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Application of adsorptive stripping differential pulse voltammetry with chemometrics in the determination of ascorbic acid, rutin and quercetin in the food and health products

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The simultaneous determination of ascorbic acid, rutin and quercetin in the food and health products on the glassy carbon electrode have been investigated by adsorptive stripping differential pulse voltammetry (AdSDPV) with the aid of chemometrics. In the pH 5.2 KH_2PO_4 – Na_2HPO_4 buffer solution, both rutin and quercetin has shown a pair of sensitive reversible oxidation-reduction peak, while ascorbic acid only has shown an irreversible oxidation peak on the glassy carbon electrode. In the range of 0.35-0.50 V, the vlotammograms of three components has shown serious overlap with peak potential of ascorbic acid, rutin and quercetin. So, it is extremely difficult to realize direct measurement for the content of single component. Chemometrics methods have been introduced to determine the admixture of the three components. In this way, we have avoided the troublesome procedures of separation and purification, and assay the artificial compound of the three components all at once. We have estimated the three components in the food and the health products with satisfactory results.

Keywords: Flavonoids, Adsorptive stripping differential pulse voltammetry, Chemometrics methods

Flavonoids are a large group of phenolic compounds and constitute one of the largest groups of secondary metabolites in plants, which belong to a class of water-soluble plant pigments.[1] They always exist companion with ascorbic acid in vegetables, fruits, foodstuffs and health products such as propolis.[2] Flavonoids that are present in herbal edibles have anti-oxidative properties and possess a remarkable spectrum of biochemical and pharmacological actions, such as inhibition of key enzymes in mitochondrial respiration, protection against coronary heart disease, regulation of cell signalling and gene expression and anti-inflammatory, antitumour and antimicrobial activities, especially in the respect of anti-cancer activity.[3] They can inhibit the proliferation, invasion and metastasis of the cancer cells, interfere with the signal transduction, induce apoptosis, anti-oxidization and so on. Flavonoids are also associated with a low incidence of osteoporosis and menopausal vasomotor symptoms such as hot flashes and night sweats.[4] As aglicone of rutin, quercetin usually coexists with rutin. Quercetin may be present as a hydrolytic product of rutin in pharmaceuticals, containing rutin and ascorbic acid as tablets and soft gelatine capsules, when they are not well stored. What is more, they can be often found together in Chinese herbs or herbal edibles, such as apple, onion, buckwheat and propolis.[5]

Hitherto, various analytical methods have been reported for the separation and determination of quercetin or/and rutin in flavonoids mixtures. High-performance liquid chromatography[6-9] and capillary electrophoresis[10-15] have been effectively used for the separation, coupled with various detection techniques, such as spectrophotometry, mass spectrometry, electrochemical detection and chemiluminescence, etc. The coupling of these techniques may provide high selectivity of the assay, but bring also some disadvantages of operating complexity, time and reagent consuming, high cost, etc. Usually, extraction or separation of active components from herb is tedious and inefficient because of poor affinity and selectivity of conventional separation materials (i.e., silica gel, modified silica gel or polyamides). The electroanalytical methods for analytes determination (mixtures of ascorbic acid with rutin, quercetin and

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rutin mixtures) as well as chemometric approaches successfully applied for the recognition and quantification of the analytes (for example, other phenolic antioxidants) in the mixtures have been reported.[16-20]

Up to now, simultaneous determination of quercetin and rutin has not reported. Hence, it is necessary to develop some simple, economical and efficient methods for the determination of rutin and other flavones in herbal drugs or plants. Because most flavonoids are electrochemically active at modest oxidation potentials, electrochemical methods are preferable[10-14] with advantages of higher sensitivity and less interference from non-electroactive substances. In this paper, we describe the research and development of an analytical method for simultaneous determination of ascorbic acid, rutin and quercetin on the glassy carbon electrode with the aid of different chemometrics methods for prediction of the analytes, including CLS, PLS, PCR, and ANN.

Materials and Methods

Reagents and experimental procedure

Rutin and quercetin were obtained from National Institute for the Control of Pharmaceutical and Biological Products and ascorbic acid was purchased from Shanghai chemical reagent purchase and supply deport. Stock solution of rutin, quercetin and ascorbic acid (0.1 mg/mL) were prepared by dissolving the appropriate amount of each compound in 50 mL ethanol and diluted to 100 mL with distilled water. Standard solutions of these compounds or their mixtures were then diluted to the required concentrations with distilled water. A KH_2PO_4-Na_2HPO_4 buffer solution (PBS) of pH 5.2 was prepared by mixing KH_2PO_4 with Na_2HPO_4 at the ratio of 32:1. All chemicals were analytical grade reagents and all the solutions were prepared with doubly distilled water.

