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A new automated approach to determine monosodium glutamate in dehydrated broths by using the flow-batch methodology

Carolina C. Acebal, Matías Insausti, Marcelo F. Pistonesi, Adriana G. Lista*, Beatriz S. Fernández Band*

FIA Laboratory, Analytical Chemistry Section, INQUISUR (UNS-CONICET), Av. Alem 1253, B8000CPB Bahía Blanca, Buenos Aires, Argentina

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ABSTRACT

The advantages of the flow-batch methodology were exploited to implement a simple system with nephelometric detection for the determination of monosodium glutamate (MSG) in food samples. The method is based on the inhibitory effect of the MSG over the crystallization of L-lysine in an isopropanol/acetone mixture. The calibration curve was prepared on-line. The method was linear over the range of 2.8×10^{-3} to 1.1×10^{-2} g L^{-1} and a detection limit of 9.7×10^{-5} g L^{-1} was achieved. It was successfully applied to determine the MSG concentration in food samples, without a previous treatment. A recovery study was carried out on real samples and the percentages were between 98 and 106%.

1. Introduction

It is well known that monosodium L-glutamate (MSG) is used to enhance the flavor of food, particularly in meat products, sauces and soups. The ingestion of food containing high amounts of MSG can produce allergic effects such as asthma [1,2]. An excess of MSG can cause some diseases of the nervous central system [3,4]. Therefore, MSG is an important analyte to be determined and its quantification is essential for quality control of some food products. The determination of MSG has been carried out by liquid chromatography [5,6], spectrophotometric [7] and fluorimetric [8] methods and electroanalytical methods using ion-selective electrodes and biosensors [9–12]. Moreover, several continuous flow procedures with spectrophotometric, fluorimetric and amperometric detection have been developed [13–15]. Also multicomponent analysis using chemometric techniques were used to determine MSG and others flavor enhancers [16].

During the crystallization of organic compounds, the presence of some substances even at such a lower concentration as trace levels can produce a delay in the precipitation process. Grases et al. have presented a nephelometric method to determine L-glutamic acid in pharmaceutical products based on the inhibitory effect of this analyte over the crystallization of L-lysine in a water/isopropanol mixture [17].

Since 1975, the use of flow techniques has provided a simple way to enhance the laboratory productivity and to reduce the reagent consumption and the production of wastes. In 1999, Araújo et al. [18] developed a new automated methodology that presents the intrinsic favourable characteristics of the flow systems such as high sampling rate, low sample and reagent consumption, low cost, etc., and the wide application inherent to batch techniques: the flow-batch systems. A remarkable advantage of this combination is the facility of carrying out the classical batch mode method in a new automatic instrumental approach. These systems are characterized by the use of three-way solenoids valves or a multi-port selecting valve and an open mixing chamber. In them, the sampling and signal processing are carried out as the flow systems, whereas chemical reactions take place in a reaction chamber similar to those found in batch systems. With a flow-batch analyzer is possible to implement different analytical processes [19–21] just by changing the operational parameters in their control software.

With the purpose to determine the MSG concentration in dehydrated broths, a new flow-batch method, based on the paper presented by Grases et al., was developed. Since the flow-batch methodology allowed a better control of the precipitation parameters, the implementation of the crystallization of L-lysine was carried out in a more efficient and simple way. Additionally, the amount of the solvent used was significantly reduced.

2. Experimental

2.1. Reagents

Analytical grade reagents and ultra pure water (>18 MΩ cm^{-1}) were used.

* Corresponding authors. Tel.: +54 291 4595100; fax: +54 291 4595160.
E-mail addresses: alista@criba.edu.ar (A.G. Lista), usband@criba.edu.ar (B.S.F. Band).

