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A critical examination of sorbent extraction pre-concentration with spectrophotometric sensing in flowing systems

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Abstract

In this paper, the solid-phase spectrophotometric concept is critically examined in flow systems exploiting a dedicated microcolumn-based optical sensor packed with octadecyl chemically-modified silica gel. The flow configuration integrates matrix separation with pre-concentration and on-column sensing of non-polar complexes resulting from analyte derivatization. The design criteria for optimum performance of both the sorbent-packed microcolumn and optosensing instrumentation are for the first time discussed in detail. Practical considerations required to warrant the maximum sorption efficiency of the reversed-phase material by avoiding chain collapse, and avenues to overcome the effects derived from its transparency changes upon solvation with solutions of different polarity are also addressed. The noteworthy features of the flow-through enrichment system involve the sensitivity enhancement with respect to common spectrophotometric procedures in the liquid-phase, as well as the capture of a large amount of scattered light, which is the most severe pitfall of conventional solid-phase absorptiometric approaches using commercially available flow-through cells.

Several common spectrophotometric assays for monitoring key inorganic and organic parameters (viz. oxidized nitrogen, ammonium, sulfite, phosphate, iron(II)/total iron, chromium(VI), nickel(II) and phenol index) in environmental samples are taken as practical examples. Plausible sorption mechanisms together with discussions for proper performance of the different investigated reversed-phase extraction optosensing methods are presented in the bulk of the text. Special emphasis is paid to problems arising in real sample analysis due to unspecific sorption of matrix constituents and potential means to circumvent them are thoroughly explored.

Keywords: Sorbent extraction; Preconcentration; Optosensing; Flow injection

1. Introduction

The use of solid-phase reactors is one of the most rapidly developing and challenging areas in flow injection analysis (FIA) research [1–4]. In the majority of applications, the solid-phase constitutes an immobilized reagent or a bonded-phase functional group, the purpose of which is to initiate an heterogeneous (bio)chemical reaction [5,6], to introduce (often unstable in solution) reagents into the flow system [7–10], to develop displacement reactions during analyte passage through the packed reactor, and further detection of the delivered species [11–13], to enrich the target compound after membrane separation [14,15], or to hold back the analyte, the reaction product of a chemical assay, or interfering matrix constituents [16–18]. In the latter case, pre-concentration and/or matrix removal is concomitantly attained. This strategy has been extensively used for the improvement of the performance of atomic spectrometric methods [19,20], but also exploited in combination with chromatographic [21] and electrophoretic separations [22].

In recent years, attention is being given to the integration of separation, concentration and detection in FIA. The basic idea behind this concept is on-the-fly monitoring of the processes occurring within the solid-phase using optical, electrochemical, thermal or mass detection [23]. Optical detection of the pre-concentration step taking place at the solid-phase has by far most commonly been employed. Historically, the fundamentals of this technique can be traced back to the pioneering work of Yoshimura et al. [24], who for the first time used the solid-phase spectrophotometric

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scheme for the direct measurement of light attenuation of a chemically-modified ion-exchanger phase after sorptive pre-concentration of elements onto it from solution. Initially, this method was performed in batch procedures [25], and it was only after the trailblazing papers of different researchers on the implementation in FIA [23,26,27] that the full potential of this approach has been widely recognized. Since then, the published material on solid-phase optosensing describes many different applications for the determination of inorganic and organic compounds, and the use of a variety of sorbent materials [28]. With respect to the selection of the sorbent, the extensive experience of solid-phase extraction (SPE) technology [29] can serve as a valuable source of information. Accordingly, the choice of the sorbent is mainly guided by the chemical structure of the target molecule to be sorbed, but sufficiently good chemical and mechanical stability, and ready availability have also to be considered. An indispensable requirement for the application of permanent packed-bed reactors in SPE-based flow systems, which involves repetitive cyclic operation, is total reversibility of the sorption/elution process. Finally, the sorbent material must possess high optical transparency to be suitable for the application in solid-phase spectrophotometric measurements.

For the concentration of charged species, ion-exchange resins are generally favored [27], but it is sometimes difficult to desorb the retained complexes completely because of too strong secondary interactions between aromatic rings of the target molecule and those of the resin. Hydrophobic interactions are generally utilized for sorption of neutral compounds containing long-chain carbon skeletons or aromatic rings. In many instances, however, the exact mechanism of retention is not known, and often, combined interactions, such as physical adsorption and partitioning, can be made responsible for the particular sorption behavior [30,31]. Modified silica gel sorbents available in widely varying polarity are characterized by high capacity, well-defined reactivity, homogeneous particle and pore size distribution and extremely good mechanical stability. In contrast to most copolymeric resins, silica materials do not exhibit undesirable shrinking and swelling upon changing the kind of solvent and/or ionic strength of the medium, thus appearing perfectly suited for at-column optical sensing schemes. The only limitation in its applicability can be seen in the limited pH-stability ranging from pH 2 to 8 [29].

The aim of this paper is to examine critically the flow-through sorbent extraction optosensing concept at octadecyl chemically-modified silica gel sorbents, currently used as a packing material of commercial available flow-through cells for the single or multiparametric determination of either non-polar active constituents in pharmaceutical formulations [32,33] or heavy metals in environmental or steel samples [34,35] following analyte derivatization. General considerations regarding aspects of the flow-through optical detector cell arrangements are presented, and the effects of experimental variables on sorption behavior and detection capabilities are thoroughly investigated. Special emphasis is also given to the fundamental requirements to be considered aiming to warrant maximum sorption efficiency via prevention the chain collapse of the reversed-phase material. Several common spectrophotometric assays for various inorganic and organic species (namely, oxidized nitrogen, ammonium, sulfite, phosphate, iron(II)/total iron, chromium(VI), nickel(II) and phenol index) are taken as examples for the inherent potential and limitations of the proposed flow-through pre-concentration assembly. The problems associated with the application to real-life environmental samples are discussed in detail, and efficient means to overcome these limitations are presented.

