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Application of multivariate curve resolution – alternating least square (MCR-ALS) method for determination of concentration profiles of flavonoid–DNA equilibrium monitored by UV-Vis data

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Abstract

Interaction of one of flavonoids namely quercetin with chicken blood ds-DNA has been investigated via spectrophotometric method at different temperatures. The equilibrium constants of flavonoid-DNA complex in several temperatures have been determined by applying MCR-ALS method on the absorption spectra. The quantitative analysis of the data of undefined mixtures, was carried out by simultaneous resolution of the overlapping spectral bands in the whole set of absorption spectra. The enthalpy and entropy of the dimerization reactions were determined from the dependence of the equilibrium constants on the temperature (van't Hoff equation).

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Introduction

Transcription and replication are not only important for cell survival and proliferation but they also help in smooth functioning of all body processes. DNA starts transcribing or replicating only when it receives a signal, which is often in the form of a regulatory protein binding to a particular region of the DNA. Thus, if the binding specificity and strength of this regulatory protein can be mimicked by a small molecule, then DNA function can be artificially modulated, inhibited or activated by binding this molecule instead of the protein. The synthetic or natural small molecule can act as a drug when activation or inhibition of DNA function is required to cure or control a Disease (Uil *et al*., 2003). Chemotherapy is the technique in which replication of DNA is stopped by these foreign molecules i.e., drugs. The molecules which interact with DNA are classified as having anticancerous properties because they can inhibit further replication of DNA. Among this large family of molecules, flavonoids have got considerable interest due to their broad pharmacological activity. In fact, they are best known for their antioxidant properties, and can act in vitro as reducing agents, hydrogen donors, free radical quenchers and metal ion chelators and this may account for the anti-tumor activities of flavonoids (Russo *et al.,* 2000).

Reactivity of deoxyribonucleic acid (DNA) offers the analytical chemist a powerful tool in the recognition and monitoring of many biologically important compounds (Palecek *et al.,* 1998). DNA contains all of the genetic information related to cellular function, including DNA replication and gene expression. However, DNA molecule can be easily damaged, e.g., by reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Oliveira *et al*., 2004). Therefore, in a health preventing perspective, there is a deep interest in identifying free radical scavengers or antioxidants that inhibit oxidative DNA damage. Flavonoids are non-nutritive compounds of plants which possess broad pharmacological activities like antioxidant, antiallergic, anti-inflammatory, antimicrobial, anticancer, antiviral, antitumor, antimutagenic and antiviral (Johnson *et al.,* 2000; Russo *et al.,* 2000). The nature and dynamics of binding of small molecules (drugs and flavonoids) to biomacromolecules like DNA represent an active area of investigation which can lead to rational drug design.

In 2011, Janjua *et al.* studied flavonoid–DNA binding interactions at physiological conditions in which spectral effects upon addition of DNA to drug were investigated. The observed spectral effects (bathochromic and hypochromic effects) were partly attributed to intercalation of the interacting chromophore between DNA bases. Generally, both the bathochromic and hypochromic effects in the absorption spectrum have been attributed to intercalation of small molecules into double helix of DNA (Janjua *et al.,* 2011).

The structures of flavonoid derivatives also provide evidence to intercalation mode. Planarity is the key structural feature for intercalation i.e., planar aromatic chromophore can insert between two base pairs in DNA double helix (Janjua *et al.,* 2011).

The investigations based on drug–DNA interactions not only help to understand the action mechanisms of some anti-tumor and anti-viral drugs but also to design new DNA-targeted drugs and to screen these drugs in vitro. This emerging field of research has got much importance in recent years (Uil *et al .,* 2003; Russo *et al.,* 2000; Janjua *et al,.* 2009) and a large number of techniques likes gel electrophoresis (Hamdan *et al.,* 1998), footprinting technique (Mauffret *et al.,* 1991), X-ray crystallography (Kennard, 1993), structural modeling (Riahi *et al.,* 2010), spectroscopy and especially fluorescent spectroscopy (Zhang *et al.,* 2010; Kanakis *et al.,* 2009) have been employed to study the interactions of some anticancer drugs with DNA.

In the present paper, the application of the multivariate curve resolution (MCR) method for the analysis of UV-Vis data is proposed and interaction of one of flavonoids namely quercetin with chicken blood ds-DNA has been investigated via application of

spectrophotometric data at different temperatures. This approach is based on the determination of formation constant of flavonoid–DNA complex and thermodynamic parameters which is showed the strength of bonding between flavonoid and DNA as well as spontaneity (enthalpy driven process) and compactness of complex. Thus the goal is to calculation of the thermodynamic parameters and equilibrium constants of formation equilibria of drug-DNA at different temperatures.

