A Combined Experimental Green Flow-Injection Procedure and Computational Analysis to Determine Amino Acids

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Authors' contributions

Author SE suggested the idea of the assay and designed the proposed analytical method. Author SE supervised the whole study. Authors TAF and ANE carried out the molecular modeling and computational analysis. Authors MK carried out the analytical experimental work, analyzed the data statistically, participated in the results and discussion, and preparing the manuscript. Author WZ participated in the experiment work, assay design, results and discussion. Author MAK participated in supervision and drafted the manuscript. All authors read and approved the final manuscript.

Research Article

ABSTRACT

Aim: Methods of pollution reduction and human health protection would be more successful if the fields of green analytical chemistry and computational analysis could

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come together. This overlap would help to minimize, or even eliminate the use and generation of hazardous substances. The goal of this study was to identify a way of further developing the fields of green analytical chemistry and computational analysis.

Place and Duration of Study: Misr International University, between April 2011 and November 2012.

Methodology: A single-line flow-injection (FIA) packed-reactor technique has been developed for the analysis of amino acids. Amino acids were injected into a flowing stream of distilled–deionized water, carried through the packed reactor of CuO followed by UV detection. The flow rate was 1.25 mL/min and the column temperature was ambient (25 °C). New mathematical models for correlations of the physicochemical parameters of the Cu^{II}-amino acid complexes with analytical parameters such as lower limit of detection (LOD), lower limit of quantitation (LOQ) as well as lower limit of linearity range (LL) and higher limit of linearity range (HL) were employed.

Results: The developed procedure can accommodate sample frequency of 60 sample/hrand a recovery% ranging from 98.800% to 100.260%. A good accordance was observed between the computed values of LOD, LOQ, LL and HL and the observed results.

Conclusion: An extremely simple environmentally friendly analytical procedure including packed-reactor FIA technique has been developed and evaluated for the routine quality control of amino acids. The developed method has excellent sensitivity and accuracy, as well as, it required relatively simple and inexpensive instrumentation. A good correlation between the computed values and the FIA results suggests that the mathematical models are reliable, and hence provide a theoretical basis for the determination of amino acids.

1. INTRODUCTION

Amino acids play an important role in maintaining the immune system, and along with their derivatives, regulate metabolic pathways by synthesizing proteins through biological processes [1]. As most common amino acids are not readily detected by UV-Vis spectrophotometric techniques, varieties of successful analytical procedures for their determination have been developed. These procedures require efficient and effective reagents that can modify the behavior of amino acids and allow their detection. These reagents included, *o*-phthaldialdehyde (OPA) [2], phenylisothiocyanate (PITC) [3-4], 1-dimethylaminonaphthalene-5-sulphonyl chloride (Dansyl-Cl) [5], 1-fluoro-2,4-dinitrobenzene (FDNB) [6-7], and ninhydrine [8]. Despite of the popularity of these reagents, various disadvantages have been reported [9]. The instability of the OPA derivatives lowers the reproducibility of the results when using manual derivatization process. Dansyl-Cl suffers from a drawback, that the reaction should be proceeded in dark. FDNB undergoes decomposition in methanol-water solution when exposed to daylight. PITC is highly toxic and the reaction condition is very harsh. Alternatively, amino acids can be analyzed by pre-column derivatization and then separated by reversed-phase high-performance liquid chromatography (HPLC) [10-14].

The flow injection analysis (FIA) technique became a versatile instrumental tool that contributed substantially to the development of automation in pharmaceutical analysis due to its simplicity, low cost and relatively short analysis time. Several FIA manifolds for the