2. Experimental

2.1. Reagents and solutions

All chemicals were of analytical grade quality (Merck, Darmstadt). Deionized, doubly distilled water was used throughout. The stock solutions of the various analytes were made by dissolution in water of appropriate amounts of sodium nitrite, sodium nitrate, sodium sulfite, ammonium chloride, sodium dihydrogenphosphate, ammonium iron(II) sulfate, nickel nitrate, potassium dichromate and phenol to give a final concentration of $1.0\,g\,l^{-1}$ of the desired species. Working standards were prepared by serial dilution of the stock solutions. In order to remove compounds that are adsorbable at the C_{18}-microcolumn and may hence interfere in optosensing measurements, all inorganic reagent solutions were forced through a cartridge filled with the reversed-phase material prior to use. Furthermore, the reagent solutions and the methanolic eluent were thoroughly degassed by ultrasonic agitation under water suction vacuum and filtered through 0.45 μm cellulose acetate membrane filters.

For the determination of nitrite, sulfite, iron(II), chromate and nickel a two-line manifold (plus two additional channels required for conditioning and elution) with sample and one reagent stream (R_1) was built, whereas for the determination of the other species a three-line manifold with an additional reagent channel (R_2) was used. The optimized reagent solutions used in the various assays investigated were composed as follows:

2.1.1. Determination of nitrite/nitrate

R_1: 5.0 g of sulfanilamide (SAM) and 0.5 g of *N*-(1-naphthyl)-ethylenediamine dihydrochloride (NED) are dissolved in 50 ml of concentrated phosphoric acid, and the solution is made up to 500 ml with water. Nitrate was reduced in-line to nitrite using a cadmium-filled microcolumn [36] inserted into an extra flow-channel connected with the assembled set-up using an ancillary switching valve. For the determination of total oxidized nitrogen (viz. sum of nitrite and nitrate) the sample stream merges with a 1.5 M

ammonium chloride solution at pH 8.5 prior to passage through the reduction column.

2.1.2. Determination of sulfite

R_1: 50 mg of 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) are dissolved in 10 ml ethanol and added to 100 ml of a solution containing 1.0 g of potassium hydrogen phthalate. This mixture is adjusted to pH 6 with hydrochloric acid and filled up to 250 ml with water.

2.1.3. Determination of ammonium

R_1: 42 g of phenol and 7.5 g of sodium tartrate are dissolved in 100 ml of water. Eighteen grams of sodium hydroxide and 0.6 g of disodium pentacyanonitrosoferrate are dissolved in 100 ml of water. When required these two solutions are mixed and diluted to 250 ml with water.

R_2: A 10% (w/v) hypochlorite solution prepared immediately before measurement is used.

2.1.4. Determination of phosphate

R_1: 1.0 g of ammonium molybdate is dissolved in 300 ml of water. Ten milliliter of concentrated sulfuric acid is cautiously added and the solution is made up to 500 ml with water.

R_2: 30 mg of tin(II) chloride and 0.5 g of hydrazine sulfate are dissolved in a mixture of 10 ml concentrated sulfuric acid and 300 ml of water. After complete dissolution the solution is made up to 500 ml with water.

2.1.5. Determination of iron(II)/total iron

R_1: 0.5 g of 3-(2-pyridyl)-5,6-bis(4-phenylsulfonic acid)-1,2,4-triazine disodium salt (Ferrozine) and 10 g of sodium acetate are dissolved in 1 l of water. Hydrochloric acid is added dropwise to give a final pH of 4.5. For the determination of total iron, an additional flow channel (R_2) was installed aiming to merge the sample stream with a 5.0 g l^{-1} hydroxylammonium chloride solution prior to react with the color-forming reagent.

2.1.6. Determination of chromate

R_1: 0.2 g of 1,5-diphenyl carbazide are dissolved in 10 ml acetone. This solution is filled up to 500 ml with a 0.2 M sulfuric acid solution.

2.1.7. Determination of nickel

R_1: 0.5 g of dimethylglyoxime sodium salt are dissolved in the minimum volume of ethanol. The solution is adjusted to pH 12 before making up to 250 ml with water.

2.1.8. Determination of phenol

R_1: 0.1 g of 4-aminoantipyrine (4-AAP) are dissolved in a 500 ml buffer solution. The buffer composes 1.0 g sodium bicarbonate, 1.2 g boric acid and 1.3 g potassium hydroxide per liter of water, the pH being precisely adjusted to 10.2.

R_2: 0.3 g of potassium hexacyanoferrate(III) are dissolved in 500 ml of water and the solution adjusted to pH 11 by dropwise addition of 0.5 M sodium hydroxide solution.

2.2. Flow system

The basic arrangement of the optosensing flow system is depicted in Fig. 1. It comprises a multichannel peristaltic pump (Ismatec, Type IPS-8, Zurich) furnished with Tygon or Viton tubing for propelling the aqueous and alcoholic solutions, respectively. The sorbent-filled tubular microcolumns were nested in a PTFE Rheodyne Type 50 six-port injection valve aiming to perform a counter-current elution scheme. Another identical six-port rotary valve was employed to direct either the conditioning solution or the derivatization product to the packed-bed reactor. The manifolds were built from PTFE tubing of 0.5 mm i.d. and Kel-F mixing tees. Two- and three-line manifolds have been designed for the application of various spectrophotometric methods as detailed in the foregoing section. In order to simplify the respective procedures, the flow rates of sample and reagent streams as well as dimensions of the reaction coils (viz. 80 cm long) were kept constant throughout, and optimization was done solely by variation of the respective reagent compositions.

2.3. Construction of the optosensing instrument

The mechanical construction of the purpose-made optosensing transducer cell is schematically shown in Fig. 2. The aluminum housing was made to accept tubular microcolumns of up to 10 mm o.d. The light emitting diode (LED) [37] and the blue sensitive silicon photodiode with integrated amplifier (Stock No. 590–963, RS Components Ltd., Corby,

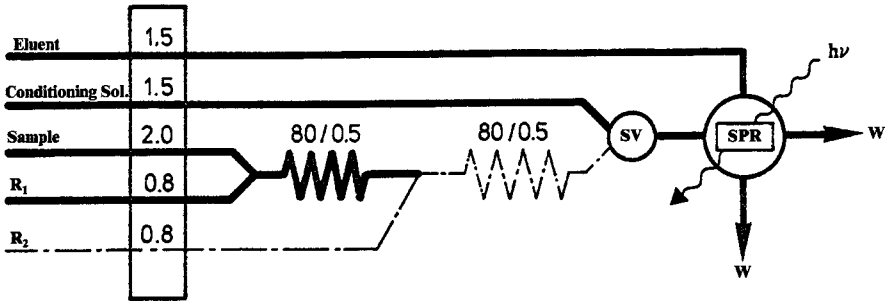

Fig. 1. Schematic representation of the flow manifold used for reversed-phase optosensing at octadecyl chemically-modified silica gel beads. SPR: solid-phase reactor; SV: switching valve; R: reagent; W: waste.