Materials and methods

Material

Chicken blood DNA extracted in lab was dissolved in autoclaved distilled water (16, 18 and 20 μM) and their concentration was determined spectrophotometrically at 260 nm using molar extinction coefficient, ε_{260} = 6600 cm^{-1} M^{-1}. Quercetin hydrate (Acros Organics) were used without purification (20, 22 and 24μM). Experiments were conducted in 0.1M sodium phosphate buffer solution with pH 7.30.

Apparatus

Absorption spectra were measured on CARY 100 UV–vis Spectrophotometer (Varian) equipped by temperature controller. Conventional quartz cell (10mm×10mm) were used throughout. The cuvettes were treated with repel-silane prior to measurements to avoid dye adsorption. The pH values were measured by a Metrohm 692 furnished with combined calomel Ag/AgCl electrode.

Computer hardware and software

All absorption spectra where digitized at five data points per nanometer in the wavelength 340-450 nm for and transferred to a computer for subsequent analysis by MATLAB (Mathworks, Version 7.8).

Data treatment: Multivariate analysis

Multivariate UV-Vis data were analyzed with the softmodeling MCR procedure to evaluate pure spectra and concentration profiles of spectroscopically active components present in the system from decomposition of experimental data matrix D according to the equation:

$$D = CS^T + E \quad (1)$$

where C and S^T are data matrices containing concentration profiles and pure spectra for each one of these components or conformations present in the experiment. E contains residual noise not explained by the proposed components or conformations in C and S^T. The MCR procedure applied in this work consisted of the following steps (for a more extensive explanation, see references (Tauler *et al.,* 1995):

1. *Data arrangement*: For an experiment monitored by UV-Vis, the recorded spectra were collected in a table or matrix D. The dimensions of this matrix were N_r rows×λ_m columns, where N_r represents the spectra recorded at successive temperature values and λ_m the number of wavelengths measured.

2. Determination of the number of conformations, N: The number of spectroscopically active conformations N was estimated by applying several methods, like singular value decomposition (SVD) or SIMPLISMA (Tauler *et al.,* 1995).

3. ALS optimization: The ALS optimization procedure is an iterative method used to solve Eq. (1) for the proposed number of conformations N. This iterative process is started with an initial estimation of the pure spectra S_T for each one of the N components or conformations proposed (Tauler *et al.,* 1995).

Concentration profiles C and pure spectra S^T resolved for each conformation in the analysis of individual data matrices may differ from the true ones because of possible unresolved underlying factor analysis ambiguities (Tauler *et al.,* 1995). This means that concentration profiles and pure spectra may be only one solution within a band of feasible solutions that are bounded by the constraints applied in the calculation. Some of these ambiguities are more easily solved by means of the simultaneous MCR analysis of multiple experiments under different conditions. The model used for MCR simultaneous analysis of several equilibrium systems at different total concentrations

is described by Eq. (2).

$$\begin{bmatrix} D_{5.15x10^{-5}M} \\ D_{5.75x10^{-5}M} \\ D_{6.35x10^{-5}M} \end{bmatrix} = \begin{bmatrix} C_{5.15x10^{-5}M} \\ C_{5.75x10^{-5}M} \\ C_{6.35x10^{-5}M} \end{bmatrix} S^T + \begin{bmatrix} E_{5.15x10^{-5}M} \\ E_{5.75x10^{-5}M} \\ E_{6.35x10^{-5}M} \end{bmatrix} \quad (2)$$

This simultaneous analysis of several matrices has already been shown to be more powerful than the separate individual analysis, and allows improvement of the resolution of complex experimental data structures. MCR analysis of column-wise augmented data matrices has been shown to give more reliable solutions, eventually removing rotational ambiguities and rank deficiency problems (Tauler *et al.,* 1995).

All MCR calculations were performed using in MATLAB (version 7.8, The Mathworks Inc., Natick, MA, USA) routines, which can be downloaded from the MCR webpage (ASCII files).

Results and discussion

Two bands appeared in the UV spectra of pure flavonoids in 1:1 ethanol–water mixture where absorbance increased with the flavonoid concentration in accordance with the Beer's law. In all flavonoids, the band I at higher wavelength is related to the n–π* transitions whereas the band II is related to the π–π* chromophoric transitions. A change in flavonoid spectra with added DNA indicates the formation of some type of flavonoid–DNA complex. Thus the equilibrium established may be:

Flavonoid + DNA ↔ flavonoid – DNA (3)

Table 1. Equilibrium constant (K) and thermodynamic parameters values at different concentrations.

Concentration	[DNA]=16μM [Quercetin]=20 μM	[DNA]=18μM [Quercetin]=22 μM	[DNA]=20μM [Quercetin]=24 μM
Log K (30°C)	4.46	4.50	4.50
ΔH° (kJ mol^{-1})	-110.3	-110.5	-110.2
ΔS° (J mol^{-1} K^{-1})	-226.8	-226.5	-227.1

The absorption spectra of the solution, at different total dye concentrations of DNA (16, 18 and 20 μM) and Quercetin hydrate (20, 22 and 24 μM), were recorded in the wavelength 340-450 nm and temperature 30–70 °C at 5 °C intervals and pH 7.30. The samples absorption spectra are shown in Fig. 1.

