Flow injection analysis with electrochemical detection

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Fließinjektionsanalyse mit elektrochemischer Detektion

Summary. Examples are given of successful combinations of flow injection analysis (FIA) with simple electrochemical detectors. FIA is especially suitable for ion-selective electrodes (ISE); stability, selectivity and speed of analysis can be significantly enhanced. A multi-ion FIA assembly for the analysis of Na^+, K^+, Ca^{2+}, NO_3^-, Cl^-, and HCO_3^- in drinking water is described, as well as the indirect ISE potentiometry of Al^{3+} combined with FIA. As an example of an amperometric detector, a secondary harmonic AC polarographic detection in an FIA arrangement which detects traces of ascorbic acid is presented and the reasons are given why FIA is also beneficial in amperometry.

Introduction

Flow injection analysis (FIA) is mainly a sample introduction/preparation technique. It is becoming the major technique in the field of automatic analysis for large quantitites of samples. FIA can be likened to high-performance liquid chromatography (HPLC) without the chromatographic column, since the instrumental set-up is almost identical. Owing to the omission of the time-consuming separation step, FIA is a very fast technique. However, it requires a specific detector with short response times. The principle of FIA was first demonstrated in 1970 by Pungor et al. [1], who used ion-selective electrodes (ISE) as the specific detector. However, Ruzicka and Hansen [2, 3] were responsible for the great commercial success of this technique.

Since several other papers in this volume of the journal have discussed the great advantages of FIA, together with the novel instrumental assemblies, details concerning the FIA of the electrochemical flow-through detectors remain to be elucidated. Some remarks concerning the advantages of using electrochemical detectors instead of photometric detectors are presented below, together with some typical examples. The main aim of this article is to demonstrate the excellent possibilities offered by FIA in connection with electro-analytical methods which upto now have scarcely been used. There are over 50 different electro-analytical methods [4] and only the two most important, namely ISE potentiometry [5] and polarography or amperometry [6, 7], are dealt with here.

FIA is a good choice of technique if the following conditions are applied: only one substance is present or is to be determined; a specific detector is available; large quantity of samples is essential; the sample matrix remains unchanged, as in the case of speciation analysis, where complex equilibria are involved.

The most common electrochemical method in ion chromatography involves a conductivity measurement, where the use of FIA is not suitable, since it is unspecific. The most widely used electrochemical detector, with superb sensitivity in HPLC, is one based on amperometry. Its limited selectivity has to be checked carefully prior to any application in FIA. However, sufficient selectivity is more easily achieved than is the separation of interfering substances which are also oxidized or reduced at or below the applied working electrode potential. One way of increasing the selectivity of this method is to use the technique of differential pulse polarography or AC voltammetry. The working electrode has to be carefully adjusted so that the potential lies within the half-wave region of the substance to be determined.

Potentiometry with ion-selective electrodes

As mentioned before, Pungor et al. [1] favoured ISE for FIA assemblies, because they offer certain advantages compared with the photometric detector normally used. These advantages are as follows:

1. ISEs are not affected by the colour or turbidity of the sample.
2. ISEs normally do not need the addition of an expensive specific reagent, since they are in many cases sufficiently selective.
3. Gas-sensing electrodes for some important volatile compounds (NH_3, SO_2, CO_2, HCN etc.) are commercially available and can be used to increase the reliability of FIA by introducing a separation step without large losses in the speed of analysis.
4. Potentiometry offers an extremely small dead volume in the detector (wetted ISE surface).
5. Potentiometry is simple and the instrumentation is not expensive.
6. ISE potentiometry gives information about the state of oxidation or binding, which are essential in speciation analysis.
7. ISE potentiometry can be used over a wide concentration range.

Flow-through arrangements with ISE have been described by Cammann [5, 8, 9] and Bailey [10]. The simplest version consist of a pair of electrodes held nearly horizontal with the upper ISE connected to the reference electrode via a small strip of filter paper. The FIA carrier solution is delivered onto this filter strip just above the ISE. Other

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simple constructions have been described by Frenzel in this volume of the journal.