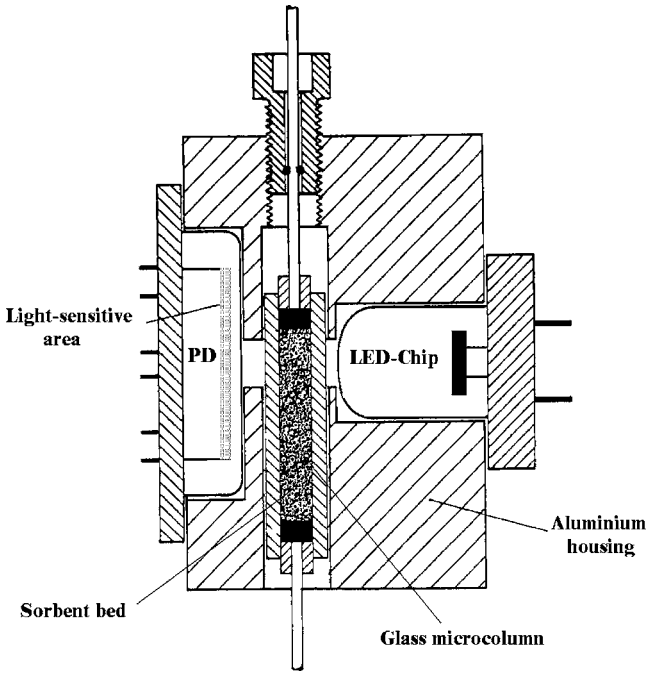

Fig. 2. Schematic view of the dedicated flow-through cell exploited for absorptiometric sensing at C_{18}-covalently-modified beads packed in tubular microcolumns. LED: light emitting diode; PD: photodiode.

UK) were arranged oppositely to each other in a way that the light is transmitted radially through the tubular microcolumn. The bores of the light-path and column reception were made to match the particular column dimensions. In axial direction, the position of the microcolumns can be precisely adjusted by means of a square-threaded cap-nut (see Fig. 2). The electronic circuits of the light-source and light detector unit were similar to those described by Betteridge et al. [38], and may be obtained from the authors upon request. The instrument provides electronic compensation of the background attenuation and variable gain setting. It was designed to permit ready exchange between various LEDs depending upon analysis requirements without troublesome electronic tuning. As no logarithmic amplifier has been used, light transmission is recorded and, therefore, absorbance values have to be calculated if needed. Output signals were transferred to a strip-chart recorder and evaluated manually.

Though not truly monochromatic light is available with LEDs, they have proven well suitable for spectrophotometric measurements [39]. The unique features of LEDs as the light source for spectrophotometers are their high signal-to-noise ratio, low power requirement, long lifetime, low price, and miniature size. The latter permits convenient implementation of LEDs into the separation/preconcentration unit without any additional optical components.

2.4. Preparation of the microcolumns

Most of the microcolumns accommodated in the devised configuration were made from glass-tubes of approximately 3 cm long with inner diameters ranging from 1 to 7 mm. In all instances, from one side a polypropylene frit (cut from that used in common solid-phase extraction cartridges) was inserted, and positioned about 8 mm from the end of the tube. A methanolic slurry of C_{18}-covalently-modified silica gel material removed from commercial solid-phase extraction cartridges (30–60 μm particle size, Bond-Elut, Varian) was pipetted carefully into the other side of the tube so that the sorbent bed got a length of about 10 mm. A second frit was inserted to embed the reversed-phase sorbent. Both ends of the glass tubes were connected to the flow-lines with tightly-fitting silicon tubing. Care was taken to minimize dead volumes. Microcolumns with inner diameters of 1 mm or below were made from transparent PTFE-tubing. Filling of the sorbent material into the tiny tubing was done by repeated tipping the opening of the tube into a small portion of the dry sorbent. Small portions of quartz wool were used to embed the active phase. This way of microcolumn preparation is much more straightforward than conventional strategies involving packing flow-through cells with sorbent beads up to a proper height [17,32,34,35], and yields surprisingly repeatable and durable reactors with minimum costs.

2.5. Manifold configuration and analytical procedure

The flow manifold devised for solid-phase optosensing measurements enables the selection of the sample stream (premixed with reagent/s), conditioning solution or eluent stream to be delivered through the column in whatever sequence. The conditioning solution matches the composition of the reagent medium aiming to prevent changes in the optical transparency of the sorbent between conditioning and loading steps, which, in turn, would cause artefact signals at the front edge of the pre-concentration signal.

In general, before on-column detection the C_{18}-silica gel filled microcolumn is pre-conditioned by repetitive switching between the conditioning stream and eluent solution for a period of 1 min each. The absence of baseline drift, and a repeatable change of the attenuation between the aqueous and alcoholic solution is taken as indication of proper reactor preparation. After a final passage of the conditioning solution through the packed-bed column for 2 min at 1.5 ml min^{-1}, the switching valve is initiated so that the sample stream merging with reagents is directed to the microcolumn. As the reaction product reaches the column, the retention on the sorbent causes an increase of light attenuation. This continues to happen as long as fresh sample solution is supplied (typically at a flow rate of 2.0 ml min^{-1}) or saturation of the material occurs. The pre-concentration time is either preset to a fixed value (usually 2.5 min) or adjusted with respect to the appearing signal, i.e. sample aspiration is terminated after recording a pre-selected signal height, aiming to avoid saturation of the observation zone. At the end of the pre-concentration time, the switching valve is moved back to allow conditioning solution to pass through the reactor for 30 s prior to column regeneration. Hence, the

rising attenuation curve is followed by a plateau region, the height of which relative to the baseline is commonly taken as analytical signal. Appropriate blank subtraction is done when required. The elution time (typically 30 s) is adjusted to warrant complete removal of the retained analyte, rendering the flow system ready for starting a new measuring cycle.

