CROATICA CHEMICA ACTA CCACAA **75** (2) 531–543 (2002)

ISSN-0011-1643
CCA-2815
Original Scientific Paper

Determination of Optimal Conditions to Achieve Maximal Response at Maximal Repeatability in FIA-AMZ Experiment through a Mathematical Modelling Approach*

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Received May 2, 2001; revised October 8, 2001; accepted October 9, 2001

The determination of optimal experimental conditions in flow injection analysis asynchronous merging zone technique (FIA-AMZ) on the basis of a mathematical model is proposed. An improved commutator enabling reagent addition with minimal frontal dispersion is used for the simultaneous determination of two species, Fe^{2+} and Fe^{3+} ions. The mathematical model used to simulate the detector response is based on a bell-shaped function. The model is well suited for the fitting of symmetrical as well as for very asymmetrical detector responses, reflecting different dispersion of the sample plugs. The influence of the frontal dispersion of the reagent, asymmetrically added to the sample plug, is studied using the proposed model. A measure for an optimal ratio between the sensitivity and repeatability is described on the basis of the parameters obtained from the simulations of the experimental curves obtained for different dispersions.

Key words: flow injection analysis, AMZ, optimization, modelling, Fe^{2+}, Fe^{3+}.

INTRODUCTION

The flow injection analysis asynchronous merging zone (FIA-AMZ) technique emerged from the FIA-synchronous merging zone technique.[1,2] Becau-

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* This paper is dedicated to Professor Milan Randić in honor of his 70th anniversary.

** Author to whom correspondence should be addressed. (E-mail: marjana.novic@ki.si) se of its simple hardware configuration and great flexibility, it may be an attractive FIA technique for speciation studies. It is a simple, accurate and general technique for the determination of the selectivity coefficients in FIA of certain components.[3] Nevertheless, some competitive techniques such as splitting of the injected sample,[4] reversed flow injection based on single or dual injection valves,[5] and internally coupled injection valves[6] are often chosen instead of FIA-AMZ. This is certainly because of the relatively low sensitivity of the detection of the second species (detected after merging the sample with the reagent), which causes large errors in the determination of the concentration of the second species.

The overall sample dispersion is recognised to play an important role in the sensitivity of the detection of the first species, (Fe^{2+} in our case), and sensitivity and repeatability of the detection of the sum of both measured species (Fe^{2+} and Fe^{3+} ions). In our study the factors influencing the sensitivity of the simultaneous detection of two species by the FIA-AMZ technique were analysed in order to find the conditions facilitating optimal detector response. Using the developed mathematical model,[7] a variety of detector responses for different sample dispersions and the behaviour of the parameters of the model function were studied. With increasing dispersion, the peak height and the slope of the tailing part of the detector signal decrease. As a consequence, the sensitivity of the detection of Fe^{2+} ions decreased. However, the diminished slope of the tailing part of the signal offered a more accurate determination of the sum of both analytes (the determinations were based on the peak height measurements). The error in the sampling time for the reducing agent addition (Δt = 0.6 s), which could not be avoided, did not influence the resulting peak height as much as in a steeper peak region. A compromise between the sensitivity and repeatability, expressed as a function of the parameters derived from the simulations of the experimental curves, was determined and tested for different dispersions.

METHODS

FIA and FIA-AMZ Technique

Flow injection asynchronous merging zone (FIA-AMZ) is a modification of simple FIA technique. The main idea is to carry out an experiment in such a way that the second species is converted to the detectable form online by the addition of an appropriate reagent. The reagent addition should be carried out in such a way that it does not influence the information about the concentration of the first detected species. The reagent is therefore mixed with the sample after a certain time delay that causes the detection of the reacted part of the sample to appear after the maximal absorption of the unreacted sample has passed the detector beam. Thus the resulting double peak contains information about the concentration of the unreacted sample (frontal part) and that of the sum of the reacted and unreacted sample (tail-part). In a mixture of Fe^{2+} and Fe^{3+} ions, Fe^{2+} can be detected by UV/Vis spectrophotometer at 512 nm (with the color-forming reagent being 1,10-phenanthroline), and so can Fe^{3+} upon reduction to Fe^{2+} through the addition of an efficient reducing agent (*e.g.,* ascorbic acid).

