tography (silica gel with hexane/ethyl acetate as eluent), to afford 0.35 g pure **1**. Identity of the compound was verified by TLC, HPLC and comparison of NMR-data with those in the literature [9].

2. High performance liquid chromatography

Commercial samples (NC-1 to NC-5) were bought from Nutrimart (Diamond Bar, CA, USA), additional plant material (NC-6 to NC-8) was grown in the greenhouse. Voucher specimens of all samples are deposited at the NCNPR. Solvents and reagents were of HPLC grade and purchased from Fisher (Fair Lawn, NJ, USA) and Sigma (St. Louis, MO, USA). All samples were analyzed in triplicate (max. standard deviation 1.67%).

Each sample (2.00 g; dried and powdered) was spiked with 1.00 ml of internal standard solution (10 mg geraniol/ml in methanol) and extracted 3-times with 10 ml methanol by sonication for 10 min. The combined extract was dried in vacuum and re-dissolved in 1.50 ml methanol. Prior to use, all samples were filtered through a 0.45 μm Nylaflo filter (Gelman, Ann Arbor, MI, USA). Liquid samples (NC-3 and NC-4) were injected directly after filtration.

Standard solutions were prepared by dissolving 10.0 mg of **1** in 10.0 ml methanol; further calibration levels (concentrations from 1000.0 to 4.1 μg/ml) were prepared by diluting the stock with methanol (resulting regression equation: $y = 89300\ x$; correlation coefficient: $R^2 = 0.9999$).

All HPLC experiments were performed on a Waters 2690 Alliance system, equipped with a 996 PDA detector, using a Synergy MAX-RP 80 Å (150 × 4.6 mm; 4 μm particle size) from Phenomenex (Torrance, CA, USA). The mobile phase consisted of water (A) and acetonitrile (B), which were applied in the following gradient elution: from 60A/40B to 55A/45B in 20 min. Each run was followed by a 5 min wash with an acetonitrile/methyl t-butyl ether-mixture (3:1) and an equilibration period of 10 min. The flow rate, column temperature and detection wavelength were set to 1.0 ml/min, 40 °C and 225 nm, respectively; the sample volume injected was 10 μl. Compound **1** was assigned by comparison with the retention time and UV-spectra of a standard.

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Micro-quantitative determination of quercetin by perturbation of a non-equilibrium stationary state in the Bray-Liebhafsky reaction system

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The aim of the present work is to acquaint a broader readership with a novel method for micro-quantitative determination of quercetin. It uses a general approach to micro-quantitative analysis [1, 2] based on specific features of nonlinear chemical systems far from thermodynamic equilibrium [3]. The method described in this particular work relies on disturbing a delicate balance between the species existing in the matrix reaction system, that is established in a stable non-equilibrium stationary state in the vicinity of a bifurcation point[1]. By introducing the analytes, this balance may be disturbed. The response of the system following the applied perturbation is then monitored. The species examined need not be intrinsic to the matrix reaction system, but it is sufficient that it reacts with it. The choice of the matrix reaction system is optional [1, 2] and, for practical purposes, it is not necessary to know the actual mechanism by which the analyte reacts with the matrix.

For the micro-quantitative determination of quercetin, we have chosen the Bray-Liebhafsky (BL) oscillatory reaction [4, 5], i.e. the reaction of hydrogen peroxide decomposition catalyzed by H^+ and IO_3^-, as the matrix system. This deceptively simple reaction proceeds through a complex mechanism comprising a number of intermediates: I^-, I_2, HOI, HIO_2 etc. [2, 4–14], which makes it suitable for such analysis.

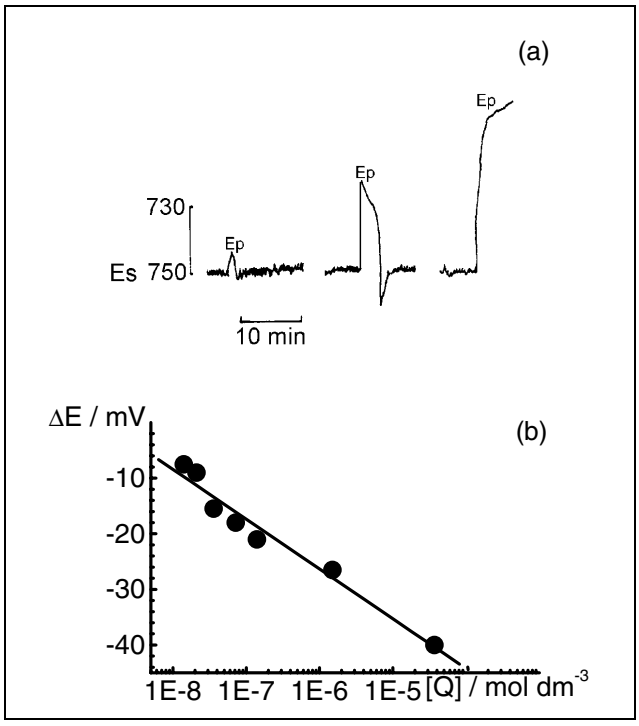

Fig. 1: (a) Typical response curves obtained after perturbing a stationary state in the BL reaction by addition of microvolumes of ethanolic solution of qercetin. The quercetin concentrations, from left to right, are: $[Q] = 1.4 \times 10^{-8}$ mol dm^{-3}; $[Q] = 1.5 \times 10^{-7}$ mol dm^{-3}; $[Q] = 3.7 \times 10^{-5}$ mol dm^{-3}. (b) A standard series calibration curve for quercetin

Typical response curves, obtained after perturbing a stable non-equilibrium stationary state established in the BL system with microvolumes of ethanolic solution of quercetin, are given in Fig. 1a. As can be seen, the change in potential, defined as the difference $\Delta E = E_p - E_s$, is proportional to the quercetin concentration. Hence, the unknown concentration of quercetin in a sample may be determined from a previously constructed calibration diagram (Figure 1b).

