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Successive projections algorithm applied to spectral data for the simultaneous determination of flavour enhancers

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ABSTRACT

A novel variable selection strategy for multiple lineal regression (MLR), the successive projections algorithm (SPA), was applied to spectrophotometric data (190–320 nm) for the simultaneous determination of monosodium glutamate (MSG), guanosine-5′-monophosphate (GMP) and inosine-5′-monophosphate (IMP) in dehydrated broths samples. This selection method uses simple operations in a vector space to minimize variable collinearity and has become an interesting variable selection strategy for multivariate calibration. In this work, nine, six and four wavelengths for MSG, GMP and IMP, respectively, were selected to construct calibrations models in order to solve successfully the serious spectral overlapping in samples containing these analytes. The relative errors of prediction (REP) for the validation set were 2.3%, 0.9% and 1.8% for MSG, GMP and IMP, respectively. Commercial samples were analysed and a recovery study was carried out to verify the accuracy of the proposed method with satisfactory results. A continuous flow system was used to develop a simple, cheap and rapid method (sample throughput: 200 h^{-1}), without any previous extraction step.

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1. Introduction

Nowadays, most of spectrophotometric determinations for multicomponent systems are carried out by using multivariate calibration methods allowing the simultaneous analysis of several analytes. Among these, multiple linear regression (MLR), principal component regression (PCR) and partial least squares (PLS) are commonly used [1]. The application of these methods requires the selection of spectral variables for building well-fitted models [2]. Several authors have presented theoretical and empirical evidence supporting the use of variable selection to improve the predictive ability of PCR, PLS and MLR models [3–6]. MLR yields models which are simpler and easier to interpret than PCR and PLS, since these calibration techniques perform regression on latent variables without a physical meaning. On the other hand, MLR calibration is more dependent on the spectral variables selection and it can be severely affected by collinearity between the regressors [7,8]. To overcome this problem, Araújo et al. [9] proposed a novel variable selection strategy for MLR calibration: the "successive projections algorithm" (SPA). The goal of SPA consists of finding a small representative set of spectral variables with an emphasis on the minimization of collinearity.

In terms of prediction ability, SPA-MLR models have shown to be comparable to or better than full-spectrum PLS and/or PCR models in a number of applications including UV–vis [10], ICP-AES [11] and NIR [12] spectrometry. Good results involving the use of SPA together with wavelet regression have also been reported [13]. Furthermore, SPA has also been favourably compared with the genetic algorithm [7,11], which is a popular tool for variable selection in multivariate calibration [14,15].

Flavour enhancers play important roles in the taste, palatability and acceptability of food, so they were widely used as additives to enhance the flavour of dehydrated broths, soups and meat [16]. Umami is a characteristic taste imparted by monosodium glutamate (MSG) and was first discovered in 1908 by K. Ikeda. The most unique characteristic of umami taste is synergism. Purinic ribonucleotides, such as inosine-5′-monophosphate (IMP) and guanosine-5′-monophosphate (GMP), can strongly enhance the umami taste intensity [17]. In human taste tests, 200 μM of IMP, which does not elicit any umami taste by itself, can increase one's umami taste sensitivity to glutamate by 15-fold [18].

Although the FAO/OMS safety evaluation not specified an Acceptable Diary Intake (ADI) [19] for these additives, some studies affirm that high concentrations of these compounds may cause health problems in people who are sensitive to them [20,21].

There are a few articles that report the determination of the three flavour enhancers in a simultaneous way. Most of them involve multi-step, time consuming or expensive procedures such chromatographic techniques [22,23].

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Table 1
Statistical parameters obtained for calibration and validation, and the figures of merit.