Mathematical Models for FIA and FIA-AMZ Detector Responses

The FIA detector response, the absorption of the sample as a function of time, is recorded from the moment of the injection of the sample ($t = 0$), being the largest at time $t = t_0$ when the maximal absorption of the sample passes the detector beam. The bell-shaped function used as a model to simulate a basic FIA signal[7] is a combination of two sigmoidal functions and has the following form:

$$f(t) = \frac{h}{1+\exp\left[-\left(\dfrac{t-t_0+p_1}{p_2}\right)\right]} \cdot \left(1-\frac{1}{1+\exp\left[-\left(\dfrac{t-t_0-p_1}{p_3}\right)\right]}\right) \qquad (1)$$

h – parameter comparable to the height of the model function
t_0 – peak position of the model function
p_1 – parameter for the general shape of the model function
p_2 – parameter for the shape (slope) of the left side of the model function
p_3 – parameter for the shape (slope) of the right side of the model function.

To obtain the typical FIA-AMZ signal with an asymmetrically split peak, the following function was utilized:

$$f^{\mathrm{Fe(II)+Fe(III)}}(t) = f^{\mathrm{Fe(II)}}(t) + S(t) \times f^{\mathrm{Fe(III)}}(t) \qquad (2)$$

where $S(t)$ is the function describing the conversion of the undetectable form of iron (Fe^{3+}) to the detectable one (Fe^{2+}). The function $S(t)$ was introduced as a cut-off function having a value 0.0 through the time-period in which the non-reduced sample passed the detector beam; it is smoothly raised to 1.0 until all the analyte is quantitatively reduced. In an ideal case (no frontal dispersion, no mixing delay, 100% conversion yield) $S(t)$ would have a form of Heaviside function[8] being 0.0 up to time t_{mix}, time at which the reduced sample – mixed with the reagent – appears at the detector beam, and 1.0 afterwards:

$$S(t) \mapsto \begin{cases} 1 \Leftarrow (t - t_{\text{mix}}) \geq 0 \\ 0 \Leftarrow (t - t_{\text{mix}}) < 0 \end{cases}. \tag{3}$$

Because of non-ideal nature of FIA-AMZ system, the non-ideal function $S(t)$ is used as shown in Eq. (4).

$$S(t) = \frac{1}{1 + \exp\left[-d \cdot (t - p)\right]}. \tag{4}$$

The two parameters, d and p determine the slope and the inflection point of the sigmoidal function, respectively. The combination of d and p gives a proper t_{mix} used in Eq. (3). The Eq. (4) describes actually the reagent frontal dispersion together with mixing efficiency and reduction yield. Eq. (2) can be further elaborated as:

$$f^{\text{Fe(II)+Fe(III)}}(t) = \frac{h^{\text{Fe(II)}}}{1 + \exp\left[-\left(\frac{t - t_0 + p_1}{p_2}\right)\right]} \cdot \left(1 - \frac{1}{1 + \exp\left[-\left(\frac{t - t_0 - p_1}{p_3}\right)\right]}\right) +$$

$$S(t) \times \frac{h^{\text{Fe(III)}}}{1 + \exp\left[-\left(\frac{t - t_0 + p_1}{p_2}\right)\right]} \cdot \left(1 - \frac{1}{1 + \exp\left[-\left(\frac{t - t_0 - p_1}{p_3}\right)\right]}\right). \tag{5}$$

The determination of parameters ($h^{\text{Fe(II)}}$, $h^{\text{Fe(III)}}$, t_0, p_1, p_2, p_3, d, and p) was performed with a simplex algorithm,[9] the optimization criterion being the difference between the experimental and simulated curve in the predefined peak region.

