Calibration free and fluorescein based fiber optic pH sensor for clinical applications

Bernard Gauthier-Manuel[1], Rutjaphan Kateklum[1], Bruno Wacogne[1, 2]

[1]FEMTO-ST institute, Univ. Bourgogne Franche-Comte, CNRS, 15B avenue des Montboucons, 25030 Besancon, cedex, France
[2]INSERM CIC 1431, Besancon University Hospital, 25000 Besancon, France

Abstract—**This communication describes how fluorescein grafted at the end of an optical fiber can be used for calibration free pH sensing in the range of physiological values. The method is based on a complete mathematical description of the fluorescence mechanisms of this pH indicator. The analysis of the shape of the fluorescence spectra allows computing values of both the pH and the pKa$_3$. Doing this, it becomes possible to access calibration pH sensing using conventional optical fibers. This is, to the best of our knowledge, the first fiber optic calibration free pH sensor reported to date.**

Keywords—pH sensing; calibration free; fluorescein; optical fiber

I. INTRODUCTION

Because pH modification of the body fluids maybe the cause or the consequence of various pathologies, clinical staff looks for minimally invasive pH sensors which can be used in the human body for diagnosis, screening or monitoring. Together with the minimal invasiveness aspect of the ideal pH sensor, the latter must be operated in a calibration free manner because clinical examination are not conducted in a laboratory environment.

In order to meet these requirements, optical fiber based pH sensors have been reported for several years [1-4]. In this technology, pH sensitive indicators are grafted at the end of an optical fiber and pH is deduced from the fluorescence behavior of the indicators (fluorescence intensity or spectra analysis). Mostly 2 kind of indicators are used. The first family concerns indicators exhibiting a single emission peak. pH is related to the intensity measured at the unknown pH normalized by the intensity at high pH values. This is the case of fluorescein for example [5]. A priori, this indicator cannot be used for calibration free measurements. However, we will see later that under certain conditions, this statement is no longer valid. The second family concerns indicators with a double emission peaks. Each peak corresponds to either fully protonated or fully deprotonated forms of the molecules. Measuring the ratio of the intensities at the 2 peak wavelengths allows calculating the pH value. This is the case of SNARF® (seminaphtarhodafluor) for example [2]. This ratiometric technique is however not calibration free and molecule manufacturers advise users to perform a pre-calibration using the acidic and basic endpoints of titration respectively. This calibration procedure requires controlling very accurately the experimental conditions and cannot be envisaged in clinical applications.

Among different reasons, calibration is required to account for degradation of the sensor probe due to aging or repetitive use. In fact, pH sensing is based on the monitoring of the acid-base reaction which occurs in the indicator layer grafted at the end of the optical fiber. Calibration is required because the pKa of the acid-base reaction cannot be considered constant. pKa variations are due to several factors like temperature and/or ionic strength of the solution to be measured, structure and thickness of the indicator layer at the end of the fiber. Indeed, pKa can differ by almost 2 units between the value in a low concentration solution and the value in a dense layer, close to a solid surface, which is the case in fiber optic pH sensors [6].

Some authors proposed a more mathematical description of fluorescence properties. In [7] for example, authors propose to extend the 2 wavelengths ratiometric method to the analysis of the whole fluorescence spectrum. Their approach consists in computing the spectra of both fully protonated and fully deprotonated forms of SNARF®. These 2 extreme pH spectra form a basis which is now used to fit a spectrum recorded at an unknown pH. However, variation of the pKa are not accounted for and calibration free measurement is not obtained. In [8, 9] authors employ the same method. Their approach is more "chemimathematic" because they consider 2 phenomena playing a role in pH determination: shifts of fluorescence wavelengths and intensities and variations of the pKa value. In order to account for the real pKa value, authors measure spectra obtained with perfectly known pH solutions. The value of pKa is subsequently computed using the Henderson-Hasselbach equation. However, computing the pKa value using solutions of known pH means conducting calibration experiments.

In this communication, we show that modeling the fluorescence properties of ratiometric indicators existing in their 2 acidic and basic forms cannot be used to access calibration free pH sensing. We then explain how fluorescein, which exhibits more complex prototropic equilibria, can be used to compute not only the pH value but also the value of the pKa at the moment when measurements are performed. We present the complete mathematical modeling of the fluorescence properties of fluorescein using a set of fibers, and we present application of the model test fibers which were not used to elaborate the mathematical model.