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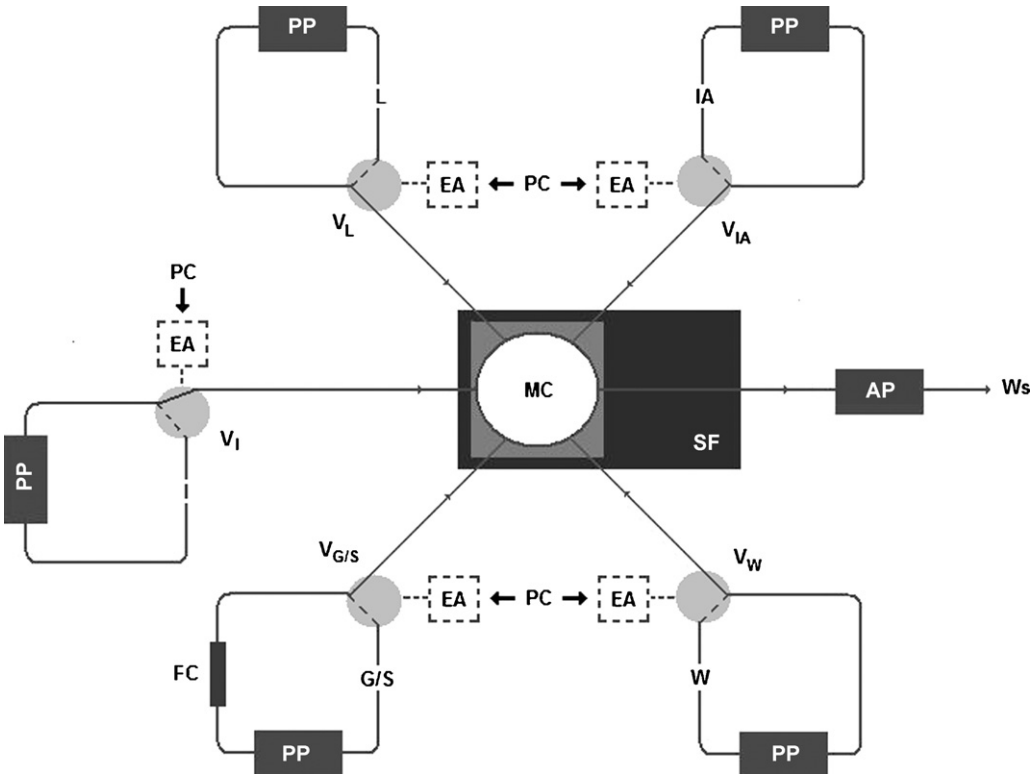

Fig. 1. Flow-batch system to determine MSG concentration in food samples—L: L-lysine solution; IA: isopropanol/acetone mixture; I: isopropanol; G/S: MSG standard solutions/samples; W: water; FC: filter column; MC: mixing chamber; SF: spectrofluorimeter; PP: peristaltic pump; Ws: waste; AP: auxiliary pump; EA: electronic actuator; PC: microcomputer; V: solenoid valves.

A 4.8×10^{-2} mol L^{-1} L-lysine (Fluka) and 1.0×10^{-2} mol L^{-1} monosodium glutamate monohydrate (Anedra, Buenos Aires, Argentine) standard solutions were prepared by dissolving a suitable amount of the solid drugs in distilled water. A 3.4×10^{-4} mol L^{-1} glutamate working solution was prepared by an appropriate dilution of the standard solution.

An isopropanol/acetone solvent mixture was prepared in a 1/1 (v/v) ratio. Both solvents were supplied by Anedra (Buenos Aires, Argentine).

Commercial samples of different trademarks were purchased in supermarkets in Bahía Blanca, Argentine and they were labeled as A, B, C and D. A, B and C samples were meat dehydrated broths and D was a vegetable dehydrated broth.

2.2. Flow-batch assembly

The nephelometric measurements were carried out in an Aminco Bowman® Series 2 luminescence spectrometer at 420 nm. Solutions were propelled by a multichannel Watson–Marley peristaltic pump (323S). A Gilson Minipuls-3 peristaltic pump was used as auxiliary pump in order to empty the mixing chamber (MC).

A 4.0 mL laboratory-made mixing chamber (MC) was constructed in PTFE and a Hanna Instruments magnetic stirrer, model HI 190M was placed underneath the MC.

Five NResearch three-way solenoid valves were used: four of them: V_L, $V_{G/S}$, V_{IA} and V_W to carry out the L-lysine standard solution, the MSG working solution, the isopropanol/acetone mixture and water into the MC. The fifth solenoid valve (V_I) was used to wash the MC with isopropanol. Tygon® pumping tubes of different inner diameters and PTFE tubes (0.8 mm i.d.) were employed.

A microcomputer supplied with a laboratory-made parallel interface was used to an automatic handling of the flow-batch manifold through a computer program developed in Labview® 5.1 graphic language.

2.3. Flow-batch procedure

The proposed flow-batch system is shown in Fig. 1. Before starting the analysis, the channels had to be filled. All the valves were initially switched OFF so the solutions were continuously pumped to load the channels and returned to their respective containers. Then, each valve was switched ON for 2 s and the solutions were pumped towards the MC to fill the channels between the valves and the MC. The excess of the solutions in MC was pumped out to the waste in 4 s by an auxiliary pump (AP). This process called "fill channels" lasted 14 s.

