Detection Of **Biogenic Amines** By The Use Of Advanced **Chemometrics Tools**

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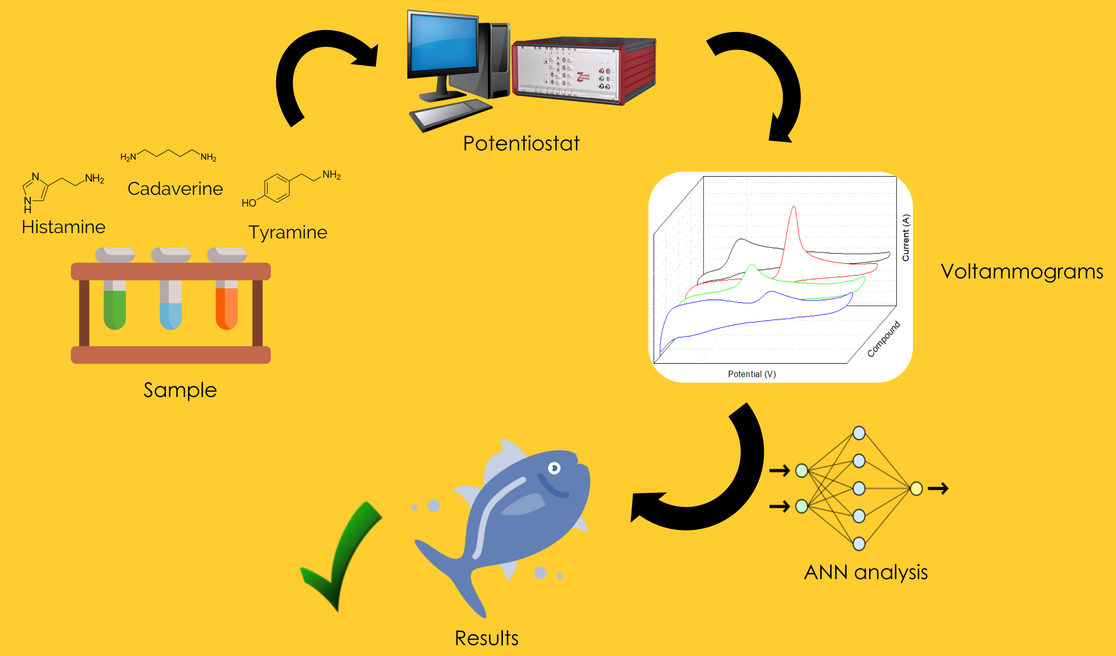
Abstract

The present work proposes an electronic tongue arrangement for the detection of biogenic amines (BAs) in ternary complex mixtures.

Since the formation of BAs is directly proportional to the increase of temperature and the presence of bacteria, the elevated concentration of that kind of compounds could be related easily with the quality of the food industry products. The most regulated field is the fish industry, which has set limits for histamine as the marker compound: 100 mg·Kg-1 in the European Union and 50 mg·Kg-1 in the United States of America.

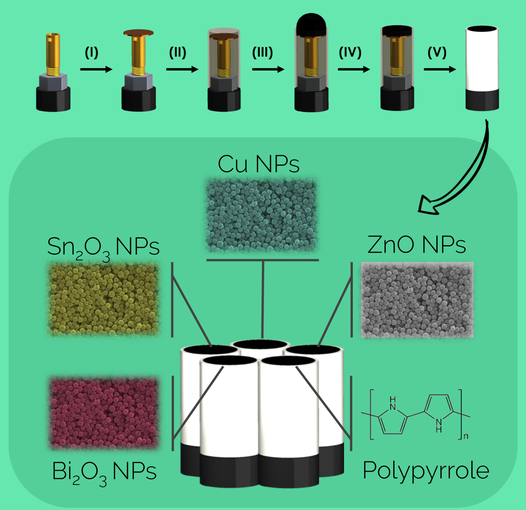
Herein it is proposed a voltammetric sensor array for the quick and efficient detection of histamine (Hys), cadaverine (Cad) and tyramine (Tyr) which, together with advanced chemometric tools such as artificial neural networks (ANN) and partial least squares (PCA), leads to models able to predict the individual concentration of each BA in the analysed samples.