determination of amino acids have been reported. Spectrophotometric FIA method has been reported for the determination of cysteine, *N*-acetyl cysteine and glutathione based on the reduction of Fe(III)/ferricyanide. The in situ reduced ions were reacted with unreduced portion of ferricyanide/Fe(III) to form soluble Prussian blue [15]. Giannousios and Papadopoulos [16] determined cysteine by FIA procedure based on the inhibitory effect of cysteine in the oxidation reaction of 4-methoxy-1,2-diamino benzene dihydrochloride by hydrogen peroxide in the presence of iron[III] as a catalyst. The reaction of methionine, cysteine and glycine with OPA has been utilized for their determination by FIA [17-18]. Optical flow-through cell-detector with incorporated transparent chemosensitive layer of Prussian blue has been applied in FIA system for cysteine analysis [19]. Chemoluminescence FIA methods for the determination of amino acids have been described [20-24]. Oxidation of cysteine with thallium[III] has been proposed for its determination by FIA with fluorimetric detection [25]. Cysteine has also been analyzed by FIA with bioamperometric [26] and potentiometric detectors [27]. Amino acids were determined without derivatization using FIA followed by electrospray ionization and tandem mass spectrometry [28]. The use of solid-phase reactors incorporated into FIA manifolds may offer certain advantages over homogeneous systems. Reagent consumption is greatly decreased and the system is simplified with fewer junctions for mixing the reagents, sample and carrier streams. A packed-bed reactor of Co[II] ions entrapped in a polymeric material has been used in FIA determination of cysteine. The released Co-complexed was monitored at 360 nm [29]. Copper carbonate has been used as a solid reactor for the determination of glycine by an indirect FIA-atomic absorption spectrometry [30]. A packed-bed reactor of Cu[II] ions has also been utilized to determine amino acids. The released Cu-complex reacted with zincon in a basic medium producing a blue color that was monitored at 600 nm [31]. Amperometric determination of tyrosine, in strongly alkaline solution, by a carbon ceramic electrode modified with CuO nanoparticles has been reported [32]. A post-column solid-state reactor of CuO in the HPLC system for the analysis of amino acids has been reported [33]. In recent years, more strict regulation related to the quality control of pharmaceuticals led to increasing demands on automation of the analytical assays carried out in appropriate control laboratories. Automated analysis promise a major reduction in manual laboratory working time, and correspondingly a significant cost reduction. Furthermore, the results tend to be more precise and reproducible. Also, it is essential to take into consideration the requirements of green chemistry while working out new analytical methods [34-35].

Computational chemistry and molecular modeling is a fast emerging area which is used for the modeling and simulation of small chemical and biological systems in order to understand and predict their behavior at the molecular level. It has a wide range of applications in various disciplines of engineering sciences, such as materials science, chemical engineering and biomedical engineering. Computational chemistry uses principles of computer science to assist in solving chemical problems. It uses the results of theoretical chemistry, incorporated into efficient computer programs, to calculate the structures and properties of the molecules and solids. Considering the importance of this discipline, computational analysis has been extensively used to understand the mechanisms, thermodynamics and kinetics of the reactions, drug-receptor interactions and metal–metal coordination in the complexes. It is also cost-effective tool in evaluating hazards without actually testing the chemicals and shows a lot of promise, which could be a powerful tool for chemical analysis. [36-38]

Therefore, our study was involved in a research effort aiming to develop and validate green analytical procedure using a packed reactor FIA for the analysis of L-cysteine (L-cys), L-proline (L-pro), L-isoleucine (L-iso), L-alanine (L-ala), L-methionine (L-met), L-arginine (L-arg), L-glycine (L-gly) and L-valine (L-val). The method is based on the reaction of amino

acids with a packed reactor of finely powdered CuO in a single line FIA manifold. The resulting Cu-amino acids complexes are measured spectrophotometrically.The second part of the current study investigated the use of computational models to analyze amino acids and determine their lower limit of detection (LOD) and quantitation (LOQ) as well as lower limit (LL) and higher limit (HL) of the linearity range. The new models are compared with experimentally FIA observed results.

