RSC Advances

PAPER

View Article Online
View Journal | View Issue

Cite this: *RSC Adv.*, 2016, **6**, 14261

Determination of blood potassium using a fouling-resistant PVDF–HFP-based optode†

Mizuki Tenjimbayashi,[a] Hirokazu Komatsu,[b] Masaaki Akamatsu,[c] Waka Nakanishi,[b] Koji Suzuki,[a] Jonathan P. Hill,[b] Seimei Shiratori*[a] and Katsuhiko Ariga[b]

Received 11th December 2015
Accepted 25th January 2016

DOI: 10.1039/c5ra26514b

www.rsc.org/advances

Monitoring potassium levels in blood is a significant aspect of clinical analysis. For this reason, polymeric bulk optodes have received much attention for their use in portable and easy-to-use analysis systems *in situ* determination without additional calibration. However, blood contamination on the detection area of the sensor can hinder accurate sensing and also increases risk of infection from the wounds. In this paper, we report a system for determination of potassium in blood which has the additional advantage of being blood-fouling resistant. We have replaced the generally used poly(vinyl chloride) (PVC) with hydrophobic fluorinated poly(vinylidene fluoride–hexafluoropropylene) (PVDF–HFP) for preparation of a polymeric bulk optode. Sensing ability in the visual range of the polymeric bulk optode was retained despite the variation of the polymer matrix. These polymeric bulk optodes are suitable for potassium determination in blood with the PVDF–HFP-based optode possessing better blood antifouling properties than the PVC-based optode. The blood monitoring system described here represents the basis for functionalization of the optode toward safe and easily implementation in blood and *in situ* sensing applications.

Introduction

Potassium cations are physiologically important species being one of the most abundant elements found in intracellular and extracellular media.[1,2] Excessive or deficient extracellular potassium levels in blood are respectively known as hyper- or hypo-kalemia, both of which are responsible for various symptoms including potentially fatal cardiac arrythmias.[3] Therefore, the monitoring of potassium levels in patients' blood is a very significant and useful aspect of clinical analysis. Various methods for quantitatively determining blood electrolytes have been developed including atomic absorption/emission spectroscopy (AAS/AES)[4] and capillary electrophoresis (CE)[5] although the most popular method involves the use of ion-selective electrodes (ISEs),[6] which are widely applied in commercial blood analyzers. However, these methods are implemented using special instruments and usually require additional calibration.[7,8]

Recent research has highlighted techniques operating based on an optical response, which may be adaptable for blood analysis.[9] Alternatively, polymeric bulk optodes have been used for quantification of blood electrolytes. Introduction of an ionophore selective for a reference ion into the sensor membrane permits monitoring of the degree of protonation of a dyestuff which can in turn be used to quantify the activity of the primary ion in analyte samples because the response mechanism is based on a competitive ion–exchange equilibrium.[10,11] Optical response of an optode device is usually observed in absorbance or fluorescence mode[12,13] and, since the visual responsive of the optode can be used to quantify the activity of the primary ion in an analyte, it can be applied for blood analysis in the absence of an electrical supply (such as occurs at the time of a natural disaster or in remote locations).[14] However, most approaches to blood analysis are based on fluorescence response optodes not visually responsive optode.[12,13,15] This is largely due to components of blood contaminating the detection area of the sensor, which can hinder accurate sensing in the visual region[16] and also increases risk of infection from the wounds.[17,18]

Here we present a visually responsive system for determination of potassium concentrations in blood.[19,20] The system has the additional property that it resists fouling by blood analyte samples. While in most previous reports PVC was used

[a]*Department of Integrated Design Engineering, Faculty of Science and Technology, Keio University, 3-14-1 Hiyoshi, Kohoku-ku, Yokohama, Kanagawa 223-8522, Japan. E-mail: shiratori@appi.keio.ac.jp*

[b]*WPI-MANA, National Institute for Materials Science (NIMS), 1-1 Namiki, Tsukuba, Ibaraki, 305-0044, Japan*

[c]*The Matile group, University of Geneva, 30 Quai Ernest Ansermet, 1211 Geneva 4, Switzerland*