Each sample solution contained a suitable amount of either one of the ascorbic acid, rutin and quercetin or a mixture of these compounds, together with a PBS buffer (2 mL, pH 5.2). Such a sample was mixed thoroughly and transferred to an electrochemical cell, and diluted to 10 mL with distilled water. The analytes were pre-concentrated at 0.3 V for 180 s. After a 10 s static period, a differential pulse voltammetric scan was run from 0.1 to 0.7 V at the glassy carbon electrode. The resulting voltammograms were sampled by a computer at 101 potential points in the range of 0.2 to 0.6 V at 4 mV intervals. Examples of voltammetric curves of each compound and their mixtures (Fig. 1) show the peak potentials for ascorbic acid, rutin and quercetin at 0.34, 0.42, and 0.51 V, respectively, as well as the heavily overlapped nature of the composite voltammograms of the mixtures.

Instrumentation

A CHI 660A electrochemical analyzer (CHI) equipped with a BAS C-1 cell stand (BAS) was used for voltammetric measurement. A three-electrode cell, containing a glassy carbon electrode as working electrode, an Ag/AgCl electrode as reference electrode and a platinum wire as auxiliary electrode was employed for the electrochemical measurements. The pH measurements were performed on an Orion SA 720 pH meter equipped with an Ag/AgCl glass combination pH electrode. All experiments were carried out at 25 °C. Data analysis was performed on a Pentium IV computer. Software of RBF-ANN was employed with the use of the Neural Network Toolbox, Matlab 6.5 (mathworks) and other chemometrics programs were written in-house.

Chemometrics methods

The nature of electrochemical data

Before discussing how data can be analyzed, it is important to consider exactly what form these data take and how they are most conveniently organized.[21] In essence, all data collected from a series of electrochemical experiments can be represented by some kind of data matrix. Let us consider the case where voltammograms have been acquired from several different samples. The voltammogram of each sample can be represented by a vector of current measurements, denoted as x_i, where i is the sample

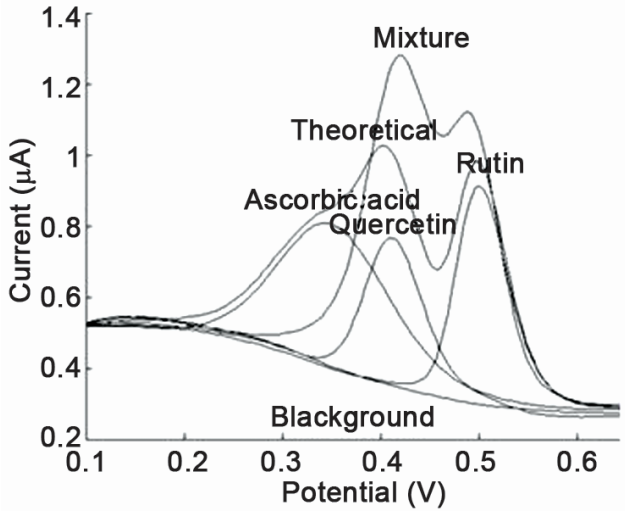

Fig. 1 — Differential pulse stripping voltammograms of rutin, quercetin, ascorbic acid and their mixtures.

number. Within this vector, each element x_{ij} represents the current measured at point j in the voltammogram. The current measurement at each potential point can be considered to be a variable hence voltammograms are multivariate. The vectors representing the voltammograms of individual samples can be amalgamated into an $I \times J$ data matrix, $\boldsymbol{X}$, where I is the total number of samples analyzed and J is the number of potential steps in the voltammogram. Each row of the $\boldsymbol{X}$ matrix is therefore an individual voltammogram, while each column indicates the current measured at a specific potential over all samples. A single data matrix is therefore sufficient to describe all the samples analyzed in a given series of experiments. In the case of an array of amperometric electrodes, the situation is very similar, with each sample having an associated matrix row. However, the columns in this case would be related to specific electrodes, rather than potentials. Once a data matrix has been constructed, there are essentially three different data analysis activities that can be of interest: a) data exploration, b) sample classification and c) calibration. Approaches for tackling these tasks are described below, along with examples of where these approaches have been used in the literature.