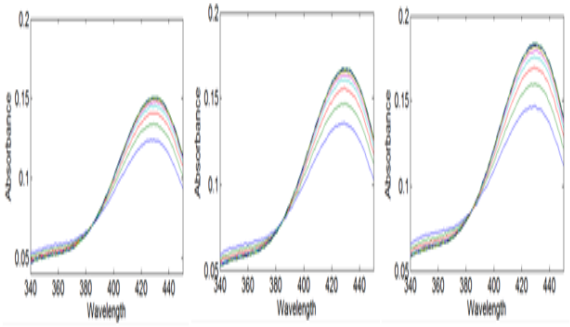

Fig. 1. Absorption spectra of system: a) [DNA]=16μM, [Quercetin]=20 μM, b) [DNA]=18 μM, [Quercetin]=22 μM and c) [DNA]=20 μM, [Quercetin]=24 μM in5 °C intervals between 30 and 70 °C at pH 7.30.

The MCR analysis was repeated with three and four components, and with several combinations of constraints, i.e. non-negativity, equality, unimodality and closure. The best (chemically meaningful) results were obtained when only three components were considered, all of them included in the closure constraint, and when the non-negativity constraint was applied to both spectral and concentration profiles. The lack of fit was 2.81% of the experimental data matrix D, which was considered good taking into account the instrumental technique and the large number of matrices simultaneously analyzed, recorded in independent experimental conditions.

The resolved concentration profiles (Fig. 2) and spectra profiles (Fig. 3) show the presence of three components.

where H° is the molar enthalpy change, S is the molar entropy change , R = 8.31 Jmol^{-1} K^{-1} the universal gas constant, and T the Kelvin temperature. A linear regression of equilibrium constants with respect to 1/T is then performed, which determines enthalpy and entropy change of the reaction (Table 1).

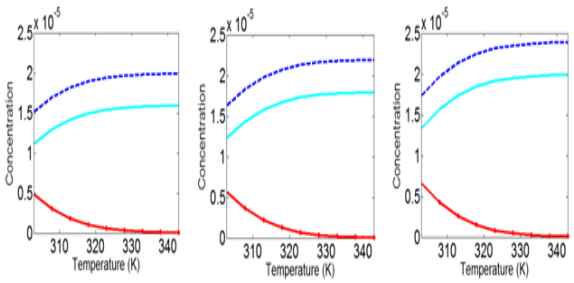

Fig. 2. Concentration profiles of Quercetine (---), DNA (—) and Quercetine-DNA (*): a) [DNA]=16µM, [Quercetin]=20 µM, b) [DNA]=18 µM, [Quercetin]=22 µM and c) [DNA]=20 µM, [Quercetin]=24 µM in 5 °C intervals between 30 and 70 °C.

The concentration profiles clearly depicts that as we increase the temperature from 303 to 343 K, the binding constants of all flavonoids decrease i.e., the association between flavonoid and DNA weakens as a result of destabilized flavonoid–DNA complex (Janjua *et al.,* 2009).

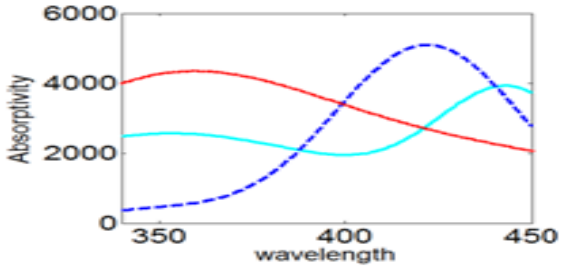

Fig. 3. Spectra profiles of DNA (—), Quercetine (---) and DNA-Quercetine (...).

As intercalation is reversible inclusion of drug in between the base pairs of DNA, so at higher temperatures the complex formed between the DNA and flavonoids molecule may not remain compact as it was at lower temperatures. Increase in temperature changes the geometrical orientation of DNA and the complex formed may become loose and the dissociation may set in. Thus the formation constant values decrease as the temperature is increased. From the negative values of ΔH, one can presume that complex formation is an enthalpy driven process (Shahabadi *et al.,* 2009). During the formation of the complex, the overall entropy of the system also decreases i.e., more compact complex is formed.

Conclusions

Multivariate analysis has been shown to be a useful tool for the analysis of UV-Vis data recorded during thermal change of equilibria. The application of MCR improved the results obtained by classical univariate analysis of UV-Vis data. The simultaneous analysis of several data matrices, corresponding to different experimental conditions, has allowed the resolution of several systems which are difficult to solve by individual analysis. Flavonoids interact with ds.DNA in a non-covalent way of interaction via intercalation due to their planarity. The negative values of enthalpy change indicate exothermic of binding of quercetine with ds.DNA.

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