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Competitive interactions of adriamycin and ethidium bromide with DNA as studied by full rank parallel factor analysis of fluorescence three-way array data

Hong-Ping Xie [a,b], Xia Chu [c], Jian-Hui Jiang [a], Hui Cui [a], Guo-Li Shen [a], Ru-Qin Yu [a,*]

[a] *State Key Laboratory for Chemo/Biosensing and Chemometrics, College of Chemometrics and Chemical Engineering, Institute for Chemometrics and Chemical Technology, Hunan University, Changsha 410082, People's Republic of China*

[b] *Department of Chemistry, Sichuan Teachers' College, Nanchong 637002, People's Republic of China*

[c] *College of Chemistry and Chemical Engineering, Hunan Normal University, Changsha 410082, People's Republic of China*

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Abstract

The competitive interactions of adriamycin (AMC) and a fluorescence probe of ethidium bromide (EB) with DNA have been studied by full rank parallel factor analysis (FRA-PARAFAC) of fluorescence excitation–emission three-way data array. The excitation and emission spectra as well as the equilibrium concentrations of co-existing species in different reaction mixtures can be directly obtained by the FRA-PARAFAC treatment. The concordance of the resolved excitation and emission spectra of AMC, EB and EB–DNA with the standard spectra of these species confirmed the reliability of the equilibrium concentrations of these components in the reaction mixtures studied. The results obtained are valuable for providing a deeper insight into the competitive interaction mechanism of AMC and EB with DNA. The conclusion was directly given out that the interaction of AMC with DNA is the intercalating model. The FRA-PARAFAC method as exemplified by the present study provides an useful approach for studying the interaction of clinical drugs with DNA in the presence of disturbance of drug assistants.

Keywords: Competitive interaction; Full rank parallel factor analysis; DNA; Adriamycin; Ethidium bromide

1. Introduction

An anti-cancer antibiotic adriamycin (AMC) [1] with the anthracycline structure linked to an amino sugar has been widely used in clinical therapy of many kinds of cancer [2]. It is believed that AMC binds to DNA by weak linkages with the base pairs of DNA [3]. The binding interaction, which inhibits the cellular DNA and RNA dependent replication and transcription processes, results in the anti-cancer activity [4–7]. Studies of binding interaction between the drug and DNA is very interesting not only in understanding the

* Corresponding author. Tel./fax: +86-731-882-2782
E-mail address: rqyu@mail.hunu.edu.en (R.-Q. Yu).

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mechanism of interaction, but also for guiding the design of new drugs.

For studying the binding interaction between AMC and DNA, Vedaldi et al. [8] utilized the quenching of the fluorescence occurring in the drug complexed with DNA by means of fluorimetric titration. With the development of chemometric algorithm for high order data, in particular for the trilinear excitation–emission fluorescence data array, it becomes possible to study the competitive interactions of many chemical species with DNA, and one can directly obtain the interaction model of the drug of interest with DNA without utilizing Scatchard plot [9] for mechanism elucidation even in the presence of unknown interferents such as drug assistants. Such a type of studies is especially valuable for guiding the use of clinical drugs and the design of new drugs.

We have studied the competitive interactions of AMC and ethidium bromide (EB) with DNA in the presence of the assistants of the clinical drug. EB was utilized to probe the interaction of AMC with DNA. A three-way data array of the complex system consisting of anti-cancer drug AMC, fluorescence probe EB and DNA has been obtained by excitation–emission fluorescence spectroscopy and resolved by a proposed chemometric algorithm of full rank parallel factor analysis (FRA-PARAFAC) [10], which is a modified algorithm for parallel factor analysis [11]. The method determines simultaneously excitation and emission spectra of AMC, EB and complex of EB and DNA and their equilibrium concentration variations with different initial concentrations of AMC. The results obtained provide valuable information for a deeper insight into the competitive interaction model of AMC and EB with DNA.