† Electronic supplementary information (ESI) available: Equations for calculating coupling constants K_{exch} and ion selectivities K_{ij}^{opt}; response curve for determination of potassium level in human blood by a standard addition method; SEM images before and after color change of the sensor; absorption spectra of KD-M13; optical response in the pH range 6 to 8; color value changes with the potassium addition in human blood obtained by color difference meter. See DOI: 10.1039/c5ra26514b

to form polymer bulk optodes,[21,22] we have attempted to use the fluorinated copolymer PVDF–HFP due to its greater hydrophobicity over PVC.[23,24] We have also investigated the influence on optical response caused by changing to a lower surface tension polymer as well as evaluating the adherence qualities of blood on the optode by measuring the contact and sliding angles on the polymer.[25] Sensing activity in the visual range of the polymeric bulk optodes was not affected by changing the polymer from PVC to PVDF–HFP with the PVDF–HFP-based optode exhibiting similar activity for potassium determination in blood as that of the PVC optode. The PVDF–HFP-based optode did however exhibit much better blood anti-fouling properties than those of the PVC-based optode. There have been only a few reports of the variation of the optode bulk polymer where PVC was not used. Alternative approaches have made use of a Si–O matrix or amphiphilic polymers for preparation of emulsion-based optodes.[26–30] Additionally, there has been scant attention paid to the relationship between the interactions involved at the optode liquid interface and its sensing ability.[31]

Experimental

Material

Host molecule bis[(benzo-15-crown-5)-4-ylmethyl]pimelate,[11,32] cation-exchanger tetrakis[3,5-bis(trifluoromethyl)phenyl]borate sodium salt (NaTFPB), and Bis-Tris were purchased from Dojindo Laboratories (Japan). DOS bis(2-ethylhexyl)sebacate was purchased from Tokyo Chemical Industry Co., Ltd (Japan). Dehydrated tetrahydrofuran (THF), KCl, NaCl, $MgCl_2$, and $CaCl_2$ were purchased from Kanto Chemical Co., Inc. (Japan). Poly(vinyl chloride) high molecular weight (PVC), and poly(vinylidene fluoride–hexafluoropropylene) high molecular weight (PVDF–HFP) were purchased from Sigma-Aldrich Co., LLC. (USA). HCl aq., and 1 M NaOH aq. were purchased from Nacalai Tesque, Inc. (Japan). KD-M13 was synthesized according to reference literature method.[33] Pig blood (including sodium citrate as anticoagulant) was purchased from Tokyo Shibaura Zouki Co., Ltd. Human blood was purchased from BIOPREDIC International Company (France).

Sample preparation

All samples were prepared with Tris-buffer, and pH was adjusted with HCl (aq.) to 7.4. KCl, NaCl, $MgCl_2$, $CaCl_2$ were dissolved in Tris-buffer with different concentrations in the range from 10^{-5} M to 10^{0} M.

Preparation of sensor membrane

PVDF–HFP (1 g) or PVC (0.79 g) was dissolved in dehydrated THF (15 mL) by stirring for 24 h at 60 °C. The sensing medium was prepared by mixing the potassium cation host molecule bis[(benzo-15-crown-5)-4-ylmethyl] pimelate (2.6 mg), cationic exchanger tetrakis[3,5-bis(trifluoromethyl)phenyl] borate sodium salt (3.0 mg), plasticizer bis(2-ethylhexyl) sebacate (40 μL), and dyestuff KD-M13 (1.8 mg) in the respective polymer solution (1 mL), then stirring for 1 h. The sensing mixture was then coated on a PET film by casting or using the squeegee method.[24]

Characterization

Absorption spectra of sensor responses were measured by using a Shimadzu UV-3600 UV/Vis/NIR spectrophotometer with each cation as its chloride salt dissolved in Tris-buffer solutions in a 1 cm quartz cell. For comparison, absorption spectra of completely deprotonated sensor prepared by applying 1 M NaOH aq. were also measured. Coupling constants K_{exch} and ion selectivities K_{ij}^{opt} were calculated from the response curve constructed using absorption spectra measured under different chloride salt concentrations (see the ESI†). Coupling constants K_{exch} and ion selectivities[34] K_{ij}^{opt} could not be calculated accurately for Ca^{2+} and Mg^{2+} since reliable approximations of their response curves could not be obtained. Potassium levels in human blood were measured by using a standard addition method (see the ESI† for details). A colour difference meter (Colour Reader CR-13, Minolta Co., Ltd., Tokyo, Japan) was used to quantify the colour change of the sensor responses. Blood contact sliding angles were measured by casting pig blood (20 μL) on a Contact-Angle Meter (CA-DT, Kyowa, Tokyo, Japan). Image analysis of the blood contamination area on the sensor was obtained using Image J software (U. S. National Institutes of Health, Bethesda, Maryland, USA). For determination of potassium level in the blood and image analysis of the blood contamination, the whole surface was exposed to blood during time periods ranging from several tens of seconds to a minute. Surface structure was observed using a scanning electron microscope (SEM, TM3030Plus Microscope, HITACHI, Japan).

