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Flow-through Spectrophotometric Sensor for the Determination of Aspartame in Low-Calorie and Dietary Products

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A very simple flow-through sensor is presented for the determination of the intense sweetener aspartame in low-calorie and dietary products. The sensor is implemented in a monochannel flow-injection system with UV spectrophotometric detection using a Sephadex CM-C25 cationic exchanger packed 20 mm high in a flow cell. This method is based on the transient retention of a cationic species of the sweetener on the solid phase when a pH 5.0 acetic acid sodium acetate buffer (0.01 M) is used as a carrier (2.6 mL^{-1} min). The carrier itself elutes the analyte from the solid support, regenerating a sensing zone. Aspartame was determined by measuring its intrinsic absorbance at 219 nm at its residence time without any derivatization. Calibration graphs were linear over the range of 5.0 – 600.0 μg mL^{-1} with an RSD of 0.55% (peak height). This sweetener was determined in several samples by measuring the height or peak area, obtaining recoveries ranging between 95 – 101% and 97.5 – 101%, respectively. The procedure was validated for its use in the determination of aspartame in low-calorie and dietary products, giving reproducible and accurate results.

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Aspartame, dipeptide *N*-L-α-aspartyl-L-phenylalanine-1-methyl ester (E-951), is a non-glucose, low-calorie product with a clean sweet taste, described as sucrose-like, and with a sweetness potency 160 – 220 times that of sucurose on a weight basis, with liquorice, caramel, and bitter flavors and a liquorice and bitter aftertaste, cooling effect and body.[1] It shows synergy with acesulfame-K or saccharin. Unlike other intense sweeteners, intestinal esterases hydrolyze aspartame to aspartic acid, methanol, and phenylalanine. The amino acids, in turn, are metabolized to provide 17 kJ g^{-1}. Thus, this sweetener provides energy, and is a nutritive sweetener; however, because of the intense sweetness of aspartame, the amount of energy derived from it is negligible. Metabolic studies show that aspartame is rapidly digested to its moieties, and that those are then absorbed, metabolized, and excreted by normal pathways, as with identical moieties in natural foods.[2] Numerous studies have shown that aspartame is non-toxic and safe for the general population (ADI 0 – 40 mg^{-1} kg body weight).[3]

Aspartame is used in a variety of beverages and foods, such as soft drinks, power soft drinks, fruit juice beverages, flavored milk, cocoa mixes, coffee, yoghurt, chewing gum and confections, gelatine desserts, frozen desserts, ice cream, chocolate, pudding, jams, topping mixes, cereals, sauces and dressings. It is also used in tabletop sweeteners, laxatives, pharmaceutical products and supplements. Aspartame cannot be used in products that are baked or fried, because it decomposes when exposed to high temperatures (~130°C);[4] nevertheless, it is possible to use heat-protected aspartame in those products.

The determination of aspartame in beverages, food products and pharmaceutical preparations has an economic and social relevance for both health and legal reasons. Different and not very selective spectrophotometric methods have been proposed for aspartame based on common reactions of amino acids, such as ninhydrin[5] or *N*-bromosuccinimide-metol-sulfanilamide.[6] Several ion-selective electrodes have been developed for aspartame: for example, a PVC membrane electrode based on the ion-pair of cetylpyridinium and 2,4,6-trinitrobenzene sulfonate for pharmaceutical formulations.[7] Monitoring the slow reaction of aspartame with 2,4-dinitro-1-fluorobenzene by means of a fluoride-selective electrode makes its kinetic determination possible.[8] Different enzymatic assays have been developed for aspartame based on different enzyme arrays: for example peptidase, aminotransferase and glutamate oxidase have been used, monitoring the oxygen consumption with a Clark electrode. This method shows a detection limit of 2.5×10^{-5} M,[9] or carboxyl esterase-alcohol oxidase with a detection limit of 5.0×10^{-8} M.[10] Other ways explored for enzymatic sensing of aspartame include the bienzyme system carboxy peptidase and L-aspartase in an ammonia electrode,[11-13] or phenylalanine decarboxilase and carboxy peptidase in a carbon dioxide electrode.[14]