It is important to underline that the detection limit for quercetin, defined as the concentration of the tested species which will produce a signal-to-noise ratio of 3, is determined to be $[Q] = 1.5 \times 10^{-8}$ mol dm^{-3}. Thus, by the proposed method the detection limit is improved considerably – about two orders of magnitude, compared to spectrophotometric methods [15]. At the same time, the amount of sample required for a complete analysis may be as small as 20 μl.

Experimental

As a first step in the micro-quantitative determination of quercetin, we have chosen the Bray-Liebhafsky (BL) oscillatory reaction [4, 5] as the matrix system. In this system, the decomposition of hydrogen peroxide, catalyzed by H^+ and IO_3^-, is being achieved:

$$2H_2O_2 \xrightarrow{H^+, IO_3^-} O_2 + 2H_2O\,.$$

Then, as the second step, a suitable stable non-equilibrium stationary state has to be determined. To achieve this, the BL reaction was conducted in the Continuously fed well Stirred Tank Reactor (CSTR) [2, 3, 11, 13]. A schematic representation of the experimental setup is given in Fig. 2a. In the CSTR, a dynamic state of the BL reaction can be achieved and sustained over an extended time by continuously supplying aqueous solutions of KIO_3, H_2SO_4 and H_2O_2 from reservoirs into the reaction vessel and by removing the surplus volume of the reaction mixture. This state may be deliberately changed by varying any of the control parameters, for example temperature. When temperature is varied, the transition from a stable non-equilibrium stationary state to sustained oscillations, i.e. a bifurcation, occurs at a certain critical value of temperature, when the temperature is increased while all other parameters remain unchanged [2, 13]. This evolution of BL system dynamics over time, monitored potentiometrically [2] is presented in Fig. 2b, and the corresponding bifurcation diagram is given in Fig. 2c. Since the bifurcation point is found at T = 49.5 °C (Fig. 2c), the stable non-equilibrium stationary state in the vicinity of the bifurcation point, i.e. at the temperature T = 46.3 °C (indicated by an arrow in Fig. 1c), is selected as the working temperature for further analysis.

Finally, to construct the calibration diagram, perturbations of the selected stable non-equilibrium stationary state were performed by addition of ethanolic solutions of quercetin. Volumes from 5 μl to 500 μl were added. (Perturbations with volumes larger than 500 μl are not reliable, under the current setup, because the dynamic pattern may be additionally changed as a result of dilution.) Since not only the amount of the injected analyte, but also the rate and the duration of the injection may affect the subsequent response [14], fast and reproducible injections are required. We used manual injections with an approximate duration of 0.5 s [2]. Quercetin (3,3′,4′,5,7-pentahydroxyflavone) was introduced as a solution of quercetin dihydrate ($C_{15}H_{10}O_7{\bullet}2H_2O$) in ethanol. (Additions of such volumes of ethanol alone do not perturb the BL system.) The linear dependence obtained between the potential shift (ΔE) and the logarithm of quercetin concentration (log [Q]) is presented in Fig. 1b. Points in the calibration curve (Fig. 1b), for a series of standard solutions, are obtained from three independent additions. The best linear fit of the experimental points is determined by the least squares method. In the concentration range investigated, the regression equation of the standard series calibration curve shown in Fig. 1b, is

$$\Delta E = -9.0 \times \log [Q] - 80$$

with a correlation coefficient r = 0.98.

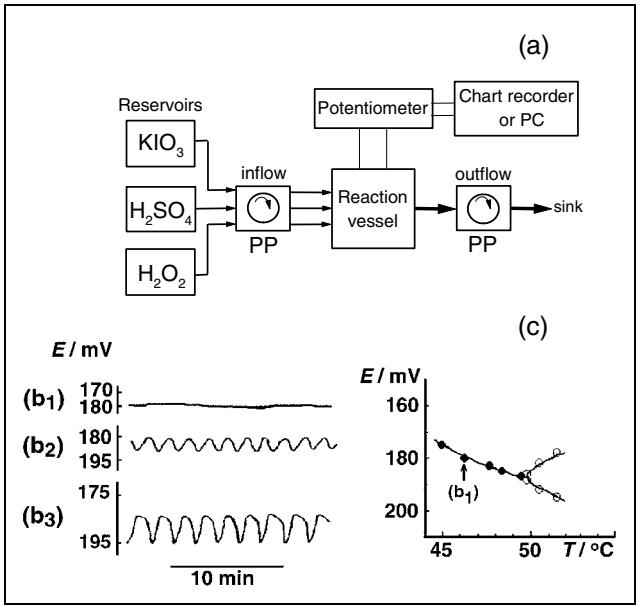

Fig. 2: (a) Schematic presentation of the applied experimental setup used. (PP stands for peristaltic pumps.) (b) BL reaction dynamics established when the mixed inflow concentrations are: $[KIO_3]_0 = 5.9 \times 10^{-2}$ mol dm^{-3}, $[H_2SO_4]_0 = 5.5 \times 10^{-2}$ mol dm^{-3}, $[H_2O_2]_0 = 2.0 \times 10^{-1}$ mol dm^{-3}, the residence time is $\tau = 33.8$ min; at (b_1) T = 48.5 °C, (b_2) T = 50.5 °C, (b_3) T = 51.5 °C. (c) Bifurcation diagram showing the transition from a stable stationary state (solid circles) to sustained periodic oscillations (open circles), when the temperature is increased. The actual operation point is indicated by an arrow

[1] A qualitative change of one dynamic structure into another caused by variation of the control parameter is called a bifurcation [3].

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