2. MATERIALS AND METHODS

2.1 Apparatus

A single line FIA manifold was employed in this study. Samples were loaded into Rheodyne 7125-081 injection valve, equipped with a 20-µl sample loop (Rheodyne, Berkeley, CA, USA), and then injected into an inert carrier stream of distilled-deionized water. A model Agilent 1100 Series Iso pump G1310A pump (Agilent Technologies, USA) was used to deliver the carrier solution at a flow rate of 1.25 mL/min. The reaction of the tested amino acids with Cu^{II} occurs during the flow of the carrier stream containing the analytes through the packed reactor of CuO (10 mm x 4.6 mm i.d.). A model Agilent 1100 Series VWD G1314A UV/Vis detector was used for the detection of the derivatization products of amino acids at 254 nm. The packed reactor was inserted between the injection valve and detector. The i.d. of the connecting tubes was 0.5 mm and a minimum distance (5 cm) was achieved between the injector and the reactor and between the reactor and the detector. Data acquisition was performed on Agilent LC ChemStation software. All experiments were carried out at ambient temperature (25 ºC).

2.1.1 Column packing

A definite weight of CuO (4 g) was suspended in isopropyl alcohol and degassed under vacuum with continuous stirring for 10 min. A stainless-steel cylinder (50 mm x 7.5 mm i.d.) was used as a reservoir for the packing materials. This reservoir was connected to a column (10 mm x 4.6 mm i.d.) and the suspended CuO supplied from it was packed into the column with the aid of an HPLC pump at flow rate of 5 mL/minwith ethanol as a purge solvent (10 min). The cylinder was then disconnected and a mixture of ethanol and distilled-deionized water (1:1) was passed through the column at a flow rate of 1 mL/minfor further 10 min. The column was equilibrated with distilled-deionized water at a flow rate of 1.25 mL/minfor 30 min.

2.2 Chemicals

Analytical grade chemicals were used without any additional purification. Distilled-deionized-distilled water in glass was used for the preparation of the standard solutions of the amino acids and as a flowing stream. L-Cys, L-Pro, L-Iso, L-Ala, L-Met L-Arg, L-Gly and L-Val were obtained from Aldrich (Milwaukee, Wisconsin, USA). Copper oxide (50 µm) was purchased from Al-Nasar Co., (Cairo, Egypt).

3. RESULTS AND DISCUSSION

The use of a packed reactor, in the described FIA assembly, as an alternative to existing reagent solutions for the determination of amino acids is dependent on optimizing the system to achieve maximum peak height, with a shorter residence time and minimum

dispersion. On the basis of the experimental results, it can be stated that the flow rate and the column dimensions are the key parameters as they affect the extent of the residence time between the solid surface of CuO and the moving sample zone solutions as well as peak width.

3.1 Flow Rate

Fig. 1 shows the negative effect of the use of increasing carrier flow rate on the reaction yield of amino acids with CuO packed reactor. The effect of the flow rate was checked over the range of 0.5–2.5 mL/min (at 0.25 mL/min interval). It was found that, the detector response in case of L-Cys and L-Arg was not significantly affected by the flow rate. On the other hand, the magnitude of decrease in detector response was more evident in case of L-Val and L-Pro. The negative effect of the use of increasing carrier flow rate on the reaction yield of L-Gly, L-Iso, L-Ala, and L-Met, with CuO was also observed. When the flow rate was reduced from 2.5 to 0.5 mL/min, a maximum increase in detector response was observed. On the same time, a reduction in the maximum number of sample analysis per hour was also observed. Although lower flow rates up to 0.5 mL/min gave higher UV intensities, the peak height reproducibility was poor and the peaks were so broad that sample throughput was very low. A compromise between analytical signal and sample frequency was established by choosing a working flow rate of 1.25 mL/min. The developed procedure can accommodate sample frequency of 60 sample/hr. Fig. 2 shows a diagram chart for FIA to determine L- proline over the concentration range of 0.8-28µg/mL.
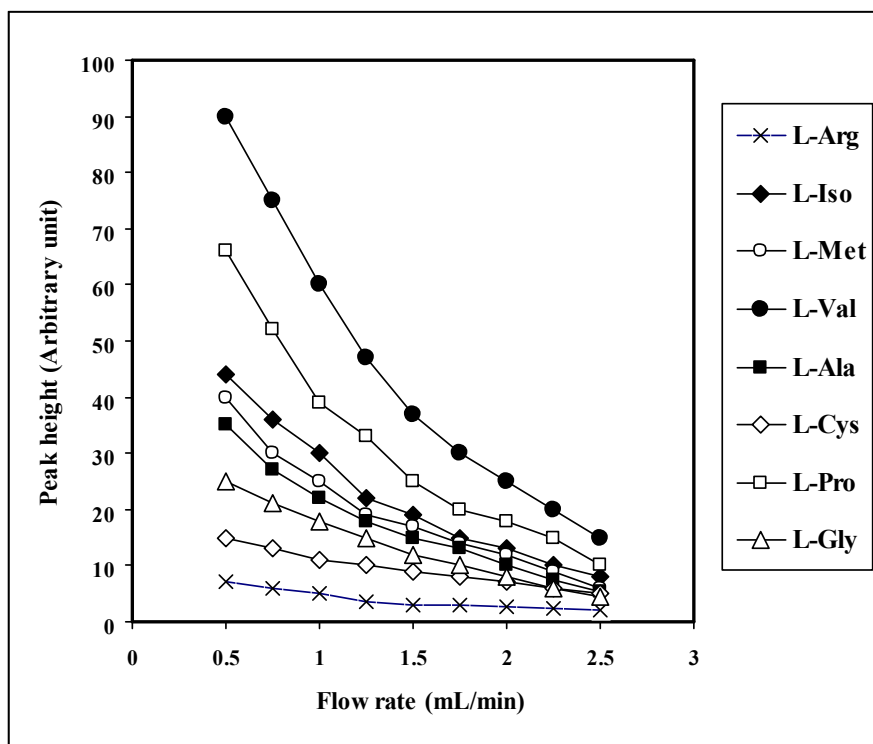