II. Calibration Free pH Sensing by Modeling the Emission Spectra

A. Indicators exhibiting only 2 prototropic forms

As we briefly mentioned, ratiometric indicators usually exist under 2 prototropic forms: fully protonated or fully deprotonated. Each of these forms exhibits different fluorescence emission spectra. Depending on their relative concentration, the resultant spectrum allows measuring the pH by calculating the ratio of the emitted intensities at 2 distinct wavelengths. More precisely, pH can be determined by studying the whole shape of the spectrum and not only the intensities at 2 wavelengths. We also mentioned that calibration is required in order to account for pKa variations which occur during the life time of the sensor. Indeed, the equilibrium between protonated and deprotonated forms is governed by the law of mass action through the dissociation degree given by:

$$\alpha = 1/(1 + [H^+]/K_a)) = 1/(1 + 10^{(pKa-pH)}) \tag{1}$$

Here, Ka is the dissociation constant. It can clearly be understood that, whatever the method used to study the shape of fluorescence spectra, it is impossible to de-correlate the value of the pH from the value of the pKa. Indeed, the only accessible variable is the ratio $[H^+]/pKa$ or the difference $(pKa-pH)$. Consequently, it is impossible to access calibration using pH indicators exhibiting only 2 prototropic forms.

B. Need for more complex prototropic equilibria

A way to overcome this problem is to use pH indicators exhibiting more complex prototropic forms. This is the case of fluorescein which exhibits cationic, neutral, monoanionic and dianionic forms. Here, there exist 3 dissociation constants, each of them concerning the conversion from one form to another one. Therefore, the laws of mass action are more complicated. Equations (2) and (3) show the concentrations in monoanion (HFlu$^-$) and dianion (Flu^{2-}) as a function of pH and pKa$_s$.

$$[HFlu^-] = \frac{[FLU_{total}]}{\frac{[H^+]^2}{Ka_1 Ka_2} + \frac{[H^+]}{Ka_2} + 1 + \frac{Ka_3}{[H^+]}} \tag{2}$$

$$[Flu^{2-}] = \frac{[FLU_{total}]}{\frac{[H^+]^3}{Ka_1 Ka_2 Ka_3} + \frac{[H^+]^2}{Ka_2 Ka_3} + \frac{[H^+]}{Ka_3} + 1} \tag{3}$$

We only give equations concerning the mono and dianion because only these 2 forms are fluorescing. It can be noted that the concentrations in fluorescent forms are related to the pH and dissociation constants in a nonlinear way. This allows de-correlating pH and pKa$_s$ when studying the shape of fluorescence spectra.

Concentrations of the different prototropic forms depend on the 3 dissociation constants. To simplify, pKa$_1$ = 2.22

principally dictates the proportion between cationic and neutral forms, pKa$_2$=4.34 the proportion between neutral and monoanionic forms and pKa$_3$=6.68 between the mono and dianionic forms. The fluorescence spectra depend not only on the concentration in mono and dianion but also on the molar absorptivities and quantum efficiencies of these forms. The dianion fluoresces about 7 times stronger than the monoanion. When used at low concentration in solutions of low ionic strengths, pKa$_s$ of fluorescein can be considered constant. At physiological pH values with pKa$_3$=6.68, mostly the dianion produces a fluorescence signal. Therefore, the shape of the fluorescence spectra remains constant for any pH value. This is why, up to now, measuring pH with fluorescein is based on the relative fluorescence intensity measurements as explained in the introduction. Indeed, in solution, studying the shape of the fluorescence spectrum is useless because it does not depends on pH. It is then usually considered that fluorescein cannot be used to access calibration free measurement.

However, when grafted close to a surface, the pKa$_s$ of fluorescein can shift of about 2 units [6]. Because we consider physiological pH values, we only consider variations of pKa$_3$. Fig.1 shows the relative fluorescence intensities of mono and dianion for variations of pKa$_3$ around its value in solution. It can clearly be seen that the fluorescence contribution of the monoanion strongly increases with pKa$_3$.
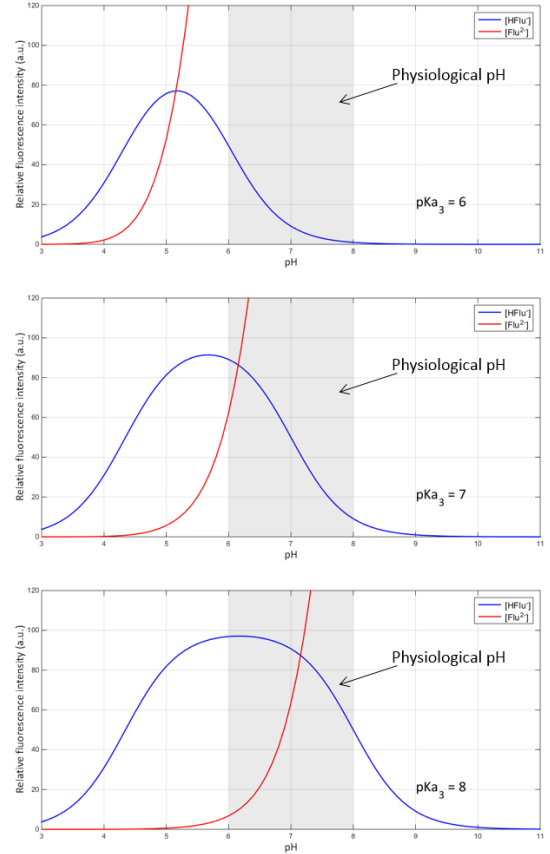