3. Results and discussion

3.1. Design criteria of the sorbent-packed flow cell and the optosensing instrumentation

Selecting a suitable flow-cell configuration for the implementation of sorbent extraction optosensing is not a simple task. In contrast to batch applications [25] where a uniformly covered sorbent material is brought into the cuvette, in flow-systems the sorbed analyte forms a longitudinally distributed concentration gradient (see Fig. 3 for graphical illustration). Under favorable conditions, the target compound is retained in a narrow zone at the column head, thus attaining a sharp profile as shown in Fig. 3. With respect to the optical measurement, the ideal situation is given when the illuminated area by the incident light beam matches the section of sorbent where the analyte is concentrated. A mismatch will cause either the reduction of the dynamic linear range when the illuminated area is smaller than the retention zone or lower sensitivity and worsening of the signal-to-noise ratio when a larger area is irradiated.

Different types of packed flow-through cells have been employed for solid-phase spectrophotometric measurements. In most reports, standard flow-cells originally designed for liquid-phase measurements have been packed with sorbent materials [23,27,33]. Except of the advantage of being compatible with common spectrophotometers such configurations can be regarded far from optimum for several reasons: (1) the packing material does not take up the entire volume of the cell cavity, so that, on the one hand, backmixing at the inlet occurs and, on the other hand, the solution within this volume may contribute to the absorbance measurements; (2) the packing material is hold in the cell by merely placing a frit at the outlet. This obviates backflush elution of the retained species to be explored, which, in turn, is generally pursued to circumvent bead settlement; (3) due to the reasonably high costs of the commercial flow cells, they must be re-filled after deterioration of the sorbent, which is inconvenient and time-consuming.

Tubular microcolumns similar to those commonly used in on-line pre-concentration techniques with eluate detection [4,16,19,20] do not suffer from the above-mentioned drawbacks, and are hence better suited. When light is transmitted radially through the column an additional advantage arises from the fact that interferences caused by refractive index changes of the interstitial solution are less pronounced [39]. Though made possible after minor modifications of the cell holder, the insertion of sorbent-filled microcolumns into common spectrophotometers is not recommendable. Difficulties in proper positioning of the column with respect to the incident light often result in a mismatch of the optical

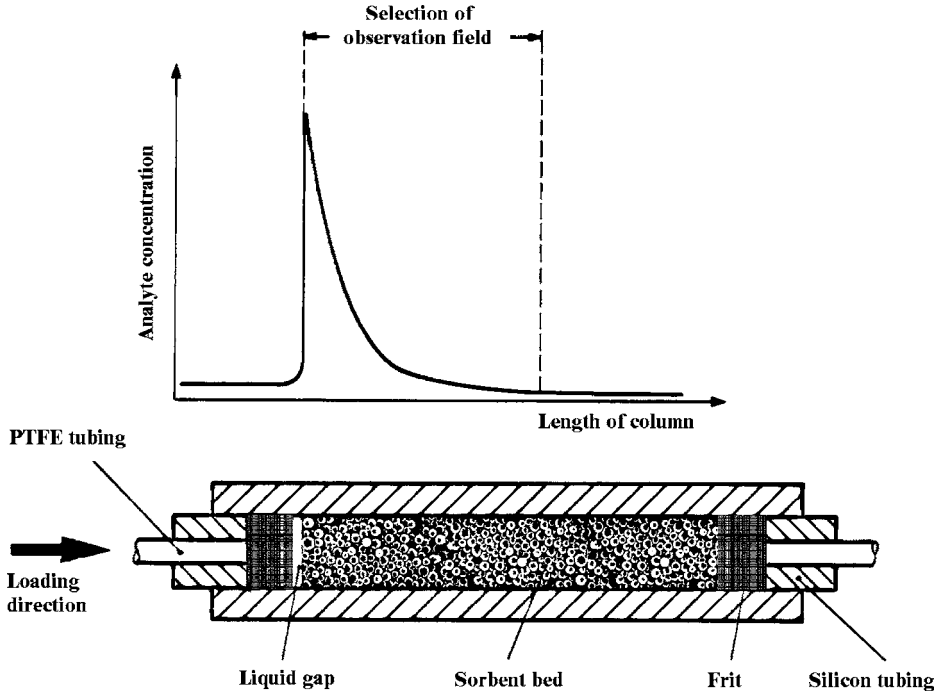

Fig. 3. Typical concentration profile of the transient sorbed analyte along the packed-bed in flow-through configurations. The basic elements of the microcolumns assembled in the optical sensor are also detailed.

observation field and the pre-concentration zone. Moreover, the replacement of the original light source from the photometer by a high-intensity fiber optic variable light-source was reported to be needed to account for the high background absorbance [26]. We have observed that the major contribution of light loss in solid-phase spectroscopy with common spectrophotometers is a result of the only small portion of light passing through the cell that is actually transferred to the entrance slit of the photodetector. Due to the wide-angle scattering at the sorbent-filled microcolumn, more than 90% of the incident light may be lost using common optical configurations. In order to capture a large amount of the scattered light, which in fact represents a significant fraction of the analytical signal, the photodiode and the sorbent bed should have immediate contact. This is to a great extend realized in our configuration (see Section 2.3).

3.2. Investigation of parameters affecting the performance of the flow-through sorbent extraction optosensing system

The pink azo-dye formed in the nitrite assay according to Shinn reaction was selected as test compound to explore the performance of the flow-through sensor. In order to avoid secondary effects resulting from a change in sample dispersion or reaction kinetics, these investigations were made by aspiration the dye solution pre-formed in a steady-state procedure using a single-line flow manifold. Phosphoric acid at the pH of the assay was used as a conditioning stream, and methanol acidified to pH 2 with hydrochloric acid served as eluent.