Because most of the methods outlined above are not very selective, and because of the need to determine this sweetener in mixtures with other additives, such as preservatives and antioxidants, separating methods are most commonly used. Plane chromatography, especially TLC, has been widely used for the identification and/or determination of this intense sweetener, because the needed equipment is simple, inexpensive and flexible.[15] HPLC is the most popular system for separating and analyzing aspartame due to its multianalyte ability, and because it adjusts itself better to aspartame's physicochemicals. Although the use of a reverse phase with UV absorption at a fixed wavelength is the most common method,[16] postcolumn derivatization procedures have also been used.[17] Ion chromatography has been used as well because it offers an alternative to the organic or hydro-organic solvent-mediated HPLC methods.[18]

The reaction of ethylchloroformate in the presence of trifluoroethanol and pyridine with amino acids ethylates the amine and carboxyl group in one step, and has been used for

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aspartame derivatization and determination by GC-MS in the positive-ion chemical ionization mode.[19] Alternatively, the determination of aspartame based on its thermal decomposition products using pyrolysis GC-MS has been proposed.[20] Different modes of capillary electrophoresis have been applied to achieve the separation of aspartame and other additives. Major protocols include CZE,[21] MEKC,[22] and CITP.[23]

Different flow-injection methodologies have been used for aspartame. As an example, its known reaction with ninhydrin has been proposed.[24] Also, a solid-phase reactor with copper(II) phosphate, based on the release of Cu(II) ions by the formation of soluble Cu(II)-aspartame complex and the reaction alizarin red S, makes the indirect determination of aspartame possible.[25] A chemiluminometric FIA procedure for aspartic acid, created by the hydrolysis of aspartame, has been proposed, although it has not been applied to food samples.[26]

In the present work we determined aspartame using a flow-injection approach based on such intrinsic properties as UV absorption and a transient retention in a solid phase placed in a flow-through cell. In this way, it is possible to develop a very simple and inexpensive methodology for the analysis of aspartame that does not need derivatization reactions, or complicated flow schemes using a monochannel manifold with a very low residence time. Under the experimental conditions proposed for this determinations, the procedure has sufficient selectivity for the intended application, *i.e*. low-calorie and dietary products. In order to assess the usefulness of the proposed flow-through sensor for aspartame, it was applied to its determination in low-calorie and dietary commercial products.

Experimental

Reagents and chemicals

All of the chemicals used were of analytical-reagent grade. Reverse-osmosis type quality water (Milli-RO 12 plus Milli-Q station from Millipore, Billerica, MA, USA) was used throughout. Aspartame stock solutions (1000.0 μg mL^{-1}) in water were prepared by exact weighing (Sigma Chemical Co. Inc., St. Louis, MO, USA). The solution was spectrophotometrically stable when protected from light and kept in a refrigerator at 5°C for up two months. Working solutions were prepared by appropriate dilutions with water. Stock solutions (5000.0 μg mL^{-1}) in water were prepared by exact weighing of glucose, fructose, sucrose, maltose, sodium citrate, sodium ascorbate (all from Panreac, Barcelona, Spain), sodium cyclamate, saccharin, acesulfame-K and lactose (Sigma Chemical Co. Inc., St. Louis, MO, USA).

C-18 bonded silica (Waters, Millipore Corporation, Milford, MA, USA) with average particle sizes of 55 – 105 μm, ion exchangers Sephadex DEAE A-25, Sephadex CM C-25, Sephadex QAE A-25 and hydrophilic adsorbent Sephadex G-25, G-15 (Sigma Chemical, St. Louis, MO, USA) were tested as a solid support. As a carrier buffer, citric acid/sodium citrate (Panreac, Barcelona, Spain) was used.

As carrier solutions, the buffers acetic acid/sodium acetate, benzoic acid/sodium benzoate, phosphoric acid/sodium dyhydrogenphosphate and citric acid/sodium citrate (Panreac) of different concentrations were used.

