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Characterisation of a gas-diffusion membrane-based optical flow-through sensor exemplified by the determination of nitrite

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Abstract

A membrane-based optical flow-through sensor is described which can be alternatively used for absorbance and reflectance detection within the receiver channel of a sandwich-type gas-diffusion separation cell. Using the common spectrophotometric detection scheme for nitrite based on azo-dye formation, the principle features of the flow-through sensor are investigated and the performance is characterised particularly with regard to selectivity and sensitivity aspects. The determination of nitrite in waste waters and meat extracts was used to demonstrate the applicability to real sample analysis. The main advantage of the proposed flow-through sensor is the absence of interferences due to sample colour and turbidity enabling direct sample admission of complex samples without tedious sample pretreatment.

Keywords: Flow-through sensor; Membrane separation; Gas diffusion; Nitrite determination

1. Introduction

The development of flow-through sensors (not to confound with probe-type sensors applied in flowing streams [1]) is a subject that emerged from miniaturisation and detector integration in flow injection analysis techniques [2–7]. Different types of flow-through sensors can be distinguished according to the respective detection principle, the way of operation and the complexity of analytical steps involved [1]. Within the optical flow-through sensor family, a basic distinction between those relying on sorbent extraction optosensing [7–10] and membrane-based separation and detection [5,6,11,12] can be made.

The latter are particularly interesting since they offer extraordinarily high selectivity and can be flexibly applied to different analytes by simply changing the chemical derivatisation reactions taking place within the flow channels of the separation unit [1,5,13]. In the present paper, the instrumental configuration of a novel optical flow-through sensor is described that can be alternatively used for absorbance and reflectance measurement. The determination of nitrite by the modified Griess reaction [14,15] was exemplarily used to critically exploit the performance of the flow-through sensor. The influence of the operational parameters was investigated and application to the analysis of waste waters and food extracts demonstrated.

2. Experimental

2.1. Reagents and solutions

All chemicals were of analytical-reagent grade and used without further purification. Deionised water from a Seradest system (specific resistivity >18 MΩ cm) was used for the preparation of solutions. The colour reagent (serving as the receiver solution in the present application) was prepared by dissolution of 10 g sulfanilamide, 0.15 g *N*-(1-naphthyl)-ethylenediamine dihydrochloride and 25 ml concentrated hydrochloric acid in 500 ml water. When stored in a brown bottle, this solution is stable for at least 1 week. Sulphuric acid (0.5 mol l^{-1}) was used as a releasing agent to set HNO_2 and NO_2 free from the nitrite solutions.

2.2. Apparatus

The basic flow system used is depicted in Fig. 1. Solutions are propelled by a variable speed multichannel

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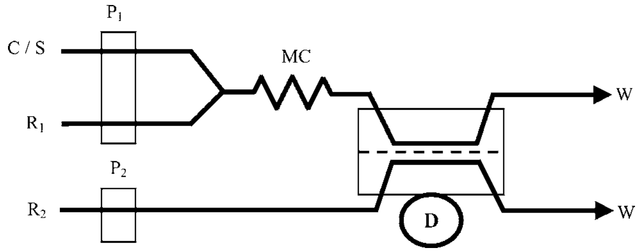

Fig. 1. Schematic depiction of the general flow system used. P_1/P_2, peristaltic pumps; MC, mixing coil; C/S, carrier or sample solution; R_1, releasing agent; R_2, receiver solution; D, flow-through sensor.

