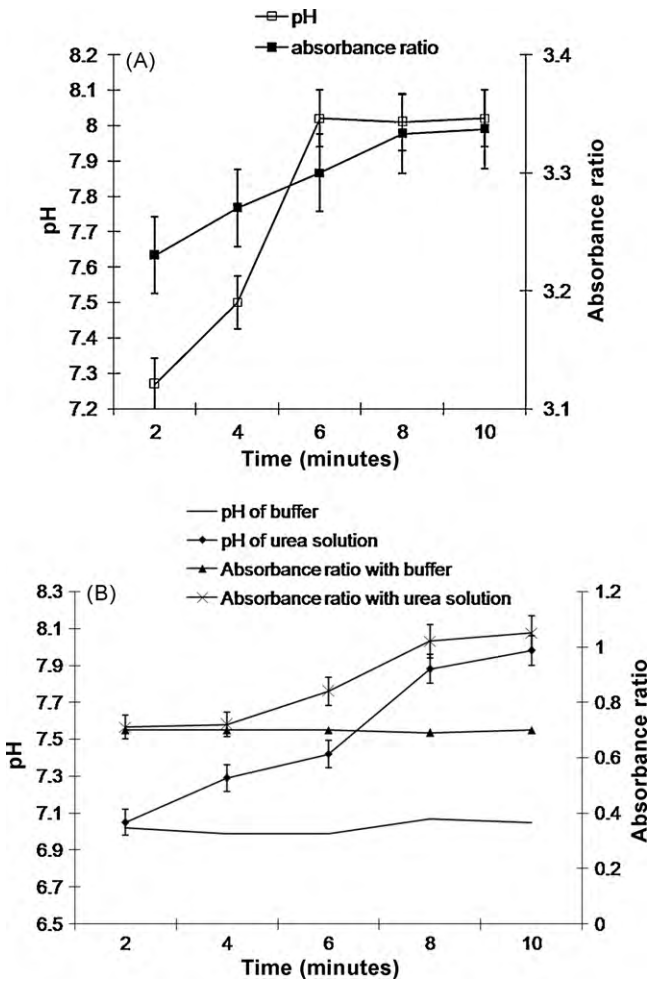

Fig. 4. (A) Response of urease in solution phase with 60 mg dL^{-1} urea solution. (B) Response of urease in immobilized phase with 60 mg dL^{-1} urea solution.

concentration was pumped for the second time through the flow cell. In this manner, four different sets of pH and absorbance ratio values were obtained from a single cuvette based flow cell in a total time period of approximately 40 min as shown in Fig. 5A and B.

Fig. 5A shows the change in pH with respect to time. The changes in the pH during the first and the second use might be due to the initial leaching out of loosely bound dye molecules in the biosensor beyond which it remained constant. Fig. 5B displays the change in absorbance ratio with respect to time and both the figures have been statistically verified by Student's *t*-test. There was no significant difference between the means of four sets of pH and absorbance ratio values at $p < 0.05$ but a significant difference was observed beyond that (represented by * in Fig. 5A and B) because of the leaching out of cresol red molecules. The relative standard deviation (RSD) calculated for given concentration of urea solution pumped four times through the sensor is 2.4%. Alqasaimeh et al., have reported that the acceptable value of RSD is approximately 9.53% [19]. This implies that the biosensor gave reproducible results when used continuously for four times in a single flow cell. The results of inter-device repeatability of twelve urea biosensors are as shown in Table 1. The average of absorbance ratio and pH was calculated and this was 1.01 ± 0.01 and 7.85 ± 0.03, respectively yielding a relative standard deviation of 4% and 1%, respectively. The difference obtained for absorbance ratio and pH values may be due to errors while immobilization of urease enzyme within the microspheres and encapsulation of cresol red during LbL process.

