CHEMICAL ENGINEERING TRANSACTIONS
VOL. 32, 2013
A publication of AIDIC
The Italian Association of Chemical Engineering
Online at: www.aidic.it/cet
Chief Editors: Sauro Pierucci, Jiří J. Klemeš

ISBN 978-88-95608-23-5; **ISSN** 1974-9791
DOI: 10.3303/CET1332278

UV Cured Boronic Acid Based Fluorescence Sensor for the Determination of Glucose

Soner Çubuk[a], Ece Kök Yetimoğlu*[a], M. Vezir Kahraman[a], Sibel Kenan[a], Melike Fırlak[b]

[a]Faculty of Art and Sci., Chemistry Dept., Marmara University, 34722 İstanbul, Turkey.
[b]School of Pharmacy, İstanbul Kemerburgaz University, İstanbul, Turkey.
ecekok@marmara.edu.tr

The use of polymers is finding a significant place in development of sensors. Better selectivity and rapid measurements have been achieved by replacing classical sensor materials with functions of polymers. Several receptors have been employed to detect glucose in fluorescence sensors, and these include enzymes such as glucose oxidase, glucose dehydrogenase and hexokinase/glucokinase, bacterial glucose-binding protein, and boronic acid derivatives. Boronic acid has a important role in the design of glucose sensors. The sensing membrane was prepared with p-Vinylphenylboronicacid (VPBA), Hydroxyethylmethacrylate (HEMA) and Poly(ethylene glycol) diacrylate (PEG-DA). The membran is capable of determining glucose between 0.1 ppm and 0.7 ppm. It can be completely regenerated by using distilled water. The sensor performance characteristics such as response time, dynamic working range and sensitivity were reported. The optical sensor was stable, cost effective, easy to prepare, rapid and simple for the determination of glucose. The results suggest that the sensor can be used as glucose sensors to determine glucose.

1. Introduction

Determination of carbohydrates in biological samples has the utmost importance since carbohydrates, especially glucose, plays crucial role in various diseases such as diabetes. Glucose plays a main role in various metabolic processes. Development of a novel selective and sensitive method for the determination of glucose concentration in body fluids is an active field of research. Several analytical techniques are currently used for determination of carbohydrates such as, optical rotation, electrochemistry, spectroscopy, and chromatographic techniques [Manju S. et al. ,2010). In addition to these techniques, enzyme or enzyme-free, colorimetric and fluorescent sensors are extensively employed to evaluate glucose concentration (James T.D. et al., 1997). Among them, fluorescent sensors have had a large success in the past 20 years with their commercial availability and applicability to detect several species and furthermore, fluorescence measurements have various advantages such as high sensitivity, and being harmless to the host system (Luque G.L. et al., 2005). Synthetic polymers are widely used to as sensor membranes because they exhibited perfect mechanical and chemical stability and can be easily prepared (Marinov et al., 2009). Sensors based on polymer films which construct to analysis of a target molecule usually contain a specific chelator. Among the chelators which used to detect glucose, boronic acid derivatives are known over a century and have an important place. There have been several boronic acid based glucose sensing membranes reported in the literature (DiCesare N. et al., 2002). Because boronic acid and its derivatives are relatively cheap and can bind with glucose reversibly, boronic acid based fluorescence membranes are promising for glucose analysis (Pickup J. C. et al.,2005).
Hereby, in our work we developed a novel boronic acid based fluorescence sensor for the determination of glucose. Characterization and standardization of the sensor has been performed and various parameters

Please cite this article as: Cubuk S., Kok Yetimoglu E., Kahraman M.V., Kenan S.., Firlak M., 2013, Uv cured boronic acid based fluorescence sensor for the determination of glucose, Chemical Engineering Transactions, 32, 1663-1668 DOI: 10.3303/CET1332278

such as dynamic linear range and effect of pH have been investigated. The results showed that synthesized glucose sensor could be used in clinical samples to determine glucose concentration.