3.2.1. Influence of column dimensions

Experimental studies on the influence of the column dimensions on the performance of optosensing measurements were carried out with tubular microcolumns of inner diameters ranging between 0.5 and 7 mm. In columns with diameters above 3 mm, a very thin colored layer (<1 mm) was observed at the column head in close vicinity to the frit. In order to make full benefit from the higher sensitivity that can be achieved when the light transmits the reasonably long path of the dye layer precise focusing of the incident light to this layer would be required. From the practical point of view, we found the adjustment to this position very difficult. Depending on the relative position of the dye-loaded zone with respect to the incident beam, either the frit was partially illuminated and contributed to light-scattering or a larger portion of non-loaded sorbent came into the observation field, leading to reduced sensitivity. To move the sorbed species into the sensed microzone, the enhancement of the pre-elution effect via addition of 10–20% (v/v) methanol to the conditioning solution was successfully exploited. Sometimes, it was also observed that due to a more dense packing of the sorbent with time, a thin liquid-layer was formed between the frit and sorbent bed (see Fig. 3 for illustration). If this gap comes into the observation field the response became highly irreproducible. A further problem with large-diameter columns is the unfavorable flow pattern at the entrance accruing from the abrupt change in flow channel size. This gives rise to reasonably large dead volumes at the column head which cause significant tailing during countercurrent elution of the retained compound, thus requiring prolonged regeneration times.

The use of columns with inner diameters below 1 mm leads to an enhanced longitudinal distribution of the target compound, which, in turn, deteriorates the dynamic range and causes low breakthrough volumes. Yet, from practical point of view, small diameter columns are readily prepared, require only a minute amount of sorbent material, and easily fit into the conduits of flow manifolds (no dead volumes, simple connection). However, taking into account both practical aspects and analytical performance, the use of glass-columns with 1–2 mm i.d. is recommended. All further investigations reported have been made with 2 mm i.d. glass columns.

3.2.2. Effect of flow-rate and pre-concentration time

The selection of proper flow rate of the solution passing through the microcolumn during sample loading and detection period requires consideration of the retention behavior of the target compound and practical aspects. For a given sample volume, increasing flow rates during the loading period concomitantly lead to faster pre-concentration and higher sample throughput. The distribution of sorbed compound in longitudinal direction, however, may be increased due to slow sorption kinetics. In the determination of nitrite, the finally reached signal obtained upon aspiration of 5.0 ml sample volume containing 0.1 μg NO_2^- remained almost unaffected for flow rates within the range 1.5–4.0 ml min^{-1}. At lower flow rates, a slightly smaller attenuation value is recorded, which can be explained by the fact that a reasonable portion of the dye is concentrated in a narrow area at the bed inlet, being thus not probed by the photodiode. The likewise smaller signal at flow rates between 4.0 and 5.0 ml min^{-1} is probably reasoned by kinetic effects. Visual inspection of the column revealed a significantly broader dye zone at high flow rates so that part of the retained colored species may have left the observation field of the optical detector. Flow rates above 5.0 ml min^{-1} could not be set as a consequence of the deterioration of the sensor performance due to the build-up of high backpressure.

At a given analyte concentration, the sampling volume determines the amount of analyte that is retained on the packed beads. If the observation field is set properly, a linear relationship between sample volume and attenuation is to be expected. Besides, the likelihood to exploit a linear mass calibration has been successfully assessed. Thus, a set of samples covering a wide range of nitrite concentrations (two to three decades) may be analyzed using the same calibration graph by proper selection of the loading time. In practice, deviations from linearity often occur for low analyte concentrations, which in fact require high sampling times. This is the result of the influence of a chromatographic effect

due to the sample solvent itself, which serves as a weak eluent. For the determination of trace levels of nitrite, sample volumes above 7.0 ml, corresponding to pre-concentration times higher than 3.5 min, provided unreliable results as a consequence of the longitudinal distribution of the azo-dye below the illuminated section of the microcolumn.

3.3. Analytical methods investigated

In general, the choice of the reaction chemistry used for determination of trace levels of a desired analyte via flow-through sorbent extraction optosensing is guided by the following considerations: (a) distribution ratio of the analyte or reaction product between aqueous and sorbent (total and fast adsorption is desirable); (b) ability of total and fast elution of retained compounds from the sorbent (reversibility); (c) high absorption coefficient of the reaction product; (d) location of maximum absorption wavelength (minimum interference from concomitantly sorbed species); (e) stability of the reaction product on the column; and (f) availability, purity and cost of reagents. In the present study, common spectrophotometric flow injection procedures were selected in order to investigate their applicability to sorbent extraction optosensing at C_{18}-silica gel columns, or in case of failure to discover possible reasons. In the following, a short survey of the results obtained for the various methods is given emphasizing both the features and problems occurred during the investigation. The optimum reagent compositions for the different assays are given in Section 2.1. The pre-concentration time for calibration, unless otherwise stated, was set to 2.5 min, corresponding to a loading volume of 5.0 ml. Longer pre-concentration times were occasionally applied to examine the capabilities of improving the concentration factors and detection limits for the selected assays.

An important requirement for the successful application of sorbent extraction optosensing using reusable reactors is the carefully selection of an eluent capable to remove quantitatively the retained analyte from the active microzone, thus avoiding carryover effects. Pure methanol was found suitable in many applications for disrupting hydrophobic interactions. It was however observed that the addition of salt or acid to the eluent generally resulted in decreased elution times. Since this diminution was more pronounced when moderately polar derivatization products had to be eluted from the sorbent, it is thus believed that polar interactions between the target molecule and free silanol groups at the silica substrate exist. The composition of the eluent was hence investigated for the respective applications.

A fundamental aspect to take into account when operating with reversed-phase materials containing octadecyl moieties is that the falling of the alkyl chains in the plane of the silica surface, which, in turn, causes a dramatic decrease of the sorbent capacity, occurs if the sorbent is not properly solvated with short-chain organic solvents [31]. In order to maintain the solid-phase wetted during the entire analytical cycle, thus preventing the chain collapse, 1% (v/v) methanol is added to both the reagents (R_1 and R_2) and the conditioning solution for the whole set of analytical methods investigated.

The analytical performance of the different methodologies explored using the devised optosensor including dynamic range, determination limit, repeatability, and practical remarks are compiled in Table 1.