2. Trilinear model and resolution algorithm of FRA-PARAFAC

2.1. Trilinear model

The K of $I \times J$ matrices of the excitation–emission fluorescence spectra are obtained by measuring K equilibrium mixtures containing N fluorescing components at I excitation and J emission wavelengths. An $(I \times J \times K)$ three-way data array $\mathbf{R}$ is formulated by the K of $(I \times J)$ matrices as following [12]:

$$\mathbf{R}_{I\times J\times K} = \sum_{n=1}^{N} \boldsymbol{x}_n \otimes \boldsymbol{y}_n \otimes \boldsymbol{z}_n + \mathbf{E}_{I\times J\times K} \tag{1}$$

Here the symbol $\otimes$ denotes a tensor product; $\boldsymbol{x}_n$, $\boldsymbol{y}_n$ and $\boldsymbol{z}_n$ are excitation, emission and concentration profiles of the nth fluorescing chemical component, respectively; $\mathbf{E}$ is a three-way array of residuals.

The excitation, emission and concentration profiles of N fluorescing species may be expressed with matrices as following:

$$\mathbf{X}_{I\times N} = (\boldsymbol{x}_1,\ \boldsymbol{x}_2,\ \ldots,\ \boldsymbol{x}_N) \tag{2}$$

$$\mathbf{Y}_{J\times N} = (\boldsymbol{y}_1,\ \boldsymbol{y}_2,\ \ldots,\ \boldsymbol{y}_N) \tag{3}$$

$$\mathbf{Z}_{K\times N} = (\boldsymbol{z}_1, \boldsymbol{z}_2,\ \ldots,\ \boldsymbol{z}_N) \tag{4}$$

where $\mathbf{X}$, $\mathbf{Y}$ and $\mathbf{Z}$ are excitation, emission and concentration matrices of N species, respectively.

The trilinear model can also be expressed as matrices along K-mode, i.e. sample mode for the data array studied:

$$\mathbf{R}\ldots_k = \mathbf{X}\mathbf{diag}(\boldsymbol{z}_{(k)})\mathbf{Y}^{\mathrm{T}} + \mathbf{E}\ldots_k \quad (k = 1,\ 2,\ \ldots,\ K) \tag{5}$$

where the superscript 'T' denotes transpose of a matrix; $\mathbf{R}\ldots_k$ and $\mathbf{E}\ldots_k$ are the kth matrix slices of $\mathbf{R}$ and $\mathbf{E}$ along K-mode, respectively; $\mathbf{diag}(\boldsymbol{z}_{(k)})$ denotes the diagonal matrix whose diagonal elements are the corresponding ones of the kth row vector $\boldsymbol{z}_{(k)}$ of the concentration matrix $\mathbf{Z}_{K\times N}$.

2.2. Trilinear algorithm of FRA-PARAFAC

Based on the trilinear model, FRA-PARAFAC algorithm utilizes alternating least squares (ALS) in an iterative way to carry out trilinear resolutions and to determine the response profiles $\mathbf{X}$, $\mathbf{Y}$ and $\mathbf{Z}$. Actually, for trilinear resolution it is only necessary that matrices $\mathbf{X}$ and $\mathbf{Y}$, i.e. fluorescence excitation and emission spectrum matrices, respectively, are full rank ones for their column spaces, that is, trilinear model shown by (Eq. (1)) would only be symmetrical with respect to x and y. FRA-PARAFAC algorithm utilizes the constrained

condition of column full rank of matrices **X** and **Y** in ALS procedure.

The objective function and constraint equations are formulated as:

$$E(\mathbf{X},\ \mathbf{Y},\ \mathbf{Z}) = \sum_{k=1}^{K} \|\mathbf{R}\ldots_k - \mathbf{X}\mathbf{diag}(z_{(k)})\mathbf{Y}^{\mathrm{T}}\|^2 \quad (6a)$$

$$\mathbf{X}^{\mathrm{T}}\mathbf{P} = \mathbf{I}_{\mathrm{N}} \quad (6b)$$

$$\mathbf{Y}^{\mathrm{T}}\mathbf{Q} = \mathbf{I}_{\mathrm{N}} \quad (6c)$$

The Eqs. (6b) and (6c) are really the condition of column full rank of **X** and **Y**, where **P** and **Q** are $I \times N$ and $J \times N$ matrices, respectively, and $\mathbf{I_N}$ is a $N \times N$ identity matrix.