peristaltic pump (Typ IPS-8, Ismatec, Zurich) furnished with Tygon tubing. For interconnections, PTFE tubing of 0.5 mm i.d. was used. The details of the membrane-based optical flow-through sensor are shown in Fig. 2. It is a kind of hybrid of membrane-based flow-through sensors presented in previous papers for either absorbance [5,6,13] or reflectance [6,11–13] measurements. The cell comprises three parts, i.e. two perspex blocks (each 43, 30, 17 mm for length, width and height, respectively) with appropriate holes (0.7 mm i.d.) for the inlet and outlet of donor and receiver solutions sandwiching a black (carbonised) PTFE-plate (2 mm thick) with a central recess of 1 cm length and 3 mm width. Two holes were drilled horizontally into the PTFE-plate to precisely extend the axis of the recess. Into these holes, 1 mm diameter plastic optical fibres (Conrad electronics, Berlin) were pressfitted serving as the two light guides from the light source and to the photometric detector, respectively. Thus, the recess forms a 1 cm path length flow-through cuvette for absorbance measurements. Into the upper (acceptor-side) perspex block, an additional central hole was drilled accepting the end of a bifurcated optical cable for reflectance measurements. This optical cable consists of 15 individual fibres of 0.5 mm diameter each, 5 serving as light inlet from the source and 10 for the transmission of the reflected light to the photodetector, respectively. Into the lower (donor-side) block, a rectangular channel was engraved of 10 mm length, 3 mm width and 0.2 mm depth. The separation membrane was placed between the carbonised PTFE-plate and the lower perspex block. The three parts were held together with a clamp. Hydrophobic microporous polypropylene membranes (75% porosity, 0.2 μm pore size, 80 μm thickness, Type 1E-PP, Enka, Wuppertal) were used throughout the work.

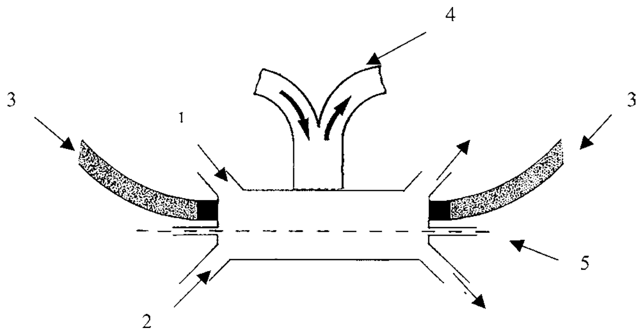

Fig. 2. Membrane-based flow-through sensor for absorbance and reflection measurements. (1) Receiver inlet; (2) sample inlet; (3) optical fibres for absorbance measurements; (4) bifurcated fibre for reflectance measurements; (5) separation membrane.

The optical parts of the system were constructed in the electronic workshop of our institute to permit optimum performance and high flexibility. The system used here basically consists of a green light-emitting diode (Rohm electronics, Munich) with an emission maximum of 563 nm as the light source and a phototransistor for light detection. The respective electronic circuits are similar to those described by Bettridge et al. [16] and can be obtained upon request, free of charge from the authors. Depending on whether absorbance or reflection measurements are intended, the respective optical fibres are connected to the light source and the photodetector. Output signals were transferred to a strip-chart recorder and signals were evaluated graphically.

2.3. *General operating procedure*

For system start-up, water (as a blank solution), sulfuric acid and the colour reagent are aspirated via the respective flow channels (see Fig. 1) and the measured light in either absorbance or reflectance mode is electronically adjusted to zero (i.e. maximum transmission or remission). Then, water is exchanged for nitrite standard solutions as the sample and at the same time, the receiver solution is stopped. Upon passage of the sample through the donor channel HNO_2 and NO_2 generated is transferred across the membrane and trapped in the receiver solution where the colour reaction takes place. This leads to a continuous increase of absorbance or decrease of remission as long as the sample solution is flowing and saturation in the receiver channel does not occur. When the flow of the receiver solution is initiated, the dye formed is flushed out, the baseline is established and a new measuring cycle can be started. When samples are injected (rather than continuously aspirated), the optical signal increases during passage of the sample zone and thereafter approaches a constant value. Evaluation of the signals can be made as in kinetic methods of analysis by fixed time, fixed signal or initial slope measurements [17].