3.3.1. Determination of nitrite and nitrate

The chemical conditions for the spectrophotometric determination of nitrite using the Shinn reaction are well established [40]. It should be stressed that both the reagent concentrations and the acidity of the assay must be carefully assessed aiming to minimize competitive reactions and accelerate the formation of the azo-dye. According to previous observations [41], the pink dye formed on-line is readily sorbed at the C_{18}-microcolumn in a narrow zone and remains stable at the column for extended periods of time. Fast elution can be accomplished with a small volume of methanol acidified to pH 2 with hydrochloric acid. A problem encountered in the determination of nitrite at the low ng/ml-level was the concomitant retention of a brownish compound formed gradually in the combined reagent, which created a significant background attenuation. This interfering species could be completely eliminated by introducing a C_{18}-precolumn of reasonable capacity, containing approximately 60 mg of material, into the reagent channel.

Preliminary investigations were carried out by rinsing the loaded column with distilled water. In this case, no plateau level was recorded. Yet, a dropping tendency was observed with time, which may be attributed to the partial wash-out of the target compound as a consequence of the dramatic pH change undergone by the sensed microzone. Therefore, the use of an acidic conditioning stream is fully justified in this assay.

The accessible working range detailed in Table 1 can be readily extended to lower concentration levels using larger sample volumes. With a loading volume of 10 ml, the detection limit amounts to 0.1 ng ml^{-1} nitrite. Yet, a strict control of reagent contamination from absorption of ambient nitrogen dioxide is required to avoid a fast saturation of the guard-column as a consequence of the immobilization of colored products from Saltzmann reaction.

For the determination of nitrate in-line reduction to nitrite using a copperised cadmium reductor has been applied [36,40]. The general features of this method are similar to the nitrite assay. The increased backpressure with two columns in series sometimes lead to flow-rate irregularities and concomitant worsening of repeatability.

3.3.2. Determination of sulfite

The reaction of sulfite with the non-carcinogenic reagent DNTB yields a strongly absorbing thiol. The reaction is instantaneous at pH 4–7. Although within this pH range the reaction product is present in ionized form, efficient sorption at the C_{18}-material was observed. It can, therefore, be

Table 1
Analytical performance of the spectrophotometric assays explored using the flow-through reversed-phase extraction optosensing system designed

Analyte	Reaction	Working range[a] (5 ml sample) ($\mu g\,l^{-1}$)	Determination limit (5 ml sample) ($\mu g\,l^{-1}$)	Elution medium	Repeatability (%, $n = 7$)	Comments/practical remarks
Nitrite	Shinn	1–200	0.5	MeOH/10^{-2} M HCl	2.3	Strict pH-control during retention. Reagent preservation against atmospheric nitrogen dioxide
Nitrate	Shinn + copperised cadmium reductor	5–500	2	MeOH/10^{-2} M HCl	3.3	
Sulfite	5,5′-Dithiobis-(2-nitrobenzoic acid)	2–100	2	MeOH/0.1 M NaCl	2.0	Contribution of polar interactions in the complex sorption
Ammonium	Indophenol blue	2–500	1	MeOH	2.6	Slow destruction of C_{18}-material due to the high pH of the reaction medium
Phosphate	Molybdenum blue	5–500	3	MeOH	3.8	Retention depends critically on sample pH
Iron(II)	Ferrozine	2–500	1	MeOH	1.5	Adjustment of the samples to a high ionic strength
Iron(III)	Ferrozine + hydroxylamine	10–500	5	MeOH	1.8	
Chromium(VI)	1,5-Diphenylcarbazide	2–200	2	MeOH	2.3	Large spectral band difference between reagent and chelate
Nickel	Dimethylglyoxime	100–1000	80	MeOH/0.1 M HCl	4.7	Adsorption on the inlet frit. Low reagent solubility in water
Phenol	4-Aminoantipyrine	5–500	1	MeOH	2.8	Careful selection of reagent concentration aiming to avoid self-oxidation products

[a] The upper limit can be extended ad libitum by the application of fixed signal measurements.

assumed that either hydrophobic interactions between the aromatic rings of the derivatization product and the alkyl chains of the reversed-phase sorbent are sufficiently strong, or polar interactions of the colored species with the residual acidic silanol moieties at the silica gel surface are responsible for retention. The latter assumption is supported by the fact that elution with methanol is significantly accelerated when sodium chloride is added, thus revealing the contribution of ionic exchange mechanisms for the temporary immobilization of the target compound. Despite the contribution of the reagent itself to the light attenuation, the proposed configuration yields determination limits as low as $2\,ng\,ml^{-1}$ for a sampling time of 2.5 min.

3.3.3. Determination of ammonium

It has been proved that the indophenol blue species resulting from the Berthelot reaction can be readily adsorbed at the reversed-phase material and reversibly eluted with methanol. Background attenuation is low so that high concentration factors and concomitantly low detection limits can be achieved. The only but serious drawback encountered in this methodology was the slow deterioration of the silica sorbent (namely, hydrolysis of the covalently attached chains to the silica support) due to the high pH of the assay. After contact with sample solution for more than 2–3 h, the sorbent bed shrinked to such a degree that a liquid gap appeared at the column head. However, during the first hours of measurement, the response to ammonia was surprisingly stable. The use of highly pH-resistant poly(styrene-divinylbenzene) copolymer beads alkylated with octadecyl groups (40 μm size average; Polysorb MP-1, Transgenomic, San Jose, CA) made available recently was not very promising so far because the analyte retention does not take place instantaneously leading to excessive longitudinal distribution and early analyte breakthrough.

3.3.4. Determination of orthophosphate

Molybdenum blue formed in the common orthophosphate assay is a heteropolyacid species the sorption of which at C_{18}-silica gel beads was found to vary dramatically with the acidity of the assay and the reaction time. The pH dependence of the sorption behavior is probably due to the fact that the complex formed can exist in both neutral and ionic form, the former being more favorably retained through partitioning and physical adsorption [26]. With decreasing acidity, retention efficiency increases but at the same time the formation of the homopolyacid, which is likewise adsorbed, also increases. Prolongation of the reaction time through the lengthening of the reaction coils up to 200 cm leads to more favorable retention. Since the formation and reduction of the molybdophosphate complex is extremely fast, the above result can be explained attending the progressive coagulation and eventual precipitation of molybdenum blue species, so that filtering rather than adsorption becomes the mechanism of retention. The proposed optosensing system enabled orthophosphate monitoring within the range $5–500\,ng\,ml^{-1}$.