In order to simplify the calculation procedure, the dimension of **X**, **Y**, **P** and **Q** matrices are reduced by transforming them into $N \times N$ matrices **A**, **B**, **G** and **H**, respectively, as follows:

$$\mathbf{X} = \mathbf{U}_X\mathbf{A} \quad (7)$$

$$\mathbf{Y} = \mathbf{U}_Y\mathbf{B} \quad (8)$$

$$\mathbf{P} = \mathbf{U}_X\mathbf{G} \quad (9)$$

$$\mathbf{Q} = \mathbf{U}_Y\mathbf{H} \quad (10)$$

where $\mathbf{U}_X$ and $\mathbf{U}_Y$ are the principal component vectors corresponding to the first N largest singular values obtained from singular value decomposition of matrices unfolded along I-mode and J-mode of **R**, respectively. The objective function and constraint equations (Eqs. (6a), (6b) and (6c)) are transformed into:

$$E(\mathbf{A},\ \mathbf{B},\ \mathbf{Z}) = \sum_{k=1}^{K} \|\mathbf{R}\ldots_k - \mathbf{U}_X\mathbf{A}\mathbf{diag}(z_{(k)})\mathbf{B}^{\mathrm{T}}\mathbf{U}_Y^{\mathrm{T}}\|^2 \quad (11a)$$

$$\mathbf{A}\mathbf{G}^{\mathrm{T}} = \mathbf{I}_{\mathrm{N}} \quad (11b)$$

$$\mathbf{B}\mathbf{H}^{\mathrm{T}} = \mathbf{I}_{\mathrm{N}} \quad (11c)$$

The constrained minimization problem of Eqs. (11a), (11b) and (11c) is equivalent to an unconstrained one as following:

$$L(\mathbf{A},\ \mathbf{B},\ \mathbf{Z}) = \sum_{k=1}^{K} \|\tilde{\mathbf{R}}\ldots_k - \mathbf{A}\mathbf{diag}(z_{(k)})\mathbf{B}^{\mathrm{T}}\|^2 + \lambda\|\mathbf{A}\mathbf{G}^{\mathrm{T}} - \mathbf{I}_{\mathrm{N}}\|^2 + \lambda\|\mathbf{B}\mathbf{H}^{\mathrm{T}} - \mathbf{I}_{\mathrm{N}}\|^2 \quad (12)$$

where λ is a penalty weight, and,

$$\tilde{\mathbf{R}}\ldots_k = \mathbf{U}_X^{\mathrm{T}}\mathbf{R}\ldots_k\mathbf{U}_Y \quad (k = 1,\ 2,\ \ldots,\ K) \quad (13)$$

The FRA-PARAFAC algorithm minimizes the loss function shown in (Eq. (12)), and then is performed by using following iterative procedure:

1) Estimate the number of chemical components N, and calculate matrices $\mathbf{U}_X$, $\mathbf{U}_Y$. Calculate $\tilde{\mathbf{R}}\ldots_k$ according to (Eq. (13)).
2) Initialize both **A** and **B** with an identity matrix and estimate penalty weight λ.
3) Calculate **Z** according to the formula:

$$z_{(k)} = \mathbf{diag}(\mathbf{A}^{\mathrm{T}}\tilde{\mathbf{R}}\ldots_k\mathbf{B})^{\mathrm{T}}[(\mathbf{A}^{\mathrm{T}}\mathbf{A})*(\mathbf{B}^{\mathrm{T}}\mathbf{B})]^{-1} \quad (k = 1,\ 2,\ \ldots,\ K) \quad (14)$$

Here $z_{(k)}$ is the kth row vector of matrix **Z**, and symbol '*' denotes a Hadamand product, that is, if $\mathbf{W}_{M\times N} = \mathbf{U}^*_{M\times N}\mathbf{V}_{M\times N}$ for $(M \times N)$ matrices **U**, **V** and **W**, then $w_{mn} = u_{mn}v_{mn}$, where w_{mn}, u_{mn} and v_{mn} are elements of the mth row and nth column of matrices **W**, **U** and **V**, respectively.
4) Calculate **G** and **H** according to following expressions:

$$\mathbf{G} = (\mathbf{A}^{-1})^{\mathrm{T}} \quad (15)$$

$$\mathbf{H} = (\mathbf{B}^{-1})^{\mathrm{T}} \quad (16)$$

5) Calculate **A** as following:

$$\mathbf{A} = \left[\sum_{k=1}^{K} \tilde{\mathbf{R}}\ldots_k\mathbf{B}\mathbf{diag}(z_{(k)}) + \lambda\mathbf{G}\right] \times \left[\sum_{k=1}^{K} \mathbf{diag}(z_{(k)})\mathbf{B}\mathbf{B}^{\mathrm{T}}\mathbf{diag}(z_{(k)}) + \lambda\mathbf{G}^{\mathrm{T}}\mathbf{G}\right]^{-1} \quad (17)$$