FIA, with its continuous and controlled flow of certain carrier solutions, is especially beneficial to ISE measurements for the following reasons:

1. The hydrodynamics in front of the sensing electrode surface responsible for the thickness of the Nernst diffusion layer, which itself determines the response time, are controlled and stable. This results in a very reproducible potential generation.

2. The liquid junction potential at the diaphragm of the reference electrode is also stabilized by the controlled hydrodynamics and can be further minimized by the proper choice of a carrier electrolyte solution. This also leads to more reproducible signals.

3. In several cases, the selectivity of an ISE can be drastically improved, since the time in which the sample segment passes the sensor surface is too short to establish the full equilibrium potential for the interfering ions. Cammann and Ilcheva [8, 9] demonstrated an improvement by some orders of magnitude in the case of Br^- and I^- as interferences on a single crystal AgCl electrode. The improvement in selectivity by this "kinetic discrimination" of interfering ions is less pronounced with PVC membrane electrodes [11].

4. Contamination problems encountered in normal "beaker analysis", in which the salt bridge solution leaks into the sample via the normally used highly diffusible sleeve diaphragm, are eliminated since the reference electrode can be positioned downstream.

5. The carrier solution of the FIA arrangement can be especially optimized for ISE application in order to perform the following additional functions:

(a) If the same solution is used as in the salt bridge of the reference electrode, the liquid junction potential is minimized. This also results in minimal variation of the latter.

(b) A steady flow of solution, past the sensing surface of the ISE keeps the surface clean. To prevent the deposition of surface layers, which have a deteriorating effect, certain special additions can be made, e.g., chelates to prevent salt precipitations, and heparin or trypsin-HCl additions to prevent the formation of bio-layers in the case of biological fluid analysis.

(c) If the ion to be sensed is already present in the carrier solution, the ISE membrane is automatically conditioned with this ion, which improves the response time and the selectivity loss with time. When working in the limit of the detection range, the base-line stability is improved dramatically. An additional feature of this technique is to make the concentration of the sensed ion in the carrier solution equal to a limiting value, which has to be controlled by analysis (environmental control). This leads to easily observed FIA peaks. Concentrations below the limiting value are recorded as negative peaks, concentrations above as positive peaks.

Some examples of FIA-ISE arrangements

Depending upon the special sample situation, which may or may not require an ionic strength adjustment buffer (TISAB), two major sample injection techniques can be used. If no TISAB is necessary, the extremely simple set-up described in Fig. 1 A can be used and leads to optimal results. If the ionic strength of the different sample solutions differs markedly, a sample injection into a small mixing chamber (see Fig. 1 B [11]) is the method of choice, since the dilution of the injected sample segment with the carrier solution is limited only to the two borders of the segment in the typical FIA set-up.

Nitrate in drinking water

In water analysis, nitrate ions are of special concern, since the main values found in ground-water are continuously increasing with time and some communities already have values above the drinking water limit of 50 mg/l. Therefore, a fast and simple screening method is required. A commercial NO_3^--ISE can be used in the arrangement shown in Fig. 1. In this special case, a 0.01 M carrier solution in NaH_2PO_4 containing exactly 50 mg/l NO_3^- was found to be optimal for the FIA-ISE of nitrate. The resultant FIA signals are shown in Fig. 2. As can be seen, the sensitivity is excellent (<5 ppm). The sample preparation was also extremely simple: 125 µl of a 4 M NaH_2PO_4 stock solution was placed in a 50 ml volumetric flask and the flask was filled with the sample to be analysed. The addition of 1–2 drops of concentrated sulphuric acid brought the final pH to about 4.5 in order to eliminate the interfering HCO_3^- ions. Only if extremely high chloride concentrations are present (normally absent in drinking water) does Cl^- have to be reduced by filtration through Ag_2SO_4 prior to analysis.

The excellent reproducibility and speed of this nitrate FIA-ISE is shown in Fig. 3. Up to 100 samples/h can be analysed by this method, with a standard deviation of only 2.4% in the ppm range. The only drawback of this method is that the calibration curve is no longer linear. A simple personal computer would be advantageous for direct and automatic on-line data evaluation.

