Asian Journal of Chemistry; Vol. 24, No. 2 (2012), 551-557

ASIAN JOURNAL OF CHEMISTRY

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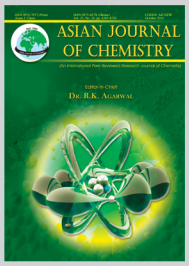

Study on Potential Toxic Mechanism of Chrysophanol Binding DNA by Saturation Value Binding DNA

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(*Received*: 19 January 2011; *Accepted*: 5 October 2011) AJC-10479

In this study, the interaction of chrysophanol and DNA is reported to reveal the toxic mechanism of chrysophanol by the resonance light scattering spectrum. Meanwhile, the saturation value binding DNA is calculated from the resonance light scattering results to evaluate the ability of intercalating into DNA and the potential toxicity of chrysophanol. As the resonance light scattering results of chrysophanol interacting with DNA is very similar to these of ethidium bromide, adriamycin and mitoxantrone, it is believed that the chrysophanol can also intercalate into the base pairs of double-helical DNA in a similar DNA intercalator way. The saturation value of the binding DNA of chrysophanol is much lower than those of mitoxantrone, adriamycin and ethidium bromide. Therefore, it can be speculated that chrysophanol shows a weak capacity of intercalating into DNA. The saturation value changed with environmental factors, such as amino acids, sodium chloride, glucose and pH. The saturation value binding DNA can be evaluated the potential toxicity of anthraquinone derivatives and the toxic effects in terms of external factors and conditions.

Key Words: Resonance light scattering, Potential toxicity, Chrysophanol, Saturation value binding DNA.

INTRODUCTION

All herbs or herbal medicines have been erroneously considered to be gentle, non-toxic and even harmless for a long time in the minds of some people because of their natural origin. However, it is well-known that the consumption of herbal medicine is capable of producing prominent adverse health effects. Due to increased morbidity and mortality, poisonings associated with the use of herbs have raised universal attention in the last few years[1-3]. Anthraquinone derivatives are the main components of rhubarb, but they are also the major components of many other herbal medicine or Chinese medicine[4-8]. Synthetic drugs anthraquinone derivatives have strong side effects and have been recognized by more and more people[9,10]. There are reports that even the natural anthraquinone derivatives, such as emodin, 1,8-dihydroxy anthraquinone, chrysophanol and physcion have a certain genetic toxicity and there are differences in toxicity while the molecular structure are different[11,12].

Lerman[13] demonstrated that acridine dye could insert or intercalate between DNA base pairs. He first proposed the model of DNA intercalator, which is a large class of planar, aromatic structures molecules and has been found to have the ability to intercalate into DNA in the space between base pairs. The effect of resonance light scattering (RLS) technique on characteristic aspects of biological macromolecules is obvious. In recent years, many scholars have used resonance light scattering technique to study the interaction between drug molecules and nucleic acid. Berberine[14] or eugenol[15] interaction with DNA have enhanced resonance light scattering signals. The operation of resonance light scattering is simple and high sensitive. In this paper, we study the interaction between chrysophanol and DNA by the resonance light scattering spectrum. The saturation value binding with DNA can be evaluated the potential toxicity of anthraquinone derivatives and its toxic effects in terms of external factors and conditions.

EXPERIMENTAL

A Shimadzu RF-5301PC fluorophotometer (Kyoto, Japan) with a quartz cell of 1 cm path length is used to measure resonance light scattering spectrum.

Natural double DNA used in this study is herring sperm DNA (fs DNA, Sigma). The stock solutions were prepared by dissolving the solid DNA in double distilled water with occasionally gentle shaking and stored at 4 ºC. The concentration of DNA solution was determined by UV absorption at 260 nm using the molar absorption coefficient ε_{260} = 6600 mol^{-1} cm^{-1}. Concentration of DNA in the stock solution was 3.78 × 10^{-4} mol L^{-1} in the experiment. Dilute to 3.78 × 10^{-5} mol L^{-1} when it is used.

The stock solution of 1.0×10^{-4} mol L^{-1} chrysophanol ethidium bromide, adriamycin and mitoxantrone (chrysophanol, ethidium bromide, adriamycin and mitoxantrone, National Institute for Control of Pharmaceutical and Biological Products) was prepared by ethanol solutions. The stock solution of 1×10^{-4} mol L^{-1} sodium chloride, glucose, leucine, valine, histidine, aspartate and tryptophan (sodium chloride, glucose, leucine, valine, histidine, aspartate and tryptophan, Fine chemicals of Tianjin Siyou Limited Company) was prepared by distilled water. Dilute to 1×10^{-5} mol L^{-1} when they are used.