and then normalize the column vectors of **A**.
6) Calculate **B** as following:

$$\mathbf{B} = \left[\sum_{k=1}^{K} \tilde{\mathbf{R}}\ldots_k^{\mathrm{T}}\mathbf{A}\mathbf{diag}(z_{(k)}) + \lambda\mathbf{H}\right] \times \left[\sum_{k=1}^{K} \mathbf{diag}(z_{(k)})\mathbf{A}\mathbf{A}^{\mathrm{T}}\mathbf{diag}(z_{(k)}) + \lambda\mathbf{H}^{\mathrm{T}}\mathbf{H}\right]^{-1} \quad (18)$$

and then normalize the column vectors of **B**.
7) Return to step (3) until convergence.

The penalty weight is estimated according to minimum model error for a given number of iteration cycles (ca. 10^{-3} for 1000 cycles). The convergence criterion of this algorithm is that the improvement of function (Eq. (12)) between the consecutive two iteration cycles is less than 10^{-10}. The response profiles **X** and **Y** would be calculated according to Eqs. (7) and (8), respectively.

A main advantage for the FRA-PARAFAC algorithm is that it is robust to the estimated component number provided the estimated number is not less than the real chemical component number of the system studied.

3. Experimental

The stock solution of AMC, made in Pharmacia and Upjohn S.P.A., Italy, was prepared by dissolving its freeze-dried power agent for clinical injection into re-distilled water. The stock solution prepared contains doxorubicin hydrochloride of 1.73×10^{-4} mol/l. Calf thymus DNA was obtained from Sino-American Bio-technology Company, China, and the concentration of its stock solution is 0.49 mg/ml of DNA. EB from Sigma was used to prepare stock solution of 1.11×10^{-3} mol/l. All the stock solutions were stored in 4 °C. A phosphate buffer solution was prepared with NaH_2PO_4 of initial concentration 5.00×10^{-3} mol/l adjusted to pH 7.0 with NaOH solution.

A set of the nine reaction mixture solutions were prepared with DNA and EB of initial concentrations of 4.45×10^{-3} mg/ml and 3.34×10^{-6} mol/l, respectively, and the initial concentration of AMC changed from 0 to 6.91×10^{-7} mol/l with an interval of 8.64×10^{-8} mol/l. The binding reaction took place in a room temperature of 24 °C. After the equilibrium of complex reaction was reached, the excitation–emission fluorescence spectra were recorded by F4500 HITACHI fluorescence spectrophoto-meter with the slit widths of 5 nm, scan wavelength intervals of 5 nm and a scan wavelength speed of 240 nm/min. The ranges of excitation and emission wavelengths for all samples were 390–560 and 570–680 nm, respectively.

The background was subtracted using a reagent blank. The FRA-PARAFAC program compiled in MATLAB was used to resolve the spectra and concentrations of co-existing species in the equilibrium mixtures.

4. Result and discussion

By studying for a large number of simulative and real three-way array data, it has been proved that when the component number estimated for a complex system is more than or equal to the real one, FRA-PARAFAC algorithm is a robust method [10]. It resulted from the algorithm utilizing the constrained condition of column full rank of excitation and emission fluorescence data matrices. FRA-PARAFAC is adaptable for studying a complex system which presents unknown interferents.

In the studied system, there obviously are three fluorescing main species, i.e. AMC, EB and a complex EB–DNA, which also are the ones concerned for the reactive system. When a component number 3 is used for the three-way data array obtained, the excitation and emission spectra of AMC, EB and EB–DNA resolved by FRA-PARAFAC algorithm are shown in Figs. 1 and 2. One notices that the excitation and emission spectra resolved for EB and AMC are obviously different from the real ones obtained. It turned out that the component number should be more than three. When FRA-PARAFAC algorithm is run with a component number of 5 for the three-way data array measured, the resolved excitation and emission spectra of EB, AMC and EB–DNA are shown in Figs. 3 and 4, respectively. The resolved excitation and emission spectra concerning the three chemical components are closely matching the real ones obtained. Similar results were obtained when the number of components was set to six. On the later case the excitation and emission spectra resolved for the sixth component were clearly the contribution of experimental noise, with the concentration values very close to zero. The aforementioned results show that the reaction of AMC and EB with DNA is a relatively complicated one. For obtaining correct fluorescence excitation and emission spectra of AMC, EB and EB–DNA one has to set the number of