Fig. 1. Evolution of the fluorescence intensities of the mono and dianion with pKa$_3$. Red curve: contribution of the dianion. Blue curve: contribution of the monoanion. The grey area represents the physiological pH window.

The key point of this communication is based on this observation. When grafted onto a fiber, fluorescein exhibits 2 fluorescent forms producing fluorescence spectra of different shapes. The shape of the global fluorescence spectrum now evolves with the pH value. Therefore, the idea is to determine a mathematical function which describes the shape of the global fluorescence spectra as a function of pH and pKa_3 which are considered as variables. Because we focus on physiological pH, the other pKa_s are considered constant. Once this $Flu(pH,pKa_3)$ function is established, it can be used to fit any fluorescence spectrum obtained at unknown pH with any fluorescein grafted fiber. pH and pKa_3 values being de-correlated as explained above, it is possible to compute their values using a simple fitting of the recorded spectrum.

Practically, fluorescence spectra obtained with 8 different fibers and with solutions of pHs in the physiological range are used to define the $Flu(pH,pKa_3)$ function. Note that each fiber exhibits its own pKa_3 value because of unavoidable variations of the fabrication process. We found out that 5 gaussian profiles were required to fully describe any fluorescence spectrum. Monoanion and dianion are described with 3 and 2 gaussian profiles respectively. The amplitudes related to both forms are fixed by the laws of mass action, molar absorptivities and quantum efficiencies. Amplitude coefficients are used to account for variations of the fluorescein concentration at the end of the fibers. Values of the pKa_1 and pKa_2 are fixed. pH and pKa_3 are considered as variables. Other parameters concern positions and widths of the spectra of both prototropic forms. They depend on the thickness and structure of the fluorescein layer at the end of the fiber. They must be considered as variables.

Once the $Flu(pH,pKa_3)$ function is fully defined with the first 8 "model" fibers, it is used to fit the spectra obtained with other "test" fibers. Fig. 2 shows an example of such a fitting for a fiber immersed in a buffer solution of pH 7. The fitting returns a pH value of 7.02 and a pKa_3 value of 7.99 (to be compared to 6.68 for the fluorescein in solution). This example shows a calibration free pH measurement which accounts for variability of the pKa_3 value due to fabrication variability and/or sensing layer aging.
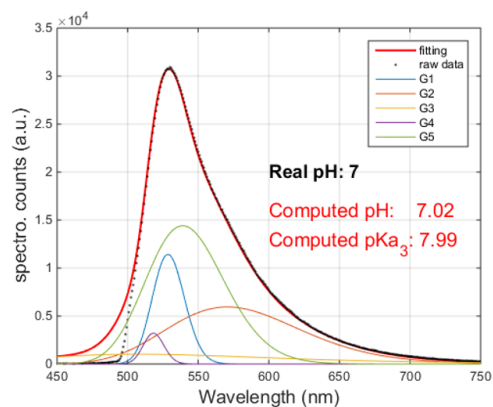
Fig. 2. Use of the Flu(pH,pKa3) function to fit a fluorescence spectrum obtained with a solution at pH 7 with a fiber of unknown pKa3. Black dots: raw data. G1…G5: gaussian profiles. Red curve: fit result = G1+…+G5.
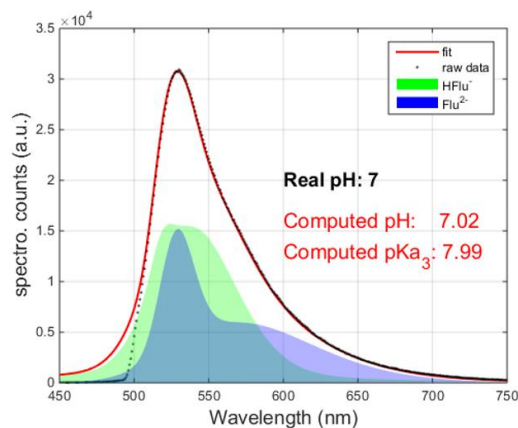
Fig. 3. Contribution of the mono and dianion to the whole fluorescence spectra. Green: monoanion. Blue: dianion.