2. Experimental

2.1 Materials and reagents

p-Vinylphenylboronic acid (VPBA), the crosslinker Poly(ethylene glycol) diacrylate (PEG-DA), The commercial monomers Hydroxyethylmethacrylate (HEMA) and the photoinitiator 2,2-Dimethoxy-2-phenylacetophenone (DMPA) were purchased from Sigma. All the other chemicals were of analytical grade and used without further purification. The pH values of the solutions were checked using a digital pH meter (WTW) calibrated with standard buffer solutions of Merck. All of the experiments were operated at room temperature, 25±1 °C. All water used in the experiments was purified using a Milli Q-water purification system (Millipore).
Boronic acids react with 1,2-diols or 1,3-diols in aqueous solution to create five- or six-membered cyclic esters Figure 1

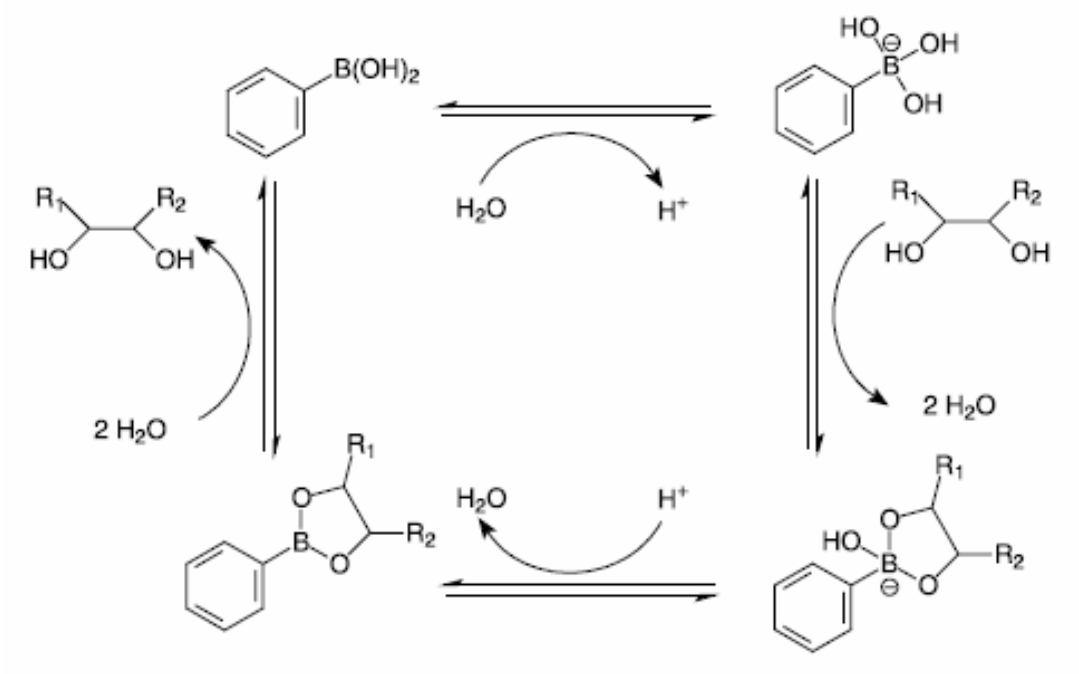
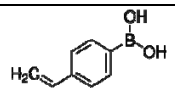
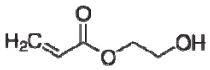
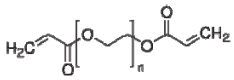
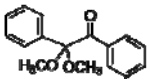


Figure.1. Schematic of binding process between phenylboronic acid and diol (Mader H. S., 2008).