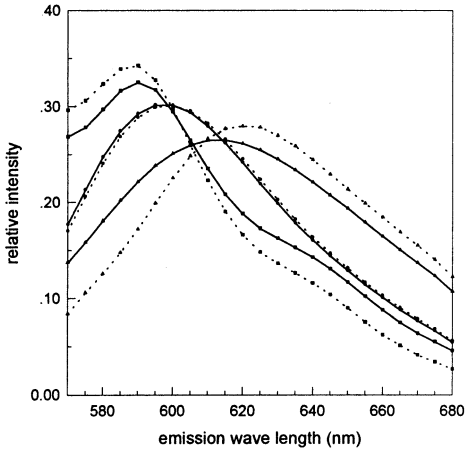

Fig. 1. Emission fluorescence spectra resolved by FRA-PARAFAC using the number of components of 3. Solid and dashed lines are the resolved and recorded spectra, respectively, of AMC (□), EB (△) and EB–DNA (○).

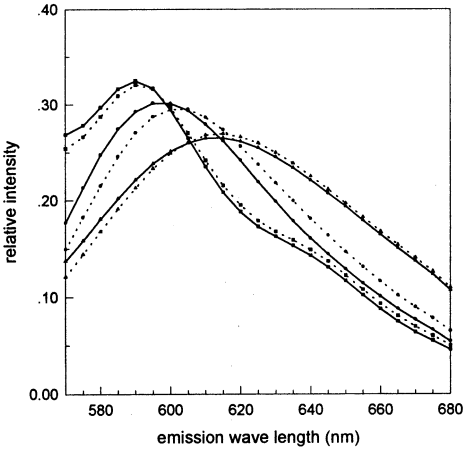

Fig. 3. Emission fluorescence spectra resolved by FRA-PARAFAC using the number of components of 5. Solid and dashed lines are the resolved and recorded spectra, respectively, of AMC (□), EB (△) and EB–DNA (○).

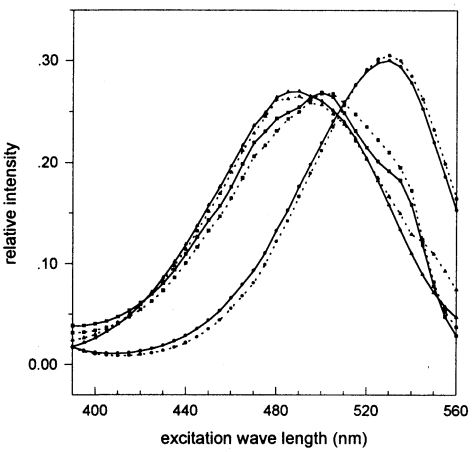

Fig. 2. Excitation fluorescence spectra resolved by FRA-PARAFAC using the number of components of 3. Solid and dashed lines are the resolved and recorded spectra, respectively, of AMC (□), EB (△) and EB–DNA (○).

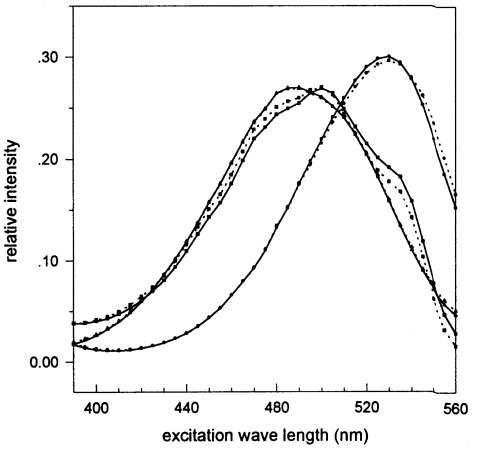

Fig. 4. Excitation fluorescence spectra resolved by FRA-PARAFAC using the number of components of 5. Solid and dashed lines are the resolved and recorded spectra, respectively, of AMC (□), EB (△) and EB–DNA (○).

components to at least 5. The two extra components seems associated with the presence of drug assistants such as lactose and hydroxybenzoate. Another possibility is the deviation of the reaction system studied from the trilinear model which need be compensated by introducing extra factor components. The concordance of the resolved three spectral profiles corresponding to the first three components with the excitation and emission spectra of AMC, EB and EB–DNA confirms that the resolved concentration values corresponding to these three components are concentrations of AMC, EB and EB–DNA in corresponding reaction mixtures. The concentration profiles concerning the three main species EB, AMC and EB–DNA are shown in Fig. 5.

