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# Study of the interactions of berberine and daunorubicin with DNA using alternating penalty trilinear decomposition algorithm combined with excitation–emission matrix fluorescence data

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**Abstract**

Studies of interactions between drugs and DNA are very interesting and significant not only in understanding the mechanism of interaction, but also for guiding the design of new drugs. However, until recently, mechanisms of interactions between drug molecules and DNA were still relatively little known. It is necessary to introduce more simple methods to investigate the mechanism of interaction. In this study, the interactions of daunorubicin (DNR) or berberine (BER) with DNA and the competitive interactions of DNR and BER with DNA have been studied by alternating penalty trilinear decomposition algorithm (APTLD) combined with excitation–emission matrix fluorescence data. The excitation and emission spectra as well as the relative concentrations of co-existing species in different reaction and equilibrium mixtures can be directly and conveniently obtained by the APTLD treatment. The results obtained are valuable for providing a deeper insight into the interaction mechanism of DNR and BER with DNA. It is proved that the fluorescence spectrum of complex DNR–DNA is different from that of DNR. Furthermore, the present method provides a new way to search for a new non-toxic, highly efficient fluorescent probe. For controversial interaction mechanism of the drugs and DNA, it can provide a helpful verification.


*Keywords:* Drug; DNA; Interaction; APTLD; Daunorubicin; Berberine

## 1. Introduction

Chemotherapy is an important part of the program for cancer treatment. A lot of compounds have been developed as potential candidates for anticancer drugs, but only a handful of them have become effective clinical drugs [1,2]. The development of new drugs requires that the underlying mechanism of the drug action at the cellular and molecular levels be better understood. The study on the interaction of small molecules with DNA is of great importance in many areas [1–11]. Many anticancer drugs are known to interact with deoxyribonucleic acid (DNA) to exert their biological activities. Generally, DNA-acting anticancer drugs can be classified into three categories. Drugs of the first category form covalent linkages with DNA. Drugs of the second category form noncovalent complexes with DNA by either intercalation or groove-binding. Drugs of the final category cause DNA backbone cleavages [2].

Daunorubicin (DNR) is an anthracycline antibiotic with antiblastic and anticancer activity, which is linked by the formation of intercalative complexes with DNA and the inhibition of both DNA and RNA synthesis [9–11]. Berberine, an alkaloid, is the active component of *Coptis chinensis*, which is a traditional Chinese medicine. It is initially used as an antibiotic and has a wide range of pharmacological activities, including antisecretory, anti-inflammatory, antimicrobial, antimalarial as well as anticancer properties and cardiovascular actions [12–18]. Despite its slightly buckled structure due to the partial saturation of the central ring, berberine has been previously characterized as a DNA intercalating agent and as a cationic ligand, electrostatic forces play an important role in its interaction with DNA [19–25]. However, the intercalation mechanism initially proposed [26] contradicted a minor groove orientation

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mechanism recently proposed on the basis of NMR analyses [27].

It is very interesting and significant that interactions between drugs and DNA are studied for understanding the mechanism of interaction and guiding the design of new drugs. The interactions between drugs and DNA have been studied with structural tools, including high resolution X-ray diffraction and NMR spectroscopy [28] and utilizing Scatchard plot [29]. However, until recently, relatively little was known as far as the detailed interactions between DNA (including some unusual structures) and drug molecules. It is necessary to introduce more simple methods to investigate the mechanism of interaction.

With the development of modern high-order analytical instruments and data collection techniques, in particular the application of chemometrics methods dealing with three-way data set [30–40], it becomes possible to study the interactions between drugs and DNA, and one can conveniently predict the interaction model of the drug of interest with DNA even when there exists a complicated chemical equilibrium in the mixtures. The attractive predominance lies in the fact that the decomposition of a three-way data array is often unique, allowing relative concentrations and profiles (in the spectral, time, pH or other domains) of individual components in a complex system to be extracted directly. The type of studies is especially valuable for guiding the use of clinical drugs and the design of new drugs.

The interactions of DNR and berberine (BER) with DNA have been studied in the present paper. Chemical structures of DNR and BER are characterized in Fig. 1. A series of three-way data arrays has been obtained by excitation–emission fluorescence spectroscopy and resolved by the APTLD algorithm [30]. The results revealed that relative concentrations, excitation and emission spectra profiles of individual components in interaction systems can be conveniently achieved and provide valuable information for a deeper insight into the interaction mechanisms of DNR and BER with DNA.