3. Results and discussion

3.1. *Chemical variables*

The composition of the colour forming reagent is similar to that used in the common Saltzman procedure for the determination of gas-phase NO_2 [18]. The respective concentrations of the two constituents have, however, been slightly modified to obtain lower blank values, a better stability and most importantly for the present application, faster reaction kinetics. The actual composition used is given under Section 2. With respect to the releasing agent, different acids were tested at various concentration levels. It was found that the kind of acid is not important as long as the concentration is sufficiently high to generate a pH well below 1 in the donor stream. A concentration of 0.5 mol l^{-1} H_2SO_4 is suitable when the sample solution and the releasing agent are mixed in a 1:1 ratio and this was used here.

3.2. Effect of flow rates

The flow rate of the acceptor stream is not very critical since it is stopped during the detection step, i.e. while the increasing absorbance is being recorded (see also Section 2.3). Too fast flow, however, can damage the membrane and too low flow rates require long wash-out times after signal evaluation. A flow rate of 0.5 ml min^{-1} was generally set. With respect to the flow rate of the donor stream, two situations have to be distinguished. When samples are continuously aspirated, a high flow provides rapid renewal of the sample solution within the donor channel maintaining a high concentration gradient across the membrane and hence a fast increasing concentration in the receiver stream (i.e. high sensitivity) [5]. When samples are injected, the residence time of the sample plug in the donor channel depends on the donor flow rate. In principal, in such case, maximum sensitivity can be achieved by exhaustive gas-diffusion which desires slow donor flow rates or even a temporarily stopped flow [5].

In the present investigation, continuous sample aspiration was generally used and a flow rate of the donor stream (sum of sample and releasing agent) of 0.5 ml min^{-1} was found to be a reasonable compromise as far as sensitivity, consumption of sample and problems with differential pressure across the gas-permeable membrane were concerned.

3.3. Evaluation of the performance

Most of the measurements have been made in the transmission mode. A typical recording obtained when a nitrite standard of 50 mg l^{-1} is continuously aspirated is shown in Fig. 3. Converting the actually recorded transmission data into absorbance shows (in accordance with Beer's Law) a linear dependence between concentration time and absorbance during the first 3 min. Comparison of the data obtained at different concentration times with that of the standard batch procedure [15] for the determination of nitrite using the same reagents revealed a dramatic dilution effect in the present measurement configuration. Partially, this is due to the gas-diffusion separation involved (which is a slow process). An additional yet even more serious reason is a conceptual problem which came out only after a thorough inspection of the events taking place in the acceptor channel. For a better understanding, reference is made to Fig. 4 showing the enlarged yet scaled view of the acceptor channel being the pseudo-cuvette of the present flow-through sensor. The analyte passing through the membrane only slowly diffuses into the acceptor solution so that a vertical concentration gradient is built up with the maximum concentration in the immediate vicinity of the membrane surface. For practical reasons, the depth of the acceptor channel cannot be made infinitively small to sense this concentrated zone because firstly, sufficient light-transfer is required through this