EXPERIMENTAL

Apparatus and Reagent

A mixture of Fe^{2+} and Fe^{3+} ions was used to study the dispersion effects in FIA-AMZ system carried out on ASIA (Ismatec) flow injection analyser. In Figure 1 a schematic flow diagram is demonstrated. Fe^{2+} ions are detected with UV/Vis spectro-

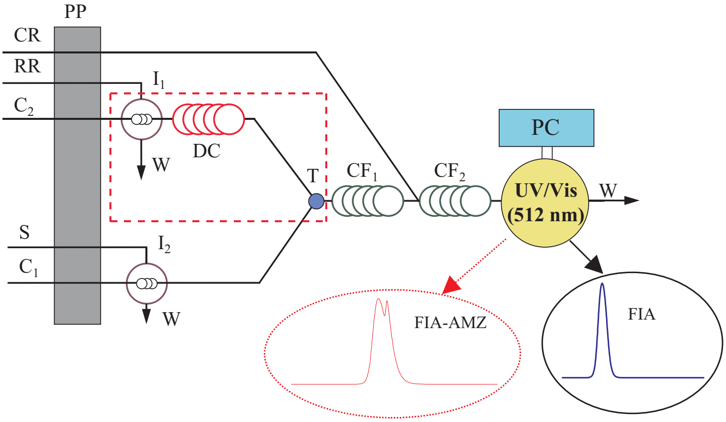

Figure 1. The scheme of the apparatus used for the simultaneous spectrophotometric determination of Fe^{2+} and Fe^{3+} ions. PP – Peristaltic Pump; I_1 and I_2 – Injectors; C_1 and C_2 – Carriers (H_2O); RR – Reductant (1% Ascorbic Acid); CR – Color Forming Reagent (1,10-phenantroline); S – Sample; CF_1 and CF_2 – Chemical Reactors; DC – Delay Coil; T – Mixing Point; UV/Vis – Spectrophotometer (512 nm); PC – Data Collecting System; W – Waste; Dotted line box – Components that Can be Replaced by the Commutator (shown in Figure 3).

photometer SpectraSYSTEM UV 2000 (Spectra-Physics) operating at 520 nm. Data were collected in the ASCII format with a PC *via* 12 bit A/D converter.

The Colour-forming reagent: 1.26 g 1,10-phenanthroline + 100 mL acetate buffer (pH value 5.3) was added to a 500-mL volumetric flask and diluted to 500 mL with 18 MΩ cm^{-1} water.

Reducing agent: 1g of ascorbic acid (Merck) was dissolved with 18 MΩ cm^{-1} water, transferred into the 100-ml volumetric flask and diluted to 100 mL with 18 MΩ cm^{-1} water. As a carrier 18 MΩ cm^{-1} water was used.

Fe^{2+}, Fe^{3+} standards: Standard solutions of Fe^{3+} were prepared from stock solutions containing 10.05 mg mL^{-1} of Fe^{3+} (Aldrich) by appropriate dilution with 0.1 M HCl. Standard solutions of Fe^{2+} were prepared from Fe^{2+} stock solution (1 mg mL^{-1}) (702.2 mg of $(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O$) was dissolved in 0.1 M HCl, transferred to a 100 mL volumetric flask and diluted to 100 mL with 0.1 M HCl and standardised against 0.0016 M dichromate standard solution.

RESULTS AND DISCUSSION

Example of a Typical Signal Obtained by FIA-AMZ Technique

In Figure 2 the experimental and simulated curves characteristic for the FIA-AMZ technique are shown. Eq. (5) was taken for a model function. The parameters (see Eq. (5)) that lead to the best fit with the experimental curve are: p_1 = 16.84 s; p_2 = 3.726 s; p_3 = 11.77 s; t_0 = 44 s; $h^{\mathrm{Fe(II)}}$ = 228; $h^{\mathrm{Fe(III)}}$ = 290; p = 0.5 s; d = 0.6 s.