## 2. Theory

The $K$ size of matrices $I \times J$ of the excitation–emission fluorescence spectra are obtained by measuring $K$ equilibrium mixtures containing $N$ fluorescing components at $I$ excitation and $J$ emission wavelengths. A three-way data array $\underline{\mathbf{X}}$ is obtained with dimensions $I \times J \times K$. According to the trilinear model, i.e. PARAFAC model [41], each element $x_{ijk}$ of the data array $\underline{\mathbf{X}}$ has the form:

$$x_{ijk} = \sum_{n=1}^{N} a_{in} b_{jn} c_{kn} + e_{ijk},$$
$$(i = 1, 2, \ldots, I; \quad j = 1, 2, \ldots, J; \quad k = 1, 2, \ldots, K) \qquad (1)$$

where $x_{ijk}$ is the element $(i, j, k)$ of $\underline{\mathbf{X}}$, $N$ denotes the number of factors, which should correspond to the total number of detectable species, including component(s) of interest and background as well as unknown interferences; $e_{ijk}$ the element of an $I \times J \times K$ three-way residual array $\underline{\mathbf{E}}$; $a_{in}$ the element $(i, n)$ of an $I \times N$ matrix $\mathbf{A}$ corresponding to excitation spectra profiles of $N$ species; $b_{jn}$ the element $(j, n)$ of a $J \times N$ matrix $\mathbf{B}$ corresponding to emission spectra profiles of $N$ species; $c_{kn}$ the element $(k, n)$ of a $K \times N$ matrix $\mathbf{C}$ corresponding to relative concentrations of $N$ species.

Regardless of scaling and permutation, the decomposition of the trilinear model proposed above will be unique and no free rotations provided that $k_1 + k_2 + k_3 \geq 2N + 2$ [41–43], where $k_1$, $k_2$ and $k_3$ are $k$-ranks of $\mathbf{A}$, $\mathbf{B}$ and $\mathbf{C}$, respectively. In other words, the profile matrices $\mathbf{A}$, $\mathbf{B}$ and $\mathbf{C}$ will be resolved in a unique way.

The APTLD algorithm [30] is an alternative one and used to solve the PARAFAC model by utilizing alternating least-squares principle and the alternating penalty constraints to minimize three different alternating penalty (AP) errors simultaneously. It assumes the loadings in two modes and then estimates the unknown set of parameters of the last mode until optimizing the residuals of the model. For detail information of the APTLD algorithm, one could refer to the literature [30]. The number of responsive factors ($N$) can be estimated by several methods. In this study, core consistency diagnostic (CORCONDIA) [44], which compares the results from the core matrix of the Tucker3 and PARAFAC models with different factors attempted, and ADD-ONE-UP [45], which unfolds the three-way data along with the two orders into two cube matrices and then runs PARAFAC algorithm twice to compare the residuals of different factors, were used to estimate the chemical rank of three-way data arrays.

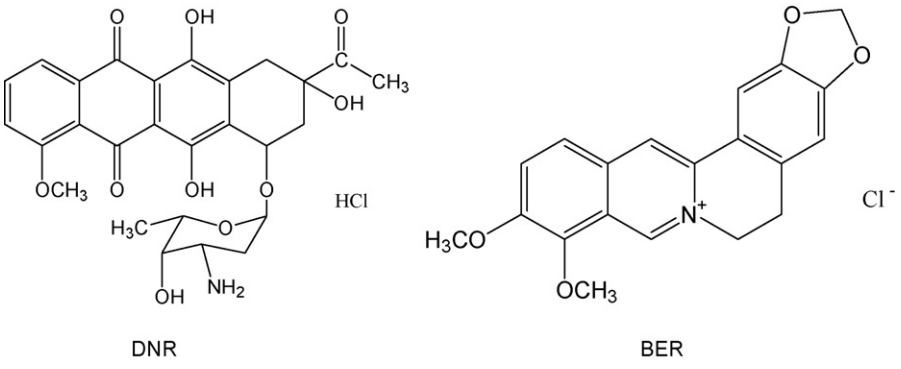


Fig. 1. Chemical structures of DNR and BER.