Fig. 3 shows the calculated contributions of the 2 fluorescing forms of fluorescein. Overall, not only the pH and the pKa_3 are fitted. It is necessary to fit 14 other parameters used to define the $Flu(pH,pKa_3)$ function. Some parameters used in the function are fixed and not considered as variables. Experiments conducted up to now show a pH and pKa_3 determination accuracy less than 0.1 unit. The accuracy can further be improved by refining parameters values using a larger number of "model" fibers and by considering other pKa_s as variables.

III.   DISCUSSION AND CONCLUSION

These results constitute what is, to the best of our knowledge, the first demonstration of a calibration free fiber optic pH sensor. It could be objected that the pH we measure is the pH in the sensing layer and not the pH in the solution to be measured. But, relation between the pH in the sensing layer and the pH in the solution is accounted for when determining the parameters of the $Flu(pH,pKa_3)$ function.

More generally, literature concerning fiber optic pH sensor is relatively old (about 2 decades) and it seems that authors did not pursue their efforts in this domain. At that time, experimental set-ups, including lasers, monochromators and bulk spectrometers were too large to envisage the use of such sensors in clinical situations. Nowadays, compact apparatuses are available and applications in medical environment can be envisioned. More importantly today, computing capabilities of conventional computers allow including efficient fitting algorithms in the proposed medical devices.

In this communication, we put to profit this current experimental environment to set-up a calibration free pH sensor. For this, we use fluorescein, a robust and cost effective indicator considered as a single emission peak indicator, unable to provide calibration free measurements. Here, we show that it can be used for calibration free measurements. The condition for this is to graft it close to a surface in order to shift the values of the pKa_s. Indeed, grafting is exactly what we need for non-invasive intracorporeal pH sensing. Our current studies also concern calibration free ion sensing, in particular Li^+ sensing for application in psychiatry.

REFERENCES

[1] Y. Kawabata, K. Tsuchida, T. Imasaka, N. Ishibashi, "Fiber-optic pH sensor with monolayer indicator", Analytical Sciences, Vol. 3, pp 7–9, 1987.

[2] K.P. Dobmeier, G.W. Charville, M.H. Schoenfisch, "Nitric Oxide-Releasing Xerogel-Based Fiber-Optic pH Sensors". Analytical chemistry, vol. 78, pp.7461–7466, 2006.

[3] I. Kasik, J. Mrazek, T. Martan, M. Pospisilova, O. Podrazky, V. Matejec, et al., "Fiber-optic pH detection in small volumes of biosamples", Anal Bioanal Chem Vol. 398, pp 1883–1889, 2010.

[4] I. Chen, Q. Yang, H. Xiao, H. Shi, Y. Ma, "Local pH monitoring of small cluster of cells using a fiber-optic dual-core micro-probe", Sensors and Actuators B: Chemical, Vol. 241, pp. 398–405, 2017.

[5] H. Diehl, R. Markuszewski, "Studies on fluorescein—VII", Talanta, Vol. 36, pp. 416–418, 1989.

[6] C. Rottman, M. Ottolenghi, R. Zusman, O. Lev, M. Smith, G. Gong, et al., "Doped sol-gel glasses as pH sensors", Materials Letters, Vol. 13, pp. 293–298, 1992.

[7] A.C. Ribou, J. Vigo and J.M. Salmon, "C-SNARF-1 as a fluorescent probe for pH measurements in living cells: two-wavelength-ratio method versus whole-spectral-resolution method", J. of Chem. Educ., Vol. 79, pp. 1471–1474, 2002.

[8] C. S. Owen, "Comparison of spectrum-shifting intracellular pH probes 5′(and 6′)-carboxy-10-dimethylamino-3-hydroxyspiro[7H-benzo[c]xanthene-7, 1′(3′H)-isobenzofuran]-3′-one and 2′,7′-biscarboxyethyl-5(and 6)-carboxyfluorescein", Analytical Biochemistry, Vol. 204, pp. 65–71, 1992.

[9] C. S. Owen, P. Carango, S. Grammer, S. Bobyock, D. B. Leeper, "pH-dependent intracellular quenching of the indicator carboxy-SNARF-1", Journal of fluorescence, Vol. 2, pp. 75–80, 1992.