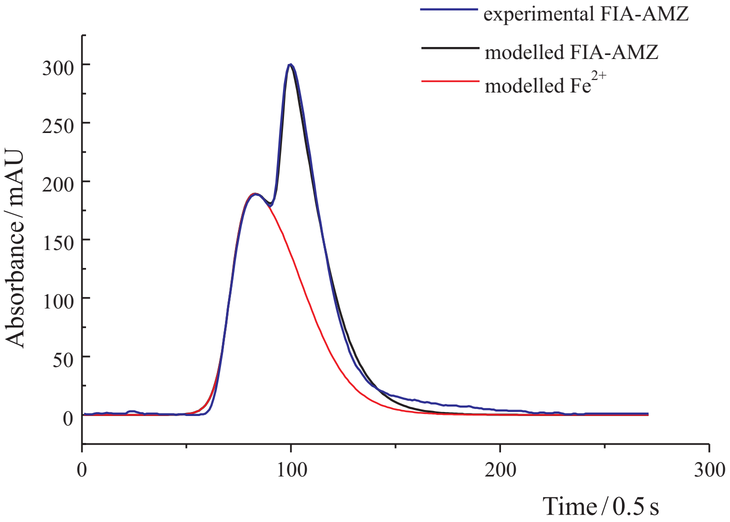

Figure 2. The detector signal obtained in FIA-AMZ analytical technique (thick line) and its best fit using the proposed mathematical model expressed by Eq. (5) (dashed line). The parameters are given in the text.

It is possible to influence two items that have a large effect on the shape of the detector response curve. One is the reagent frontal dispersion associated to the $Fe^{2+} \rightarrow Fe^{3+}$ conversion efficiency and the other is the analyte profile reflecting the overall sample dispersion. In the applied mathematical model the frontal dispersion of the reagent plug is accounted for by the parameter d in Eq. (4). The overall sample dispersion is simulated with the parameters p_1, p_2, and p_3 (Eqs. (1) and (5)).

Optimisation of the Reagent Frontal Dispersion

Reagent frontal dispersion is caused by the implementation of the delay coil controlling the delayed merging of the reducing agent with the sample. In other words, the reducing agent reaches the mixing point T (see Figure 1) after the sample plug has partially passed it. In order to diminish the frontal dispersion of the reagent plug, an improved commutator enabling the addition of the reagent to the carrier stream practically without any frontal dispersion, was developed. In Figure 1 the components of the FIA-AMZ system replaced by the commutator are marked by a dotted line box. The commutator was constructed from one electrically driven standard injection valve (ASIA, Ismatec), on which the original PTFE stator was modified as it is shown in Figure 3. Originally, non-exploited stator's central hole was connected to one of the outer holes (sample-in, Figure 3) through in-laboratory engraved micro channel (i.d. = 0.5 mm). In addition, a PTFE tubing (i.d. = 0.5 mm) was added to the mentioned central hole, enabling the connection to the chemifold. Carrier, water and reagent were connected to the commutator as shown in Figure 3.

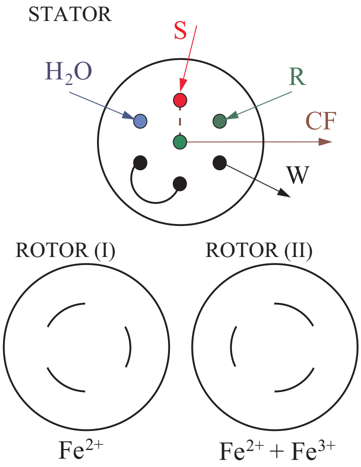

Figure 3. The scheme of the commutator with marked external connections, which enables minimal frontal dispersion of the reducing reagent. S – Sample; R – Reducing agent, CF – chemifold; W – waste.

In the first rotor position (I, Figure 3), sample plug and water merged on the commutator. Water merged the sample plug. Diluted sample was delivered by carrier through the central hole to the chemifold CF (Figure 3) and consequently, detector response was obtained corresponding to the concentration of Fe^{2+}. Reagent was delivered to waste. In the second rotor position (II), water was re-directed to waste, while reagent R was merged with the sample. Because of the operation principle of the developed commutator, reagent merged sample plug with zero frontal dispersion.

The influence of the frontal dispersion of the reducing agent on the FIA-AMZ detector response, more precisely on the second part of the split peak arising from the sum of the Fe^{2+} and Fe^{3+} ions, is demonstrated in Figure 4. The pure Fe^{2+} peak is plotted in dash-dot line (lower peak), while the peak corresponding to the sum of the Fe^{2+} and Fe^{3+} ions is gray (upper peak). The dashed line indicates an ideal FIA-AMZ signal, which should appear in the absence of all disturbing factors such as frontal dispersion of the reducing agent and the mixing intensity. It is also evident that the signal obtained with the commutator is closer to an ideal one than the one obtained with the delay coil.