Parameter	Flavour enhancer		
	MSG	GMP	IMP
Selected variables (nm)	188, 190, 192, 194, 212, 248, 284, 296, 318	190, 194, 202, 250, 284, 296	196, 244, 278, 318
Calibration			
Concentration range ($\mu g\,mL^{-1}$)	447.6–1398.6	5.028–34.190	5.028–34.190
RMSE ($\mu g\,mL^{-1}$)	3.589	0.196	0.466
REP (%)	0.389	0.997	2.377
Validation			
Concentration range ($\mu g\,mL^{-1}$)	503.5–1202.8	10.056–30.168	10.056–30.168
RMSE ($\mu g\,mL^{-1}$)	18.83	0.187	0.364
REP (%)	2.29	0.908	1.763
Figures of merit			
Limit of detection ($\mu g\,mL^{-1}$)	168	2.0	1.1
Sensibility ($mL\,\mu g^{-1}$)	1.79×10^{-5}	1.5×10^{-3}	2.6×10^{-3}

Because of the demand for fast, inexpensive and friendly environmental methods, there is a great interest in searching alternative analytical methods using chemometrics tools [24,25].

In this work, the spectrophotometric simultaneous determination of MSG, GMP and IMP in dehydrated broths is proposed. Small calibration matrixes were constructed for each analyte, based on the spectra variable selection by SPA. Then, simple calibrations models were obtained applying MLR to these matrixes. A continuous flow system was used to develop a cheap and rapid method without any previous separation and/or derivatization reaction.

2. Experimental

2.1. Apparatus

All spectra were obtained by using a Hewlett Packard 8452A diode array spectrophotometer, with a spectral bandwidth of 2 nm.

A Hellma QS flow cell of 18 μL and 1 cm optical path, and a Gilson minipuls-3 peristaltic pump were used.

All the reaction coils were made of PTFE tubing (i.d. 0.5 mm).

MLR-SPA calculations were performed using a routine developed by Araújo et al. [9] in MATLAB® 7.0 (The MathWorks) software.

2.2. Reagents

Analytical grade reagents and ultra pure water (>18 MΩ cm^{-1}) were used. 0.50 g L^{-1} IMP (Fluka), 0.49 g L^{-1} GMP (Fluka) and 14.03 g L^{-1} MSG (Anedra) stock solutions were prepared by dissolving the appropriate amount of their solid drugs in water.

A pH 10.0 buffer solution was prepared by mixing 50.0 mL of 0.03 M $Na_2BO_4{\cdot}10H_2O$ (Mallinckrod) and 18.3 mL of 0.1 M NaOH (Anedra) and diluting to 100 mL with water.

2.3. Sample preparation

Six commercial dehydrated broths samples were purchased in different local supermarkets. Taking into account the enhancers' concentration in these samples, a suitable amount of them was weighed and dissolved in 25.0 mL of water.

On the other hand, to validate the proposed method a recovery study was performed. So, an appropriate sample amount was weighed, spiked with the three enhancers and dissolved in 25.0 mL of water.

2.4. Calibration and validation sets

A calibration set of nine standard solutions was prepared following a full factorial design. The concentration range for each analyte was selected considering the components ratio present in this kind of food.

The external validation of the calibration models was achieved by using another full factorial design. Nine synthetic mixtures were prepared with concentrations within the range used for the calibration set.

The concentration ranges for calibration and validation sets are shown in Table 1.

2.5. Procedure

Fig. 1 shows the continuous manifold used for MSG, GMP and IMP determination. The system had a packed column (length 4.0 cm; internal diameter 0.7 cm) filled with acetate to filter the sample on line. A selection valve (SV) was used to introduce the standard solution or the filtered sample solution into the system. These solutions merged with the buffer solution stream and reached the flow cell. In that moment, the flow was stopped and the absorption spectrum was recorded between 190 and 320 nm. Then, the flow was restored.