Results and discussion

Ion-selective optodes usually make use of the selective interaction between hydrogen ions and a lipophilic dyestuff. The films can be made to respond to cations by buffering of the pH of the fluid analyte sample under a competitive ion–exchange equilibrium which is given as

$$M_W^{z+} + zS_O + zD_OH^+R_O^- \leftrightarrow S_{zO}M_O^{z+}R_O^{z-} + zD_O + zH_W^+ \quad (1)$$

as shown in Fig. 1. M^{z+} is the analyte cation, S is the ionophore, D is the dye, and R is the cationic ion-exchanger. The subscript W denotes "existing in the analyte solution" and the subscript O denotes "existing in the polymer film". When M^{z+} comes into contact with the optode, they are extracted into the film and exchanged with hydrogen ions in order to conserve electrical neutrality within the film. This results in the release of protons from the optode and the deprotonation of the dye. For a specific analyte ion, the dynamic range of optodes can be tuned by adjusting the pH of the analyte, by using ion carriers with different binding constants, or by incorporating dyestuffs of different basicities. There was no observable change in the membrane structure of the ion-selective optode after deprotonation (see Fig. S2 of the ESI†).

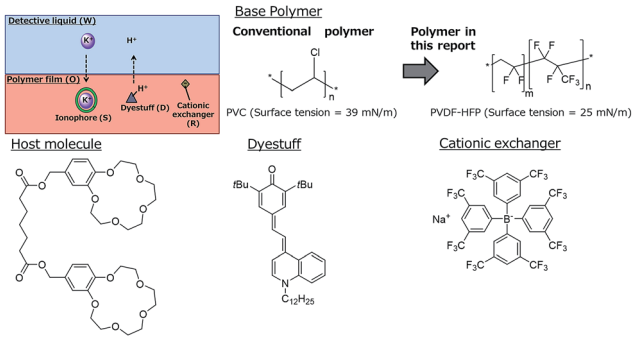

Fig. 1 At top left, operation mechanism of polymeric bulk optode. Chemical structures of the polymers, host molecule, dye and cation exchanger used in this research. In this research, PVDF–HFP is used as bulk polymer instead of PVC.

Here, KD-M13 was chosen as the responsive dye.[33] As described in the preceding paragraph, the absorbance spectrum of this dye varies according to its degree of protonation (see Fig. S3 of the ESI†). Absorption spectra of the protonated state contain an absorption peak at 470 nm while increasing degrees of deprotonation result in the appearance and gradual increase in intensity of an absorption peak at 625 nm with a concurrent decrease in intensity of the absorption peak at 470 nm. These spectral changes are accompanied by a gradual colour variation from yellow to blue; a response that can be use in ratiometry.

Bis[(benzo-15-crown-5)-4-ylmethyl] pimelate was selected as the K^+ ion selective ionophore and this combination of ionophore and dye showed a visual response in the pH[26] range between 6 and 8, which includes the physiologically important pH value of 7.4 (ref. 35) (pH of human blood), and responses in this range were included in the linear region of the chemical equilibrium curve (see Fig. S4 of the ESI†).