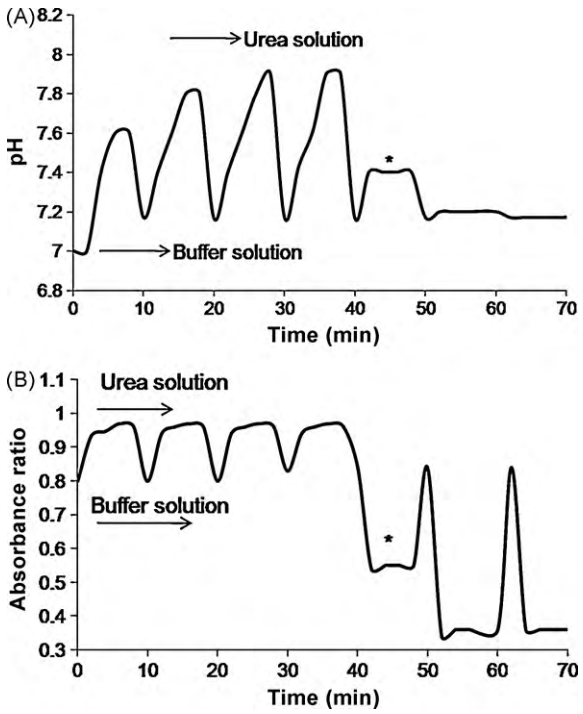

Fig. 5. (A) Analytical response of the biosensor: graph displaying pH change with time (*$p < 0.05$ as compared to fourth set of pH values). (B) Analytical response of the biosensor: graph displaying absorbance ratio change with time (*$p < 0.05$ as compared to fourth set of absorbance ratio values).

During hemodialysis procedure, it is preferred to measure urea levels frequently. For instance, in the first hour of dialysis, samples can be measured at 5, 10, 15, and 30 min intervals and then after every hour [20]. Due to the 8 min response time of the present biosensor it is not possible to take urea readings at 5, 10, 15 and 30 min interval with a single biosensor but Fig. 5A and B clearly shows that the current biosensor is also capable of taking at least four readings at an interval of 8 min beyond which the dye leaches out and a new sensor can be used for subsequent readings as per the requirement. Another option is that a separate sensor can be used for each measurement of urea. Since urea follows an exponential fall during hemodialysis, the urea levels in the first half of the procedure is crucial to determine. It may not be necessary to measure urea levels continuously during the entire hemodialysis session (which lasts for about 4 h) which may otherwise exhaust both the sensor and its optical detection unit. In our cuvette based urea biosensor the operational stability is determined as 40 min and the reason behind this is to highlight the usage of this sensor in the first hour of hemodialysis.

Table 1
Inter-device repeatability of the sensor with 60 mg dL^{-1} urea solution.

Absorbance ratio	pH
1.02	7.6
1	7.98
1.09	7.8
1.1	7.9
1.05	7.8
0.96	7.9
0.96	7.92
1	7.872
0.997	8.01
1.053	7.8
1.083	7.8
1.07	7.9
Mean = 1.03	Mean = 7.85
SE = 0.01	SE = 0.03
RSD = 4%	RSD = 1%

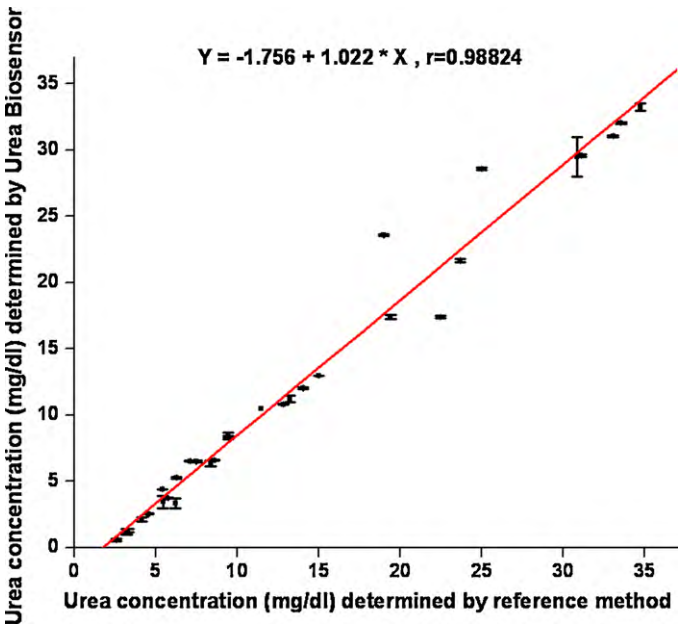

Fig. 6. Correlation of the results of spent dialysate urea determination obtained using the present biosensor and the reference method.