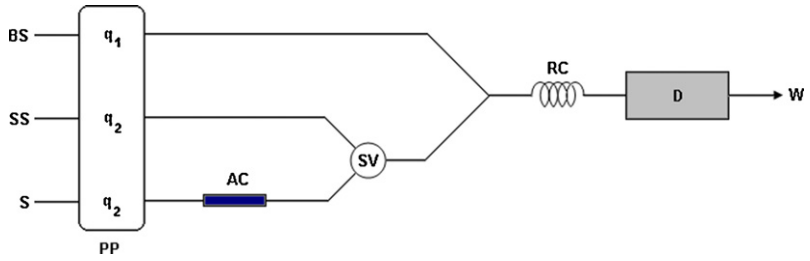

Fig. 1. Flow system proposed for the simultaneous determination of MSG, GMP and IMP. AC, acetate column; BS, buffer solution; D, detector; PP, peristaltic pump; q_1, buffer solution flow rate (2.06 mL min^{-1}); q_2, standard and sample solution flow rate (1.08 mL min^{-1}); RC, reactor coil; S, sample; SS, standard solution; SV, selection valve; W, waste.

3. Results and discussion

3.1. Optimization of chemical and flow system variables

In a previous work, a study of the pH influence on the absorption spectra of the analytes was carried out [24]. A pH 10.0 $Na_2BO_4{\cdot}10H_2O$/NaOH buffer solution was selected for the simultaneous determination of the three analytes. This buffer solution was chosen by considering the least spectral overlapping. Furthermore, in that work the flow system variables were optimized in order to obtain the optimum values for reactor length (600 mm), buffer and sample flow rate (2.06 and 1.08 mL min^{-1}, respectively).

3.2. MLR-SPA application to the absorption data

The absorption spectra for the different mixtures of MSG, GMP and IMP were recorded from 190 to 320 nm. Previous to the application of MLR-SPA, the data were mean centred in order to remove constant background effects.

MLR-SPA uses a calibration (Xcal) and a validation (Xval) set consisting of instrumental response data and parameter values measured by a reference method (y). The essence of SPA consists of projection operations carried out on the calibration matrix. A detailed explanation of the projection operations is given elsewhere [9,11]. Starting from each of the J variables (columns of Xcal) available for selection, SPA builds an ordered chain of K variables where each element is selected in order to present the least collinearity with the previous ones. The collinearity between variables is assessed by the correlation between the respective column vectors of Xcal. It is worth to point out that, according to this selection criterion, no more than K variables can be included in the chain [9,11]. It is possible to extract K subsets of variables from each of the J chains constructed by using one up to K elements in the order in which they were selected. Thus, a total of $J \times K$ subsets of variables can be formed. In order to choose the most appropriate subset $J \times K$, MLR models are built using the calibration samples set and compared in terms of the root-mean square error (RMSE) obtained for the validation set,

$$\mathrm{RMSE} = \left[\frac{\sum_{i=1}^{I}(c_{\mathrm{nom}} - c_{\mathrm{pred}})^2}{I}\right]^{1/2} \quad (1)$$

where c_{nom} and c_{pred} represent the nominal and predicted concentrations, respectively, and I is the total number of validation samples.

Previously, Acebal et al. [24] proposed the simultaneous determination of MSG, GMP and IMP in stock cube samples using partial least squares (PLS). In that work, three different spectral ranges were used: 190–320 nm for MSG, 248–288 nm for GMP and 252–302 nm for IMP. Considering a step size of 2 nm, the PLS models employ 65, 20 and 25 spectral variables, respectively.

As can be seen in Table 1, only nine, six and four variables were selected by SPA for MSG, GMP and IMP, respectively. It can be verified that some of these variables are in agreement with the highest absorbance regions for each analyte (Fig. 2a and b). Thus, MSG appears to be better predicted if absorptions at 192, 194, 196 and 214 nm are included while GMP is better predicted if absorptions at 192, 196, 204, 252 and 286 nm are selected. A better prediction for IMP is obtained if absorptions at 198, 246 and 280 nm are considered. However, some variables outside these regions are important and have also been identified by SPA, for example, the MSG absorptions at 250 and 286 nm. Probably, it must be the way in which SPA resolves the spectral overlapping. At 250 and 286 nm the regression coefficients in the MSG model were negative, corroborating this assumption.