Apparatus, software and flow diagram

Absorption measurements were made with a Hewlett Packard HP-8453 diode array spectrophotometer (Norwalk, CT, USA), interfaced to a Pentium MMX 200 microcomputer *via* an HP IB interface board, and equipped with a Hellma 138-QS flow glass

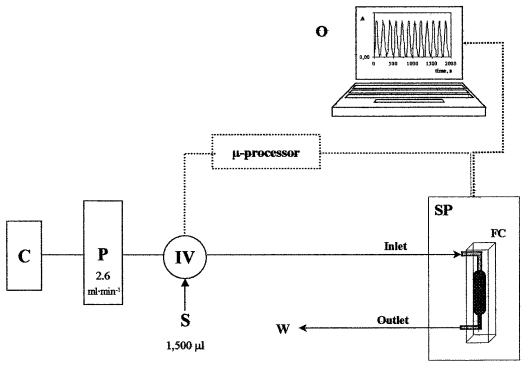

Fig. 1 Manifold. P, pump; C, carrier; IV, injection valve; S, sample loop; SP, spectrophotometer; W, waste; FC, flow cell.

cell with 1 mm light path. A flow-through cell with a 50 μL inner volume was packed with a solid support by introducing it as a water suspension with the aid of a syringe; it was then placed in the cell compartment of the spectrophotometer with the aid of a plastic spacer that was painted black. A Hewlett-Packard HPLC Model 1090 equipped with fluorescence detector (Waldbronn, Germany) and connected to a Pentium 200 PC fitted with ChemStation HPLC^{3D} (Win95) software were used. A Crison Digit pH-meter equipped with a combined glass-calomel electrode was used.

The flow analysis set-up (Fig. 1) consisted of a Gilson Minipul-2 four channel peristaltic pump working at a constant flow-rate and three variable volume Rheodyne 5041 Teflon rotary valves controlled electromechanically.[27] Both were connected with the spectrophotometers cited above to a conventional microprocessor that controlled the pump, the valves and the spectrophotometer using software designed by us, written in BASIC language. This set-up was interfaced using RS-232C interfaces to a microprocessor. PTFE tubing (Omnifit, Cambridge, England) (0.8 mm i.d. and 1.6 mm o.d.) and various end-fittings and connectors of different diameters (Omnifit) were used.

As software for the acquisition and manipulation of spectral data, the UV visible Chemstation software package supplied by HP was used. Software programs used for the measurements of FIA peak and area were: Statgraphics software package, ver.6.0 STSC Inc. Statistical Graphics Corporations, Englewood Cliffs, NJ, USA, 1993, and Grams/386 software package ver.1.0, Add. Galactic Industries, Salem, USA and software program used for validation purposes was Data Leader software package, Beckman, Fullerton, CA, USA, 1987.

Procedures

Basic procedure. A sample solution (1500 μL) containing between 5.0 – 600.0 μg mL^{-1} of aspartame, with the same pH as the carrier, was inserted into the carrier stream (pH 5.0 acetic acid–sodium acetate buffer 0.01 M to pH 5.0) at a flow-rate of 2.6 mL min^{-1}. The aspartame was retained in the solid phase and the absorbance was measured at 219 nm upon reaching a flow cell containing Sephadex CM C-25. Once the maximum absorbance was reached, the system was conditioned by passing the carrier for 10 s until the absorbance value returned to the baseline. The relationship between the concentration and height (measured in absorbance units) or peak area was established by conventional calibration with external standards.

Reference procedure. As reference method the HPLC method proposed by Lawrence[17] was used. A 5 μm C18 silica into a

100 × 2.1 mm column and acetonitrile/phosphate buffer 0.1 M pH 4.5 (20:80% v/v) at a constant flow rate of 1.0 mL min^{-1} were used as a stationary phase and a mobile phase, respectively. Chromatograms were obtained at a wavelength of 217 nm. In order to obtain the calibration function, 5 different concentration levels and 3 replicates of each one of the standard solutions were injected into the chromatograph using the peak area as the analytical parameter.

Treatment of samples. For the analysis of aspartame in chewing gum (composition: xylitol, sorbitol, mannitol, maltitol syrup, base gum, malic acid, citric acid, sodium citrate, aroma, arabic gum, lecithin, titanium dioxide, aspartame, butylated hydroxytoluene, and carnauba wax) an adequate amount (typically 15 g) was weighed, cut into small chunks with a blade, and then treated with 150 mL of water heating gently and with magnetic stirring to favor sweetener extraction. The solid was filtered first through filter paper and then through a 0.45-μm Millipore filter, and finally the solution volume was brought up to 200 mL with water.

For analysis in lemon flavor sugarless sweets (isomalt, citric acid, natural aroma, aspartame) an adequate amount (typically 40 g) was weighed and thoroughly crushed in a glass mortar then dissolved in water (75 mL) with the aid of an ultrasonic bath and filtered through a 0.45-μm Millipore filter; finally, the solution volume was brought up to 100 mL with water.