3.2. Clinical evaluation: dialysate urea nitrogen (DUN) determination

Urea sensing in the range of 0.1–60 mg dL^{-1} have been done previously and reproducible results for the sensitivity of the biosensor were achieved after one month of storage. After completing the sensing studies with urea standards, the next step was to determine the efficacy of the biosensor to be used for clinical samples and for this purpose spent dialysate samples were obtained from KEM Hospital, Mumbai, India to validate the results with a reference method using the urea assay kit. The urea values obtained using the reference method and the urea biosensor is statistically same as calculated using Student's *t*-test. The correlation equation for analysis of spent dialysate samples was: $y = 1.0008x - 0.1703$ with regression coefficient $r = 0.988$ as shown in Fig. 6.

3.2.1. Monitoring and modeling of hemodialysis

Fig. 7A–C represents the progress of hemodialysis sessions of Subjects K, D and S, respectively. The coefficient of variation (CV) between the values was observed to be 0.15, 0.06 and 0.07 for K, D and S, respectively. For most hemodialysis sessions the urea concentration profiles can be fitted to an exponential equation [21,22]. The equations for calculation of the hemodialysis parameters were adapted from Koncki et al. [1]. Kt/V parameter was calculated from Eq. (2) where $C_{(0)}$ and $C_{(t)}$ are the initial and final urea concentrations of spent dialysate:

$$C_t = C_0 \exp - \left(\frac{Kt}{V}\right) \quad (2)$$

The $C_{(t)}/C_{(0)}$ ratio was applied for calculation of urea reduction ratio from Eq. (3):

$$\text{PRU} = \text{URR} \times 100\% = \left(1 - \frac{C_t}{C_0}\right) \times 100\% \quad (3)$$

Nutritional protein catabolic rate or PCR_n is grams of urea nitrogen per kilogram body weight per day and is calculated from