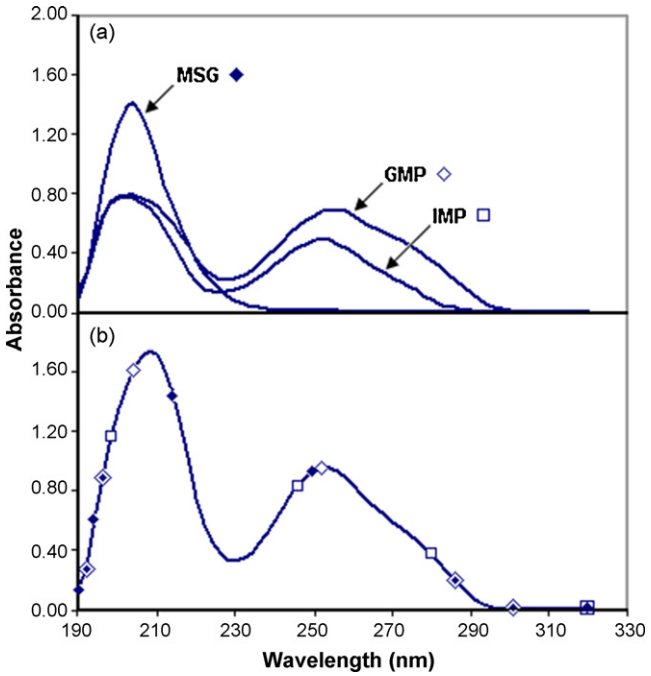

Fig. 2. (a) Pure spectra of MSG (923.1 μg mL^{-1}), GMP (19.6 μg mL^{-1}) and IMP (19.6 μg mL^{-1}). (b) Spectrum of a mixture containing MSG (923.1 μg mL^{-1}), GMP (19.6 μg mL^{-1}) and IMP (19.6 μg mL^{-1}). In this spectrum the selected variables by MLR-SPA are indicated.

Then, calibration models were constructed applying MLR. Table 1 summarizes the statistical parameters: RMSE, calculated as Eq. (1), and relative root-mean square error (REP) calculated as,

$$\mathrm{REP} = \frac{100}{c_{\mathrm{mean}}}\left[\frac{\sum_{i=1}^{I}(c_{nom} - c_{pred})^2}{I}\right]^{1/2} \quad (2)$$

where c_{nom} and c_{pred} have the same meaning as that of Eq. (1), I is the total number of calibration samples and c_{mean} is the mean concentration. Both values were reasonably low for the three models and comparable with the reported PLS method [24].

The validation set containing nine artificial samples was analysed and the statistical parameters are also quoted in Table 1. They indicate that the proposed method is accurate in the prediction of artificial samples, as suggested by the low RMSE and REP values.

With regard to the figures of merit, Table 1 shows satisfactory values for the detection limit and the sensibility for each analyte.

3.3. Analysis of real samples

The proposed method was applied to the simultaneous determination of MSG, GMP and IMP in meat dehydrated broths. The results of the analysis of six different commercial samples are

Table 2
Analysis of real samples.

Sample[a]	Flavour enhancers content (g dm^{-3})		
	MSG	GMP	IMP
1	8.93 ± 0.01	nd[b]	0.102 ± 0.003
2	5.56 ± 0.37	nd[b]	0.062 ± 0.005
3	4.43 ± 0.10	nd[b]	0.019 ± 0.003
4	16.3 ± 0.2	nd[b]	0.022 ± 0.003
5	14.2 ± 0.2	nd[b]	0.080 ± 0.002
6	11.4 ± 0.3	0.037 ± 0.001	0.134 ± 0.001

[a] The samples were analysed by triplicate.
[b] No detected.