## 3. Experimental

### 3.1. Reagents and solution

Berberine hydrochloride (BER) was purchased from National Institute for Control of Pharmaceutical and Biological Products in Changsha (China). Daunorubicin hydrochloride (DNR) and Calf thymus DNA were purchased from Sigma–Aldrich. All experiments were performed with analytical reagent chemicals. The water was doubly distilled and used in all experimental preparations. The stock solution with a concentration of 19 μg ml$^{-1}$ was prepared by dissolving the BER in water. The stock solution prepared contains daunorubicin hydrochloride of 10 μg ml$^{-1}$. Calf thymus DNA was used to prepare stock solution of 76.4 μg ml$^{-1}$. All the stock solutions were stored in glass at 4 °C, protected from light, for a maximum period of 10 days. A phosphate buffer solution of pH 7.0 was prepared.

### 3.2. Apparatus

All samples were measured on an F-4500 fluorescence spectrophotometer (HITACHI) fitted with an Xenon lamp and connected to a PC Pentium IV microcomputer running under Windows XP operating system. In all cases, a 1.00 cm quartz cell was used.

### 3.3. Computer programs

All computer programs were in-house written in Matlab, and all calculations were carried out on a personal computer with Pentium IV processor and 256 MB RAM under the Windows XP operating system.

### 3.4. Analytical methodology

In order to study of interaction between BER and DNA and interaction between DNR and DNA, the concentrations of sample Nos. 1–14 and Nos. 15–26 are prepared in accordance with Table 1 and Table 2, respectively. Similarly, aiming to study of competitive interactions of DNR and BER with DNA, the concentrations of sample Nos. 27–37 are given in Table 3. All samples contained a phosphate buffer solution (pH 7.0). All chemical reaction took place in a room temperature of 25 °C. After the equilibrium of complex reaction was reached, the samples were measured. The excitation–emission fluorescence spectra were recorded at excitation wavelengths from 400 to 520 nm at regular steps of 3 nm and the emission wavelengths from 532 to 652 nm at 3 nm steps. The slit width was 5.0/5.0 nm. The scan rate was 1200 nm/min.

## 4. Results and discussion

### 4.1. Interaction of BER and DNA

A data array produced by Nos. 1–8 samples was analyzed using APTLD. The analysis using CORCONDIA and ADD-ONE-UP demonstrated that two components are obtained for the model. The mixture of BER and complex BER–DNA contributes one component to the model, because the excitation–emission fluorescence spectra of them are very similar. Another compo-

Table 1
BER and DNA concentrations (μg ml$^{-1}$) of the samples of Nos. 1–12

| | Samples | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| BER | 0.76 | 0.95 | 1.14 | 1.33 | 1.52 | 1.71 | 1.90 | 2.28 | 1.90 | 2.28 | 2.66 | 3.04 |
| DNA | 3.82 | 3.82 | 3.82 | 3.82 | 3.82 | 3.82 | 3.82 | 3.82 | 0 | 0 | 0 | 0 |

Table 2
DNR and DNA concentrations (μg ml$^{-1}$) of the samples Nos. 13–28

| | Samples | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 |
| DNR | 0.02 | 0.04 | 0.07 | 0.10 | 0.15 | 0.20 | 0.25 | 0.35 | 0.40 | 0.45 | 0.50 | 0.55 | 0.10 | 0.20 | 0.30 | 0.40 |
| DNA | 3.82 | 3.82 | 3.82 | 3.82 | 3.82 | 3.82 | 3.82 | 3.82 | 3.82 | 3.82 | 3.82 | 3.82 | 0 | 0 | 0 | 0 |

Table 3
BER, BER and DNA concentrations (μg ml$^{-1}$) of the samples of Nos. 29–39

| | Samples | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 29 | 30 | 31 | 32 | 33 | 34 | 35 | 36 | 37 | 38 | 39 |
| DNR | 0.05 | 0.10 | 0.15 | 0.20 | 0.25 | 0.30 | 0.35 | 0.40 | 0.45 | 0.50 | 0.55 |
| BER | 3.80 | 3.80 | 3.80 | 3.80 | 3.80 | 3.80 | 3.80 | 3.80 | 3.80 | 3.80 | 3.80 |
| DNA | 3.82 | 3.82 | 3.82 | 3.82 | 3.82 | 3.82 | 3.82 | 3.82 | 3.82 | 3.82 | 3.82 |

