[ENV09] Fabrication of a novel salicylic acid optical fibre sensor and optimisation using artificial neural network

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Introduction

Salicylic acid (SA) is one of the most important active principles of many pharmaceutical products. It is widely used as kerotalic, antimicrobial and antifungal agent and as external therapeutical agent (keratolytic agent) in many pharmaceutical preparations (Martin & Domínguez, 1999). SA has keratoplastic activity in low concentration and keratolytic activity in high concentration (Ruiz-Medina, 2001). At the same time it had been found to cause nausea, vomiting and other untoward gastrointestinal symptoms (Gross & Greenberg, 1948).

Methods reported for the determination of SA including: spectrophotometric (Trinder, 1954); (Saha & Baksi, 1985); (Glombitza & Schmidt, 1994); (López Fernández *et al*., 1990), spectrofluorimetric (Graham & Rowland, 1972); (Muñoz de la Peña *et al*., 1988); (Villari *et al*., 1994); (Rowland & Riegelman, 1967), colorimetric (Adams & Miller, 1978); (Muñoz de la Peña *et al*., 1995); (Muni *et al*., 1978), liquid membrane electrodes (Choi & Fung, 1982); (Hassan & Hamada, 1988), immunoassays (Hendeles & Edwards, 1998); (Lu-Steffes *et al*., 1982), amperometric (Newmayr *et al*., 1993), chromatographic (Galante *et al*., 1981); (Belanger *et al*., 1983) and enzymatic methods (Bouvrette & Luong, 1996). However, optical fibre spectrophotometric method was used in this study for SA determination due to the simplicity and low cost of this method compared with conventional methods such as high performance liquid chromatography (HPLC) (Blanke & Decker, 1987) and other chromatographic methods (Galante *et al*., 1981); (Belanger *et al*., 1983). Even though gas-liquid chromatography is sensitive, it requires prior extraction and silyl-type derivatisation (Rainsford, 1984). Thus makes the measurement procedures become more complicated**.**

In this study, ferric(III) nitrate was used as a reagent in SA determination. It is an inveterate chemical reagent for SA determination as its usage started before 1930 (Trinder, 1954). SA has long been reported to form a stable purple complex with ferric(III) nitrate at pH 2.45 in solution (Trinder, 1954) and has been utilised spectrophotometrically for the determination of SA at wavelength of 525 nm (Saha & Baksi, 1985). Naturally, salicylate is not synthesized by *Mycobacterium* when iron (Fe^{3+}) is present in abundance, there being feedback control by Fe^{3+} on salicylate synthesis (Ratledge & Hall, 1971); (Young *et al*., 1967).

The potential of artificial neural networks (ANNs) application as a predictor of malignancy has now been widely recognised. They were applied to many problems in the areas of pattern recognition, control and optimisation (Naguib & Sherbet, 2001). The application of ANNs embraces of many fields like medical (Andrea, 1996); (Dumitra *et al*., 1995), engineering, chemistry, physic, agriculture, economy and industry (Yan *et al*., 2000); (Kolanoski, 1995); (Yang *et al*., 2000); (Hubick, 1992).

ANNs are computational models that share some of the properties of the brain. They process information by their dynamic state response to external inputs (Wasserman, 1989). The basic components of an ANNs are "neurons", weights and learning rules (Huang & Zhang, 1995). Thus, ANNs are also described as data processing systems that simulate the human brain by building on information through "learning" (Rouvray, 1993).

For ANNs training purpose, a variety of algorithms can be used. These include Kohonen network (Heyden *et al*., 2000); (Kiss *et al*., 2000), radial basis function (RBF) (Yao *et al*., 2001), probabilistic neural network (PNN) (Shaffer *et al*., 1999), recursive prediction error (RPE) (Taib *et al*., 1996); (Taib & Narayanaswamy, 1997) and back

propagation (BP) algorithm (Suah *et al*., 2003); (Suah *et al*., 2003); (Ahmad & Narayanaswamy, 2002). However BP was applied in this study due to the application of ANNs in analytical chemistry are mostly carried out by using this algorithm (Suah *et al*., 2003).

Experimental

Reagents and buffer solutions

Stock solutions of SA (BDH) range from 0.01 g/L – 2 g/L and ferric(III) nitrate (Sigma) with various concentrations were prepared by dissolution of appropriate amount of these salts in deionised water. The Tris-HCl buffers solutions of different pH values were prepared by mixing 0.05 M tris(hydroxymethyl) aminomethane (Fluka) with HCl solution (Merck) and adjusting to the desired pH. Resin of Dowex-50x8 (Na form; mesh size of 20-50 mesh) from BDH was used as support material to immobilise reagent. The support material was washing with ethanol (BDH) before usage. Phenol for interference study is from JT Baker.