Fig. 1. Effect of the flow rate on the detector response of tested Cu[II]-amino acids complexes
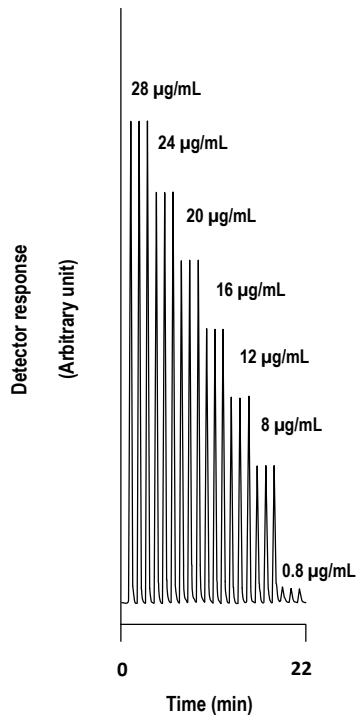
Fig. 2. Diagram chart for FIA to determine L-proline over the concentration range of 0.8-28µg/mL

3.2 Column Dimensions

As the described FIA manifold is a low-pressure system and as the purpose of the packed reactor column is not to separate the sample components, the column can be short and the reagent particles relatively large, so that the flow resistance will be low. The influence of the packed reactor internal diameter on the sample frequency and sensitivity of the described method was studied at 4.6 mm and 7.5 mm (i.d.). The developed FIA system with a packed reactor (10 mm x 4.6 mm i.d.) is capable of operating at a rate of 60 sample/hr. When this system is equipped with a wider packed reactor (7.5 mm i.d.) the sample frequency is significantly decreased (35 sample/hr). Although wider column can increase the residence time for the reaction to be complete, there is a decrease in a peak height signal due to diffusion of the resulting product over a larger area. Thus, it can be deduced that the effect of dispersion became more influential than the effect of the chemical reaction when the column i.d. of 7.5 mm was employed. A diameter of 4.6 mm i.d. was chosen in order to minimize the dispersion and maintain an efficient residence time. The positive effect of the packed reactor on peak width can also be observed with increasing column length up to 20 mm. This can be explained by the fact that an increase in dispersion gives a wider peak and subsequently a decrease in a peak height. On the other hand, the peak was so broad that sample throughput was low without a significant improvement in the detector response. The previous set of experiments led to the following results: (a) the longest columns were not suitable, due to peak height was decreased; (b) the i.d. was the main predominant parameter influencing sample dispersion. A packed column reactor of 4.6 mm i.d. and 10 mm length was therefore chosen.