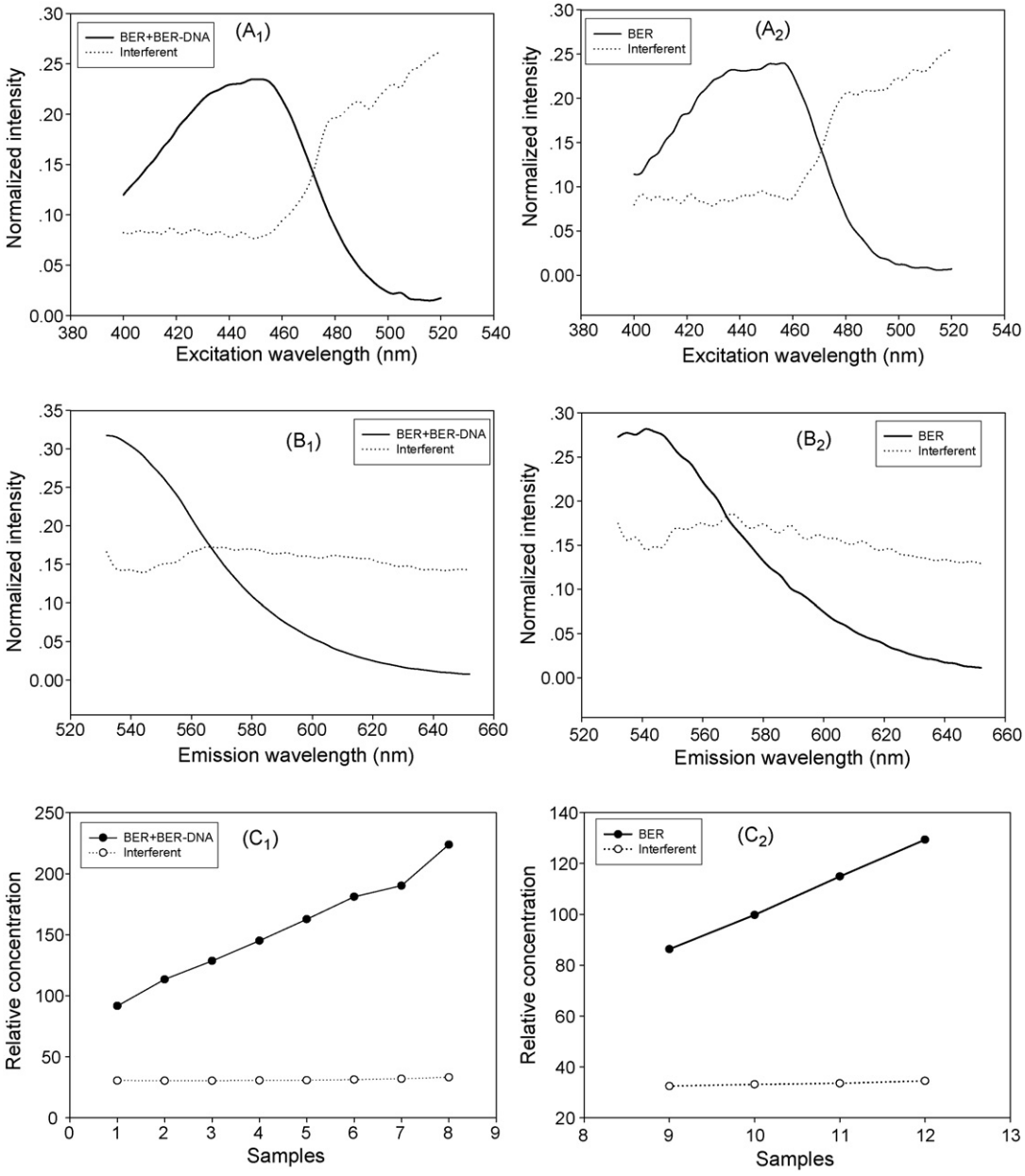


Fig. 2. Resolved excitation–emission spectral and relative concentration profiles of each component using APTLD when the chosen factor number was two.

nent is possibly contributed to the model for interferent. In order to confirm it, a data array produced by the samples of Nos. 9–12 was analyzed using APTLD. The profiles resolved by APTLD are shown in Fig. 2. One observes that the excitation and emission spectra of BER and BER–DNA are almost the same and the interferent is assuredly present in chemical BER purchased, but its fluorescence is very weak. Furthermore, it is seen from Fig. 2($C_1$) and ($C_2$) combined with Table 1 that the fluorescence is largely enhanced when BER binds to DNA and the fluorescence yield of BER in the absence of DNA is poor.