2.2 Preparation of boronic acid based fluorescence sensor

The polymer film were synthesized using p- Vinylphenylboronic acid (VPBA) and 2-Hydroxyethylmethacrylate (HEMA) by free radical crosslinking copolymerization with Poly(ethylene glycol) diacrylate (PEG-DA) as a crosslinker and 2,2-Dimethoxy-2-phenylacetophenone (DMPA) was added as a photoinitiator. The feed compositions are given in Table 1. The UV- cured membranes were taken out from the mold and immersed in a large excess of deionized water for a 1 day to wash out any unreacted monomers and initiators and then dried in a vacuum oven at 30˚ C until reaching a constant weight.

Table.1. Feed composition of polymeric sensor.

Chemicals	Formula	mmol
VPBA		**0,68**
HEMA		**6,03**
PEG-DA		**0,29**
DMPA		**0,12**

2.3 Characterization

The characterization of synthesized membrane was examined by Attenuated Total Reflection Infrared Spectroscopy (Perkin- Elmer ATR-FTIR spectrophotometer). SEM imaging of the sensor was performed on a Philips XL30 ESEMFEG/EDAX. The membranes were initially dried in vacuum air at 30 °C for 3 days before being analysed. The specimens were prepared for SEM by freeze fracturing in liquid nitrogen and applying a gold coating of approximately 300 Å. The surface of the sample was then scanned at the desired magnification to study the morphology of the membranes.

3. Results and discussion

3.1 Characterization of the prepared membrane

3.1.1 FTIR spectroscopic studies

Fig 1. FTIR spectrum of P(VPBA/ HEMA/PEG-DA) membrane FTIR spectrum of P(VPBA/ HEMA/PEG-DA) membrane was taken (Figure 2.) These can be summarized as the peak at 3396 cm^{-1} shows the hydroxyl group of HEMA and VPBA, the peak at 2924 cm^{-1} which is due to the presence of –C–H band, the strong peak at 1717 cm^{-1} indicates the carbonyl group of HEMA, the peak at 1607 cm^{-1} shows C=C stretch of aromatic ring, the peak at 1414 cm^{-1} corresponding to aromatic rings with boron attached directly, the peak at 1322 cm^{-1} indicates the B-O stretch, the peak at 1067 cm^{-1} shows C-B bond, the peaks at 1019 cm^{-1} and 896 cm^{-1} indicate for vinyl groups stretch in the case of FTIR spectrum of P(VPBA/ HEMA/PEG-DA) membrane.

3.1.2 SEM Investigation

The surface morphology of the membrane is an important factor. Figure. 2a, 2b and 2c demonstrate the SEM images of the glucose sensing membrane at different magnification. As it could be seen from the SEM images, a homogenous and non-porous glucose membrane was obtained.

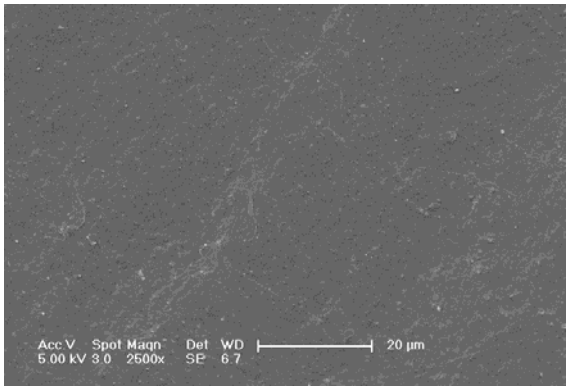


Figure 2a. SEM micrograph of glucose sensing membrane.

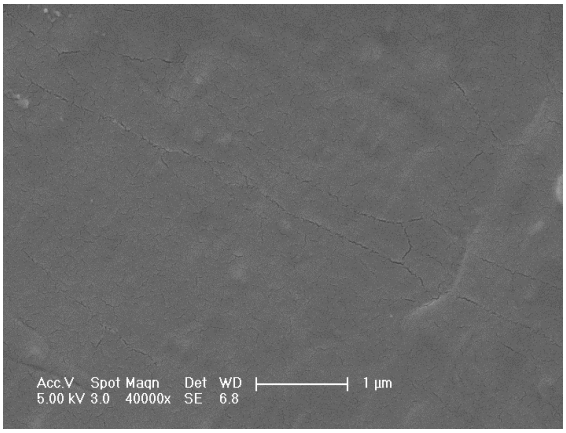


Figure 2b. SEM micrograph of glucose sensing membrane.