3.3 Dispersion of the Sample Zone

In order to obtain maximum sampling frequency it is necessary to prevent peak broadening as otherwise the sample solution would occupy undue length of the carrier stream and would intermix with the next oncoming sample zone. It is known that the packed reactor is the most practical way to achieve low axial dispersion. In another word, the use of packed reactor will restrict the broadening of the sample zone. Generally, when the sample zone reaches the packed reactor column, the derivatization product disperses further before passing to the detection system. Therefore, it is necessary to design the CuO packed column in such a way that dispersion of the zone is kept at a minimum. Also, limited dispersion is obtained by injecting a sample into a manifold consisting of a shorter connecting tube (5 cm x 0.5 mm i.d.) between the injector and the packed reactor and between the reactor and the detector. The dispersion (D) of the sample zone in the described FIA manifold was calculated simply by comparing the peak height of the signal (H) obtained by injecting 20 μL of 25μg/mL of L-Ala and the signal (H_o) recorded when the flow cell has been completely filled with the same compound. The signal (H_o) may be obtained by pumping the sample of L-Ala (25μg/mL) through the whole system. The ratio of the signal heights (H_o/H) yields a D value of 3.09.

3.4 Validation

3.4.1 Linearity

The calibration curves were constructed by plotting the measured peak heights versus concentrations. There was a linear relationship between peak height and concentration over the examined concentration range. The coefficient for the linear equation $Y = a + bC$ was calculated using linear regression least square method, where Y is the peak height and C denotes the concentration in μg/mL of amino acids.

3.4.2 Limit of detection and quantification

The limit of detection (LOD), defined as the lowest concentration of amino acid that can be clearly detected above the base line signal, is estimated as three-times the signal-to-noise ratio. The LOD was determined (n = 3) by injecting amino acid in decreasing concentrations. The LOQ is often defined as 10 times the signal-to-noise ratio. The LOQ was determined (n = 3) by injecting amino acid in decreasing concentrations. The precision was calculated for each concentration. Then, the LOQ was calculated as the concentration, where the precision was less than or equal to 15%. Detailed information regarding the calibration curves, linear ranges, LOD and LOQ are listed in Table 1.

3.4.3 Accuracy and precision

The relative standard deviation (RSD %) and the relative error (RE %) of the mean measured concentration were served as measures of accuracy and precision for validation of the assay procedure. The intra- and inter-day assay precision and accuracy for amino acids are summarized in Table 2. Within the examined range, the intra-day precision and accuracy of the assay were excellent, with RSD being in the range of 0.178-1.655% and with RE ranged from 0.260 to -1.200%. The inter-day RSD wasin the range of 0.186-2.226% and the mean RE ranged from 1.100 to -2.750%(Table 2). RE was evaluated by back-calculation and expressed as the percent deviation between concentration added and

concentration found according to the following:

RE = ((Conc. found - Conc. added)/Conc. added) x 100 [39]

Table 1. Characteristic parameters for the regression equations of the proposed FIA method for the determination of the amino

Amino acid	Calibration range (µg/mL)	LOD (µg/mL)	LOQ (µg/mL)	Slope (b)	Intercept (a)	Correlation coefficient (r)
L-Gly	1.4 -140	0.310	1.020	0.3029	0.2260	0.9996
L-Ala	1.2 - 120	0.290	0.950	0.2772	-0.0618	0.9997
L-Iso	1.0 - 100	0.240	0.720	0.2693	-0.3913	0.9995
L-Val.	0.5 - 50.	0.150	0.460	0.0972	-0.0681	0.9996
L-Met	1.0 - 100	0.250	0.850	0.2647	0.1417	0.9996
L-Cys	1.6 - 160	0.390	1.280	0.3118	0.1875	0.9997
L-Arg	6.3 – 630	1.750	5.750	1.3913	-0.8230	0.9997
L-Pro	0.8 - 80	0.210	0.690	0.1561	0.0997	0.9997