Construction of probe

The probe construction was simply made by encapsulating the immobilised reagent with nylon mesh. (Fig. 1).

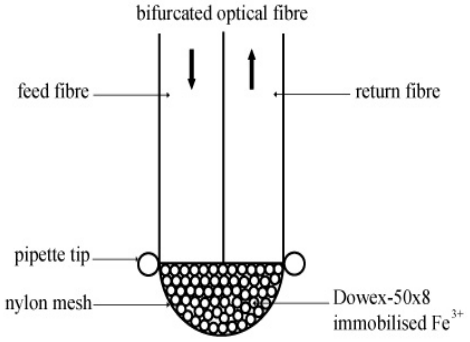

FIGURE 1 The probe design based on immobilised ferric(III) nitrate

Measurement system of the reflectance spectra

The principle of the SA measurement for the SA sensor developed in this study is shown in Fig. 2. It consists of light source, optical fibre (bifurcated) and light detector.

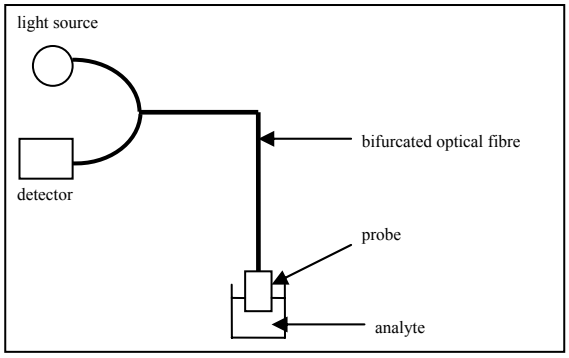

FIGURE 2 Instrumentation set up used for sensor measurement

Data treatment and analysis

A feed-forward ANN having a single hidden neuron layer with a BP training algorithm was employed for data treatment. The ANN training and data treatment were realised using a Matlab program (Suah *et al*., 2003). The training parameters used was set to the values shown in Table 1.

TABLE 1 The setting of the back-propagation specific parameters used during network training

Specific parameters	Values
Frequency epochs display in training	500
Maximum number of cycles to train	20,000
Sum-squared error (SSE) goal	0.001
Learning rate	0.00000001
Limits for weight randomisation	-0.1, 0.1

Results and discussions

The optical fibre accessory in this study gave reflectance spectra for the SAFe(III) complex. The reflectance spectra of immobilised ion Fe^{3+} in Dowex-50x8 before and after reacted with SA are shown in Fig. 3.

The optimum response of the probe was obtained at pH 2.1 (Fig. 4) when the reflectance was measured at wavelength of 786 nm. This pH was similar with what has been reported in the literature (Saha & Baksi, 1985) for the solution work. The complex is stable more than 24 hours.

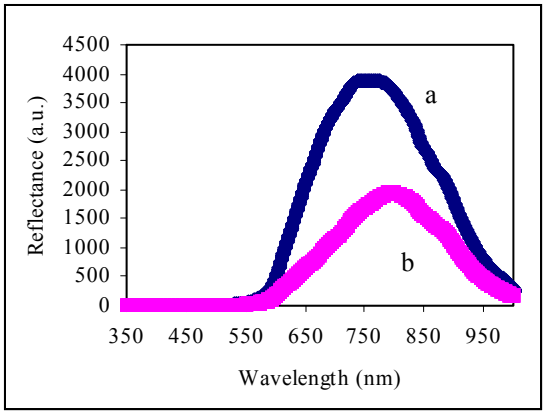

FIGURE 3 Reflectance spectra for immobilised Dowex-50x8 (a) before and (b) after reacted with SA

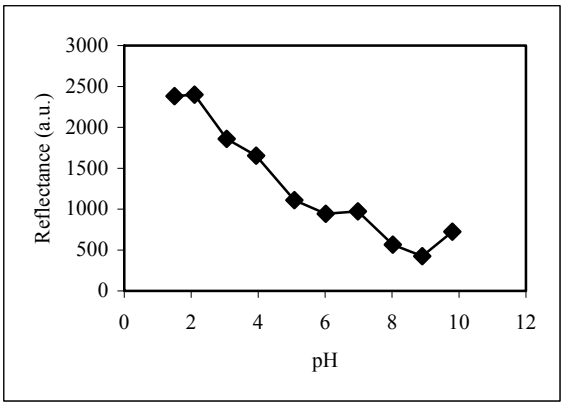

FIGURE 4 The effect of pH on the reflectance of SAFe(III) complex at wavelength of 786 nm