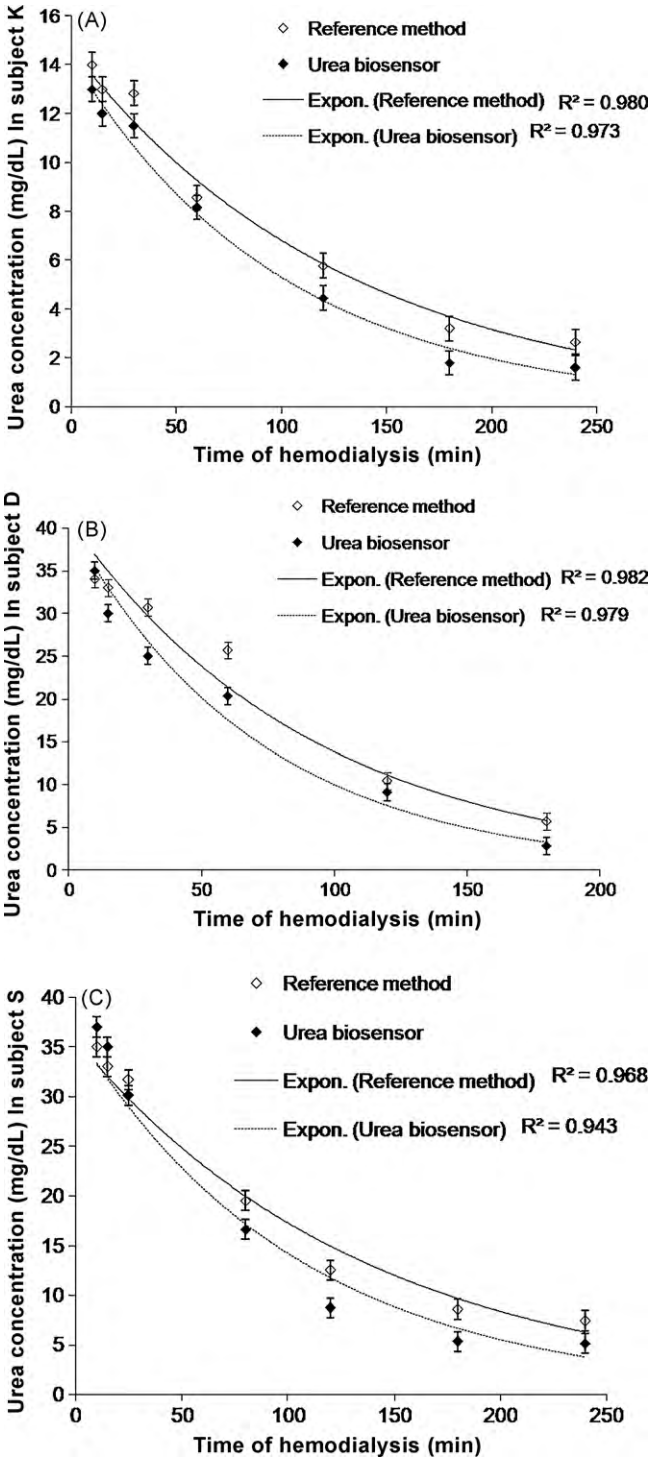

Fig. 7. (A–C) Progress of hemodialysis sessions of subjects K, D, S shown as changes of spent dialysate urea concentration, respectively.

Eq. (4):

$$\mathrm{PCR}_n = \frac{0.22 + (0.036 \times \text{intradialytic rise in urea} \times 24)}{\text{intradialytic interval}} \quad (4)$$

The discussed parameters describing both intra- and interdialytic intervals of hemodialysis can be evaluated using the biosensor presented in this work. The results of the successively monitored hemodialysis sessions are collected in Table 2 which display that the values of the parameters are high at the beginning of the week and low at the end of the week. These results obtained from nanoengineered urea biosensor clearly highlight the adequacy of hemodialysis treatments when compared to the previous works. Koncki et al. [1] have reported an average value of 68% PRU corresponding to ≈1.09 *Kt*/*V* value whereas Radomska et al. [6], Tymecki and Koncki [23], Uhlin et al. [2] have reported 0.88, 1.15, 1.23 *Kt*/*V* values, respectively.

Table 2
Biomedical parameters of successive hemodialysis sessions monitored using our nanoengineered urea biosensor.

Day of week	DUN (t = 0) (mM)	DUN (t = T) (mM)	PUR (%)	TUR (mg)	PCR_n (gm/kg/day)	*Kt*/*V*
K						
Thursday	13	1.6	87	141	0.64	2.0
Monday	12	1.2	90	109		2.3
D						
Thursday	35	2.8	92	1307	1.9	2.5
Saturday	34	2.6	92	675		2.5
S						
Thursday	37	5.1	86	1074	1.1	1.9
Saturday	23	2.6	89	574		2.2

This biosensor has the ability to determine urea levels as low as 0.1 mg dL^{-1}. Urease enzyme present in the biosensor displayed a residual activity of approximately 65% after one month of storage [24] and could sense both the physiological and dialysate urea range. Our studies have also shown that the biosensor can be used multiple times (up to four times) without the biosensor getting exhausted but there is a possibility of sensor getting exhausted once the enzyme activity is completely lost and the dye is completely leached out.

4. Conclusions

With the droplet generator technique microspheres in the size range 50–80 μm were achieved. This method was simple, took lesser time for preparation and required fewer reagents. On the other hand the immobilization of cresol red dye molecules was performed using the LbL approach which is a simple technique of multilayer deposition of polyelectrolytes based on the electrostatic force of attraction. Promising results were achieved in terms of response to changes in the pH produced by different urea concentrations which can be easily measured by cresol red. The detection range for urea is 0.1–60 mg dL^{-1} and the urea biosensor gave reproducible results within this range. Predicted urea levels in spent dialysate samples correlated well with the reference method further leading to the possibility of the system to be used as online device connected with hemodialysis machine. It proved useful for the estimation of various parameters of urea kinetic modeling helpful in the control and the assessment of adequacy of hemodialysis therapy. The achievements of this work are the enhanced urease activity upon immobilization in alginate and a storage stability of at least one month and urea determination based on ratiometric analysis of cresol red. Thus nanoengineered urea biosensor presented here is likely to be useful for both qualitative and quantitative analysis of urea.