3.4.4 Molecular modeling and computational analysis

Amino acids chelate with transition metal ion like Cu^{II} by coordinating through the lone pair of electrons of α-amino and carboxyl groups to form a complex. The phenomena of chelation were well known [40-42] and have been used for the derivatization of α-amino acids. The mechanism of the reaction between Cu^{II} and amino acids has been early investigated [31,42]. In the present work, molecular modeling of the tested amino acids with CuO was studied. The geometries of each amino acid alone, and its complex with CuO were fully optimized at full self-consistent field (SCF) levels by using MOPAC; a general molecular orbital package implemented with molecular mechanics software MMX-PC [43]. In this study, the minimization of amino acids with CuO showed that, α-amino and carboxylic groups chelate with Cu^{II} in CuO and the reaction then ends at the formation of Cu-complex according to Scheme 1.
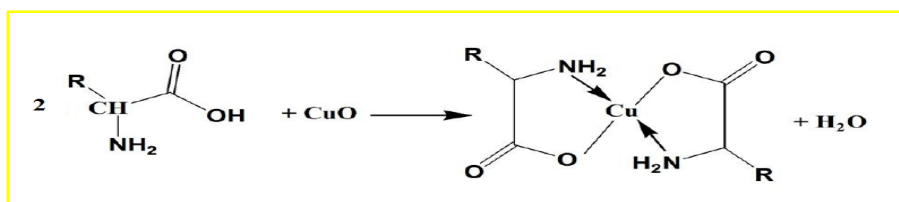
Scheme 1: The reaction between Cu^{II} in CuO and amino acid

Each Cu^{II}-amino acid complex was minimized and the physicochemical parameters were determined. These parameters were MMXE (force field); STR (stretching energy); BND (bending energy); SB (stretching: bending); TOR (torsional energy); VDW (Van der Waals forces); DD/QQ (core-core interaction); and HF (heat of formation) (Table 3).

Table 2. Precision and accuracy validation of the amino acids by the proposed FIA method

Amino acids	Nominal conc. (µg/mL)	Intra-day [a]Observed conc. (µg/mL) ± SD	RSD (%)	[a]Mean RE (%)	Inter-day [a]Observed conc. (µg/mL) ± SD	RSD (%)	[a]Mean RE (%)
L-Gly	2	1.985 ± 0.018	0.907	-0.750	1.965 ± 0.031	1.578	-1.750
	30	29.935 ± 0.242	0.808	-0.217	29.796 ± 0.264	0.886	-0.680
	120	119.450 ± 0.464	0.388	-0.458	119.425 ± 0.510	0.427	-0.479
L-Ala	2	1.990 ± 0.028	1.407	-0.500	1.983 ± 0.037	1.866	-0.850
	30	29.858 ± 0.203	0.680	-0.473	29.596 ± 0.326	1.102	-1.347
	120	119.727 ± 0.571	0.477	-0.228	119.643 ± 0.624	0.522	-0.298
L-Iso	2	1.989 ± 0.030	1.508	-0.550	1.982 ± 0.041	2.069	-0.900
	20	19.916 ± 0.217	1.090	-0.420	19.848 ± 0.277	1.396	-0.760
	80	79.504 ± 0.526	0.662	-0.620	79.369 ± 0.602	0.758	-0.789
L-Val	2	1.977 ± 0.022	1.113	-1.150	1.963 ± 0.031	1.579	-1.850
	10	10.026 ± 0.094	0.938	0.260	10.110 ± 0.116	1.147	1.100
	40	40.063 ± 0.447	1.116	0.158	40.159 ± 0.504	1.255	0.398
L-Met	2	1.994 ± 0.033	1.655	-0.300	1.977 ± 0.044	2.226	-1.150
	20	19.844 ± 0.214	1.078	-0.780	19.695 ± 0.258	1.310	-1.525
	80	79.395 ± 0.702	0.884	-0.756	79.274 ± 0.747	0.942	-0.908
L-Cys	2	1.993 ± 0.022	1.104	-0.350	1.986 ± 0.031	1.561	-0.700
	30	29.710 ± 0.356	1.198	-0.967	29.630 ± 0.436	1.471	-1.233
	150	149.775 ± 0.528	0.353	-0.150	149.614 ± 0.624	0.417	-0.257
L-Arg	10	9.880 ± 0.153	1.549	-1.200	9.725 ± 0.167	1.717	-2.750
	100	98.950 ± 0.477	0.482	-1.050	98.516 ± 0.713	0.724	-1.484
	500	496.103 ± 0.881	0.178	-0.779	495.613 ± 0.922	0.186	-0.877
L-Pro	2	1.990 ± 0.018	0.905	-0.500	1.976 ± 0.028	1.417	-1.200
	20	20.040 ± 0.154	0.768	0.200	20.140 ± 0.171	0.849	0.700
	80	80.110 ± 0.382	0.477	0.138	80.150 ± 0.516	0.644	0.188