A Britton-Robinson buffer was used to control the pH of the reaction system. All reagents were of analytical reagent grade and double distilled water was used throughout the experiments.

Resonance light scattering spectra of ethidium bromide, adriamycin, mitoxantrone or chrysophanol with DNA: Put 0.1 mL working solution of ethidium bromide, adriamycin, mitoxantrone interacting or chrysophanol and 1 mL buffer solution into a 10 mL volumetric flask, vortex and then add appropriate DNA solution, respectively. The mixture was diluted to 5 mL scale mark by double distilled water and mixed thoroughly. The resonance light scattering spectrum was obtained by scanning simultaneously the excitation and emission monochromators of the RF-5301PC spectrofluorometer from 220 to 700 nm. The extent of light-scattering was measured at the maximum wavelength with slit width at 15 nm for the excitation and emission.

RESULTS AND DISCUSSION

There are three modes for non-covalent binding of interaction of small molecules with DNA, electrostatic binding, groove binding and intercalative binding[16]. Furthermore, the modes of interaction are affected by the medium acidity, ionic strength and molar ratio of small molecules to DNA. When conditions is changed, the role of the two forms is also changed, which is the reversible interaction. And small molecules binding DNA contains more than one mode of action.

The interaction procedure of chrysophanol, ethidium bromide (EB), adriamycin or mitoxantrone with fs-DNA in pH 7.4. Britton-Robinson buffer was characterized by the resonance light scattering spectrum. It is shown that the resonance light scattering signal of DNA itself is weak and chrysophanol-DNA interaction results in strong enhanced resonance light scattering signals characterized by three peaks at 360, 470 and 560 nm (Fig. 1). Ethidium bromide-DNA interaction result in strong enhanced resonance light scattering signals characterized by four peaks at 248, 360, 470 and 580 nm respectively (Fig. 2). Adriamycin-DNA interaction result in strong enhanced resonance light scattering signals characterized by three peaks at 290, 468 and 545 nm (Fig. 3). Mitoxantrone-DNA interaction result in strong enhanced resonance light scattering signals characterized by three peaks at 290, 468 and 570 nm (Fig. 4). Resonance light scattering of liquid particles show that illuminant, molecular absorption and resonance scattering effect of associating particles are three main factors of synchronous scattering peaks[17]. Taking ultraviolet absorption and sensitive peak into consideration, we selected resonance light scattering peak of chrysophanol,

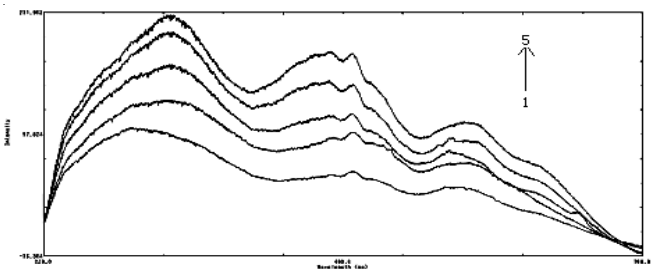

Fig. 1. Resonance light scatting spectra of DNA and chrysophanol; $C_{chrysophanol}$ 2-5: 2×10^{-7} mol L^{-1}; C_{DNA} 1-7: 1.134×10^{-7}, 0, 1.512×10^{-7}, 2.268×10^{-7}, 3.024×10^{-7}, 3.402×10^{-7}, 3.78×10^{-7} mol L^{-1}

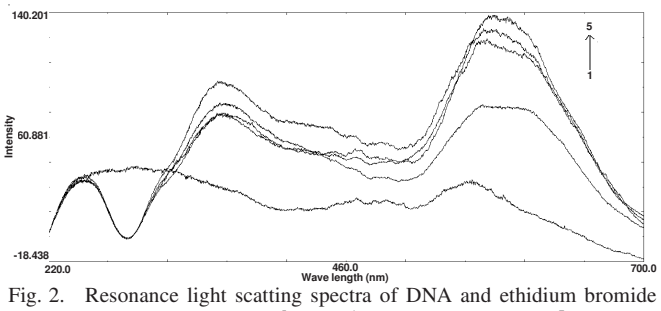

Fig. 2. Resonance light scatting spectra of DNA and ethidium bromide (EB); C_{EB} 2-5: 2×10^{-5} mol L^{-1}, C_{DNA} 1-5: 1.134×10^{-7}, 0, 7.56×10^{-7}, 1.058×10^{-6}, 1.361×10^{-6} mol L^{-1}