Classical least squares method

Classical least squares (CLS), [22, 23] which has often been labeled as the K matrix method, is based on multiple linear regression (MLR). This method was frequently used for quantitative voltammetry analysis to obtain selective information from the unselective data, which has generally presumed that there is a linear relationship between the response signal and the component concentration. This step is followed by prediction in which the calibration model is used to estimate the component concentrations from the 'unknown' sample data. Although it is a full-spectrum method and can provide significant improvement in precision over other methods that are restricted to a small number of potential points, e.g. inverse least squares (ILS), it encounters some main disadvantages, for example, it is a rigid model that needs the knowledge of all the components in the mixture, and that there should be no chemical or physical interaction between the components in the mixture or with other compounds present in the matrix [24].

Principal component regression and partial least squares methods

Principal component regression (PCR) [24-26] and partial least squares (PLS) [27] are powerful multivariate statistical tools based on factor analysis which have many of the full-spectrum advantages of the CLS method and have been successfully applied for analysis of the overlapping voltammetric signals of complex multicomponent mixtures. Like CLS method, they assume that there is a linear relationship between the peak current and the concentrations of the components in the mixture. Both methods need a calibration step, where the relationship between the peak current and the concentrations of the components is deduced from a set of reference samples, followed by a prediction step in which the results of the calibration are used to determine the concentrations of the components from the peak current of the analysed samples. As regards the application of the PLS and PCR methods, they are more flexible and do not need those requirements that the CLS method encounters owing to their capacity to reduce interference problems or background noise, together with their ability in resolving overlapping signals. It is necessary for both of these methods to make previous original data matrix decomposition. Their objective is to obtain the voltammogram of the mixture from a determined number of variable voltammograms named loadings and the different amounts of each of them that must be added to reconstruct the original data matrix and that are known as scores. The major difference between the PCR and PLS methods is that, for PCR method, only the information in the matrix of peak currents is used in the data matrix decomposition, but in the PLS method the concentration data matrix is also used in this step. [24]

$$\Delta\omega_{ij(n+1)} = \Delta\eta\delta_j o_j + \alpha\Delta\omega_{ij}(n) \quad \ldots (1)$$

where δ_j is the error term, o_j is the output of node j, η is the learning rate, α is the momentum and n is the iteration number. Iteration is completed when the error of prediction reaches a minimum. A non-linear transformation, in the form of a sigmoidal transfer function was applied between the input and output of node. Optimal values of η and α were taken as those, which minimised the error of prediction. In the networks, the process of adapting the weights to an optimum set of values is usually optimised by means of supervised training. The weights are optimised by means of a number of training input samples together with their associated desired outputs. The weight updates are based on the difference between the actual and the desired output of the network. The weight updating can be done after each sample or it can be done after all training samples have been processed. The two procedures are strictly equivalent.

Radial basis function artificial neural networks method

Radial basis function neural networks (RBF-ANN) architecture is similar to back-propagation artificial neural networks. It offers some advantages over the BP-ANN by improving the robustness and sensitivity when dealing with noisy data. Its basic theory and application to chemical problems are also found in the literature.[28,29] It is also comprised of three layers feed forward network. The first layer is made up of input nodes that transmit unweighted inputs to each node in the hidden layer. Each hidden node contains a radial basis function as the transfer function. The outputs of these nodes are weighted and summed to produce the final output. In contrast to the sigmoid function, the kernel or basis function is classified as a local activation function. The main difference between the transfer function in the BP networks and the kernel function in the RBF networks is that the latter (usually a Gaussian function) defines an ellipsoid in the input space. The key to a successful implementation of the RBF networks is to find suitable centers for such a Gaussian function, which is characterized by two parameters, i.e. center (cj), and peak width (σj). The output from the *j*th Gaussian neuron for an input object x can be calculated by the following equation:

$$output_j = O_j(x) = exp[-(\frac{|x-c_j|}{b_j})^2] \quad \ldots (2)$$

where $|x - c_j|$ is the calculated Euclidean distance (other distance measures are also possible) between *x*, and c_j, and σ_j determines the portion of the input space where the *j*th RBF will have a non-significant zero response. After selection of the center and peak width, the connections between the radial basis units and the output node are weighted. The output of the net is consequently given by:

$$y_i = \sum_{i=1}^{n} \omega_{ji} o_j(x) \quad \ldots (3)$$

where ω_{ji} represents the weights of the connections between the hidden layer *i* and output layer *j*, and $o_j(x)$ is obtained from above equation.