Multi-ion drinking water analysis

The main ions to be determined in a small drinking water analysis are: Na^+, K^+, Ca^{2+}, HCO_3^-, NO_3^-, and Cl^-. Since, for all of these ions, sufficient specific ISE are commercially available, a multi-ion FIA was developed. Figure 4 shows the 7-electrode holder used and Fig. 5 shows the results for drinking and mineral waters. Since the carrier solution contained 0.1 mM $NaHCO_3$, KNO_3, and $CaCl_2$, negative signals were expected when the actual concentrations of the corresponding ions were low. Despite certain limitations at the detection limits by some peaks, resulting from flow instabilities due to a cheap injection block (the Tecator company offers better devices for this purpose), the speed of analysis (6 ions/min), sample amount of only 200 µl and the cost of this assembly is without competition. It is extremely useful for routine screening analysis in this field. Since ISE has a tendency to detect more in case of high concentrations of interfering ions, the screening analysis has a certain safety device built-in. Samples above a certain limit can then be analysed by a more elaborate method.

Indirect aluminum FIA-ISE without reference electrode

As mentioned before, FIA is also a very valuable method in the wide field of indirect potentiometry. In this case, the carrier solution consisted of 0.01 M F^- in a pH 4 buffer. All ions which bind fluoride ions can be determined. An FIA set-up with a mixing chamber, as shown in Fig. 1, was used. The ISE detector was built with two identical fluoride

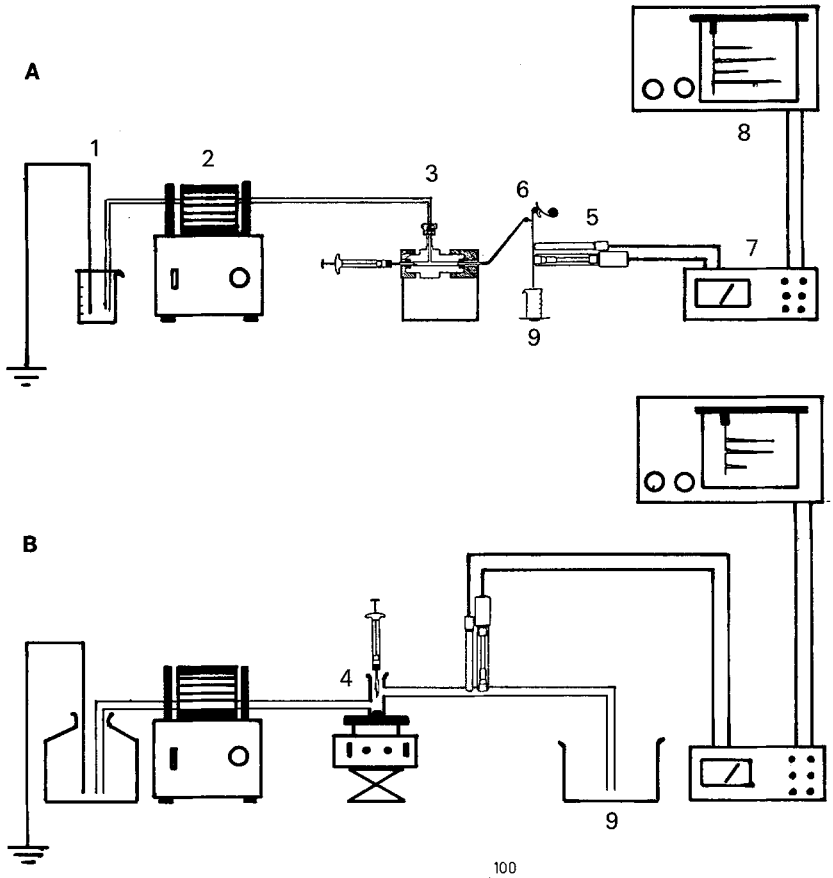


Fig. 1 A, B
Flow-injection analysis (FIA) set-up used in this work. **A** Set-up with injection block: *1* carrier solution reservoir (grounded to earth); *2* peristaltic pump (Ismatec MS-4/8); *3* injection block (Omnifit): *5* ISE reference (double junction) electrode pair; *6* strip of filter paper: *7* pH-meter; *8* recorder; *9* waste. **B** Set-up with a small mixing chamber (5 ml): *4* mixing chamber with magnetic stirrer; otherwise as **A**