Lower concentrations may be analyzed by addition of masking agents such as oxalic acid (0.25% (v/v)) able to convert the excess of molybdate into a non-reducible form [42].

3.3.5. Determination and speciation of iron

Iron(II) forms stable colored complexes with several organic reagents, such as phenanthroline derivates and certain triazine compounds. Although well retained at the octadecyl alkylated silica gel sorbent and offering high sensitivity, 1,10-phenanthroline could not be used in the present flow-through optosensor because the reaction product was found not eluable in reasonably short time with several water miscible solvents (e.g. methanol, acetonitrile, acetone). The use of 2,4,6-tripyridyl-1,3,5-triazine (TPTZ) as a chelating agent for iron(II) leads to a different problem. The reagent has much higher affinity for the sorbent than the derivatization compound, so that the excess of reagent delivered to the column during the loading period behaves as a reasonably strong eluent, causing pre-elution effects. Thus, the colored dye zone initially formed at the column head moves gradually downwards, eventually leaving the observation field of the detector. The finally tested Ferrospectral agent (synonymous with ferrozine) was found well suitable for the desired application. Retention takes place in a narrow layer of the solid bed, and methanol can be used for instant elution. Yet, it was necessary to adjust the samples to a reasonably high ionic strength since, for instance, distilled water behaves as an efficient eluent, preventing high concentration factors to be attained. This observation, though not well understood, was also made by King et al. [43]. Total iron can be determined from 10 to 500 ng ml^{-1} following in-line reduction of Fe(III) with a hydroxylammonium chloride stream.

3.3.6. Determination of chromium(VI)

The chromogenic agent 1,5-diphenylcarbazide is a widely employed reagent for hexavalent chromium determination due to its high selectivity, since only molybdenum was reported to yield a similar reaction in acid medium. The strongly colored red-violet chelate formed in the redox reaction is readily retained at the C_{18}-microcolumn, and can be eluted instantly with pure methanol. No interference of concomitant reagent adsorption occurs due to the large difference in the respective absorption maxima. Thus, extension of the working range to extremely low concentration levels is possible using large sampling volumes. With a 20 ml loading volume, the signal recorded for 0.2 ng ml^{-1} chromium(VI) was still well to distinguish from $3\sigma_{blank}$, which represents a detectability improvement of more than one order of magnitude with regard to bead-injection strategies relied upon the diffuse reflectance measurement of chelate-loaded C_{18}-beads [44].

3.3.7. Determination of nickel

The formation of nickel dimethylglyoxime (DG) is a well established gravimetric and, following solvent extraction, also spectrophotometric method with high selectivity and sensitivity. Attempts to apply this method to sorbent extraction optosensing yielded to several problems. The solubility of the dye in water is very low, and sorption of the precipitate took place already in the reaction coil and at the inlet frit of the microcolumn. Slight improvements could be achieved by the addition of water miscible organic solvents, such as methanol or acetone, to the reagent. The higher the content of solvent the lower unwanted sorption of the colored species prior to reaching the packed microcolumn was observed. Yet, the retention on the reversed-phase reactor was adversely affected by the presence of high percentages of organic solvents (>15–20%), causing a longitudinal distribution of the analyte along the column, which results in determination limits above 80 ng ml^{-1}. This analytical feature is, however, better than that recently reported for the batch determination of the analyte using cellulose membrane-based sensing approaches [45]. The immobilization of either DG or the non-carcinogenic α-benzyldioxime on the sorbent beads by repetitive treatment with saturated reagent solutions in organic medium prior to sample loading is currently being investigated in our laboratory as a promising alternative [46].

3.3.8. Determination of phenol

The spectrophotometric standard method for the determination of phenol and phenol index is based on the oxidative coupling reaction using 4-aminoantipyrine. The red reaction product is well retained at the hydrophobic sorbent, and is readily eluted with pure methanol. Though also retained, 4-AAP itself does not significantly interfere due to the spectral resolution. The yellow ferricyanide oxidant contained in the interstitial volume led to a small signal, however, it was not concentrated at the column. Thus, the observed positive baseline shift prior to the pre-concentration step could be successfully overcome by addition of the same amount of oxidant in R_2 to the conditioning buffer solution. In preliminary investigations with relatively high concentrations of 4-AAP and oxidizing agent, a large background signal was detected. Visual inspection of the column during the loading period evidenced the formation of a brownish compound which was difficult to elute with methanol. This species is presumably a side-reaction product stemming from the oxidation of the reagent itself, as recently described in a flow injection solid-phase extraction system with eluate detection [47]. Using more diluted reagent solutions detailed in Section 2, the blank value considerably decreased without significant change of the sensitivity of the method. Determination limits of phenol at the 0.1 ng ml^{-1} level were accessible when the loading volume was increased up to 10 ml.

3.4. Problems in real sample analysis and effective means to overcome interferences

Of the various methods developed and described herein, some have already been applied to the analysis of real

samples, such as different kinds of waters, eluates of filter collected airborne particulates, soil extracts, and beverages. During the elaboration of the respective procedures, we repeatedly encountered problems either specific to a particular assay or, of more general nature, regarding the composition of the sample matrix. As a rule, the interferences owing to the inherently limited selectivity of the liquid-phase spectrophotometric method in question persisted when sorbent extraction optosensing has been applied. For instance, certain amines also form indophenol blue and, hence, positively interfere in the determination of ammonium. In the determination of sulfite with DNTB, sulfide and cyanide give similar response, leading to an overestimation of results. Another example is the determination of chromium(VI) with 1,5-diphenylcarbazide, which is disturbed in the presence of excess of iron(III), since it yields a yellow-brown reaction product, which is likewise retained at the C_{18}-sorbent, and could not be resolved spectrophotometrically when the LED-based photometer has been employed.