It has been reported that the intensity of optical response varies according to the surface roughness of the polymeric bulk optode,[36] or absorption peak shifts occur by adding nanorod structures into the polymeric bulk optode.[7] Those changes were reported also to influence the binding constant of the ionophore or values of pK_a.[37,38] In this paper, we have investigated the effect of changing the surface tension of the polymer matrix. While surface roughness or morphology influences the interaction area between optode and fluid analytes, the surface tension (or surface energy) influences the osmotic action of the molecules between optode and the analyte.[39] Fig. 2 shows the sensing property of the K^+ selective membrane where the base polymer was (a and b) PVDF–HFP or (c and d) PVC. The responses lie in the range from 10^{-4} M to 10^{-1} M, which includes the physiological level of K^+ in blood.[7] There was no apparent difference in the optical response or any shift of absorption peaks between the PVDF–HFP-based optode, with $\log K_{exch} = -5.19$, and PVC-based optode, with $\log K_{exch} = -5.46$. Thus, the surface tension does not influence optical response properties or cause shifts in absorption peaks (there was also no apparent change in response velocity).

The response curve of the PVDF–HFP-based optode for fluid analytes containing physiologically important cations as their chloride salts (K^+, Na^+, Mg^{2+}, Ca^{2+}) is shown in Fig. 3(a). The

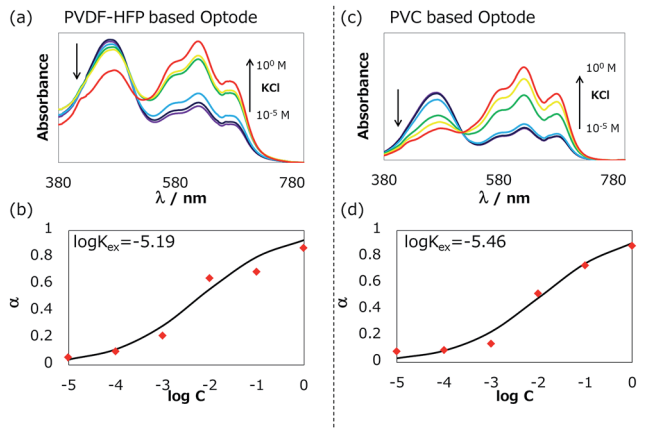

Fig. 2 (a) Absorption spectra of the PVDF–HFP based optode (b) response curve of the K^+ selective PVDF–HFP based optode (c) absorption spectra of PVC based optode (d) response curve of the K^+ selective PVC based optode.

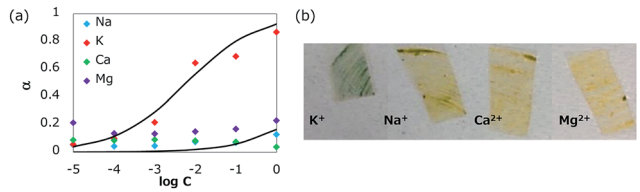

Fig. 3 (a) Response to KCl, NaCl, $CaCl_2$, and $MgCl_2$ (b) photograph of PVDF–HFP based optodes after immersion in 1 M KCl, NaCl, $CaCl_2$, or $MgCl_2$ contained in Tris-buffer.

PVDF–HFP-based optode turned blue in 1 M KCl solution, but maintained its yellow colour in 1 M NaCl, $MgCl_2$, or $CaCl_2$ solution as shown in Fig. 3(b). From the response curve, we calculated the coupling constants K_{exch} as shown in Table 1 and ion selectivities K_{ij}^{opt} as shown in Table 2. The results indicate that the sensor has sufficient selectivity for use in the analysis of blood samples (the ion selectivities required for blood are given in the literature[7]). As shown in Fig. 3(a), a linear response was obtained in the range from 10^{-4} M to 10^{-1} M indicating that these optodes are sufficiently selective to operate under a background of other blood electrolytes and so are suitable for physiological measurements.

Fig. 4 shows the response curve obtained by the standard addition method. A linear response was obtained in the range from 10^{-4} M to 10^{-1} M. Hence, the potassium level in human blood measured by using the standard addition method based

Table 1 Coupling constants (K_{exch})[a]

Ion	K_{exch}
K	6.47×10^{-6}
Na	1.68×10^{-9}
Mg	Unmeasurable
Ca	Unmeasurable

[a] These coupling constants were calculated from the equation listed in ESI.

Table 2 Selectivity (K_{ij}^{opt}, i = K^+)[a]

Ion	log K_{ij}^{opt}	log K_{ij}^{opt} (required for 10-fold diluted blood)	log K_{ij}^{opt} (required for undiluted blood)
Na	−3.59	−3.14	−3.09
Mg	At least less than −3	−0.92	−0.88
Ca	At least less than −3	−1.39	−1.31

[a] Selectivities were calculated from the equations listed in ESI. Selectivities required for blood analysis were obtained from ref. 7.