The effect of the reagent concentration on the sensor response was studied by using different initial concentrations of the reagent. Fig. 5 shows the effect of reagent concentration of ferric(III) nitrate used during immobilisation of the reagent on the reflectance intensity of the complex. As the concentration of Fe^{3+} is increased, the measured reflectance intensity was also increased. This is due to the more SAFe(III) complex have been formed when more immobilised reagent is available. The curvature at higher concentration is expected due to all adsorption sites of the Dowex-50x8 have been fully occupied by SA. The same curvatures have been reported by Bouvrette and Luong (Bouvrette & Luong, 1996) as well as Ahmad and Narayanaswamy works (Ahmad & Narayanaswamy, 2002).

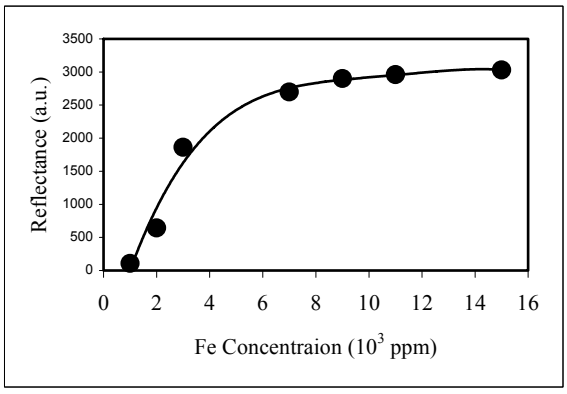

FIGURE 5 Effect of reagent concentration in SAFe(III) complex at 786 nm (SA = 1 g/L)

Three different concentration of SA, i. e. 0.1, 0.5 and 1.0 g/L were chosen for reproducibility study. The results give relative standard deviation (RSD) values of 0.38 %, 0.39 % and 0.90 % for 0.1g/L, 0.5 g/L and 1.0 g/L of SA, respectively. The study showed a very promising RSD value. Therefore this method has a good potential to be adopted as analytical method for SA determination because it is highly reproducible.

The interference of phenol has been carried out by using three different mole ratio of SA: phenol i.e. 1:1, 1:10 and 1:100. Fig. 7 showed low interfering effect for all ratios of 1:1, 1:10 and 1:100 with 0 %, 2 % and 10 % of the interfering percentage respectively. Hence, no obvious interference is caused by phenol if compared with other SA detection method which is suffered from interference by benzoic acid (Saha & Baksi, 1985).

The dynamic range of SA concentration determined using the probe is shown in Fig. 6. The plot of SA concentration against reflectance was linear for SA concentration in the range of 2.0×10^{-2} g/L to 5.0×10^{-1} g/L SA. The linear range is rather narrow. However this problem has been overcome by applying artificial neural network (ANN) like what have been reported in the previous works (Ahmad & Narayanaswamy, 2002). The useful dynamic linear range has been extended to a wider range of 0.02 – 2.00 g/L and this broadening range is rather similar with the developed ISFET SA sensor (Suah *et al.*, 2003). However, the fabrication cost of this sensor is far cheaper that the ISFET SA. Besides, the sensor developed in this study also free from suffering of plasticiser leaching problem.

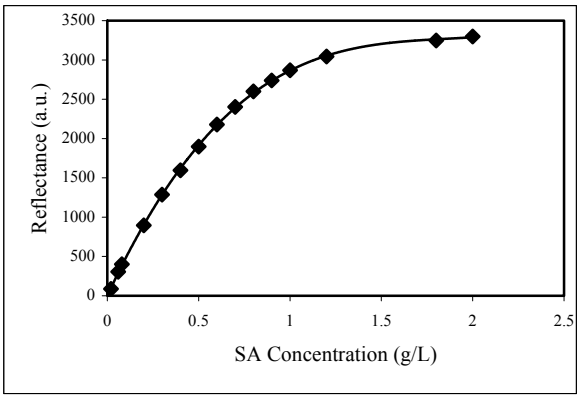

FIGURE 6 Dynamic range of SA concentration in SAFe(III) complex at 786 nm with Fe (10000ppm)

The three-dimensional reflectance spectra of the optical fibre SA sensor is shown in Fig. 7. As shown, Fig. 6 demonstrates the non-linear characteristics that lies beneath the sensor's data. ANN is suitable to be used for non-linear modelling purposes. It was linear for SA concentration in the range of 2.0 x 10^{-2} g/L to 5.0 x 10^{-1} g/L SA. Therefore the beneficial linear range is rather narrow.