[a]*Mean and S.D. for five determinations*

Table 3. Physicochemical parameters of minimized Cu^{II}-amino acids complexes at full SCFa

	L.Gly	L-Ala.	L-Isoleu	L-Val.	L-Meth.	L-Cys.	L-Arg.	L-Pro.
MMXE	-34.49	-32.97	-24.89	-101.48	-4.97	-100.1	-53.54	32.66
STR	0.21	0.24	32.44	27.28	5.23	3.81	18.96	40.53
BND	0.87	1.06	8.43	2.18	21.13	1.89	6.86	8.64
SB	-0.01	0.02	0.77	0.66	-0.23	0.2	0.6	1.01
TOR	-0.28	0.051	74.72	0.69	59.36	-0.36	2.68	5.5
VDW	-31.8	-31.11	137.88	-130.83	-86.78	-102.4	-74.68	-21.78
DD/QQ	-3.48	-3.69	-3.37	-1.47	-3.67	-3.21	-7.96	-1.24
HF	-128.18	-135.4	-148.25	-218.42	-110.25	-194.9	-95.58	-68.67

^{a}*Parameters are: MMXE (force field); STR (stretching energy); BND(bending energy); SB (stretching:bending); TOR (torsional energy); VDW (Van der Waals forces); DD/QQ (core-core interaction); HF (heat of formation)*

The effect of the multiple physicochemical parameters of each amino acid on its reactivity with CuO, and consequently on the sensitivity, expressed as LOD was investigated. A multiple-regression analysis was performed to derive an equation (**Equation 1**) by which a calculated value of LOD could be determined. The results are given in Table 4, and the derived equation was:

LOD = -0.112 x 10^{-1} MMXE + 0.151 x 10^{-1} STR + 0.212 x 10^{-1} BND - 0.222 SB - 0.068 x 10^{-1} TOR + 0.013 x 10^{-1} VDW - 0.162 DDQQ + 0.083 x 10^{-1} HF + 0.467   (Equation 1)

A good correlation between the computed values and the FIA results suggests that the mathematical model is reliable, and hence provides a theoretical basis for the determination of LOD. The residual percent was ranged from 4.167 to -12.857 % indicating good prediction of the LOD (Table 4). The residual percent was calculated as follow:

Residual percent = [(C$_{obs}$- C$_{cal}$)/C$_{obs}$)] 100.

where (**C$_{obs}$**) was the mean value of the observed concentration and (**C$_{cal.}$**) was the calculated concentration.

The correlations between the LOQ and the physicochemical parameters of Cu^{II}-amino acids complexes were examined and the possibility of their use to derive an equation for the calculation of LOQ was presented. It is shown that the LOQ can be accurately determined by equation 2.

LOQ = -0.369 x 10^{-1} MMXE + 0.498 x 10^{-1} STR + 0.699 x 10^{-1} BND - 0.819 SB - 0.225 x 10^{-1} TOR + 0.042 x 10^{-1} VDW - 5.344 x 10^{-1} DD/QQ + 0.274 x 10^{-1} HF + 1.542
                                                                              (Equation 2)

The validity of this equation was proved by comparing the calculated values of LOQ for each amino acid with the experimental FIA results (Table 4). The residual percent was ranged from 6.806 to -9.216% indicating perfect prediction of the LOQ.