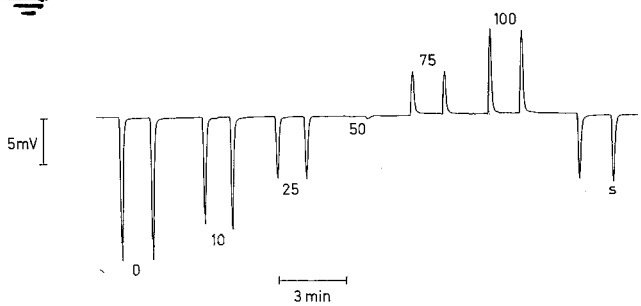


Fig. 2. FIA with NO_3^- electrode. Carrier solution: 50 ppm NO_3^- in 0.01 mol/l NaH_2PO_4; flow rate: 3.0 ml/min; injection: 300 µl NO_3^- solutions containing the numbers shown in ppm units in 0.01 mol/l NaH_2PO_4; s, Tap water sample (University of Ulm, February 1987)

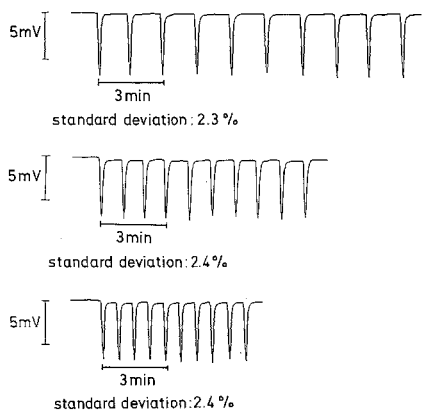


Fig. 3. Reproducibility of NO_3^- in FIA. Injection: 300 µl of a solution with 25 ppm NO_3^- in 0.01 mol/l NaH_2PO_4; other conditions as in Fig. 2

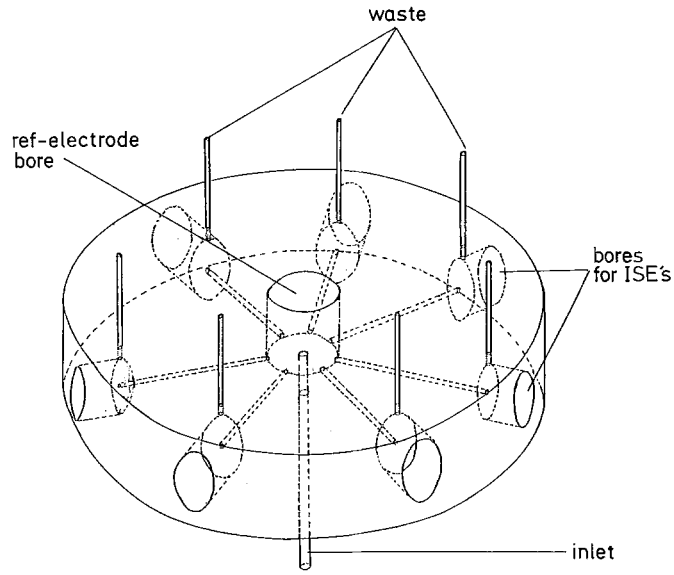
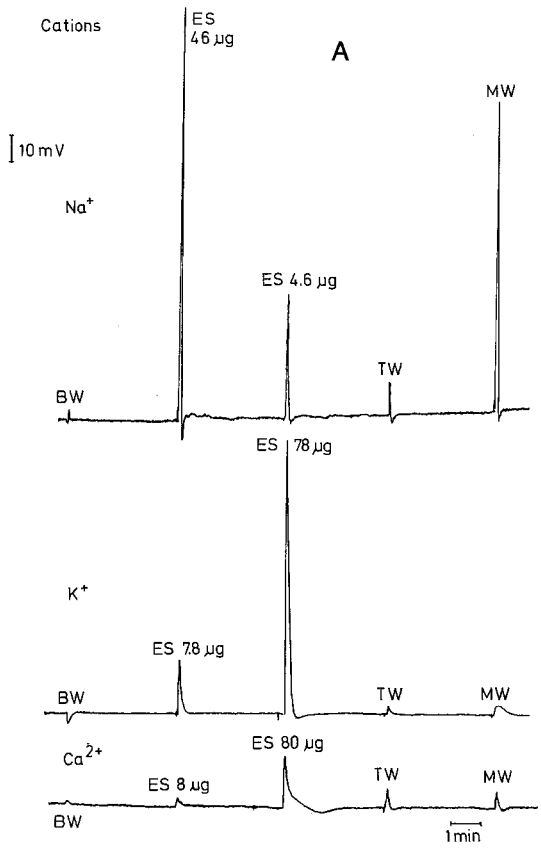