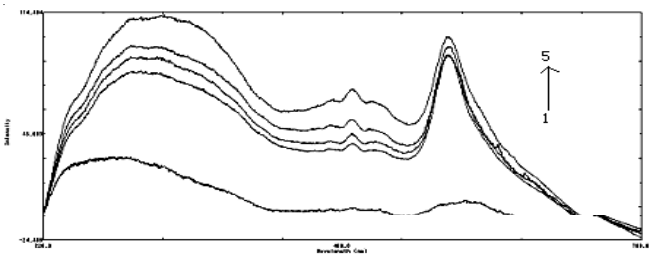

Fig. 3. Resonance light scatting spectra of DNA and adriamycin; $C_{adriamycin}$ 2-5: 4×10^{-5} mol L^{-1}, C_{DNA} 1-5: 3.024×10^{-7}, 0, 2.268×10^{-7}, 3.024×10^{-6}, 3.78×10^{-6} mol L^{-1}

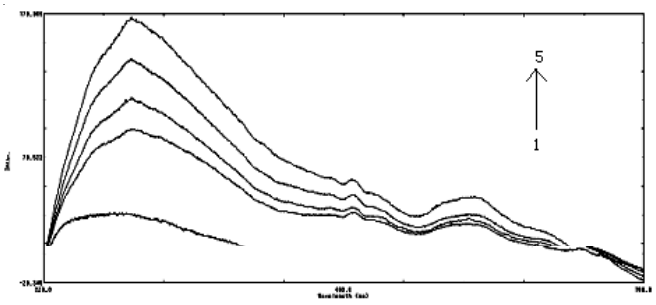

Fig. 4. Resonance light scatting spectra of DNA and mitoxantrone; $C_{mitoxantrone}$ 2-5: 2×10^{-5} mol L^{-1}; C_{DNA} 1-5: 3.024×10^{-7}, 0, 1.512×10^{-6}, 3.024×10^{-6}, 6.048×10^{-6} mol L^{-1}

adriamycin or mitoxantrone at 468 nm to investigate drug-DNA interactions and the resonance light scattering peak of ethidium bromide at 580 nm to investigate ethidium bromide-DNA interaction.

As the resonance light scattering results of chrysophanol interacting with DNA are similar to those of ethidium bromide, adriamycin and mitoxantrone, it is believed that chrysophanol also intercalate into the base pairs of double-helical DNA in a similar DNA intercalator way. Resonance light scattering

enhancing phenomenon shows that chrysophanol may combine with DNA to form a compound, resulting in the formation of super-helical structure of nucleic acid. As the DNA concentration increases, the resonance light scattering signal also gradually enhances.

Saturation value binding with DNA of chrysophanol: Efficacy or potential toxicity is closely related to the molecular structure of compounds, especially to the binding site number and binding intensity of drug molecule interacting with DNA. As the molecular structure and substituent group is certain for a specific drug molecule, the binding site number and binding strength of drug molecule interacting with DNA is also certain. When the concentration of drug is overdosed along with the increase of DNA concentration, the resonance light scattering signal is gradually enhance too, but when the concentration of DNA reach the limit, the resonance light scattering signal is no longer enhanced. The limit DNA concentration at this time is called as the saturated DNA concentration of drug binding DNA. The ratio of drug concentration to saturation concentration of DNA is defined as the saturation value binding with DNA. The saturation value of the binding with DNA = drug molecular concentration / the saturated concentration of DNA. The saturation value binding DNA of drug can correctly express the ability of drug molecule intercalating into DNA molecule. So it can be used as an indicator to evaluate the intensity of drug and DNA. Furthermore, it can be used for evaluating the potential toxicity of drug too. The greater the saturation value, the stronger the ability of intercalating into DNA and the potential toxicity of anthraquinone molecule will be, the greater the inhibitions of DNA replication, transcription and translation will be.