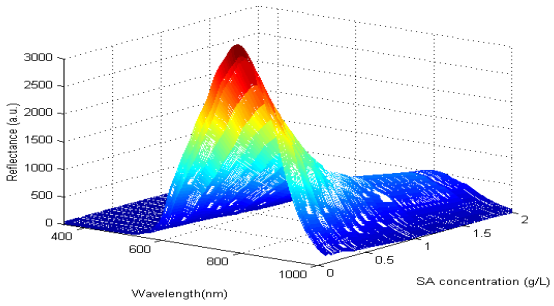

FIGURE 7 Three-dimensional reflectance spectra of the optical fibre SA sensor response measured at different SA concentrations.

In this study, ten wavelengths points (550, 650, 707, 747, 786, 793, 848, 870, 900 and 1000 nm) from each spectrum were selected to represent the input data for the ANN. These points were selected due to the general outline of the original spectra were represented and the variations in the sensor response were significant. The wavelength selection is aimed to avoid several problems during network training; including long training period (Ahmad & Narayanaswamy, 2002) and large matrices are entailed for the network connection (Taib & Narayanaswamy, 1997); (Suah *et al*., 2003). The same training method has been used and reported (Taib & Narayanaswamy, 1997); (Suah *et al*., 2003); (Suah *et al*., 2003).

A total of 15 spectra (0.02, 0.06, 0.08, 0.20, 0.30, 0.40, 0.50, 0.60, 0.70, 0.80, 0.90, 1.00, 1.20, 1.80, 2.00 g/L) were used for ANN training. The optimisation of network was performed by changing the number of hidden neurons. The SSE was measured at the end of each training and being recorded. Fig. 8. shows the SSE values of each networks undergone 20,000 epochs. The number of hidden neurons when arranged in declining SSE order is 24, 3, 15, 10, 20, 5, 22, 25 and 8. The results indicated that an optimised and suitable network can be obtained with network contains of 3, 10, 15, 20, 24 neurons in the hidden layer.

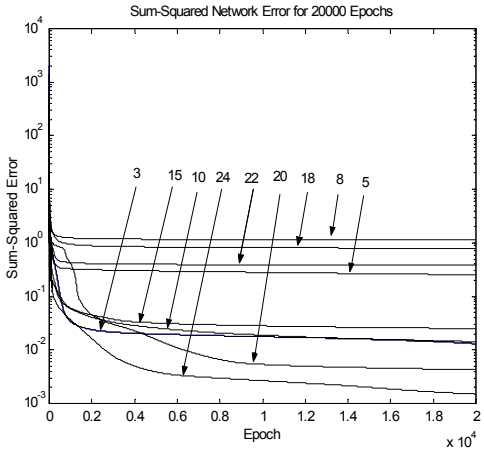

FIGURE 8 Sum-Square error (SSE) plots over 20,000 epochs for networks with 3, 5, 8, 10, 15, 20, 22, 24 and 25 hidden neurons.

All of these networks were presented with three calibration spectra (0.10, 1.40 and 1.60 g/L) in order to improve the process in choosing the best architecture of network and to establish their prediction capability. Table 2 displays the predicted values of SA concentrations with the measured SA concentrations (with optical fibre spectrophotometer).

TABLE 2 Network prediction with 15 hidden neurons

Measured SA concentration (g/L)	Predicted SA concentration (g/L)	Error (g/L)
0.10	0.098	0.002
1.40	1.521	0.121
1.60	1.682	0.082

Average calibraion error = |predicted value - measured value|/3

The networks with 15 neurons in the hidden layer gave the best predictions, with average calibration errors of 0.0683 g/L. The network prediction capability test was carried out both in linear response range and non-linear response range of the sensor.

The useful dynamic linear range is now extended to a wider range of 0.02 – 2.00 g/L. The developed ISFET SA sensor (Qu, 1991) demonstrated rather same useful dynamic liner range (5×10^{-5} – 1.5×10^{-2} M or 0.01 – 2.07 g/L).

Conclusion

The studies carried out in this work indicate that Fe^{3+} immobilised on Dowex-50x8 can be successfully used as reagent phase in the development of SA sensor based on reflectance measurement. The reflectance measurement was carried out at pH 2.1 and Fe^{3+} concentration of 1×10^4 ppm was used for immobilisation of the reagent. A good reproducibility (0.9%) of measurement was obtained with this probe. A linear relationship was obtained between 2.0×10^{-2} g/L to 5.0×10^{-1} g/L and 2.0×10^{-2} g/L to 2.0×10^2 g/L of SA before and after optimised with ANN. The average prediction error of 0.0683 g/L.

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