Fig. 4. Seven electrode holder: the reference electrode is placed from the top into the central bore. ISEs for Na^+, K^+, Ca^{2+}, NO_3^-, Cl^-, and HCO_3^- (NH_3 electrode with internal buffer of 0.1 mmol/l $NaHCO_3$) are placed horizontally around the reference electrode; the metallic waste tubes are connected to the waste via filter-paper strips

electrodes. With such an arrangement, the FIA signal looks like the derivative of the normal peak signal, since the sample changes the potential of both indicator electrodes with a certain time delay, depending on the distance between both electrodes (see Fig. 6). The corresponding calibration curve for Al^{3+} is shown in Fig. 7. Such an electrochemical detector



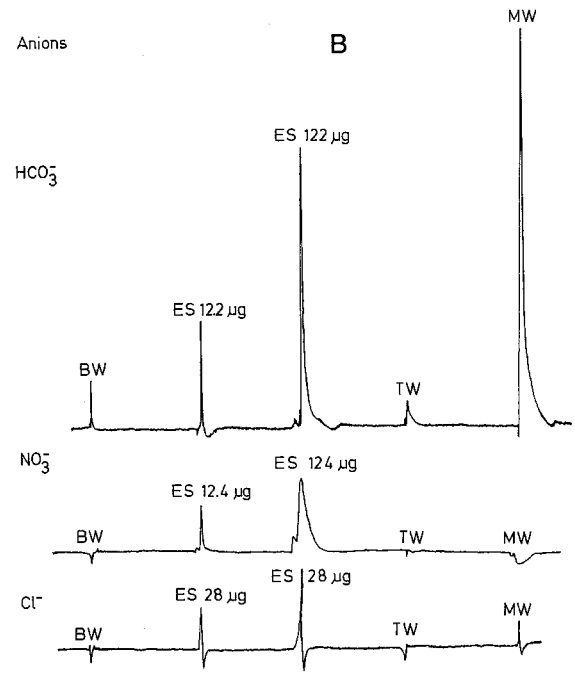


Fig. 5A, B. Multi-ion detection in FIA. **A** Cations: *BW* blank; *ES* calibration standard with the amount of element shown: *TW* tap water (TU Garching, March 1987); *MW* mineral water; sample carrier solution: see text; flow rate: 1 ml/min hydrostatically driven. **B** Anions: conditions as in **A**

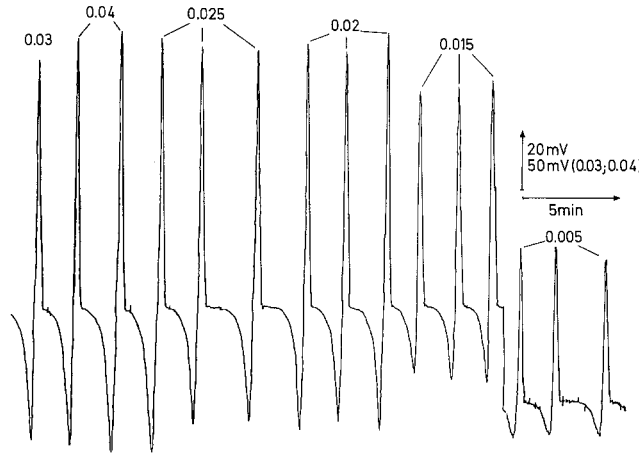


Fig. 6. FIA of Al^{3+} with 2 F^--electrodes. Carrier solution: 0.01 mol/l F^-; flow rate: 1.4 ml/min; electrode separation 10 cm; injection: 100 µl with mixing chamber; Al^{3+} concentration shown by numbers in mol/l, evaluation peak to peak value