Table 3
Recovery study in real samples.

Sample		MSG			GMP			IMP		
		Added[a]	Found[a]	R (%)[b]	Added[a]	Found[a]	R (%)[b]	Added[a]	Found[a]	R (%)[b]
1	*A*	2.76	3.02	109 (1)	0.099	0.102	103 (3)	0.099	0.083	84 (2)
	B	2.76	2.78	101 (3)	0.196	0.196	100 (1)	0.195	0.188	96 (3)
	C	5.49	4.99	91 (3)	0.098	0.102	104 (1)	0.098	0.102	104 (1)
	D	5.42	5.47	101 (1)	0.195	0.191	98 (2)	0.194	0.193	99 (2)
	E	5.47	5.65	103 (1)	0.097	0.096	99 (3)	0.195	0.194	99 (1)
2	*A*	1.38	1.42	103 (3)	0.049	0.048	98 (3)	0.049	0.050	102 (1)
	B	1.38	1.50	109 (1)	0.098	0.089	91 (2)	0.098	0.090	92 (1)
	C	2.73	2.39	88 (3)	0.049	0.050	102 (1)	0.048	0.050	104 (2)
	D	2.71	2.74	101 (2)	0.096	0.088	92 (2)	0.097	0.095	98 (1)
	E	2.73	2.55	93 (2)	0.048	0.049	102 (1)	0.098	0.100	102 (2)
3	*A*	1.36	1.49	110 (1)	0.048	0.045	94 (1)	0.049	0.046	94 (2)
	B	1.36	1.26	93 (2)	0.097	0.092	95 (1)	0.098	0.095	97 (2)
	C	2.75	2.54	92 (2)	0.049	0.047	96 (2)	0.049	0.051	104 (1)
	D	2.74	2.80	102 (4)	0.098	0.100	102 (2)	0.098	0.098	100 (1)
	E	2.74	2.75	100 (2)	0.049	0.050	102 (4)	0.098	0.099	101 (2)
4	*A*	2.62	2.62	100 (2)	0.094	0.093	99 (1)	0.094	0.094	100 (1)
	B	2.62	2.93	112 (3)	0.192	0.175	91 (1)	0.192	0.188	98 (3)
	C	5.17	4.98	96 (4)	0.093	0.087	94 (2)	0.093	0.098	105 (3)
	D	5.35	5.38	101 (2)	0.192	0.178	93 (1)	0.191	0.191	100 (2)
	E	5.35	4.88	91 (1)	0.096	0.098	102 (4)	0.191	0.176	92 (1)
5	*A*	2.67	2.55	96 (2)	0.096	0.104	108 (2)	0.096	0.101	105 (3)
	B	2.72	2.79	102 (3)	0.194	0.202	104 (2)	0.195	0.194	99 (2)
	C	5.45	5.17	95 (4)	0.098	0.092	94 (1)	0.098	0.100	102 (1)
	D	5.33	4.36	82 (2)	0.194	0.182	94 (1)	0.193	0.180	93 (3)
	E	5.32	5.05	95 (1)	0.095	0.086	90 (3)	0.193	0.187	97 (1)
6	*A*	2.75	2.34	85 (2)	0.099	0.086	87 (3)	0.099	0.089	90 (1)
	B	2.71	2.73	101 (1)	0.195	0.187	96 (1)	0.194	0.206	106 (2)
	C	5.33	5.77	108 (1)	0.096	0.088	92 (2)	0.095	0.086	91 (1)
	D	5.51	5.03	91 (2)	0.194	0.181	93 (1)	0.191	0.194	102 (2)
	E	5.44	5.58	102 (1)	0.098	0.093	95 (3)	0.195	0.205	105 (3)

1–6: meat dehydrated broths of different trade marks.
A, *B*, *C*, *D*, *E*: different added ratio concentrations of the three enhancers.
[a] Expressed in $g\,dm^{-3}$.
[b] The results are averages of three replicates.