For analysis in strawberry dietetic jam (strawberry, water, sorbitol syrup, sodium cyclamate, aspartame, pectin, sodium sorbate, and Ponceau 4R) an adequate amount (typically between 100 and 150 g) was weighed and treated with 200 mL of water with magnetic stirring for 30 min. The suspension was filtered first through filter paper and then twice through a 0.45-μm Millipore filter to remove any turbidity. The solution volume was then brought up to 250 mL with water.

For the analysis of an iron supplement (powdered skimmed milk, sugar, decaffeinated coffee extract, cereals, chicory, xanthan gum, ascorbic acid, iron and sodium pyrophosphate, aspartame, aroma, folic acid, B12 vitamin) and hipocaloric custard (calcium caseinate, inulin, hydrogenated vegetal oil, starch, glucose syrup, xanthan gum, aroma, mono- and di-glicerides of fatty acids, mineral complex, aspartame, vitaminic complex, ascorbyl palmitate and ascorbyl stearate) adequate amounts (typically 20 and 5 g, respectively) were treated in the same way as the dietetic jam, but using 75 mL of water for extraction purposes, and levelling off to 100 mL with water.

All of the samples were adjusted to pH 5.0 before being injected in the flow system.

Results and Discussion

Aspartame is an absorbing species in the UV region with an absorption maximum at 216 nm in a water solution (molar absorptivity ε: 5.73 × 10^2 L mol^{-1} cm^{-1}) and 219 nm in Sephadex CM C-25 solid phase (ε: 1.89 × 10^4 L mol^{-1} cm^{-1}). To obtain adequate experimental conditions for the retention and elution of aspartame, different influencing parameters were individually studied and optimized. The variables influencing the system can be divided into three groups: those related to the retention-elution unit, chemical and FIA variables.

Variables of retention-elution unit

Taking into account the dipeptide nature of aspartame and its isoelectric pH value (5.25), we tested both ion exchangers and adsorbents as solid-phase types in the flow-through cell in order to obtain a transient retention of aspartame. Namely, we tested Sephadex DEAE A-25, Sephadex QAE A-25, and Sephadex CM C-25 ion-exchangers; hydrophilic Sephadex G-25, and Sephadex G-15; and hydrophobic C_{18} silica adsorbents.

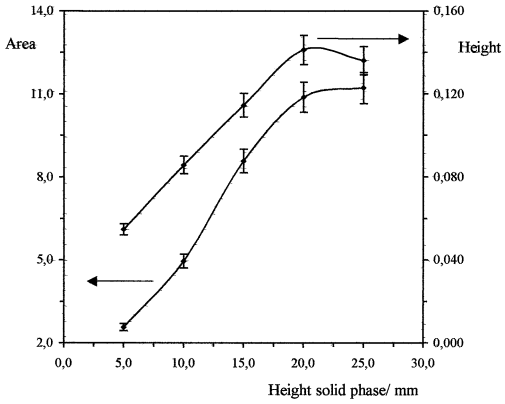

Fig. 2 Influence of the height of Sephadex CM C-25. Carrier, pH 5.0 acetic acid/sodium acetate buffer; sample pH, 5.0; flow-rate, 1.8 mL min^{-1}; loop sample, 1500 μL; aspartame, 200 μg mL^{-1}.

Hydrophilic Sephadex G-15, and Sephadex G-25 and hydrophobic silica C_{18} adsorbents give off very low analytical signals, because of their poor retention and elution on solid phases, irrespective of the carrier pH. Anionic exchangers (Sephadex QAE A-25 and DEAE A-25) retain aspartame at pH > 5.25, but the sampling frequency is very low, because of their strong retention, and the need to use an eluent to regenerate the support. On the contrary, cationic exchangers (Sephadex CM C-25) at pH < 5.25 show good retention and elution of the positively charged analyte by the carrier, itself. We chose Sephadex CM C-25 as solid phase to fill the flow-through cell, because it gives off higher signals and sampling frequency.