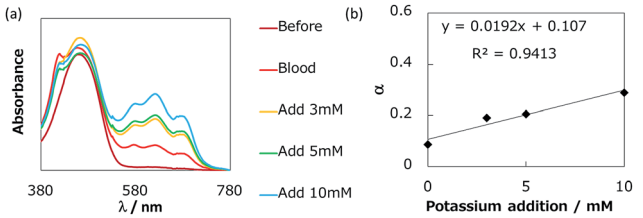

Fig. 4 Determination of potassium level in human blood by a standard addition method (a) absorption spectrum of before response (brown), blood response (red), blood with 3 mM potassium added (yellow), blood with 5 mM potassium added (green), and blood with 10 mM potassium added (light blue) (b) response curve of blood with potassium addition for the standard addition method.

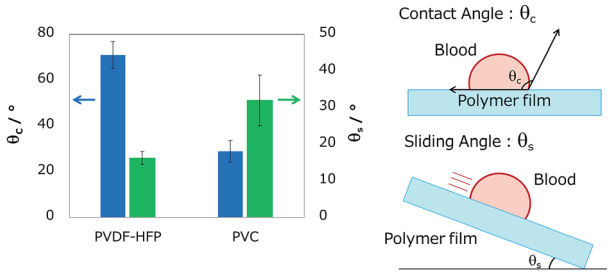

Fig. 5 Contact angle (θ_c, blue bar) and sliding angle (θ_s, green bar) of 20 μL pig blood on PVDF–HFP or PVC-based optode. Scheme at right illustrates the meaning of contact angle and sliding angle.

on response curve was 5.57 ± 0.09 mM in Fig. 4(b), which is a valid level for human blood. For determination of blood potassium levels under emergency conditions or in remote areas, UV-vis absorption measurements are not convenient for quantification of colour variation of the sensor. Thus, we performed the quantification of colour variation using a colour difference meter, which is a highly portable device useful for instant measurements (see Fig. S5 of the ESI†). Colour difference meter measurement enabled the quantification of colour changes of the human blood by adding 3 mM potassium or 5 mM potassium. In addition, the response curve obtained by using the color difference meter was linear within the range from 10^{-4} M to 10^{-1} M similarly to that obtained using UV-vis spectrophotometry. In short, by using the optode in conjunction with a color difference meter allows measurement of millimolar potassium levels in blood by a simple protocol.

Although there was no apparent difference in optical response or absorption peak shift between the PVDF–HFP-based optode and PVC-based optode, we found that an antifouling property of the optode was introduced by changing the base polymer. Fig. 5 shows the blood contact angle and sliding angle of each polymer optode, and indicates that the PVDF–HFP-based optode ought to exhibit superior resistance to fouling by blood than the PVC-based optode due to the formers larger contact angle and smaller sliding angle. The surface tension of PVC is 39 mN m^{-1} while that of PVDF–HFP is 25 mN m^{-1}.[23] Young's equation indicates that lower surface tensions of solids lead to higher contact angles and weaker adhesion of the blood.[40] Therefore, blood sliding angle is also reduced with increasing contact angle. When a blood droplet slides on the PVC based optode, the pinning of blood on the responding surface is observed (and some small contaminating material remains on deprotonated films based on PVC).[41] For this reason, we constructed a colour histogram for each polymer optode (deprotonated by blood) just following sliding of a blood sample, as shown in Fig. 6. The red area indicates remaining contaminating blood. The blue coloured area indicates where the optode has reacted with blood. Blood contamination on the PVC-based optode was greater than that on the PVDF–HFP-based optode as can be seen from the histogram of red coloured area. These results indicate that the PVDF–HFP-based optode has improved properties of blood fouling resistance over the PVC based optode. Hence, this system can be used to realize accurate sensing of potassium concentrations in blood. In addition, we believe that this system can be adapted for use in other similar applications such as for determination of ions in other contaminated fluids by varying the combination of host