Table 4. Observed and computed values for LOD, LOQ, LL and HL of Cu^{II}-amino acids complexs

Amino acids	LOD (µg/mL)			LOQ (µg/mL)			LL (µg/mL)			HL (µg/mL)		
	Obs.	Calc.	Residual (%)	Obs.	Calc.	Residual (%)	Obs.	Calc.	Residual (%)	Obs.	Calc.	Residual (%)
L-Gly	0.310	0.338	-9.032	1.020	1.114	-9.216	1.400	1.403	-0.214	140.000	140.295	-0.211
L-Ala	0.290	0.291	-0.345	0.950	0.958	-0.842	1.200	1.206	-0.500	120.000	120.623	-0.519
L-Iso	0.240	0.230	4.167	0.720	0.671	6.806	1.000	0.999	0.100	100.000	100.062	-0.062
L-Val.	0.150	0.166	-10.667	0.460	0.493	-7.174	0.500	0.502	-0.400	50.000	50.198	-0.396
L-Met	0.250	0.264	-5.600	0.850	0.892	-4.941	1.000	1.004	-0.400	100.000	100.058	-0.058
L-Cys	0.390	0.413	-5.897	1.280	1.347	-5.234	1.600	1.598	0.125	160.000	159.947	0.033
L-Arg	1.750	1.746	0.229	5.750	5.711	0.678	6.300	6.297	0.048	630.000	629.743	0.041
L-Pro	0.210	0.237	-12.857	0.690	0.698	-1.159	0.800	0.803	-0.375	80.000	80.000	0.000

The applicability of the physicochemical parameters was also tested for the lower limit (LL) and higher limit (HL) of the linearity range of Cu^{II}-amino acids complexes (Table 4). Multiple-regression analyses were performed to drive equations **3** and **4** by which calculated values of LL and HL could be determined, respectively. The derived equations were,

LL = -0.447 x 10^{-1} MMXE + 0.529 x 10^{-1} STR + 0.960 x 10^{-1} BND -10.169 x 10^{-1} SB - 0.296 x 10^{-1} TOR + 0.077 x 10^{-1} VDW - 5.529 x 10^{-1} DD/QQ + 0.318 x 10^{-1} HF + 21.451 x 10^{-1}

(Equation 3)

HL = -4.475 MMXE + 5.290 STR + 9.597 BND -101.687 SB - 2.961 TOR + 0.772 VDW - 55.295 DD/QQ + 3.181 HF + 214.510 (Equation 4)

The excellent agreement between the observed and the calculated values is shown in Table 4. The residual percents were ranged from 0.125 to -0.500% and 0.041 to -0.519% for LL and HL, respectively. These excellent correlations proved the validity of the proposed computational equations **3** and **4** that were derived for calculating LL and HL of amino acids.

3.4.5 Column lifetime and long-term precision

The column lifetime, in terms of being able to derivatize amino acids quantitatively was investigated as a function of the volume of the amino acids that were injected into the column. It was found that, the packed reactor could be used successfully for the analysis of at least 2000 times of a 20 µL-injection volume of tested amino acids (50µg/mL). As the reactivity of CuOcan not be influenced by distilled-deionized water, packed reactor retained its efficiency over the period of the study.

3.CONCLUSION

Low cost and sensitive FIA procedure exploiting CuO as a packed reactor was demonstrated to determine eight amino acids. The proposed method offers many significant improvements over the other FIA procedures for the determination of amino acids. These improvements are simplicity of the procedure, no dangerous reagents used, very mild reaction conditions, no mixing problems, less pump noise, no need for an extra pump and the reaction is achieved in a fairly short time. The use of distilled-deionized water as a flowing stream and the minimum generation of waste offered by the proposed method made this work a contribution to environmental friendly analytical chemistry. We successfully correlated the physicochemical parameters of the investigated amino acid-copper complex with the analytical parameters (LOD, LOQ, LL and HL). Comparative verification between the observed analytical parameters and the computed ones revealed significant correlations.

COMPETING INTEREST

The authors declare that they have no competing interests.

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