Both the absorbance (peak height) and the peak area increase with an increase in the level (from 5 to 25 mm) of the solid in the flow cell used (Fig. 2). With a height above 10 mm, the radiation beam only passes through the solid phase, but if the level is lower, the beam passes partially through the solution. The increase in the height and area to 20 mm is due to an increase in the retention because of the higher amount of solid phase in the cell. A Sephadex CM C-25 height of 20 mm, measured from the glass-wool plug in the outlet, was selected. The analytical signals remain constant for higher levels of the solid phase in the flow cell.

Chemical variables

The influence of the pH on the retention and elution of aspartame was studied in the range 2 – 10, adjusted with HCl or NaOH, for the solid phase selected. For the cationic exchanger Sephadex CM C-25, a high transient retention was observed when the pH of the carrier was of medium acidity, especially between 4.0 and 6.0, but fixing dramatically decreased for 4.0 < pH < 6.0 conditions. This could be due in the pH > 6.0 case to deprotonation of the carboxylic group of aspartame and Donnan exclusion of the anionic analyte from the ion-exchanger, and in the pH < 4.0 case to the competition of protons with the cationic form of the analyte for anionic sites of the ion-exchanger. Figure 3 indicates that between pH 4.5 and 5.5, *i.e*. near the isoelectric point of the analyte, both the height and the peak areas are higher. We selected 5.0 as the working pH of the carrier/eluent using Sephadex CM C-25 as a solid support for reasons of sampling frequency and sensitivity.

For the carrier we tested several buffers, namely acetic acid/sodium acetate, benzoic acid/sodium benzoate,

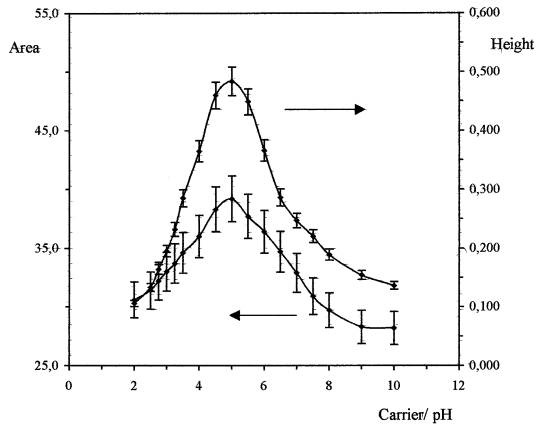

Fig. 3 Influence of the carrier pH on the height and peak area. Solid phase, Sephadex CM C-25; flow-rate, 2.6 mL min^{-1}; loop sample, 800 µL; aspartame, 600 µg mL^{-1}.

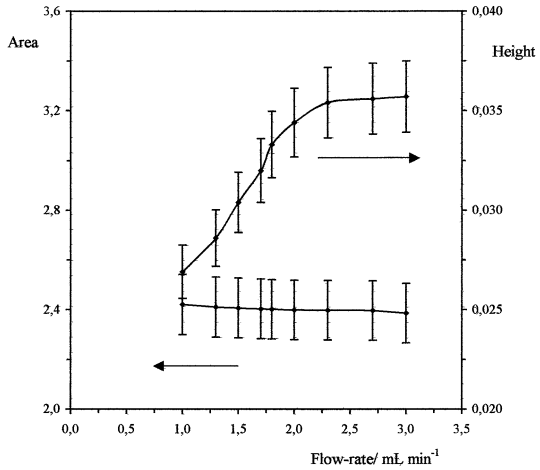

Fig. 4 Dependence of the flow-rate on signals. Solid phase, Sephadex CM C-25; carrier, acetic acid/sodium acetate buffer pH 5.0; sample pH, 5.0; loop sample, 1000 µL; aspartame, 35 µg mL^{-1}.

dihydrogenphosphate/monohydrogenphosphate and citric acid/sodium citrate. We selected an acetic acid/sodium acetate buffer of pH 5.0, because it increases both the height and the area of the peaks around 25% more than the rest. Additionally, an increase in the ionic strength with the same buffer increases the signal up to 0.01, then decreasing considerably (30% from 0.01 to 0.06) due to a higher competence of sodium by the ionic sites. The concentration of the buffer was adjusted to 0.01 M.

The pH of the samples injected into the flow system was an important variable with regard to obtaining a good FIA record, because this modified the retention of aspartame in the solid phase due to a modification of the pH in proximity to the sample plug, which modified the absorbance value. Thus, the samples were conditioned with the same pH buffer as that used in the carrier.