### 4.2. Interaction of DNR and DNA

Nos. 13–24 samples were measured and a three-way data array was produced. Two components are obtained for the model using CORCONDIA and ADD-ONE-UP. A component is contributed to the model for DNR. A possible component is complex DNR–DNA. When APTLD algorithm runs with two components for the three-way data array measured, the resolved profiles are shown in Fig. 3($A_1$)–($C_1$). The results revealed that the resolved DNR spectral profiles are nearly the same as actual ones.

To distinguish whether another component is complex DNR–DNA, not the impurity from chemical DNR, a data array produced by the samples of Nos. 25–28 only containing DNR was analyzed using APTLD. One factor is obtained for the model using CORCONDIA and ADD-ONE-UP. It has been proved that when the component number estimated for a complex system is more than or equal to the real one, the APTLD algorithm is a robust method. Therefore, in order to fully illuminate that

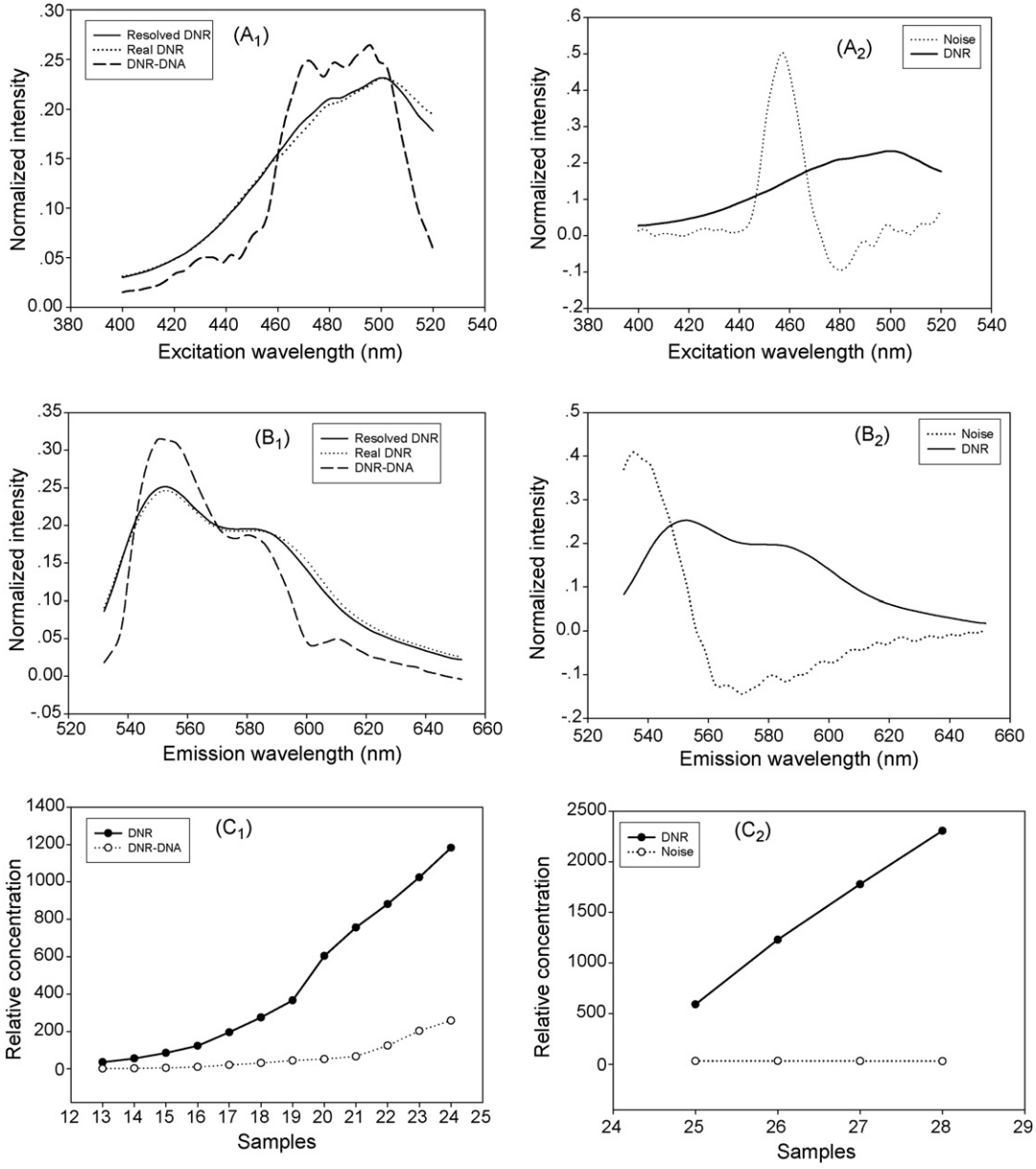


Fig. 3. Actual spectral profiles of DNR and resolved excitation–emission spectral and relative concentration profiles of each component using APTLD when the chosen factor number was two ($N = 2$).

another component is not the impurity from chemical DNR, two factors are still chosen. It is seen from Fig. 3($A_2$)–($C_2$) that relative concentrations of another component are nearly equal to zero and it is fitting pseudo component. Fig. 3 combined with Table 1 shows that the fluorescence spectrum of complex DNR–DNA is similar to and different from that of DNR. Furthermore, its fluorescence is far weaker than that of DNR, which is also consistent with the conclusion that the interaction of DNR with DNA is a complex reaction with quenching of the fluorescence [46].

Interestingly, Angeloni et al. [47] studied the interaction between adriamycin and DNA and reported that the fluorescence spectrum of complex adriamycin–DNA is different from that of adriamycin. However, for DNR, similar conclusion can be simply obtained by three-way analysis.

### 4.3. Competitive interaction of DNR and BER with DNA

Aiming to study competitive interaction of DNR and BER with DNA, Nos. 29–39 samples contain the same initial amount of DNA and BER, and only the initial concentration of DNR varied and increased gradually (see Table 3). The samples were measured on fluorescence spectrophotometer and produced a three-way data array. After CORCONDIA and ADD-ONE-UP were used to estimate the chemical rank, two components were acquired. However, from Fig. 4, it is obviously seen that there are three components in the system. It is possibly owed to the fact that the two methods estimating the chemical rank are required to run PARAFAC which bears two-factor degeneracy [48,49] and is not satisfactory in dealing with high multicollinearity. It can be the optimum number of components for PARAFAC. Interest-