Other kinds of interferences which occasionally occurred were reasoned by the high variability of ionic strength and/or pH of standards and samples and in between samples. The sample pH was found not only to influence to a certain degree the chemical derivatisation step in solution, but in several cases also to alter the retention behavior of the target compound. Appropriate buffering and ionic-strength adjustment is therefore indispensable and can be conveniently done by proper choice of the composition of the respective reagents or, if required, by the introduction of an extra channel into the flow system.

The most serious limitation in the applicability of sorbent extraction optosensing to real samples is the concomitant adsorption of dissolved high molecular weight organic matter, which is often irreversible. As a result, a stepwise increase of the baseline attenuation occurs after each sampling period. Although this itself not necessarily detracts from accurate measurements because attenuation relative to the baseline or the slope of the rising curve is used for signal evaluation, the distribution pattern of the desired reaction product along the sorbent bed is sometimes altered during consecutive analytical cycles. This makes frequent recalibration necessary. Another serious effect is that the retention of colored dissolved organic matter (even if reversible) creates a blank signal that can far exceed the attenuation due to the retention of the formed species. This was, for instance, observed in the determination of sulfite (forming a light yellow reaction product) in orange juice. A possible solution to overcome interferences caused by colored matrix constituents is the application of dual wavelength or diode array detection, which permit effective background subtraction. This has recently been demonstrated in the determination of nitrite in surface waters by eliminating the light attenuation due to unspecific and reversible sorption of humic and fulvic acids [48].

A more universal solution to this problem, which has proven useful in several methods described above, is the insertion of a guard column (filled with C_{18}-beads) into the sample aspiration line, i.e. prior to the addition of reagent(s) and formation of the colored compound. For different types of samples and analytes, it must, however, be carefully investigated whether sorptive losses of analyte occur. Though this was generally not a problem in the determination of ionic species if the ionic strength of the sample solution was sufficiently high, this strategy failed in the determination of phenolic compounds in neutral samples, since they were almost completely removed by the guard column. In this particular case, adjustment of the sample pH to a value of 11.5 could solve successfully the drawback through the conversion of the target species into the ionic form.

Another general problem in sorbent extraction optosensing is caused by suspended particulate matter, often present in real samples, since the frit at the entrance of the minicolumn only serves as coarse filter, and fines are deposited at the initial part of the sorbent bed which is optically probed. Hence, the background attenuation generally increased when turbid samples were analyzed, even without the addition of a color forming reagent. The insertion of a chromatographic in-line microfilter (0.5 μm stainless steel frit, Upchurch) was not successful due to the severe pressure build-up created in the proposed arrangement.

Yet, a very effective means to overcome interferences caused by both particulate matter and high molecular weight organic compounds was the implementation of a parallel-plate dialysis unit (12″-Technicon dialyser, 30 cm pathlength) furnished with a cellulose regenerated membrane (Premount dialysis membranes, Technicon-Type C) into the conduits of the flow system. The dialyser was placed in the sample aspiration line, so that the clean dialysate was injected into a secondary flow system [49] comprising the optosensing device. This has the advantage over the positioning of the dialysis unit within the manifold that flow rates and composition of the donor and acceptor streams can be more flexibly altered according to the optimization of the dialysis process, irrespective of the selected detection chemistry. The long-term stability of the dialysis membrane was proven excellent, even when samples with high matrix burden (e.g. fruit juices with pulp and industrial effluents) are analyzed. The sensitivity of the dialysis-sorbent optosensing hyphenation is, however, reduced since only a relatively small fraction of analyte is transferred across the membrane under given kinetic conditions. From the point of view of dialysis separation, the in-line pre-concentration capabilities of sorbent extraction are, in turn, a generally interesting feature to reconcentrate the diluted dialysate [14,15].

4. Conclusions

In this paper, optical sensing on reversed-phase materials is critically explored using a flow-through microtubular-

based sensor configuration. As opposed to conventional flow cells packed with sorbent materials, the proposed optosensor features a remarkable improvement in the signal-to-noise level as a consequence of the capture of a large fraction of scattered light, and facilitates the observation field to match the retention zone for the different assays. This, in turn, leads to, at least, comparable or, in many instances, improved sensitivity and detection limits in comparison to previously reported methods, whose analytical features are outlined in the review article [27] and monograph [6]. Practical considerations aiming to warrant maximum retention efficiency of the C_{18}-packing material and avoid the chain collapse are also discussed in detail. With regard to the optical detection, the assembled light emitting diodes are demonstrated to be really suitable for many applications. However, improved performance and enhanced capabilities would arise by implementation of optical fibers in combination with, e.g. diode-array detection, yet maintaining the tubular packed-bed sorbent configuration designed.

Various analytical methods for different species (i.e. nitrite/nitrate, sulfite, orthophosphate, iron(II)/total iron, chromium(VI), nickel, and phenol), all based on the formation of colored hydrophobic reaction products, have been examined, and particular problems related to the chemical conditions and retention behavior of the derivates have been discussed. Plausible retention mechanisms are also proposed attending the experimental data obtained. The beauty of the proposed optosensing methodology—as it appears to us—is that besides the examples presented, many other well-established spectrophotometric methods (several of them being official methods of analysis) can be straightforwardly adapted.

General problems occurring in real sample analysis are addressed, and various solutions to overcome the sorption of high molecular weight matrix constituents are proposed. Implementation of separation techniques prior to sample injection into the optosensing module, using either guard columns or sandwich-type dialysers, are proven effective to avoid poisoning of the sorbent during repetitive analytical cycles.

The major limitation of the present approach is the irreversible retention exhibited by some analyte chelates, as previously discussed for the iron(II)-1,10-phenanthroline chemistry. The concept of renewable surfaces in flowing systems, so-called bead-injection [50,51], appears a powerful scheme to overcome this drawback, thus broadening the application field of the solid-phase optosensing technique. Yet, the bead injection approach requires special instrumentation (namely, jet-ring or lab-on-valve cells) and the solid surfaces should fulfill basic requisites to be suitable for the renewable fashion, i.e. size homogeneity and perfect spherical shape, as recently described [52]. Therefore, the octadecyl chemically-modified silica-gel beads used in this work, which in fact are rather lumps, are not being really exploited as renewable surfaces, Sephadex-type ion-exchangers are preferred.

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