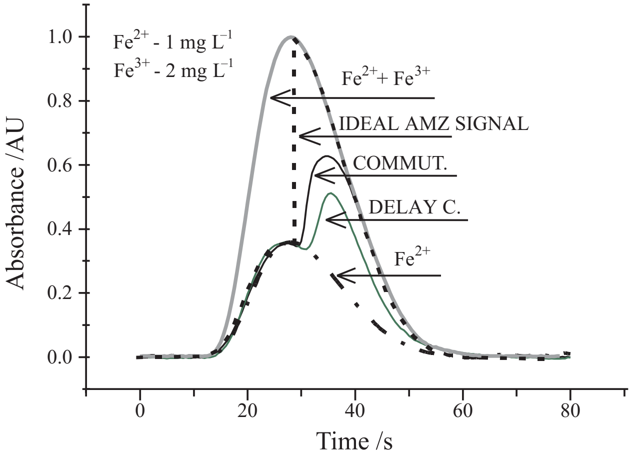

Figure 4. Comparision of the detector signals obtained in FIA-AMZ simultaneous determination of Fe^{2+} (1 mg L^{-1}) + Fe^{3+} (2 mg L^{-1}) using conventional delay coil and proposed commutator. A theoretically ideal signal is added to the plot (dashed line) showing a sharp transition from the signal of the non-reduced and of the reduced sample.

The Overall Sample Dispersion

The analyte profile in FIA experiment is dependent on the overall sample dispersion. It is known that the overall sample dispersion reduces the sensitivity of the method because it lowers the analyte concentration in the sample plug, therefore it increases the detection limit for Fe^{2+} ions. Because of the nature of FIA-AMZ speciation (non-separation based procedure), the mentioned phenomenon influences the detector signal depending on the concentration of the Fe^{2+} ions as well as on the sum of the Fe^{2+} and Fe^{3+} ions. Increase of the detection limit with an increased dispersion is obvious because at higher dispersion of the sample the peak height decreases. For the FIA-AMZ experiment it is important that together with the decreased peak height, caused by the overall sample dispersion, the slope of the tailing part of the detector signal used for the determination of the sum of both species is decreased. The smaller slope of the tailing part of the detector signal enables the determination of the sum of both species with improved precision (decreased standard deviation). The injection of the sample and reagent can be reproduced with certain precision. In our experiments an overall time span in which the reagent was added to the sample plug, was accepted to be 0.6 s. In the case that the sample dispersion is low, the repeatability (expressed as standard deviation) is low, but the sensitivity of the detection of both analytes is good, and *vice versa*. For these reasons the overall dispersion of the sample plug has to be optimised.

Criterion for the Determination of the Optimal Overall Sample Dispersion

Seven different lengths of the coil were used to show the overall sample dispersion effect. Resulting experimental curves for different dispersions are shown in Figure 5. The dispersion is expressed as a ratio h_0/h. The quantity denoted as h is the particular peak height, while h_0 is the maximal peak height (steady state signal) which can be measured if an infinite sample plug is injected and the signal is detected long enough to obtain no increase of absorbance. The lowest dispersion obtained with the shortest coil was 1.8, whereas with the longest coil a value of 7.1 was obtained.

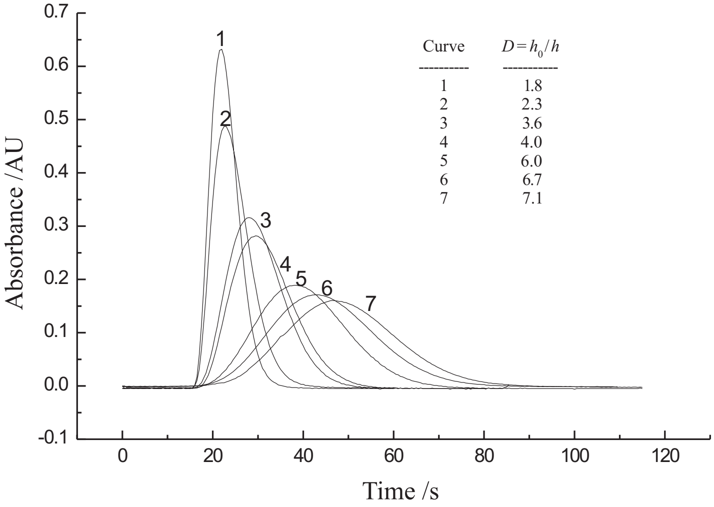

Figure 5. FIA detector signals obtained in systems inducing the overall sample dispersion as marked on the plot.