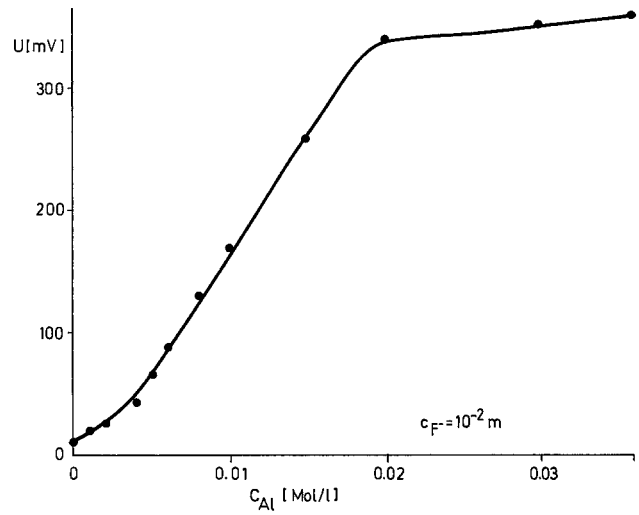


Fig. 7. Calibration curve for indirect Al^{3+} FIA

with two identical ISEs may also be useful in chromatography with gradient elution. As long as the gradient ist linear, a constant signal will be obtained by this method.

SO_2 in grape juices

As an example of the successful integration of a separation step into an FIA set-up, the determination of SO_2 in grape juices is described. A single gas-sensitive electrode was used, as depicted in the set-up shown in Fig. 1 A. A commercially available NH_3 electrode was changed into an SO_2 sensing device by changing the internal solution from NH_4Cl to a 0.001 M $NaHSO_3$ solution, which was also used as the external carrier solution. Figure 8 shows the detector response of the different samples with differing SO_2 contents. In this case, it is not necessary to wait for the complete gas separation equilibrium across the gas-permeable membrane due to the controlled dispersion. The carrier solution flow rate and the injected sample volume are mainly determined by the sensitivity which is needed to give reproducible signals.

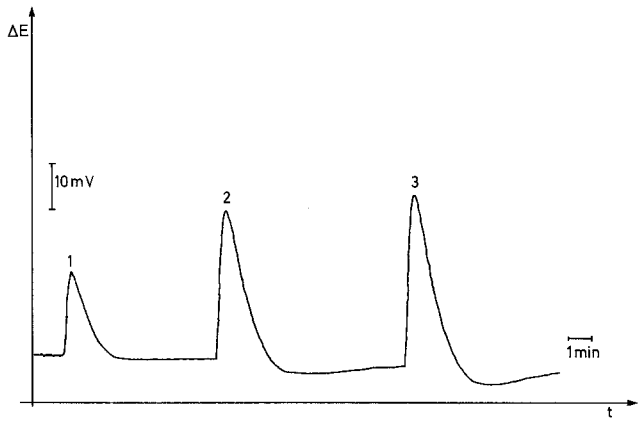


Fig. 8. SO_2 in grape juices by FIA. Carrier solution: 0.001 mol/l $NaHSO_3$; flow rate: 1 ml/min; injection: 300 µl; *1* Optima (550 ppm SO_2); *2* Müller-Thurgau (750 ppm); *3* Dornfelder (1050 ppm)

In this example, the flow rates are somewhat lower than in the other cases described so far.

Another example of the combination of FIA with a gas-sensing electrode is the determination of NH_4^+. In this case, it is better to inject the sample into a mixing chamber (Fig. 1 B) in order that mixing with the 0.01 M NaOH carrier solution takes place. The resultant NH_3 gas is then sensed by an ammonia electrode in the ppm range. The additional separation step leads to a larger increase in the selectivity without the elaborate water vapour distillation step which is required in the normal Kjeldahl method.