The nine samples measured contain the same initial amount of DNA and EB, and only the initial concentration of AMC is varied from 0 to 6.91×10^{-7} mol/l with a interval of 8.64×10^{-8} mol/l. With increase of the initial concentration of drug AMC the resolved equilibrium concentration of the complex EB–DNA decreases and that of the free EB increases gradually (referring as Fig. 5). This shows clearly that EB in the complex EB–DNA is gradually replaced by free AMC with increasing concentration of AMC added into the reactive system with the formation of a non-fluorescing complex AMC–DNA. It is well known that the interaction of EB with DNA follows the intercalation model and then that EB is an intercalator [9]. EB and the EB–DNA complex are all fluorescing species and the latter has stronger fluorescence. EB is usually used as a standard agent for probing structure of DNA and the interaction of small molecules with DNA. In the study of drug interaction with DNA, in particular, EB has been widely applied as a fluorescence probe [13,14]. The property was utilized to probe the interaction between DNA and AMC in the form of the clinical drug in the presence of drug assistants in this study. From the concentrations resolved for the components involved one can assume that the interaction of AMC with EB–DNA possesses a competitive

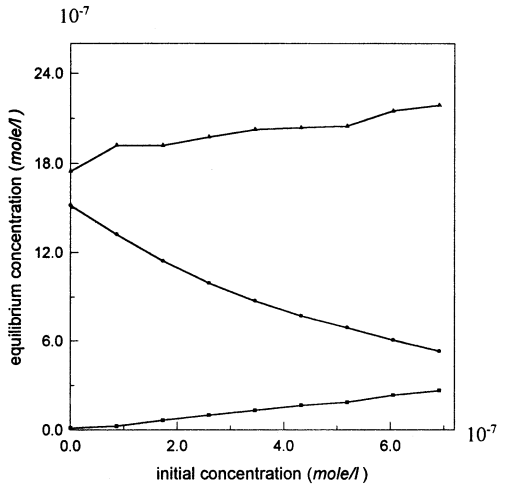

Fig. 5. Equilibrium concentrations of AMC (□), EB (△) and EB–DNA (○) resolved by FRA-PARAFAC using the component number of 5.

mechanism, that is, the interactions of AMC and EB with DNA are a pair of the parallel competitive reactions, and AMC intercalates into the same base sites of DNA as the bound EB.

The interaction of AMC with DNA is also assumed to process the same interaction model with EB, that is, AMC being an intercalator and intercalating between the base pairs of double-stranded DNA. This is consistent with the results obtained using pure chemical agents by resonance Raman spectroscopy [15]. The resolution of excitation–emission fluorescence three-way data array can provide more direct visualization of the equilibrium state of the system studied, as compared with the traditional method using Scatchard plot [9]. The results obtained by studying directly the drug formulations used in cancer therapy in the presence of possible assistants should better represent the real clinical situation of the drug use than those given by using chemically pure doxorubicin hydrochloride. Angeloni et al. [16] also studied the interaction between AMC and DNA and reported that the fluorescence spectrum of AMC–DNA is different from that of AMC. From the resolved spectra obtained by FRA-PARAFAC it seems that the appearance of the species with excitation and emission spectra similar to and at the same time different from the corresponding spectra of AMC was a result of underestimation of the number of factors in the reaction system. The reaction product of AMC and DNA is a non-fluorescing species, which is consistent with the conclusion that the interaction of AMC with DNA is a complex reaction with quenching of the fluorescence [8].

5. Conclusion

The excitation and emission spectra resolved by FRA-PARAFAC algorithm is consistent with the real spectra of AMC, EB and EB–DNA. FRA-PARAFAC algorithm directly gives out the equilibrium concentration variations of these three chemical components in the reaction samples by resolution of the trilinear data array measured. The conclusion is directly obtained that AMC is an intercalator of DNA. The results obtained shed

more light on the interaction process of AMC and DNA in the presence of EB. Trilinear decomposition algorithm has been shown to be efficient in interpretation of excitation–emission type high-dimensional analytical data array.

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