FIA variables

The shape of FIA for aspartame, both the peak width and the height, depended on the flow-rate and sample volume. An increased flow rate (1.0 – 3.0 mL min^{-1}) resulted in decreased residence times, while maintaining the area values and an increase in the peak heights up to a maximum of 2.6 mL min^{-1},

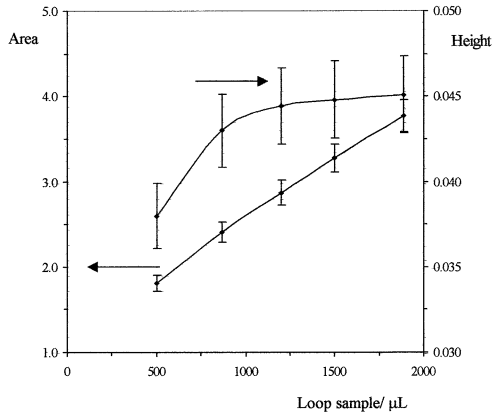

Fig. 5 Influence of the size of the sample loop. Solid phase, Sephadex CM C-25; carrier, acetic acid/sodium acetate buffer; pH, 5.0; sample pH, 5.0; flow-rate, 2.6 mL min^{-1}; aspartame, 50 µg mL^{-1}.

and remaining constant from there on. The plateau in the peak heights is due to saturation of the ion-exchanger. The peak area remains nearly constant because at high flow-rates, the band width decreases and at low flow-rates the opposite effect occurs. The appearance of a plateau in the peak compensates for the drop in the band width, with the peak area, and therefore remaining constant (Fig. 4). We selected a flow-rate of 2.6 mL min^{-1}, since the compensation between fixing and auto-elution of aspartame means a compromise between the sample frequency and the sensitivity.

The influence of the size of the sample loop used, *i.e.*, the sample volume, was studied in the range of 500 – 2000 µL (Fig. 5). The analytical signals increased linearly up to 1500 µL as the result of a larger amount of analyte in the flow system, remaining constant for higher volumes due to ion-exchanger saturation. The breakthrough concentration for the flow-through cell used filled with Sephadex CM C-25 (68 mg) was 600 µg mL^{-1} of aspartame for a volume of 1500 µL. A volume sample of 1500 µL was used for subsequent experiments. In the established conditions, it was possible to achieve a sampling frequency of 24 h^{-1}.

Analytical features

As analytical signals, the height or areas of the FIA peaks could be used. The calibration graphs were linear for aspartame in the range of 5.0 – 600.0 µg mL^{-1} using the areas or peak height as an analytical parameter, respectively. An adjustment of those analytical data was carried out by linear regression, with the lack-of-fit test being applied to test the linearity, using three replicates of each standard and five standards for each calibration graph.[28] The standard deviation of the background signal measured for the blank, which is necessary for estimating the IUPAC detection limit (K = 3) and the quantification limit (K = 10),[29] was taken as the average of ten determinations, and noted as RSD units. Table 1 shows how the use of areas makes better sensitivity and reproducibility possible.

Effect of foreign species

The effect of several common concomitants of aspartame in low-calorie and dietary commercial products was studied in order to assess the potential for this flow-through optosensor. Different synthetic sample solutions containing 100 µg mL^{-1} of aspartame and variable excess amounts of each foreign species were studied. The maximum concentration of foreign species

Table 1 Analytical parameters for the aspartame method

Parameter	Unit	Area	Height
b	μg^{-1} mL	0.066	8.0×10^{-4}
S_b	μg^{-1} mL	1.6×10^{-3}	2.0×10^{-5}
a	—	0.055	5×10^{-3}
S_a	—	0.323	4×10^{-3}
r	—	0.999	0.999
PL	%	57.30	65.69
LDR	$\mu g\ mL^{-1}$	5.0 – 600.0	5.0 – 600.0
DL	$\mu g\ mL^{-1}$	0.82	0.75
QL	$\mu g\ mL^{-1}$	2.75	2.50
RSD	%	0.54	0.55

b, Slope; S_b, standard deviation of slope; a, intercept; S_a, standard deviation of intercept; r, correlation coefficient; PL, probability level of lack-of-fit test; LDR, linear dynamic range; DL, detection limit; QL, quantification limit; RSD, relative standard deviation.