Results and Discussion

The oxidation of ascorbic acid, quercetin and rutin on GCE is well-known and widely discussed in the literature.[30-33] The effect of scan rate on peak current and peak potential for ascorbic acid, rutin and quercetin was examined by cyclic voltammetry (CV). It is found that the process at the electrode of rutin and quercetin was mainly adsorption controlled.

For a reversible reaction: $D_{ox} = D_{Red}$, so $i_{p,a} / i_{p,c} = 1$ ($i_{p,a}$ and $i_{p,c}$ denote respectively peak current of anodic and cathodic).[34] Under the condition of low scanning speed, rutin and quercetin showed good reversibility, but along with the increase of scanning speed, $i_{p,a} / i_{p,c}$ increased to more than 1. It showed that the reversibility was depravation with fast scan speed. Besides, according to $\Delta E_p = E_{p,a} - E_{p,c} = 2.2RTm/nF = 58m/n\ (mV)$ ($E_{p,a}$ and $E_{p,c}$ denote the anodic and cathodic peak potentials, respectively), it was calculated he difference of $E_{p,a}$ and $E_{p,c}$ increased to greater than 58 m/n (mV) , which verified that the reversibility was depravation with the increase of scanning speed.

The half-peak-width equation for an irreversible electrochemical reaction is $\omega_{1/2} = 62.4/\alpha n$.[35] where α is the transfer coefficient and n is the number of electrons transferred. From the cyclic voltammogram, the half-peak-width value, $\omega_{1/2}$, was estimated to be 55 mV, which on substitution into the above equation gave a value of 1.13 for αn. In general, α is taken as 0.5 for an irreversible reaction, and thus by comparison the number of electrons transferred in the reaction studied was equal to 2.

As described above, E_p of ascorbic acid varied linearly with increasing pH ($E_p = -0.0992pH + 1.0790$). According to the Nernst equation: $E_p = E^0 - 2.303(RTm/anF)$ pH (where m is the number of hydrogen ions involved in the reaction), a value of 1.9 for m can be calculated from the slope of the E_p-pH plot. Therefore, it can be concluded that two hydrogen ions were involved in the electrode reaction of ascorbic acid. The mechanism of this electrochemical reaction can be represented by a chemical equation involving single two-electrons, two-protons change.

Continuous three times cyclic voltammetry scan (scan rate was 50 mV/s) was carried on for rutin and quercetin. From the cyclic voltammogram, at the low scan rate, their oxidation processes were approximate to reversible and the peak currents declined along with the increase of scan numbers. This declared that the electrode reaction was controlled by adsorbability. On condition that there are protons take part in the oxidation-reduction reaction, the electrode reaction can be expressed as: $O + ne^- + mH^+ = R$ (where O is on behalf of oxidation, and R represent reduction).

According to the Nernst equation: $E=E^0 + \mathrm{RTln}([O][H^+]^m / [R]) / nF$,

$E=E' + \mathrm{RTmln}[H^+] / (nF)$,

$E=E'-2.303RTm\,\mathrm{pH}/nF=E'-(0.059\ m/n)\,\mathrm{pH}$,

In the light of $E_{Rutin}= -0.0541\mathrm{pH} + 0.7427$; $E_{Quercetin}=-0.0584\mathrm{pH} + 0.7227$, we can deduce that m/n = 1. So, the number of hydrogen ions involved in the reaction was equal to the number of electrons transferred.