The final ANN structure had 51 input neurons, 5 neurons in the hidden layer, and 3 neurons in the output layer. The functions used for the hidden and output layers were *Tansig* and *Purelin*, respectively. The results show that this is a valid model with slopes near to 1 and intercepts close to 0. Moreover, it is important to remark that the worst correlation has a value of almost 0.900.



Description of analysis process.

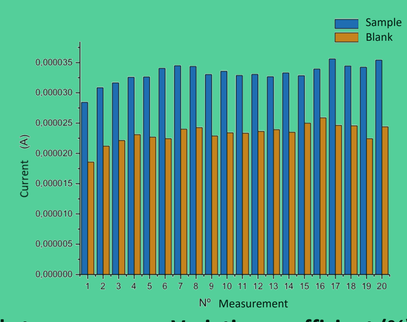
Sensor array and characterization



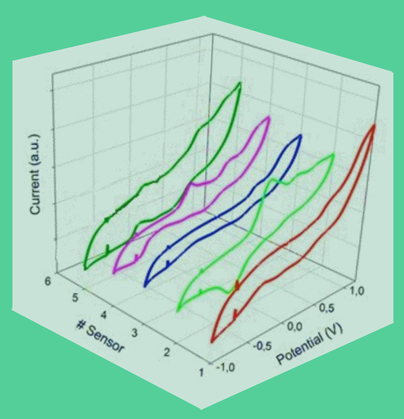
Graphite-epoxy composite electrodes were built using a copper disk soldered to a Au connector. Next, a PVC tube, used as electrode body, was filled with the composite. In this case, each electrode incorporate a 2% of different bulk modifiers. The final array was: **Bi_2O_3 NPs, Si_2O_3 NPs, Cu NPs, ZnO NPs and Polypyrrol.**

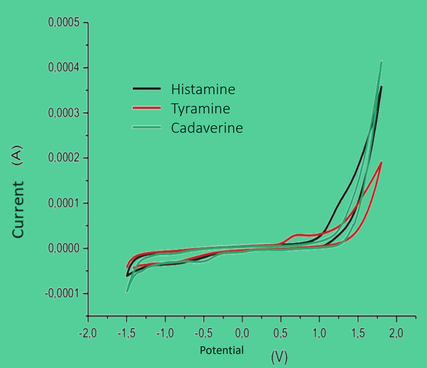
Some of the most important parameters in electronic tongue (ET) settings are the electrodes **(A) long-term stability**, **(B) cross sensitivity** and **(C) non specificity**.

Voltammograms:
-1.5 → 1.8 V vs. Ag/AgCl
50 mV/s



Substance	Variation coefficient (%)				
	ZnO	CuO	Pyrrol	Bi_2O_3	SnO_2
Histamine	6.41	8.59	2.86	5.37	6.29
Tyramine	5.17	8.53	2.85	6.93	4.86





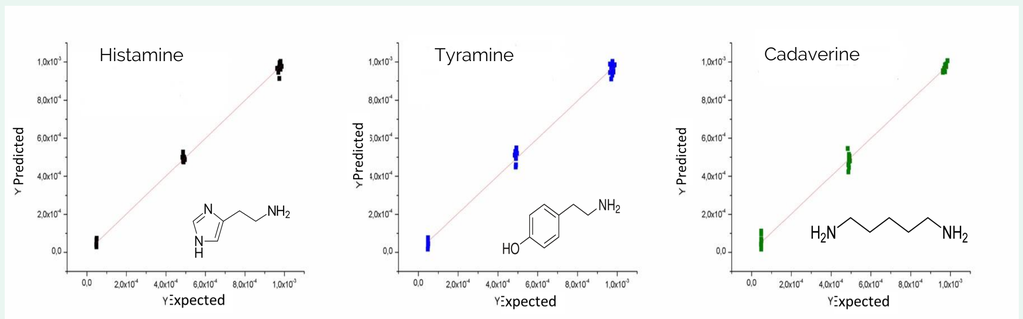
(A) 20 consecutive measurements alternating sample and blank were done in order to test the **long-term electrodes response**. The test was carried out using a sample that contains 0.1 mM histamine and **0.1 mM** tyramine in phosphate buffer solution (pH=7.0).

(B) The comparison of histamine (0.8 mM in phosphate buffer solution at pH 7.0) voltammograms for the 5 selected electrodes shows the **complementarity and non-redundancy** of the electrodes.