Acknowledgements

The authors wish to acknowledge Indian Council for Medical Research, New Delhi, India for funding this project; Sophisticated Analytical Instrument Facility (SAIF), IIT Bombay for using various instruments for this work and the Department of Biotechnology, New Delhi, India for providing fellowship to Swati M. Authors would also wish to thank Dr. N.K. Hase and the subjects at the

Dialysis Unit and the staff of KEM Hospital in Parel, Mumbai for providing the dialysate samples.

References

[1] R. Koncki, A. Radomska, S. Glab, Anal. Chim. Acta 418 (2000) 213–224.
[2] F. Uhlin, I. Fridolin, L.G. Lindberg, M. Magnusson, Nephrol. Dial. Transplant. 20 (2005) 2458–2464.
[3] T.A. Depner, T. Greene, F.A. Gotch, J.T. Daugirdas, P.R. Keshaviah, R.A. Star, Kidney Int. 55 (1999) 635–647.
[4] F. Lopot, P. Kotyk, J. Blaha, A. Valek, Artif. Organs 19 (1995) 832–836.
[5] T.A. Depner, Prescribing Hemodialysis: A Guide to Urea Modeling, Kluwer, Boston, 1991.
[6] A. Radomska, S. Głab, R. Koncki, Analyst 126 (2001) 1564–1567.
[7] M. Jurkiewicz, S. Sole, J. Almirall, M. Garcia, S. Alegret, E. Martinez-abregas, Analyst 121 (1996) 959–964.
[8] R. Koncki, A. Radomska, S. Glab, Talanta 52 (2000) 13–17.
[9] P. Calzavara, G. Calgoni, G.D. Rin, E. Canini, F. Paolini, Int. J. Artif. Organs 21 (1998) 147.
[10] L. Chiari, A. Cappello, R. Tartarini, F. Paolini, P. Calzavara, Int. J. Artif. Organs 21 (1998) 526–534.
[11] A. Orellana, E. Martinez-Fabregas, S. Alegret, J. Pharm. Biomed. Anal. 11 (1993) 921–926.
[12] M. Jurkiewicz, M. del Valle, S. Alegret, E. Martinez-Fabregas, Anal. Chim. Acta 327 (1996) 243–251.
[13] P.J. Jacobs, J. Suls, W. Sansen, R. Hombrouckx, ASAIO J. 39 (1993) M353–M358.
[14] P.J. Jacobs, W. Sansen, R. Hombrouckx, ASAIO J. 40 (1994) 393–400.
[15] R. Seitz, Optical ion sensing, in: O.S. Wolfbeis (Ed.), Fiber Optic Chemical Sensors and Biosensors, Florida, 1991.
[16] O.S. Wolfbeis, Fibre Optic Chemical Sensors and Biosensors, second ed., CRC Press, Florida, 1991.
[17] R.D. Jayant, R. Srivastava, J. Biomed. Nanotechnol. 3 (2007) 245–253.
[18] M. Swati, N. Nisha, R. Srivastava, J. Bionanosci. 1 (2007) 1–6.
[19] M.S. Alqasaimeh, L. Heng, M. Ahmad, Sensors 7 (2007) 2251–2262.
[20] J.T. Olesburg, M.A. Arnold, M.J. Flanigan, Clin. Chem. 50 (2004) 175–181.
[21] S.W. Smye, P.E. Hyden, E. Will, Phys. Med. Biol. 38 (1993) 115–122.
[22] J.P. Gabriel, G. Fellay, E. Discombes, Kidney Int. 49 (1994) 789–796.
[23] L. Tymecki, R. Koncki, Sens. Actuators, B 113 (2006) 782–786.
[24] M. Swati, R. Srivastava, Anal. Lett. 42 (2009) 1–17.