With reference to the formula: $\Delta E_p=E_{p,a} - E_{p,c}=2.2RTm/nF=58m/n$ (mV) and $\Delta E_{Rutin}=567 - 523=58m/n$ (m/v), m/n = 1.32 ≈ 1; $\Delta E_{\mathrm{Quercetin}}$ = 471-420 = 58m/n(mV), m/n = 1.14 ≈ 1. So to speak, the number of hydrogen ions involved in the reaction was equal to the number of electrons transferred. That showed a good agreement with the above-cited conclusion. Toward adsorption reversible oxidation-reduction: 25 ºC, $\omega_{1/2}$ = 3.53RT/nF = 90.6/n(mV), $\omega_{1/2\ \mathrm{Rutin}}$ = 90.6/n_{Rutin} = 40 (mV), n_{Rutin} = 2.26 ≈ 2, m_{Rutin} = n_{Rutin} = 2; $\omega_{1/2\ \mathrm{Quercetin}}$ = 90.6/$n_{\mathrm{Quercetin}}$ = 50(mV), $n_{\mathrm{Quercetin}}$ = 1.81 ≈ 2, $m_{\mathrm{Quercetin}}$ = $n_{\mathrm{Quercetin}}$ = 2. It is concluded that there was two-electrons, two-protons change reaction took place on the glassy carbon electrode. So the mechanism of this electrochemical reaction can be denoted by a chemical equation.

Sensitivity for the three compounds

Parameters of the calibration plot for each component were evaluated. The relationship of peak current and concentration of each component was fitted to a linear regression model (Fig. 2). The linear calibration parameters were calculated.[36] The limits of detection were estimated to be 364.2, 24.5 and 6.60 ng/mL[36] (Table 1) for ascorbic acid, rutin and quercetin, respectively, which compare well with the detection limits obtained for the HPLC method with a UV detector (quercetin 7.0 ng/mL).[7] Thus, these results clearly indicate that the proposed electrochemical method of analysis is reliable for the determination of individual compounds.

Prediction of three compounds in synthetic mixtures

For quantitative analysis of mixtures of ascorbic acid, rutin and quercetin, a calibration set was prepared according to the orthogonal array design method, in order to extract maximum quantitative information efficiently. A four-level orthogonal array design, denoted by OA_{16} (4^3) was selected,[37] and Table 2 shows the composition of the calibration samples. The DPSV voltammograms for each of the mixture samples were recorded between 0.1 and 0.6 V to produce a data matrix with 16 rows and 125 columns. Another set of samples consisting of 12 synthetic mixtures (Table 3) was then used to test and evaluate the prediction ability of the calibration models and submitted for prediction by each of the calibration models. The voltammograms of these samples containing mixtures of the compounds show

Table 1 — Parameter of linear calibration models for Ascorbic acid, Rutin and Quercetin

Parameters	Rutin	Quercetin	Ascorbic acid
Sample (n)	11	7	7
Linear range (μg/mL)	1-20	0.063- 0.75	0.02- 0.24
Intercept (nA)	0.0836	-0.03	0.3107
Slope (nA mL/μg)	0.0539	0.840	15.1
Correlation coefficient	0.9998	0.9996	0.9997
Standard deviation of intercept ($\times10^3$)	3.80	4.90	23.9
Standard deviation of slope ($\times10^3$)	0.319	10.9	165.8
Standard deviation of regression ($\times10^3$)	6.50	6.9	33.3
Limit of detection (ng/mL)	364.2	24.5	6.60

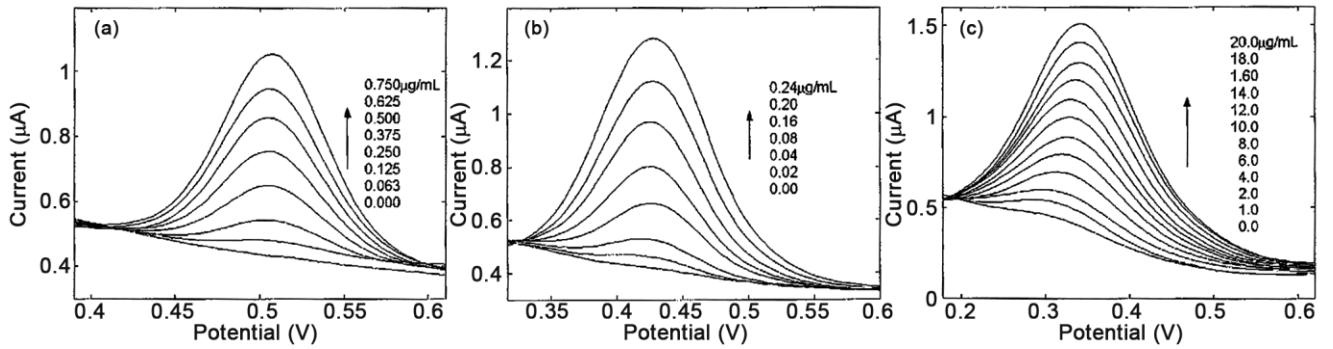

Fig. 2 — Voltammogram of (a) rutin, (b) quercetin and (c) ascorbic acid for different concentrations (μg/mL).