The baseline was recorded when the V_W, V_L and V_{IA} valves were switched ON during their respective optimum times (t_W, t_L and t_{IA}). When all the solutions were in the chamber it was necessary to wait 30 s before stirring, in order to reach the solution stability and to ensure the precipitation. Then, the magnetic stirrer was switched ON during 9 s, the precipitation began and the intensity of the scattered light began to be monitoring. The measures were done at 300 s when the precipitate has already formed and the chemical equilibrium was attained.

In order to obtain different standard solutions on-line for the calibration curve, different volumes of the MSG working solution were added to the MC. The final volume in the MC must be constant, so the added volumes of water and MSG standard solution must be modify in order to achieve the same water volume that was added when the baseline was obtained. Thus, t_L and t_{IA} were kept constant, while t_W diminished and $t_{G/S}$ increased. The V_W, $V_{G/S}$, V_L and V_{IA} valves were sequentially switched ON during t_W, $t_{G/S}$, t_L and t_{IA}, respectively. The decrease on the intensity of scattered light was directly proportional to the MSG concentration. The same

Table 1
Optimum values for flow-batch parameters.

Flow rate (mL min^{-1})	q_W	q_L	$q_{G/S}$	q_{IA}	q_I	q_{AP}
	0.61	2.32	0.61	2.93	5.9	7.8
Valve switching time intervals (s)		V_W	V_L	$V_{G/S}$	V_{IA}	V_I
Baseline signal		8	3.5	0	116	0
Standard solution		4.0–7.0	3.5	1.0–4.0	116	0
Samples		5.0	3.5	3.0	116	0

W: water; L: L-lysine solution; G/S: MSG standard solution/sample; IA: isopropanol/acetone mixture; I: isopropanol; q: flow rate; V: solenoid valve; AP: auxiliary pump.

procedure for the analyte determination in the dehydrated broths samples was performed just by changing the MSG working solution for the sample solution.

A wash cycle with isopropanol had to be carried out between measurements. For that purpose, the V_I valve was switched ON for 41 s. After this time, the AP was turned ON to empty the MC.

2.4. Sample preparation

Taking into account the quantity of MSG presents in dehydrated broths, a suitable amount of the samples were weighed and diluted to 25.0 mL with water. The solution was stirred for 10 min and filtered on-line with a packed column with acetate.

3. Results and discussion

3.1. Optimization of the flow-batch parameters

The flow-batch system for the MSG determination was optimized and the optimum values were selected as a compromise between sensitivity and reproducibility of the analytical signals.

Table 1 shows the optimum values for the different flow rates and the switching time intervals of the solenoid valves.

3.1.1. Time before stirring and stirring time

When the solutions went into the MC a delay time before stirring was necessary to obtain a good reproducibility of the L-lysine precipitation. Thus, the delay time was optimized from 20 to 40 s and the optimum time was 30 s.

Different stirring times were tested between 3 and 15 s. The best signal and the highest reproducibility were obtained when the solutions in the MC were stirred for 9 s. Lower times were not enough to form the precipitate or to achieve a good reproducibility, and at higher times the analytical signal was not improved.

3.1.2. Stirring speed

The stirring speed was tested over the range of 250–900 rpm. The optimum speed was 700 rpm. At lower values the precipitate did not form and at higher values than 700 rpm no changes were observed.

3.2. Optimization of chemical variables

3.2.1. Chemical composition of solvents mixture

In a previous study, Grases at al. [17] have proved that supersaturated solutions of L-lysine could be obtained in an isopropanol/water mixture. So, the organic solvent was added to a fixed water volume to suppress the L-lysine solubility and a colloidal precipitate was generated.

When isopropanol was used as the organic solvent, the required time for precipitating was considerably high and the time of analysis was very long. On the other hand, the time for obtaining the L-lysine precipitation was reduced when acetone was selected, but agglomerated particles were formed and the precipitate did not remain in suspension.

Taking this into account, a mixture of isopropanol/acetone was used to obtain a colloidal precipitate and to reduce the precipitation time. Different isopropanol/acetone ratios were tested and the best was 1/1 (v/v). At higher acetone amounts, the precipitate trended to form agglomerates so the measured could not be done.

3.2.2. Order for reagents addition

The order for addition of the different solutions into the MC was optimized. The isopropanol/acetone solvent mixture had to be adding after that the L-lysine and MSG solutions were placed into the MC. If the solvent mixture was added before the MSG solution, the L-lysine precipitation would start and no inhibition effect of the MSG would be observed. Different orders of addition of the L-lysine solution, MSG solution and water were proved and no significant differences were observed.