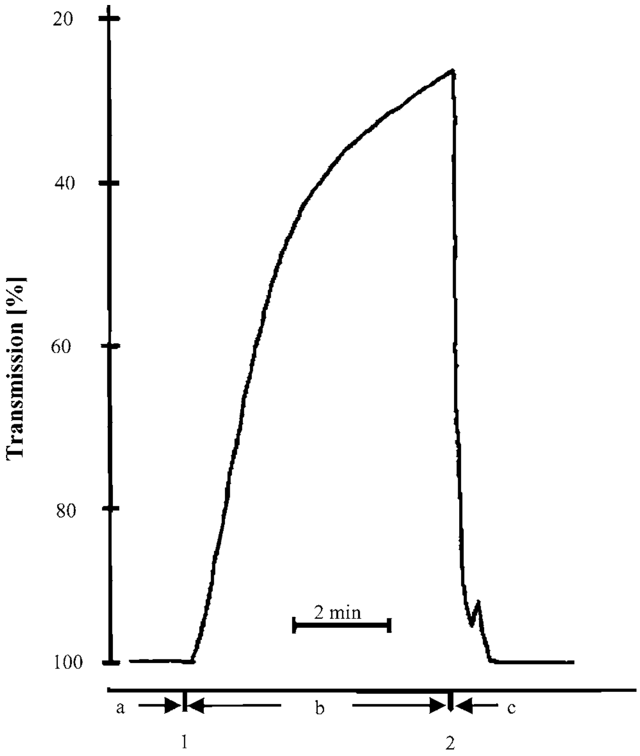

Fig. 3. Typical detector readout for a complete measurement cycle. (1) Stop of the receiver flow; (2) re-start of the receiver flow. (a) Baseline before sample introduction; (b) continuous decrease of transmission due to analyte concentration; (c) decrease to baseline through washing the receiver solution out of the flow-through sensor (see text for further details).

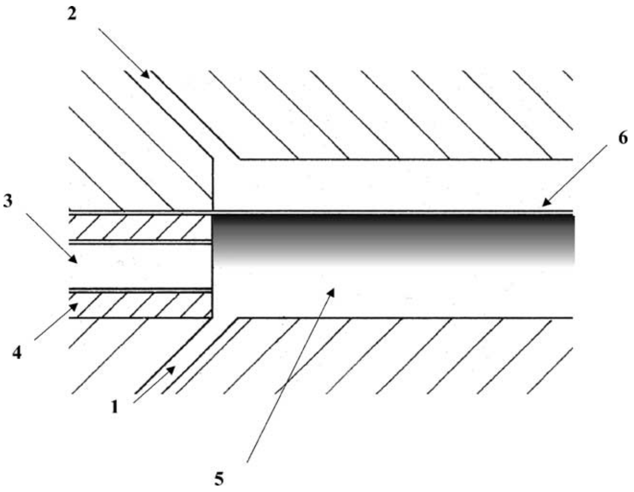

Fig. 4. Enlarged, yet scaled view of the acceptor channel of the developed sensor illustrating the conceptual problem of spectrophotometric measurement of the analyte passing the membrane and diffusing into the bulk of the channel volume. The shadowed area indicates the coloured part of the acceptor solution. (1) Receiver inlet; (2) sample inlet; (3) optical fibre; (4) PTFE spacer; (5) acceptor channel; (6) separation membrane.

channel; secondly, membrane bulging into this channel can lead to disturbances of the transmission measurements; and thirdly, the differential pressure across the membrane gets too high when the acceptor solution flows. As a result of the unduly large receiver channel dimensions, only a fraction of the reaction product formed in the acceptor channel is actually detected and most of the light-path is filled with non-absorbing reagent solution.

Reflectance measurements with the membrane being the reflective surface [12,13] is an obvious way to sense the product rich zone since the light is travelling twice through this layer in this measurement mode. Our comparative investigations which due to the hybrid-sensor could be made under identical conditions gave some interesting insights. When a standard solution is aspirated, the reflectance signal can be detected a few seconds before the absorbance signal occurs. This evidences the slow diffusional transport from the membrane surface into the bulk of the acceptor solution. After this initial period, the sensitivity (in terms of transmission change) of the reflection measurements is slightly worse compared to the measurement of absorbance. This can be explained by the short actual path length available in the reflection mode. An additional problem with reflectance measurements is the movement of the membrane which creates an unfavourable signal-to-noise ratio.