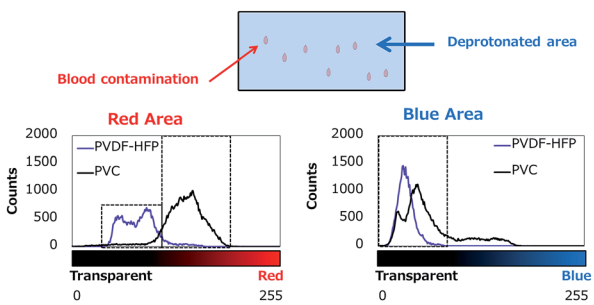

Fig. 6 Colour histogram for each optode polymer (deprotonated by blood) analysed from captured polymeric bulk optodes just after sliding the pig blood. The red coloured area indicates the remaining blood contamination of the optode. The blue coloured area indicates that the optode reacted with blood. The amount of contaminating blood on the PVC-based optode is greater than that on the PVDF–HFP-based optode based on the histogram of the red coloured area.

molecule and dyestuff. Thus, it might be developed for environmental monitoring purposes.

Conclusions

In conclusion, we have fabricated a polymer optode system for the determination of potassium in blood samples with improved blood fouling resistance. Although there was no apparent difference in optical response or absorption peak shifts between the PVDF–HFP-based optode and PVC-based optode, by changing the optode bulk polymer from PVC to the lower surface energy polymer PVDF–HFP, we have introduced the possibility of improved antifouling properties for the preparation of more effective and versatile polymer bulk optodes. This latter feature is advantageous for the development of safe and facile blood monitoring systems. This anti-fouling optode has potential for use in functional analytical systems applied to fouling analyte samples such as polluted water or slime, by varying the combination of polymer and dye components.

Acknowledgements

We are deeply grateful to Dr Kosuke Minami and Mr Atsuki Matsuda, whose meticulous comments were an enormous help.