So, the selected order for addition was: water, L-lysine solution, MSG solution and isopropanol/acetone mixture.

3.2.3. Concentration of the L-lysine standard solution

The L-lysine concentration was tested over the range of 2.4×10^{-2} to 2.0×10^{-1} mol L^{-1} and an appropriate signal was obtained with a 4.8×10^{-2} mol L^{-1}. At lower concentrations the precipitate did not form and at higher concentrations no significant changes in the signal were observed.

3.2.4. Concentration of MSG working solution

As the calibration curve was carried out on-line, the concentration of MSG working solution had to be optimized taking into account the inhibition of the L-lysine precipitation. Working with the optimum L-lysine concentration, the studied range was 0.0500–0.1500 g L^{-1} and the optimal MSG concentration was 0.0640 g L^{-1}. At lower concentrations the inhibition effect was not observed and at higher concentrations the L-lysine signal was thoroughly inhibited.

3.3. Analytical performance

By using the proposed flow-batch system and the optimized values for the chemical and flow-batch variables, a calibration graph for the MSG determination was carried out over the range of 2.8×10^{-3} to 1.1×10^{-2} g L^{-1}. The obtained regression line was $A = (-3495.6 \pm 77.6)$ [g MSG L^{-1}] + $(69{,}913 \pm 0.590)$. The detection limit (LOD) was 9.7×10^{-5} g L^{-1} calculated from the calibration curve [22]. The relative standard deviation (RSD%) was 3.8 and it was obtained from 9 replicates of real samples of 1.1×10^{-2} g L^{-1} MSG concentration. The sample throughput was 9 h^{-1}.

3.4. Analysis of real samples

The Argentine Food Code [23] establishes what kind of food can contain MSG, but only dehydrated broths has an allowed maximum value.

Table 2
Analysis of real samples and recovery study.

Sample	Added ($g\,dm^{-3}$)	Found ($g\,dm^{-3}$) ± SD[a]	R (%)
	–	0.17 ± 0.01	–
A	2.13	2.30 ± 0.02	100
	3.12	3.30 ± 0.02	100
	–	1.08 ± 0.02	–
B	0.55	1.73 ± 0.02	106
	1.01	2.19 ± 0.02	105
	–	1.65 ± 0.02	–
C	1.13	2.82 ± 0.01	102
	2.27	3.82 ± 0.01	98
	–	0.84 ± 0.03	–
D	1.10	1.95 ± 0.03	101
	2.15	2.95 ± 0.02	99

A, B, C: meat dehydrated broths of different trade marks; D: vegetable dehydrated broth.

[a] The samples were analyzed by triplicate.

So, the proposed method was applied to the determination of MSG concentration in this kind of samples. Dehydrated broths are commercial products resulting from the combination of meat and meat extracts or vegetables, fat, edible salt (NaCl), other flavor enhancers like inosine 5′ monophosphate (IMP) and guanosine 5′ monophosphate (GMP), stabilizers, emulsifiers and spices. In the present work, two varieties of these products were studied: vegetables and meat.

Table 2 shows the analyte concentration in real samples of different trade marks, expressed as the Argentine Food Code [23] requires (maximum value allowed: $8\,g\,dm^{-3}$).

The reference method to determine MSG concentration, in this kind of samples, involved a chromatographic separation with several elution steps for the analyte extraction and then, a potentiometric titration. This method required a laborious task and its selectivity is poor. Therefore, to validate the proposed method a recovery study was performed. For that purpose, an appropriate amount of the samples was weighed, spiked with two different enhancer concentrations, diluted to 25.0 mL and analyzed with the flow-batch method. In Table 2 the added concentrations to each sample and the obtained results for the recoveries are shown. As can be noticed, satisfactory recoveries percentages were obtained for this kind of products.

The results obtained with the recovery study also showed that no interferences of any of the sample compounds were found.

4. Conclusions

The proposed strategy to determine MSG in dehydrated broths represents a good alternative to conventional flow-based procedures.

The variables that usually have an effect on the formation of the precipitate (order of reagents addition, stirring time, and stirring speed) can be controlled in a most appropriate way by implementing the flow-batch methodology. Some advantages of the proposed system are that it allows the automatic preparation of the calibration curve and the samples not required a previous treatment. Moreover, it is possible to reduce significantly the amounts of reagents consumed and wastes generated in order to contribute with the green chemistry.

The new method was applied to real samples and the recovery study showed satisfactory results.

On the other hand, the method can be applied to any kind of sample with an adequate treatment.

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