3.4. Calibration

For calibration of the proposed membrane-based flow-through sensor, standard solutions of different concentrations were aspirated and the signals obtained were recorded. Conditions were set as outlined above. Though evaluation can be made in various manners (fixed time, fixed signal or initial slope [17]), the former technique was used here. In the concentration range 0.1–50 $\mathrm{mg\,l^{-1}}$ nitrite, signal heights obtained after 3 min stopped flow time strictly followed Beer's-law. The regression equation obtained (1 cm cuvette) was $y = (0.004 \pm 0.006) + (0.092 \pm 0.004)x$ $\mathrm{l\,mg^{-1}}$ $\mathrm{NO_2{}^-}$, $r = 0.9997$. The typical precision of repetitive measurements is 5% R.S.D. Lower concentrations are accessible by extending the measurement period, yet on the expense of increasing sample consumption and reduced sample throughput. In order to accommodate highly concentrated samples, the readout can simply be made at an earlier stage or initial slope evaluation can be used.

3.5. Interferences

The main reason for developing the proposed sensor was the inherent selectivity enhancement due to analyte separation by the gas-diffusion process. Only few analytes are sufficiently volatile to pass the membrane and they are hence effectively separated from the sample matrix. Therefore, species known to interfere in the Griess reaction [19,20] are almost entirely excluded. In particular, coloured and turbid samples can be analysed without problems and interferences due to extreme pH values or the presence of high salt content in the samples are eliminated so that the proposed sensor is almost specific to nitrite.

3.6. Application to real samples

The relatively low sensitivity (compared to the common spectrophotometric method [15,21]) of the developed sensor definitely limits its applicability. Among environmental samples, only certain waste waters may contain nitrite in the upper $\mu\mathrm{g\,l^{-1}}$-level and above. Other samples with high nitrite content are meat products treated with nitrite to prevent bacterial food poisoning and to confer the characteristic colour. Samples of waste waters from different water treatment plants in Berlin have been analysed. All samples were directly aspirated without any further treatment. In none of the samples nitrite was found at concentration levels accessible with the present method, i.e. above 100 $\mu\mathrm{g\,l^{-1}}$. Spiking of the samples with known amounts of nitrite (in the range 0.5–5 $\mathrm{mg\,l^{-1}}$) was made to check the recovery and to exploit potential interferences on the gas-transfer. In all but one sample, the recovery ranged from 85 to 105%. The exception was a waste water sample with high biological activity where nitrite degradation was extremely fast so that significantly low and variable recoveries were obtained. Comparative measurements with the recently published standard method utilising flow injection analysis [21] were made. The results obtained showed reasonable agreement (largest deviation $<$12%). However, using the standard method, due to the presence of high molecular weight substances and microparticulate matter, tedious and expensive microfiltration and/or solid-phase extraction was generally required prior to analysis. Homogenised meat samples were also analysed by the proposed method. After treatment with boiling water and centrifugation, direct admission (without Carrez precipitation) of the (dark redbrown) extract was possible. Recovery tests on spiked samples confirmed the reliability of the developed method.

4. Conclusions

The proposed flow-through sensor is a miniaturised optical detector with interesting features. Compared to common probe-type sensors relying on membrane separation for selectivity enhancement [22], reversibility is simply obtained by flushing out of the sensing zone after signal evaluation rather than establishment of equilibrium within that zone with a zero-concentration analyte solution. The device presented here can be alternatively used for absorbance and reflectance measurements, and hence, provide additional information. The membrane separation process involved leads to significant selectivity enhancement. In the present work, only gas-diffusion has been utilised but obviously any other membrane separation process (e.g. different forms of dialysis, liquid-membrane extraction) can be implemented. Also, with respect to the derivatisation reaction, a wide variety of common spectrophotometric methods can in principle be adopted. The only perceivable restriction is that assays requiring the sequential addition of two or more reagents cannot be used. Since the progress in time of the transfer process across the membrane is sensed, kinetic information about the chemical reaction and the separation process is obtainable. The latter has recently been utilised in the characterisation of dialysis membranes with respect to their ion-transfer characteristics [23]. The present study, however, also revealed a general conceptional problem of such type of optical flow-though sensors which is of importance when high sensitivity measurements are envisaged.

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