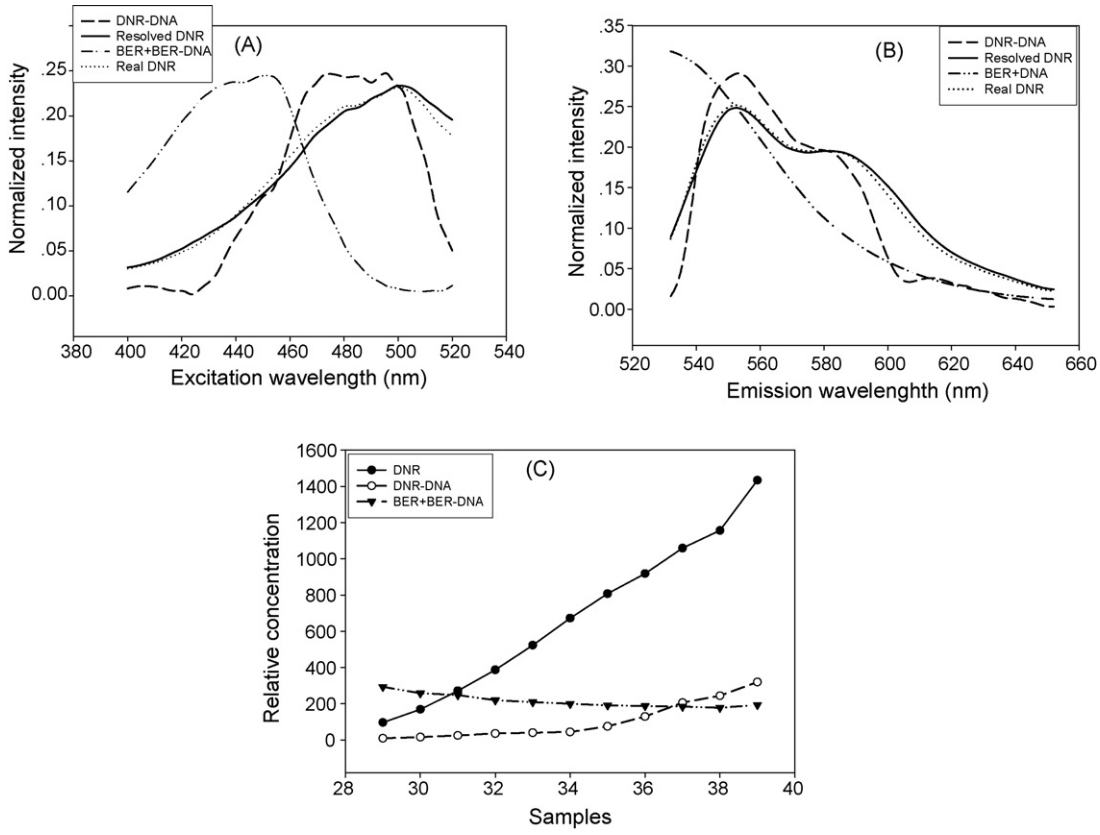


Fig. 4. Actual spectral profiles of DNR and resolved excitation–emission spectral and relative concentration profiles of each component using APTLD when the chosen factor number was three ($N = 3$).

ingly, when APTLD replaces PARAFAC in the two methods, the estimated number of components was three. The result reveals APTLD can overcome high multicollinearity to some extent. Therefore, it is reasonable that the number of components was chosen as three for APTLD, i.e. DNR, DNR–DNA and BER–DNA. As for the impurity from chemical BER, the poor fluorescence results in its burial in strong fluorescence environment.

Fig. 4 shows resolved profiles of each component using APTLD and actual excitation and emission spectra profiles of DNR. Fig. 1 demonstrates that a possible interaction between DNR and BER can be ruled out. Combined with above results, one can confirm that the resolved spectral and concentration profiles correspond to that of DNR, DNR–DNA and BER–DNA (Fig. 4). It can be seen from Fig. 4(C), the relative concentration of DNR and DNR–DNA increased gradually and the relative concentration of BER–DNA decreased gradually when the initial concentration of DNR increased gradually. The phenomena illuminated that the equilibrium concentration of complex DNR–DNA and free BER increased gradually with the increase of initial concentration of DNR. One can assume that the interaction of DNR and BER with DNA possesses a competitive mechanism, that is, the interactions are a pair of the parallel competitive reactions. It is well known that the interaction of DNR with DNA follows the intercalation model and then that DNR is an intercalator [9,10]. Consequently, one can think that DNR intercalates into the same base sites of DNA as the bound BER and the interaction mechanism of BER and DNA is the same interaction model of DNR and DNA, that is, BER being an intercalator and intercalating between the base pairs of doublestranded DNA, which is also consistent with the conclusions from the literatures [19–25].

Interestingly, Krey et al. [26] studied the interaction between BER and DNA and reported that the interaction mechanism belonged to the intercalation mechanism. However, it contradicted a minor groove orientation mechanism recently proposed on the basis of NMR analyses [27]. In present study, the results demonstrate that the interaction mechanism of BER and DNA is the intercalation model. In addition, all results display that the analysis of 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 [29] or others. It is significant to apply clinical drugs and design new drugs.

## 5. Conclusions

Several data arrays have been analyzed by APTLD combined with excitation–emission matrix fluorescence. The results presented demonstrated that the method can conveniently achieved and provide valuable information from complex systems. Accordingly, the strategy of using this chemometric tool can redound to study of the interaction mechanisms between drugs and DNA. It can be proved that the fluorescence spectrum

of complex DNR–DNA is different from that of DNR. Moreover, for controversial interaction mechanism of BER and DNA, the method can provide a helpful proof that BER is an intercalateor. Consequently, BER can be widely applied as a non-toxic fluorescent probe to replace highly toxic fluorescent probe, such as ethidium bromide. To search for a new non-toxic, highly efficient fluorescent probe, the present method can provide a new way.

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