shown in Table 2. The Código Alimentario Argentino (Argentine Food Code, CAA) [26] establishes the maximum allowed quantity that can be added in this kind of food: $8\,g\,dm^{-3}$ for MSG and $0.5\,g\,dm^{-3}$ for GMP and IMP. As can be seen in Table 2, samples 4, 5 and 6 contained a MSG concentration higher than the concentration recommended by the CAA. On the other hand, the CAA does not demand the information of the enhancers' added quantity in the product label, being the declaration of their presence the only requirement. In the analysed samples, the addition of the three enhancers is declared in sample number 6, whereas in the other five samples the presence of MSG and IMP is only declared. Bearing in mind the label information about the analytes presence/absence, the obtained results were in agreement with those specified by the manufacturers, as can be observed in Table 2.

3.4. Validation of the proposed method

To validate the proposed method, five different concentration ratios (*A*, *B*, *C*, *D* and *E*) were added to each sample, by considering the enhancers quantities that could be present in the samples. Table 3 shows the recovery percentage for each addition. The obtained values were satisfactory for this kind of products.

In order to corroborate the accuracy of the proposed method, a regression between the added concentrations and the recovered concentrations by MLR-SPA for the three analytes ($n = 90$) was carried out by applying the ordinary least squares (OLS) method. The obtained regression line was $y = 0.986x + 0.003$ with a correlation coefficient of 0.994. The estimated intercept and slope were compared with the theoretical values of 0 and 1, respectively, using the elliptical joint confidence region (EJCR) test [27].

As can be seen in Fig. 3, the EJCR, centred on the slope and intercept values of the regression line, included the theoretical point. These results indicate that there is no significant statisti-

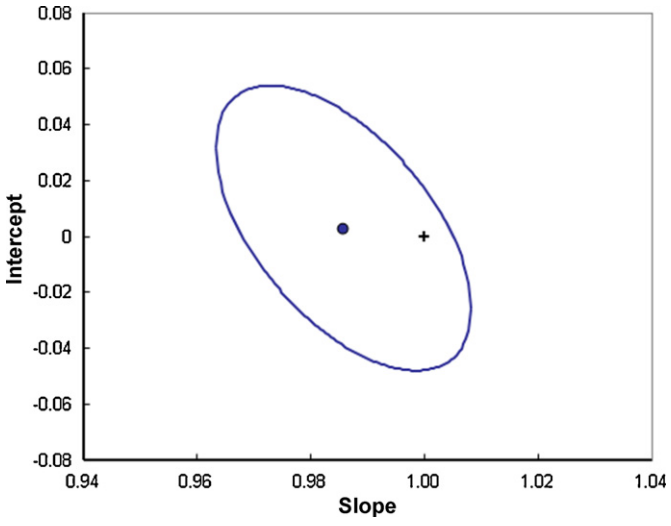

Fig. 3. Elliptical joint confidence region (EJCR) corresponding to the added concentrations and the recovered concentrations by MLR-SPA for the three analytes ($n = 90$). (+) The obtained values of slope and intercept. (●) The theoretical value of zero intercept and unity slope.

cal difference between the nominal values and the recovered ones, considering 5% as significance level.

4. Conclusions

The simultaneous determination of MSG, GMP and IMP in dehydrated broths is feasible applying MLR-SPA to the absorption spectral data. MLR-SPA models were constructed with only nine, six and four wavelengths for MSG, GMP and IMP, respectively. These models resolved successfully the serious spectral overlapping in samples with high concentration ratios of MSG/GMP and/or MSG/IMP. A relatively small calibration set, based on a full factorial design, was required.

The proposed flow system is very simple and rapid (sample throughput: 200 h^{-1}) due to none sample pre-treatment was achieved. Thus, it can be useful as a possible alternative method for the quality control analysis of this kind of food.

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