Table 2 Recovery study of aspartame in low-calorie and dietary sample

Sample	Added/ mg kg^{-1}	Found/mg kg^{-1}		Recovery, %	
		Height of peak	Area of peak	Height of peak	Area of peak
Chewing gum	50.0	48.5	49.0	97.0	98.0
	200.0	195.0	196.0	97.5	98.0
Hipocaloric custard	50.0	49.0	49.0	98.0	98.0
	200.0	198.0	197.0	99.0	99.0
Sugarless sweets	50.0	50.5	50.2	101.0	100.4
	200.0	201.0	202.0	100.5	101.0
Strawberry dietetic jam	50.0	50.2	50.1	100.4	100.2
	200.0	199.0	200.5	99.5	100.3
Iron supplement	50.0	48.0	49.0	96.0	98.0
	200.0	190.0	195.0	95.0	97.5

producing an error of ≤ 5% was taken as the tolerance level. Interfering substances, such as cyclamate, were tolerable up to 1200 mg/l, 1400 for acesulfame, 1500 for sucrose, lactose and maltose, 1600 for saccharin, 1800 for fructose, 3000 for glucose, and 4000 for citrate and ascorbate. The tolerance is slightly higher by using the height than the area as the analytical parameter.

No interference was caused by glucose, sucrose, lactose, maltose, fructose, sodium citrate or sodium ascorbate, even when they were present in concentrations much higher than those commonly found in the low-calorie and dietary products analyzed. Acesulfame and saccharin, usually present in mixtures with aspartame, because they exhibit synergy, did not interfere in ratios higher than the usual ratio in commercial products.

Applications

The flow-through sensor proposed was applied to the determination of aspartame in low-calorie and dietary commercial products that indicate its presence on their labels. Prior to the analysis, as described under *Basic procedure*, identification was carried out with a quality color test using ninhidrin as the reagent[30] and by comparing the retention time of the problem with that of a standard solution using the HPLC reference method.[17]

From the different extracting procedures proposed, we selected water for the extraction of aspartame in low-calorie and dietary commercial products.[31] We demonstrated that the extraction step used gives quantitative results through the addition of known amounts of aspartame. The recovery experiments produced results ranging between 95 – 101% and 97 – 101% using the height and the area, respectively (Table 2).

The quality and accuracy of the proposed methods for aspartame were tested using a statistical protocol based on standard addition methodology.[32,33] This validation methodology involves three different calibrations: a calibration with standards (SC), a calibration by mean standard addition (AC) and a Youden calibration (YC). The variances of the calibrations SC and AC (variances of slopes and intercepts and the standard deviation of the linear regression) were compared statistically, accepting them as being equal if the P-values were > 5%. Next, the analyte content in a real sample obtained from SC was statistically compared to the same content obtained from AC. If the P-value was > 5%, it was concluded that the method was accurate for the determination of the analyte in the analyzed sample. From the Youden calibration, we could know and evaluate the potential systematic error. The P-values of the statistical comparison between the results obtained with AC and SC for each sample demonstrate that the method can be used for the determination of aspartame in these kinds of samples. The results are given in the Table 3. Additionally, the results obtained by our method have been validated by a statistical comparison (P-value) with a reference HPLC method[17] (Table 4). The proposed method shows several advantages against the usual HPLC procedure:[17] it uses very simple, fast instrumentation that is cheaper, and has a similar precision. The disadvantage is that the other procedure has a better resolution than our procedure, as expected with HPLC, although ours is sufficient for a determination. Compared with other proposed FIA procedures, ours is more sensitive and more precise than most published procedures but, as could be expected, its sampling frequency is lower (36 – 70 h^{-1}) against 24 h^{-1} in our case.

In conclusion, the proposed flow-through spectrophotometric sensor is simple, rapid, inexpensive, and as a chemical only needs the carrier pH 5.0 acetic acid/sodium acetate buffer. The retention of aspartame as a cation increases its selectivity over other intense anionic sweeteners usually present in blends with aspartame, such as acesulfame or saccharin. The procedure has been validated for use in the determination of aspartame in low-calorie and dietary commercial products, giving reproducible and accurate results.