Ethidium bromide is a kind of small molecules with planar structure, and is recognized as a kind of DNA intercalator. It can parallel intercalate into the base pairs of double-helical DNA and then change the DNA molecule configurations. Ethidium bromide can inhibit the replication of DNA and show it's carcinogenicity[18]. Adriamycin is anthracycline antibiotics and can intercalate into DNA. Therefore, it inhibits DNA synthesis and DNA-dependent RNA synthesis. Adriamycin is a potent and broad-spectrum antineoplastic agent that plays a major role in cancer chemotherapy. Unfortunately, its use has been hampered by conventional toxicities and cardiotoxicity manifested by congestive cardiomyopathy. It also causes nausea, vomiting, alopecia and hematopoietic suppression[19]. Mitoxantrone is a synthetic anticancer agent that exhibits broad antitumor activity and it has been used effectively against breast cancer, acute leukaemia and malignant lymphomas with minimal side-effect. In contrast to the clinically useful anthracycline drugs such as daunorubicin and adriamycin, mitoxantrone appears to exhibit significantly lower cardiotoxicity[20].

From the results in Table-1, it can be speculated that chrysophanol shows a weak capacity of intercalating into DNA and toxicity, but it is much lower than those of mitoxantrone, adriamycin and ethidium bromide.

Influence of histidine on the system of chrysophanol-DNA: Chrysophanol (2×10^{-7} mol L^{-1}) and DNA (3.78×10^{-7} mol L^{-1}) are fixed and resonance light scattering in the 468 nm gradually enhance with the increasing concentration of histidine (Fig. 5). Influence on the system of chrysophanol-DNA is greatest when concentration of histidine is 2×10^{-4} mol L^{-1}. Histidine (2×10^{-4} mol L^{-1}) and chrysophanol (2×10^{-7} mol L^{-1}) are selected, resonance light scattering in the 468 nm gradually enhance with the increasing concentration of histidine (Fig. 6). When the concentration of DNA is greater than 1.134×10^{-6} mol L^{-1}, resonance light scattering remain unchanged with the increasing concentration of DNA. The limit DNA concentration at this time is called as the saturated DNA concentration of chrysophanol binding with DNA. It follows that the saturation value binding with DNA of histidine is 0.18. Alkaline histidine combine with phosphate of DNA, which chrysophanol can not be combined with phosphate of DNA in electrostatic interaction. So, chrysophanol hinder its further embedded DNA.

TABLE-1
SATURATION VALUE OF DRUG BINDING DNA

Drug	Saturation value of drug binding with DNA	Saturation value of drug/Saturation value of ethidium bromide (%)
Ethidium bromide	14.70	100
Adriamycin	10.58	72.0
Mitoxantrone	3.31	22.5
Chrysophanol	0.53	3.6

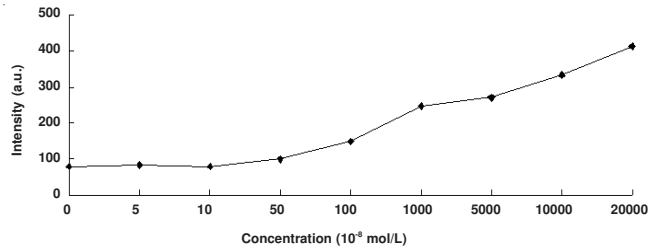

Fig. 5. Resonance scattering intensity of different the concentration of histidine on the system of chrysophanol-DNA

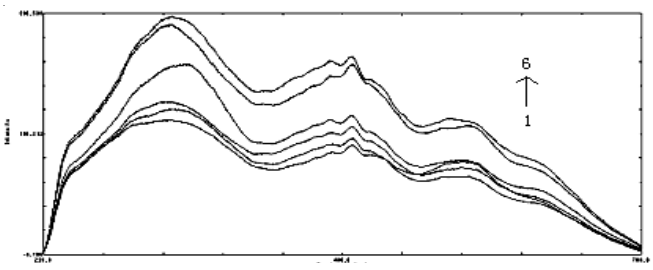

Fig. 6. Resonance light scatting spectra of DNA, histidine and chrysophanol; $C_{chrysophanol}$ 1-6: 2×10^{-7} mol L^{-1}; $C_{histidine}$ 2-6: 2×10^{-4} mol L^{-1}; C_{DNA} 1-6: 0, 0.756×10^{-8}, 3.78×10^{-7}, 6.804×10^{-7}, 9.828×10^{-7}, 1.134×10^{-6} mol L^{-1}