As can be seen from Figure 5, with increasing dispersion the two quantities influencing the peak shape, that is the height and the slope of the tailing part, are decreasing. It was already mentioned that for a good FIA-AMZ analysis both quantities could not be improved simultaneously because they act in opposite directions. By decreasing the error for them (the peak height, for example), the other (peak slope) automatically deteriorates in accuracy. In order to find a criterion for the compromise between the two quantities, a small section (corresponding to 1.2 s time interval) of the tailing part at 80% of the peak height was analysed. For each of the 7 experimental curves of different dispersions, two quantities labeled as h^{80} and NP (detailed description is given below) are determined.

Here h^{80} is the absorbance at the position which is defined as the time at which the tailing part of the peak reaches approximately 80% of maximal absorbance (time = t^{80}). Also NP denotes normalised precision and is calculated from »normalised« standard deviation as $1 - S_{\mathrm{norm}}$. The so called normalised standard deviation is scaled from 0 to 1 in the set of values obtained for seven different dispersion experiments by the following procedure: the injection time error is considered to be $\pm$ 0.6 seconds and for this time error standard deviations in the simulated detector responses are determined. Equation (1) is applied to model seven different dispersion signals. Function parameters are determined for each of the seven experimental curves separately. The error in the injection time ($\pm$ 0.6 s) is simulated by 30 absorbance values calculated in the 1.2 seconds time interval around t^{80}. More precisely, the interval $\{t^{80}$–0.6s... t^{80}+0.6s$\}$ is divided into 13 equidistant points with $\Delta t = 0.1$ s $\{t^{80}$–0.6s, t^{80}–0.5s,..., t^{80},... t^{80}+0.5s, t^{80}+0.6s$\}$. Using Eq. (1), 30 absorbances (h) are calculated at 30 different positions (time values) chosen randomly among the 13 possible time values in the predefined time interval. The standard deviation of the k-th dispersion signal (k = 1–7) is determined for i = 30 absorbances by

$$S_k = \sqrt{\frac{\sum_{i=1}^{30} (h_{i,k} - \overline{h}_k)^2}{29}} \ . \tag{6}$$

All seven standard deviations determined for experimental curves of different dispersions are scaled between 0 and 1 to obtain S_{norm}. Then NP is calculated as $1 - S_{\mathrm{norm}}$.

The product of h^{80} and NP is calculated for each of the 7 dispersions. To obtain the optimisation criterion for an optimal dispersion (compromise between the sensitivity and repeatability of FIA-AMZ experiment) a polynomial curve is fitted to these 7 product values. The obtained polynomial func-

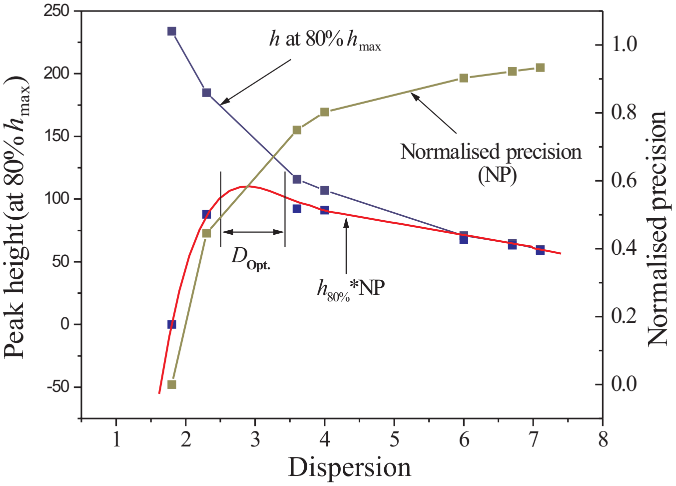

Figure 6. The influence of the sample dispersion on the peak height (h^{80}) and normalised precision NP ($1 - S_{norm}$). The product of the curves describing the detector response (h^{80}) and normalised precision NP with respect to the dispersion is found to be an optimisation criterion for the ideal dispersion in FIA-AMZ technique.

tion has a maximum at the dispersion between 2.5 and 3.0 as it is illustrated in Figure 6.