Glucose in biological fluids

The enzyme glucose oxidase (GOD) is a very specific catalyst for the oxidation of glucose by O_2, which produces gluconic acid and H_2O_2. Normally, the hydrogen peroxide is measured amperometrically. The current flowing during the oxidation of H_2O_2 is proportional to the glucose concentration in the sample. However, such an amperometric glucose sensor is very sensitive to deposition layers, which trend to decrease the rate of diffusion of the substrate into the enzyme layer in front of the working electrode. Another approach would be to detect the gluconic acid by its local pH variation in front of a potentiometric pH electrode, which does not consume the measured species and hence is not dependent on the thickness of the diffusion layer as far as the signal level is concerned. The only effect would be a longer response time. This could also be confirmed experimentally [12].

Figure 9 shows an FIA set-up for the enzymatic analysis of glucose and Fig. 10 the resulting signals [12]. The FIA technique is especially valuable in this two-substrate (glucose + O_2) reaction, which tends to be limited at higher glucose levels by the availability of oxygen. If the carrier solution is saturated with pure oxygen, the analytical range is extended more than threefold. In clinical applications, the carrier solution contains heparin. For a long life-time of the GOD reactor, addition of peroxidase would be beneficial, since H_2O_2 acts as a poison to GOD. The described FIA for glucose (Fig. 11) has the great advantage that the zero value is always measured. Hence, variations of the base line are automatically corrected for.

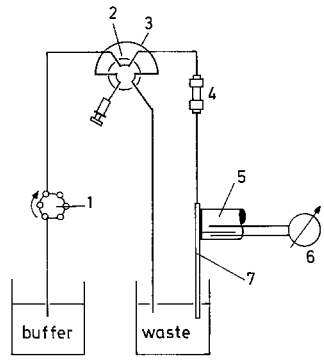


Fig. 9. FIA set-up for glucose. *1* peristaltic pump; *2* 6-way valve; *3* sample loop; *4* enzyme reactor; *5* pH-combination electrode with flat surface; *6* pH-meter; *7* filter-paper strip

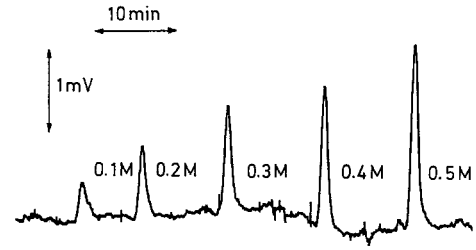


Fig. 10. FIA signals of a potentiometric glucose detector. Carrier solution: phosphate buffer, pH 7 (Merck); flow rate: 0.3 ml/min; injection: 100 µl of the glucose sample

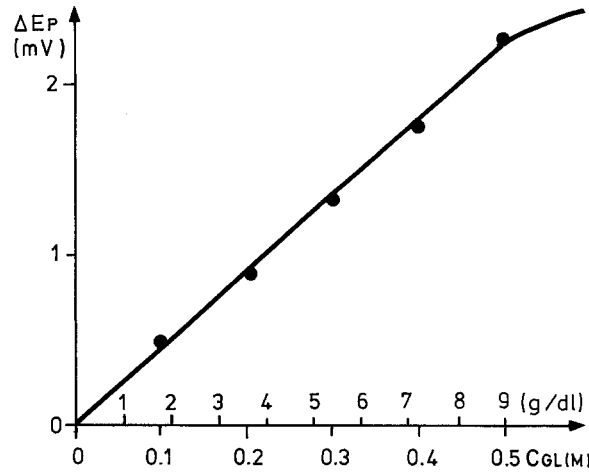


Fig. 11. Calibration curve for FIA of glucose

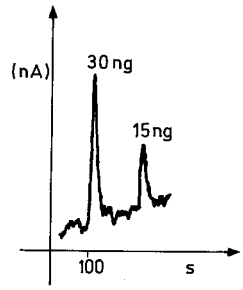


Fig. 12. FIA of ascorbic acid with AC-polarography. Carrier solution: buffer pH 4.7 (Merck); hanging mercury drop electrode, model 303 (P.A.R); injection: 20 µl of a standard solution

Further combinations with potentiometry

Most organohalogen compounds can be destroyed by strong UV radiation, giving rise to the appearance of halogen anions in a properly chosen solvent. The halogen anions are easily sensed by the corresponding ISE, as was shown by Cammann and Ilcheva [13]. If only one organohalogen compound is to be determined, FIA is the best method.