serious overlapping of individual responses, and there are only small differences between these curves (Fig. 1). Such voltammograms were analysed by DPSV according to the experimental procedure previously described. Generally, there are two types of calibration models in multicomponent analysis, i.e. for each analyte or for all analytes. In this work, PLS, PCR and RBF-ANN models were established for allthe analytes simultaneously (i.e. a global model) to simplify the calibration procedures. Three factors were selected as significant ($p = 0.05$) by cross validation,[38] for PCR and PLS, respectively, and used to build calibration models for prediction. The prediction ability was expressed in terms of the relative prediction errors (RPE_S) [39] for individual compounds, and RPE_T for total compounds (Table 3). For CLS, PCR and PLS calibrations, the $\%RPE_T$ values were around 8%, and thus, unsatisfactory. Even the PLS method, which sometimes can account for some non-linearity in the measured responses,[40] was apparently unable to cope adequately with the non-linearities of the voltammograms from the three antibiotic analytes.

It was found that the performance of the RBF-ANN calibration was acceptable for the simultaneous prediction of the fluoroquinolones with a $\%RPE_T$ of 8.1% and an average recovery of 101%. These figures of merit compare well with our other previous work, on the voltammetric determination of three organophosphorus pesticides with the aid of PLS and RBF-ANN.[41]

Over the last few years, we have been particularly interested in the research, and development of relatively uncomplicated analytical methods for the simultaneous determination of analytes of industrial,

Table 2 — Composition of the calibration samples (μg/mL)

Sample	Ascorbic acid	Rutin	Quercetin
1	1.000	0.09375	0.0400
2	1.000	0.3125	0.0800
3	1.000	0.5000	0.1400
4	1.000	0.6250	0.2000
5	4.500	0.09375	0.0800
6	4.500	0.3125	0.0400
7	4.500	0.5000	0.2000
8	4.500	0.6250	0.1400
9	7.500	0.09375	0.1400
10	7.500	0.3125	0.2000
11	7.500	0.5000	0.0400
12	7.500	0.6250	0.0800
13	10.00	0.09375	0.2000
14	10.00	0.3125	0.1400
15	10.00	0.5000	0.0800
16	10.00	0.6250	0.0400

Table 3 — Prediction results for 12 synthetic mixtures by different chemometrics methods (μg/mL)

Sample Added			Found (CLS)				Found (PCR[a])Found (PLS[a])			Found (RBF-ANN[b])					
	Ascorbic acid	Rutin	Quercetin	Ascorbic acid	Rutin	Quercetin	Ascorbic acid	Rutin	Quercetin	Ascorbic acid	Rutin	Quercetin	Ascorbic acid	Rutin	Quercetin
1	2.500	0.1500	0.0550	3.568	0.1280	0.0671	3.036	0.1504	0.0559	3.030	3.030	0.0561	2.835	0.1452	0.0576
2	2.500	0.2000	0.0950	3.088	0.1908	0.0954	2.703	0.2014	0.0834	2.694	0.2029	0.0838	2.483	0.1874	0.0859
3	2.500	0.3750	0.1300	2.310	0.3949	0.1423	2.170	0.3865	0.1325	2.160	0.3884	0.1331	2.116	0.3624	0.1338
4	3.500	0.1500	0.0950	3.798	0.1158	0.0994	3.394	0.1262	0.0886	3.388	0.1267	0.0889	3.171	0.1468	0.0976
5	3.500	0.2000	0.0550	3.826	0.1534	0.0608	3.270	0.1743	0.0514	3.269	0.1742	0.0513	3.171	0.1890	0.0527
6	3.500	0.3750	0.1800	3.806	0.3306	0.1630	3.844	0.3088	0.1540	3.838	0.3097	0.1545	3.851	0.3483	0.1689
7	3.500	0.4250	0.1300	3.858	0.3867	0.1250	3.733	0.3808	0.1179	3.725	0.3824	0.1183	3.999	0.3999	0.1289
8	6.000	0.1500	0.1300	5.874	0.1264	0.1217	5.592	0.1390	0.1147	5.588	0.1392	0.1148	5.874	0.1890	0.1289
9	6.000	0.2000	0.1800	5.670	0.2299	0.1750	5.724	0.2170	0.1677	5.715	0.2184	0.1683	5.936	0.2593	0.1865
10	6.000	0.4250	0.0950	5.963	0.4036	0.0867	5.701	0.4113	0.0854	5.701	0.4115	6.304	0.0851	0.4561	0.0957
11	9.000	0.2000	0.1300	9.308	0.1400	0.1461	9.128	0.1570	0.1473	9.135	0.1554	0.1467	9.304	0.1936	0.1494
12	9.000	0.3750	0.0950	9.119	0.3734	0.0977	9.024	0.3762	0.1016	9.029	0.3752	0.1013	9.194	0.4108	0.1045
RPE_S (%)		--	--	7.790	12.17	7.950	5.51	10.02	--	5.51	10.00	10.03	5.58	9.36	6.45
Recovery (%)[d]		--	--	--	107.7	90.23	102.6	100.7	93.68	95.49	100.6	93.89	100.9	103.	101.5
RPE_T (%)[c]		--	--	8.84	--	--	6.82		--	6.81	--	--	6.81	--	--