As can be seen from Figure 6, the optimal dispersion of the sample plug at the mixing point T (see Figure 1) should be between 2.5 and 3.5. This conclusion is general and it doesn't seem to be dependent on the concentration of the second species.

CONCLUSIONS

The aim of the present work was not only to show that the lowest dispersion of the sample plug was not optimal in FIA/AMZ analytical technique, but also to obtain further knowledge about the nature of simple FIA detector response and about the processes influencing the reproducibility and sensitivity of the simultaneous detection of two analytes in the applied analytical technique. The equations developed to model FIA detector response are of great help when we study the influence of the dispersion (h_0/h) of the sample plug on the sensitivity and reproducibility of the detection of Fe^{2+} and the sum of Fe^{2+} and Fe^{3+}, respectively. The modelling results suggested us a separation of the effects due to the dispersion of the sample plug and the one due to the dispersion of the reducing agent. The most important improvement based exclusively on the knowledge obtained by modelling FIA-

AMZ signals, was the development of a commutator enabling reagent addition with actually no frontal dispersion. Since the resulting detector response still deviates when close to t_0 from the theoretical one as is shown in Figure 4, the further step will be an improvement of the mixing of the sample and reducing agent.

The Flow injection asynchronous merging zone technique has now been described by the mathematical model, based on the linear combination (the sum) of a semi-Gaussian curve I (Fe^{2+} concentration profile) and a semi-Gassian curve II (Fe^{3+} concentration profile) multiplied by sigmoidal function, describing Fe^{3+} to Fe^{2+} conversion yield. Using the proposed mathematical model, an essential effect on the detector signal, *i.e.* the frontal dispersion of the reducing agent influencing the sensitivity of the detection of the sum of Fe^{2+} and Fe^{3+} ions was explained.

With the applied mathematical model it is possible to study the influence of the dispersion of the sample plug on the sensitivity and reproducibility of the detection of the sum of the analytes and of the first analyte, respectively. The influence of the slope of the tailing part of the dispersed sample on the reproducibility of the detection of the sum of both iron species was demonstrated. A measure is proposed for an optimal ratio between the sensitivity and reproducibility on the basis of the parameters obtained from the simulations of the experimental curves for different dispersions. The optimal dispersion of the sample plug was determined to be between 2.5 and 3.5.

Acknowledgment. – The financial support of the Ministry of Science and Technology of Slovenia obtained by the Projects: P0 – 0509–0104 and P0 0507–0104 is gratefully acknowledged.

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SAŽETAK

Određivanje optimalnih uvjeta za dobivanje maksimalnog odziva pri maksimalnoj ponovljivosti u FIA-AMZ pokusu, s pomoću matematičkog modeliranja

Milko Novič i Marjana Novič

Na temelju matematičkog modela predloženi su optimalni eksperimentalni uvjeti za FIA-AMZ. Poboljšani komutator, koji omogućuje dodavanje reagensa s minimalnim frontalnim raspršenjem, uporabljen je za istovremeno određivanje iona Fe^{2+} i Fe^{3+}. Matematički model, uporabljen za oponašanje odziva detektora, temelji se na zvonolikoj funkciji. Model je vrlo prikladan za simetrične kao i za vrlo asimetrične odzive detektora, odražavajući različito raspršenje uzoraka. Predloženim modelom proučavan je utjecaj frontalnog raspršenja reagensa asimetrično dodanom uzorku. Mjera optimalnoga omjera osjetljivosti i ponovljivosti zasniva se na parametrima dobivenim iz simulacija eksperimentalnih krivulja određenih za različita raspršenja.