References

1 J. J. Dillon, C. V. DeSimone, Y. Sapir, V. K. Somers, J. L. Dugan, C. J. Bruce, M. J. Ackerman, S. J. Asirvatham, B. L. Striemer, J. Bukartyk, C. G. Scott, K. E. Bennet, S. B. Mikell, D. J. Ladewig, E. J. Gilies, A. Geva, D. Sadot and P. A. Friedman, *J. Electrocardiol.*, 2015, **48**, 12–18.
2 L. Zhang, S. Chen, Q. Zhao and H. Huang, *Anal. Chim. Acta*, 2015, **880**, 130–135.
3 J. C. Hollander-Rodriguez and J. F. Calvert, *Am. Fam. Physician*, 2006, **73**, 283–290.
4 V. A. Fassel and R. N. Knlseley, *Anal. Chem.*, 1974, **46**, 1110A–1120A.
5 D. J. Harrison, K. Fluri, K. Seiler, Z. H. Fan, C. S. Effenhauser and A. Manz, *Science*, 1993, **261**, 895–897.
6 M. A. Arnold and M. E. Meyerhoff, *Anal. Chem.*, 1984, **56**, 20–48.
7 L. Xie, Y. Qin and H. Y. Chen, *Anal. Chem.*, 2013, **85**, 2617–2622.
8 N. Thakur, S. A. Kumar, K. S. A. Kumar, A. K. Pandey, S. D. Kumar and A. V. R. Reddy, *Sens. Actuators, B*, 2015, **211**, 346–353.
9 Y. Liu, J. Zhu, Y. Xu, Y. Qin and D. Jiang, *ACS Appl. Mater. Interfaces*, 2015, **7**, 11141–11145.
10 E. Bakker, P. Bühlmann and E. Pretsch, *Chem. Rev.*, 1997, **97**, 3083–3132.
11 P. Bühlmann, E. Pretsch and E. Bakker, *Chem. Rev.*, 1998, **98**, 1593–1688.
12 G. A. Crespo and E. Bakker, *Analyst*, 2012, **137**, 4988.
13 M. Akamatsu, H. Komatsu, T. Mori, E. Adams, R. Shin, H. Sakai, M. Abe, J. P. Hill and K. Ariga, *ACS Appl. Mater. Interfaces*, 2014, **6**, 8208–8211.
14 A. Desai and S. Milner, *IEEE J. Select. Areas Commun.*, 2005, **23**, 1556–1563.
15 J. Wu and Y. Qin, *Sens. Actuators, B*, 2014, **192**, 51–55.
16 A. Kerr, M. J. Cowling, C. M. Beveridge, M. J. Smith, A. C. S. Parr, R. M. Head and J. Davenport, *Environ. Int.*, 1998, **24**, 331–343.
17 E. T. Newman, T. S. Watters, J. S. Lewis, J. M. Jennings, S. S. Wellman, D. E. Attarian, S. A. Grant, C. L. Green and T. P. Vail, *J. Bone Jt. Surg.*, 2014, **96**, 279–284.
18 K. Manabe, S. Nishizawa and S. Shiratori, *ACS Appl. Mater. Interfaces*, 2013, **5**, 11900–11905.
19 M. Tenjimbayashi and S. Shiratori, *J. Appl. Phys.*, 2014, **116**, 114310.
20 A. De Los Santos Pereira, T. Riedel, E. Brynda and C. Rodriguez-Emmenegger, *Sens. Actuators, B*, 2014, **202**, 1313–1321.
21 A. A. A. Aziz, S. F. Mohammed and M. M. El Gamel, *J. Fluoresc.*, 2014, **24**, 859–874.
22 M. Shamsipur, T. Poursaberi, M. Hassanisadi, M. Rezapour, F. Nourmohammadian and K. Alizadeh, *Sens. Actuators, B*, 2012, **161**, 1080–1087.
23 N. Angulakshmi and A. M. Stephan, *Electrochim. Acta*, 2014, **127**, 167–172.
24 I. Okada and S. Shiratori, *ACS Appl. Mater. Interfaces*, 2014, **6**, 1502–1508.
25 N. Yokoi, K. Manabe, M. Tenjimbayashi and S. Shiratori, *ACS Appl. Mater. Interfaces*, 2015, **7**, 4809–4816.
26 X. Xie, J. Zhai and E. Bakker, *Anal. Chem.*, 2014, **86**, 2853–2856.
27 P. C. A. Jerónimo, A. N. Araújo and M. C. B. S. M. Montenegro, *Sens. Actuators, B*, 2004, **103**, 169–177.
28 A. Mujahid, P. A. Lieberzeit and F. L. Dickert, *Materials*, 2010, **3**, 2196–2217.
29 A. Samadi-Maybodi and V. Rezaei, *Sens. Actuators, B*, 2014, **199**, 418–423.
30 Y. Hiruta, N. Yoshizawa, D. Citterio and K. Suzuki, *Anal. Chem.*, 2012, **84**, 10650–10656.
31 I. H. A. Badr, R. D. Johnson, M. J. Madou and L. G. Bachas, *Anal. Chem.*, 2002, **74**, 5569–5575.
32 H. Hifumi, A. Tanimoto, D. Citterio, H. Komatsu and K. Suzuki, *Analyst*, 2007, **132**, 1153–1160.
33 H. Hisamoto, M. Tani, S. Mori, T. Yamada, T. Ishigaki, H. Tohma and K. Suzuki, *Anal. Chim. Acta*, 1999, **71**, 259–264.
34 E. Bakker and W. Simon, *Anal. Chem.*, 1992, **64**, 1805.
35 S. Hideshima, R. Sato, S. Inoue, S. Kuroiwa and T. Osaka, *Sens. Actuators, B*, 2012, **161**, 146–150.
36 V. Bychkova and A. Shvarev, *Anal. Chem.*, 2009, **81**, 7416–7419.
37 H. Hisamoto, Y. Manabe, H. Yanai, H. Tohma, T. Yamada and K. Suzuki, *Anal. Chem.*, 1998, **1**, 1255–1261.
38 H. Hisamoto, H. Tohma, T. Yamada, K. Yamauchi, D. Siswanta, N. Yoshioka and K. Suzuki, *Anal. Chim. Acta*, 1998, **373**, 271–289.
39 T. Markovich, D. Andelman and R. Podgornik, *Europhys. Lett.*, 2014, **106**, 16002.
40 H. Huxley and A. Weeds, *Philos. Trans. R. Soc. London*, 1966, **95**, 65–87.
41 K. Manabe, K.-H. Kyung and S. Shiratori, *ACS Appl. Mater. Interfaces*, 2015, **7**, 4763–4771.