[a]Three factors were selected for both PLS and PCR

[b] Parameters for spread coefficient and the number of neurons in the hidden layer are 19 and 5, respectively

[c] RPE_S (%) and RPE_T (%) are relative prediction errors for single and total components, respectively

[d] Recovery (%) = $100 \times \sum (c_{ij,pred}-c_{ij,added})/n$, where n is the number of samples, c_{ij} is the concentration of the *j*th component in the *i*th sample

environmental, pharmaceutical and pesticides importance in real mixtures. Chemometrics methods for prediction have played a central role in facilitating the simultaneous analysis, and we have compared the performance of many chemometrics methods for prediction abilities of analytes from responses obtained by spectrophotometric or voltammetric means. In this context, we have found that sometimes PLS, PCR and several ANN methods perform about equally ut we noted that the RBF-ANN method was performing consistently better or at least as well as others.[21, 41-43] The underlying reasons for this efficient performance were not obvious. However, in this work, we were able to demonstrate quite clearly the significant non-linear behaviour of the analytes' responses, which could be responsible for the poor performance of the PLS calibration models.

Artificial Neural Network is a kind of information processing system, which can imitate human brain structure and function and reflects some essence features of cerebral. It's not the lifelike description but the abstraction and simulation of creature neural system. It's not in an attempt to use nonlinear model to depict nonlinear system, but utilizing its nonlinear structure and its handling capacity of nonlinear system to solve the problem of nonlinear calibration.

The basic constituent part of neural network is neuron (also known as node). It's the fundamental processing unit. Neuron is the elementary unit of receiving, producing and conveying information in network. It includes three portions: input, sum transforming and output. Accepted neuron will carry out weighting sum to it. Furthermore, compare the sum with threshold value, and finally make sure whether activate it to export or restrain it to go on next training. Assumed x_i (i=1,2,…,p) is the ith signal to input the neuron, ω_I (i=1,2,…,p) is the weight of the ith signal, b is the bias of neuron. The import of biasing value can make figure of enabled function possible to shift the lift and right sides. After incoming signal entering into neuron and processing weighting, we can get $\sum x_i\omega_I$ by summing. This signal outputs again and get into next neuron after dealt by transition function f. The expression of output signal is

$$y=f\left(\sum \chi_i\omega_i+b\right) \quad \ldots (4)$$

RBF-ANN is the variation of three layers feed forward network, and contains input layer, hidden layer and output layer. Input layer doesn't handle signal, but distributes incoming message merely. Every neuron on hidden layer represents a suite radial basis function (RBF). Generally speaking, hidden layer exists several ordinary neurons and one biasing neuron. In this paper, we take kernel function for RBF neuron. The kernel function exported by hidden layer node is defined as

$$output_j = O_j(x) = exp\left[-\left(\frac{|x-c_j|}{b_j}\right)^2\right] \quad \ldots (5)$$

In the formula x is output vector, c_j is the center of the jth hidden layer node, b_j is the width of jth hidden layer node, $|x-c_j|$ is the Eucilidean of x and c_j, c_j and b_j are called as activation space. Admittedly, when the input data approach to c_j, the output o_i produced by hidden layer node is superior. After transform processing, the o_i is transmitted to output layer node. The transform function of output layer is linear function.

$$y_i = \sum_{i=1}^{n} \omega_{ji} o_j(x) \quad \ldots (6)$$

In the formula ω_{ji} is weight to link hidden layer node i and output layer node j.