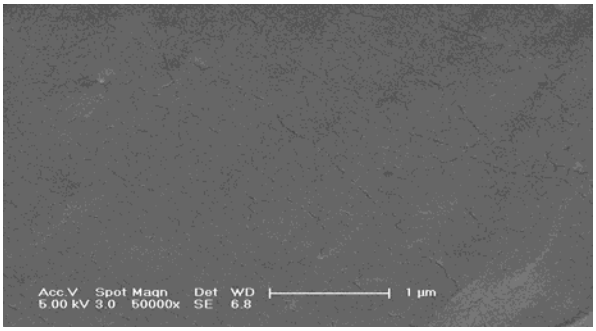


Figure 2c. SEM micrograph of glucose sensing membrane.

3.2 Spectral characterization studies

All fluorescence measurements were carried out on a Varian Eclipse spectrofluorimeter with a Xenon short arc lamp. The binding process of phenylboronic acids with glucose diols is illustrated in Figure 1. The fluorescence spectra were recorded at $\lambda_{ex.}$= 211 nm and $\lambda_{em.}$ = 424 nm. The values of the fluorescence intensities of the membrane increased with increasing glucose concentrations up to 01 ppm, a fluorescence determination was carried out in the glucose concentration range from 0.1 ppm to 0.7 ppm. A

significant decrease in fluorescence intensity of the sensor was observed upon increasing glucose concentration in this range.

3.3 Effect of pH

Measurement of pH was carried out with a pH-meter (WTW Multiline P4). All of the experiments were carried out at room temperatures in Britton Robinson (BR) buffer solutions. The fluorescence intensity measurements were made in the presence of 0.5 ppm glucose solution at different pH values, in the range of 6.0– 8.0. As it is seen from Fig. 4, the fluorescence intensities of the sensor decrease with increase solution pH from 7.0 to 8.0, increase with increasing solution pH from 6.0 to 7.0. Its incremental behavior being continued until pH 7.0 and begin to decrease when the solution pH increase further (Figure .4).

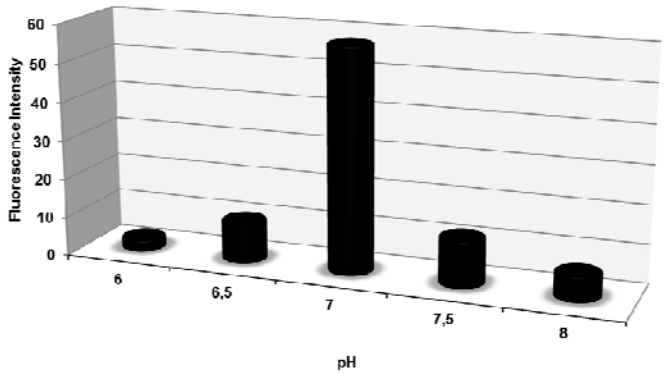


Figure 4. Effect of pH of the glucose solution (05 ppm) on the fluorescence intensity of the glucose sensor.