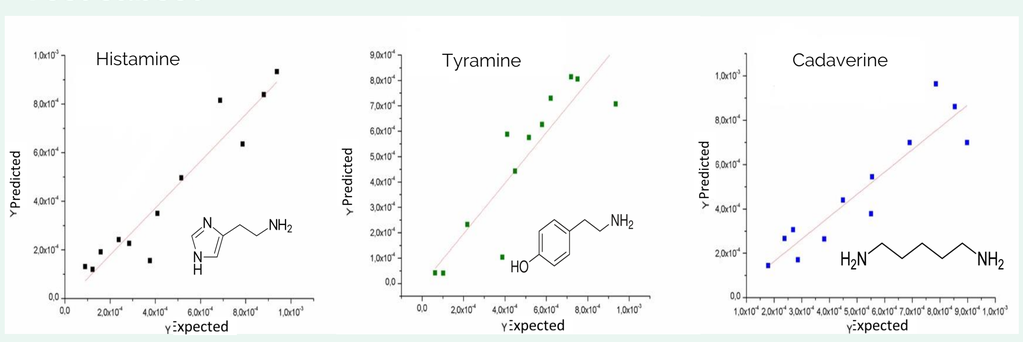
(C) The responses of ZnO NPs modified electrode towards histamine, tyramine and cadaverine 0.8 mM samples (diluted in phosphate buffer at pH 7.0) show the desired **cross-response** for the ET application.

Response model

Train subset



Test subset

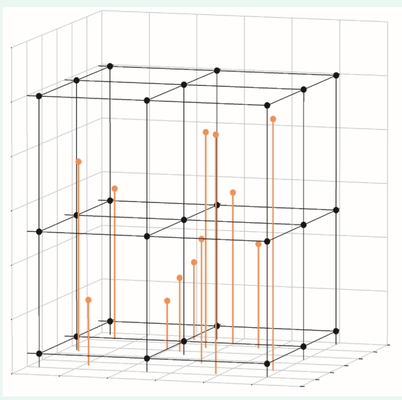


Real Sample

	OPA reaction (M)	Electrochemical (M)	% Recovery yield
MR1	$2.88 \cdot 10^{-3}$	$2.55 \cdot 10^{-3}$	89.4%
MR2	$2.43 \cdot 10^{-3}$	$2.25 \cdot 10^{-3}$	92.8%

Train				
	Slope	**Intercept (M)**	**Correlation**	**RMSE (M)**	**Total RMSE (M)**
Histamine	0.993	3.36e-6	0.999	1.01e-5	
Tyramine	0.991	4.41e-6	0.998	1.50e-5	4.27e-5
Cadaverine	0.984	8.59e-6	0.998	1.54e-5	

Test				
	Slope	**Intercept (M)**	**Correlation**	**RMSE (M)**	**Total RMSE (M)**
Histamine	0.964	-1.36e-5	0.956	5.35e-5	
Tyramine	0.998	-3.72e-6	0.891	7.52e-5	1.11e-4
Cadaverine	1.01	-3.77e-5	0.925	6.51e-5	



48 samples = **36** train + **12** test
+
2 real samples

A **fractional factorial design (L36)** was used to build the experimental layout (train subset). The model performance was evaluated with an extern set of samples defined randomly (test subset).

The recorded information was **compressed (ca. 92%)**, to avoid the overfitting and reduce the data dimension, using a pruning filter for each channel. The final model had 51 input, 5 neurons in the hidden layer and 3 neurons in the output layer. The transfer functions for hidden and output layers were *tansig* and *purelin*, respectively.

In the above tables the regression parameters for the comparison graphs can be observed, where as expected, the results for both subsets present **values near to ideal ones** (slope 1, intercept 0 and correlation coefficient 1).

The application of the model to real samples gives feasible results in comparison to **OPA reference method**. Leading to propose the use of **ETs as an alternative method** for the analysis of BAs in complex samples. Moreover, it must be emphasized that the current approach can quantify **individually each compound**, contrary to the official one.

Conclusions

The present approach presents a voltammetric ET based on the use of simple modified electrodes for individual quantification of biogenic amines in complex mixtures, demonstrating the powerful effect of ANN as an analysis tool for the determination of key compound in food industry.

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