Acknowledgements

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Table 3 Validation of the procedure for aspartame by addition standard calibration methodology

Sample	Parameter	SC	AC	CY
Chewing gum	b	8.0×10^{-4}	8.0×10^{-4}	6.9×10^{-3}
	n	15	10	5
	S_b	2.0×10^{-5}	5.7×10^{-5}	5.3×10^{-4}
	S_a	4.0×10^{-3}	6.0×10^{-3}	6.5×10^{-4}
		$S_p = 3.0 \times 10^{-5}$	$b_p = 8.0 \times 10^{-4}$	
	a	5×10^{-3}	0.018	0.0108
	a'	5×10^{-3}	0.018	—
	YB	—	—	—
		$t_b = 1.376$		
	C_x	25	25	
		$t_c = 0.649$		
Sugarless sweets	b	8.0×10^{-4}	8.0×10^{-4}	0.046
	n	15	10	4
	S_b	2.0×10^{-5}	2.9×10^{-5}	2.0×10^{-3}
	S_a	4.0×10^{-3}	3.0×10^{-3}	2.9×10^{-3}
		$S_p = 2.4 \times 10^{-5}$	$b_p = 8.0 \times 10^{-4}$	
	a	5×10^{-3}	0.061	0.020
	a'	5×10^{-3}	0.061	—
	YB	—	—	0.015
		$t_b = 1.064$		
	C_x	228	228	
		$t_c = 1.640$		
Hipocaloric custard	b	8.0×10^{-4}	8.0×10^{-4}	6.9×10^{-3}
	n	15	10	4
	S_b	2.0×10^{-5}	6.1×10^{-5}	5.3×10^{-4}
	S_a	4.0×10^{-3}	0.013	6.0×10^{-4}
		$S_p = 4.1 \times 10^{-5}$	$b_p = 8.0 \times 10^{-4}$	
	a	5×10^{-3}	0.02	0.0120
	a'	5×10^{-3}	0.02	—
	YB	—	—	—
		$t_b = 0.129$		
	C_x	25	25	
		$t_c = 0.962$		
Strawberry dietetic jam	b	8.0×10^{-4}	8.0×10^{-4}	0.028
	n	15	10	4
	S_b	2.0×10^{-5}	5.6×10^{-5}	3.0×10^{-3}
	S_a	4.0×10^{-3}	4.0×10^{-3}	4.0×10^{-3}
		$S_p = 4.0 \times 10^{-5}$	$b_p = 8.0 \times 10^{-4}$	
	a	5×10^{-3}	0.094	0.012
	a'	5×10^{-3}	0.094	—
	YB	—	—	—
		$t_b = 0.623$		
	C_x	505	505	
		$t_c = 0.996$		
Iron supplement	b	8.0×10^{-4}	8.0×10^{-4}	0.102
	n	15	10	4
	S_b	2.0×10^{-5}	4.9×10^{-5}	9.8×10^{-3}
	S_a	4.0×10^{-3}	0.012	0.018
		$S_p = 3.4 \times 10^{-5}$	$b_p = 8.0 \times 10^{-4}$	
	a	5×10^{-3}	0.08	0.06
	a'	5×10^{-3}	0.08	—
	YB	—	—	0.056
		$t_b = 1.232$		
	C_x	386	386	
		$t_c = 1.151$		

SC, Standard calibration; AC, addition calibration; YC, Youden calibration; b, slope; b_p, average slope; a, intercept; a', corrected intercept; S_b, standard deviation slope; S_a, standard deviation intercept; S_p, average standard deviation of slopes; YB, Youden blank; C_X, calculated concentration for standard and addition calibration; t_b, t-student value of comparation slopes; t_c, t-student value for accurate concentration.

In all cases, $t_b < t_{tab}$ (α: 0.05, 21) = 1.721 and $t_c < t_{tab}$ (α: 0.05, 22) = 1.717.

Table 4 Determination of aspartame in low-calorie and dietary samples by proposed and reference procedures (mg kg^{-1})

Sample	Proposed method[a]	HPLC[a]	P-value, %
Chewing gum	3286 ± 30	3280 ± 27	82.95
Hipocaloric custard	1211 ± 15	1226 ± 15	25.58
Sugarless sweets	571 ± 5	570 ± 4	92.39
Strawberry dietetic jam	1009 ± 13	1010 ± 14	97.03
Iron supplement	1927 ± 26	1921 ± 20	37.02

a. Average for three determinations ± SD.

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