Influence of aspartate on the system of chrysophanol-DNA: Chrysophanol (2×10^{-7} mol L^{-1}) and DNA (3.78×10^{-7} mol L^{-1}) are fixed and resonance light scattering in the 468 nm gradually enhance with the increasing concentration of aspartate (Fig. 7). Influence on the system of chrysophanol-DNA is greatest when concentration of aspartate is 1×10^{-3} mol L^{-1}. Aspartate (1×10^{-3} mol L^{-1}) and chrysophanol (2×10^{-7} mol L^{-1}) are selected, resonance light scattering in the 468 nm gradually enhance with the increasing concentration of aspartate (Fig. 8). When the concentration of DNA is greater than 1.361×10^{-7} mol L^{-1}, resonance light scattering remains unchanged with

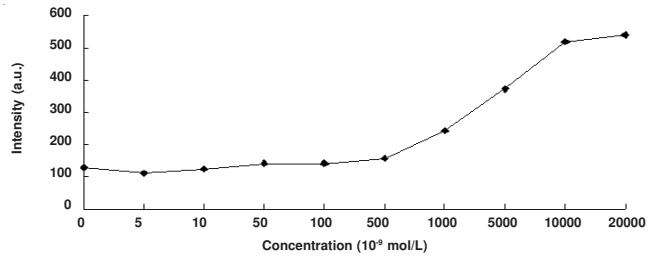

Fig. 7. Resonance scattering intensity of different concentration of aspartate on the system of chrysophanol-DNA

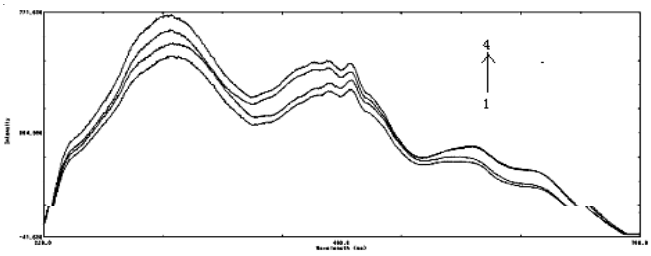

Fig. 8. Resonance light scatting spectra of DNA, aspartate and chrysophanol; $C_{chrysophanol}$ 1-4: 2×10^{-7} mol L^{-1}; $C_{aspartate}$ 2×10^{-4} mol L^{-1}; C_{DNA} 1-4: 0, 6.05×10^{-8}, 9.072×10^{-8}, 1.361×10^{-7} mol L^{-1}

the increasing concentration of DNA. The limit DNA concentration at this time is called as the saturated DNA concentration of chrysophanol binding with DNA. It follows that the saturation value binding with DNA of aspartate is 1.49. Acid aspartate make chrysophanol-DNA system more stable and enhance the ability of chrysophanol interacting with DNA. The reason might be that DNA double helix become loose and reduce the DNA embedded in the space chrysophanol resistance because of hydrogen bonding between carboxyl of polarity and the DNA minor groove and its hydrogen spatial configuration.

Influence of tryptophan on the system of chrysophanol-DNA: Chrysophanol (2×10^{-7} mol L^{-1}) and DNA (3.78×10^{-7} mol L^{-1}) are fixed and resonance light scattering in the 468 nm gradually enhance with the increasing concentration of tryptophan (Fig. 9). Influence on the system of chrysophanol-DNA is greatest when concentration of tryptophan is 1×10^{-4} mol L^{-1}. Tryptophan (1×10^{-4} mol L^{-1}) and chrysophanol (2×10^{-7} mol L^{-1}) are selected, resonance light scattering in the 468 nm gradually enhance with the increasing concentration of tryptophan (Fig. 10). When the concentration of DNA is greater than 2.646×10^{-7} mol L^{-1}, resonance light scattering remain unchanged with the increasing concentration of DNA. The limit DNA concentration at this time is called as the saturated DNA concentration of chrysophanol binding with DNA. It follows that the saturation value binding DNA of tryptophan is 1.49. Tryptophan, which is containing benzene ring or a heterocyclic structure, is similar to structure of anthraquinone. Such conjugated π-π interaction is easy to interact with the DNA base pairs, which will increase competitive combination of chrysophanol with DNA.