Besides input and output vector, there are several important parameters in the design of RBF-ANN, for example, spread coefficient of radial basis functions, mean squared error goal and the number of hidden layer node. Spread coefficient is the main parameter. Bigger the spread coefficient is, smoother the function approximation. But if the spread is too great, massive neurons must fit fast transitional function and if the spread is too small, a great deal of neurons must be used to polyfit, which will result in worse capability of generalization. So, it's an important key to select appropriate spread coefficient of radial basis functions. Mean squared error goal is discrepant degree of actual and target output. If the error is too big, the train of network is not complete and if the error is too small, the polyfit may be excessive. In RBF-ANN, the quality of network is determined by the selection of the number hidden layer nodes to the extent. Processing the training of network, the selected spread coefficient is 19, mean squared error goal is 0.001, the number of hidden layer node is 5 and iteration.

Analytical application in real samples

In conventional methods, the extraction of rutin and quercetin was accomplished by heating, boiling

Table 4 — Compare RBF-ANN with HPLC for detection of ascorbic acid, rutin and quercetin in the health food (μg/g)

Sample [b]	Found (RBF-ANN [a])			Found (HPLC)		
	Ascorbic acid	Rutin	Quercetin	Ascorbic acid	Rutin	Querceti
Propolis	1.69±0.02[c]	169.79±0.03	47.45±0.03	1.7±0.1	170.0±0.3	50.0 ±0.2
Apple pulp	22.50±0.03	27.87±0.02	4.20±0.03	22.3±0.2	28.0±0.1	4.3±0.3
Apple peel	7.98±0.01	220.93±0.01	200.24±0.02	8.0±0.2	220.9±0.1	200.1±0.2
Onion	36.97±0.03	50.96±0.02	8.61±0.02	37.0±0.3	51.0±0.2	8.6±0.1
Buckwheat	0.15±0.02	173.88±0.01	170.21±0.01	0.2±0.1	174.0±0.2	170.1±0.2

[a] Parameters used for RBF-ANN were the same as in Table 3
[b] All the samples were obtained from a supermarket in Nanchang city except propolis which was provided by Wang's bee garden
[c] mean value ±S.D. (n = 3)

or refluxing. The disadvantage of this procedure was the loss of rutin and quercetin due to ionization, hydrolysis and oxidation during extraction. Moreover, the methods brought the consumption of a large amount of solvent and the long extraction time.

The main improvements of sonication for the extractions were related to the yield and shortening of the extraction time. Ultrasounds produce cell disruption, particle size reduction and ultrasonic jet towards solid's surfaces leading to a greater contact area between solid and liquid phase, better access of solvent to valuable components.[44] Considering the above results, the experimental conditions were optimized with aqueous ethanol (70%), solvent/sample weight ratio 40/1 (v/w) and extraction time for 45 min.

In this work, five samples were chosen for analysis. 5.0 g of each sample was constant volume to 250 mL. Then, 2mL pH 5.2 KH_2PO_4-Na_2HPO_4 buffer solution, 0.03 M EDTA 100 μL, and 25 μL of the extract were transferred to the electrochemical cell and constant volume to 10 mL for analysis. The RBF-ANN method was applied for the analysis of the samples because this method was clearly superior to the others on the basis of the %RPE criterion. The results (Table 4) showed that the efficacy of the procedure was further confirmed by comparing with HPLC method.

Conclusions

An analytical method has been developed for the simultaneous voltammetric determination of ascorbic acid, rutin and quercetin. It was based on their anodic peaks observed by differential pulse stripping voltammetry on the glassy carbon electrode. The voltammograms of the individual analytes overlapped heavily, and non-linear response effects of their mixtures, various chemometrics calibration models were applied, e.g. CLS, PCR, PLS and RBF-ANN to facilitate simultaneous prediction of the analytes. Investigation of these models with simulated data and prediction measurements from synthetic mixtures of the analytes showed that RBF-ANN was the most effective calibration method on the basis of the %RPEs and %recovery criteria. Independent analysis of several different food and health products samples without spike confirmed the satisfactory performance of the method. Importantly, it was noted that consecutive studies of chemometrics calibration modeling is now indicating that RBF-ANN is a particularly well performing method.

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