Several immunoreactions between an antigen and an antibody can be transferred into the phase boundary of an ISE with a resulting potential change (if a reaction takes place). A typical example is that of digoxin antibody determination by potentiometry developed by Rechnitz [14]. FIA could be advantageous in this case, since the controlled dispersion would allow a faster speed of analysis together with an optimal surface regeneration step in between the sample injections.

FIA and amperometry

Another important and well-developed electrochemical technique which can easily be combined with the FIA principle is amperometry. In this technique, the current is measured while the working electrode potential is maintained at a certain fixed potential suitable for an elctrochemical oxidation or reduction of the substance to be determined. Compared with ion-selective potentiometry, this technique allows for the determination of neutral molecules, which increases its applicability. Since excellent electrochemical detectors [15–18] are commercially available, their superior sensitivity (pg range) can be used to advantage in FIA work. Therefore, only a few remarks concerning the specificity of the detector are made here.

Since FIA is based on a specific detector, the working conditions for an amperometric detector should be carefully chosen. If the substance to be sensed is the only electroactive (oxidizable or reducible) compound in the sample, as is often the case, e.g. in pharmaceutical analyses, the specificity is not a major problem. However, if interfering compounds which are electroactive, are present in the rather large "potential window" of classical amperometry at a fixed and controlled voltage, modern pulse or alternating current (AC) techniques have to be applied in order to narrow this potential window [19, 20].

Only one example will be given here. Figure 12 shows the results of an FIA set-up for ascorbic acid. The electrochemical detector consisted of the P.A.R. static mercury electrode equipped with the corresponding flow-through assembly. The potential of the mercury drop electrode was kept at zero volts with respect to the Ag/AgCl electrode and 25 mV peak-to-peak of a 10-Hz AC signal was superimposed. With an instrumental set-up as described by Cammann and Andersson [20], the second harmonic signal at 20 Hz was followed.

Glucose can also be determined by FIA with amperometric detection [12, 21]. In this case, the speed of analysis can be much higher than in the potentiometric example mentioned above, since the slow hydrolysis of the first reaction product, gluconolactone, into gluconic acid limits the speed of the potentiometric detector.

The benefits of applying FIA instead of a constant flow-through arrangement are as follows:

1. A clean working electrode surface is absolutely essential for this consumptive technique, in which the analytical signal depends upon the active electrode area and the diffusion layer thickness. In order to maintain the cleanliness of the surface, the carrier solution should contain some additives (e.g. heparin, in the case of blood samples) besides the ground electrolyte.

2. If organic compounds are to be detected electrochemically, normal HPLC detectors, working at a constant electrode potential, tend to be covered by polymers produced by the electrochemical reaction of certain compounds at the working electrode surface. If a non-stationary potential is used, together with a rather short contact time of the compound, this problem in the amperometric cells can be eliminated.

3. If a certain dilution can be tolerated, by mixing the sample in a mixing chamber with the carrier solution, the time normally used for de-aeration can be reduced, when the carrier solution is maintained oxygen-free and the injected volume is in the µl range.

Conclusion

As previously reported, the combination of FIA with electrochemical detectors has several advantages [22–26]. Mainly amperometric devices, developed primarily as sensitive HPLC detectors, are commercially available. However, compared with any other device, potentiometry with ISE is the most economic choice. The construction of a flow-through cell is so easy that one may be justified in wondering, why certain chromatographic separations have not been followed by applying two independent detection principles, e.g., conductivity or photometry together with ISE potentiometry. The latter can be applied, in many cases, prior to the sample waste. Two independent analytical results would significantly increase the reliability of the mean result calculated.

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