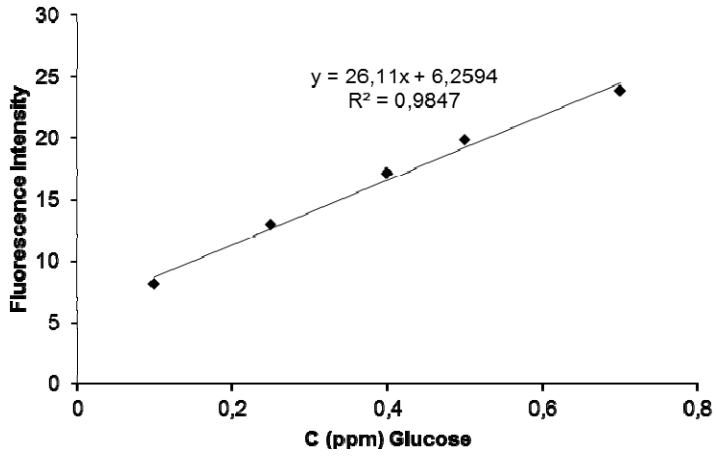


Figure 5.Calibration curve of the sensing membrane for determination of glucose ($\lambda_{ex.}$/ $\lambda_{em.}$= 211 nm/ 424 nm).

3.4 Chosen of reaction time

The short-time stability of the membrane was tested by recording the fluorescence intensity of 0.5 ppm glucose at different reaction time for seven measurements. After comparison, a 3 minute reaction time was chosen due to its higher fluorescence intensity.

3.5 Measuring range, detection limit, quantification limit

The optical response of the proposed glucose sensor at different glucose concentrations, under optimal experimental conditions is shown in Figure 5. This curve can be suitably used as a calibration plot for the determination of glucose over a concentration of 0.1 ppm – 0.7 ppm.

4. Conclusion

A novel UV cured boronic acid based fluorescent glucose sensor were synthesized using p-Vinylphenylboronic acid and characterized. The sensor is easy to prepare, cost effective and rapid, also it is fully reversible, as it can be easily regenerated by treatment with water. Method have enabled the determination of glucose in solution over a wide concentration range (0.1 ppm- 0.7 ppm) the relative standard deviation (RSD) was 0.22 % (95 % confidence levels, n= 7) for an average exposure time of 3 minute at pH 7.0. For further studies, application of this sensor to the determination of glucose in biological samples is ongoing in our laboratory.

Acknowledgement

This work was supported by Marmara University, Commission of Scientific Research Project (M.Ü.BAPKO) under grant FEN-D-090113-0014.

References

Manju S., Hari P.R., Sreenivasan K., 2010, Fluorescent molecularly imprinted polymer film binds glucose with a concomitant changes in fluorescence, Biosensors and Bioelectronics, 26, 894–897.

Okutucu B., Önal S., 2011, Molecularly imprinted polymers for separation of various sugars from human Urine, Talanta 87, 74– 79.

James T.D., Shinmori H., Shinkai S., 1997, Novel fluorescence sensor for “small” saccharides, Chem. Commun, 1, 71–72.

Zhang Y., He Z., Li G., 2010, A novel fluorescent vesicular sensor for saccharides based on boronic acid–diol interaction, Talanta, 81, 591–596.

Luque GL, Rodríguez MC, Rivas GA., 2005, Glucose biosensors based on the immobilization of copper oxide and glucose oxidase within a carbon paste matrix, Talanta, 66, 467–471.

Marinov I, Gabrovska K, Velichkova J, Godjevargova T., 2009, Immobilization of acetylcholinesterase on nanostructure polyacrylonitrile membranes, International Journal of Biological Macromolecules, 44, 338-345.

DiCesare N., Adhikari D.P., Heynekamp J. J., Heagy M. D., Lakowicz J. R., 2002, Spectroscopic and Photophysical Characterization of Fluorescent Chemosensors for Monosaccharides Based on N-Phenylboronic Acid Derivatives of 1,8-Naphthalimide, Journal of Fluorescence, 12, 147-154.

Pickup J. C., Hussain F., Evans N. D., Rolinski O. J., Birch D. J. S., 2005, Review Fluorescence-based glucose sensors, Biosensors and Bioelectronics, 20, 2555–2565.

Mader H. S., Wolfbeis O. S., 2008, Boronic acid based probes for microdetermination of saccharides and glycosylated biomolecules, Microchimica Acta,62, 1–34.