Influence of non-polar amino acids on the system of chrysophanol-DNA: Chrysophanol (2×10^{-7} mol L^{-1}) and DNA (3.78×10^{-7} mol L^{-1}) are fixed and resonance light scattering in the 468 nm gradually enhance with the increasing concentration of leucine (Fig. 11). Influence on the system of chrysophanol-DNA is greatest when concentration of leucine

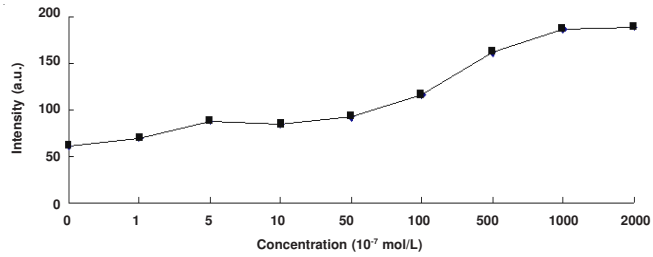

Fig. 9. Resonance scattering intensity of different the concentration of tryptophan on the system of chrysophanol-DNA

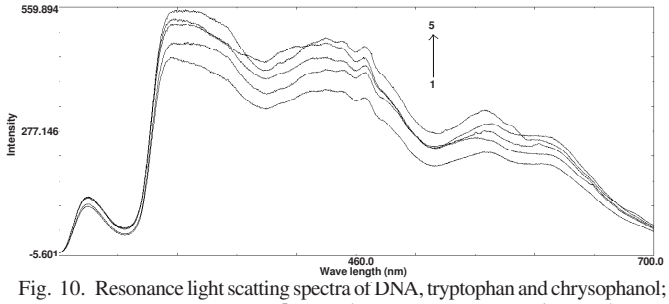

Fig. 10. Resonance light scatting spectra of DNA, tryptophan and chrysophanol; $C_{chrysophanol}$ 1-5: 2×10^{-7} mol L^{-1}; $C_{tryptophan}$ 2-5: 1×10^{-4} mol L^{-1}; C_{DNA} 1-5: 0, 7.56×10^{-8}, 0, 1.512×10^{-7}, 2.268×10^{-7}, 2.646×10^{-7} mol L^{-1}

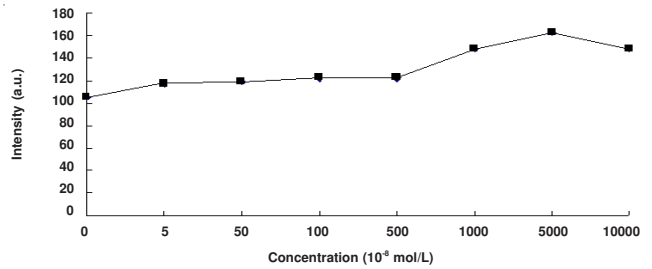

Fig. 11. Resonance scattering intensity of different the concentration of leucine on the system of chrysophanol-DNA

is 5×10^{-5} mol L^{-1}. Leucine (5×10^{-5} mol L^{-1}) and chrysophanol (2×10^{-7} mol L^{-1}) are selected, resonance light scattering in the 468 nm gradually increase with the increasing concentration of leucine (Fig. 12). When the concentration of DNA is greater than 3.78×10^{-7} mol L^{-1}, resonance light scattering remain unchanged with the increasing concentration of DNA. The limit DNA concentration at this time is called as the saturated DNA concentration of chrysophanol binding DNA. It follows that the saturation value binding with DNA of leucine is 0.53. Chrysophanol (2×10^{-7} mol L^{-1}) and DNA (3.78×10^{-7} mol L^{-1}) are fixed and resonance light scattering in the 468 nm little change with the increasing concentration of valine (Fig. 13). Leucine and valine of neutral aliphatic amino has no effect on the system of chrysophanol-DNA and the saturation value binding with DNA. Although the saturation value binding DNA are the same, resonance light scattering spectrum is different.

Influence of pH on the system of chrysophanol-DNA: Chrysophanol (2×10^{-7} mol L^{-1}) and DNA (3.78×10^{-7} mol L^{-1}) are fixed and resonance light scattering in the 468 nm change with the increasing pH value (Fig. 14). In the pH range of 2.4-11.4, resonance light scattering signal of chrysophanol-DNA system reached maximum at pH = 7.4. It shows that DNA is relatively stable in nearly neutral environment.The interaction of chrysophanol and DNA is inserted in acidic or alkaline environment. The saturation value binding with DNA of pH

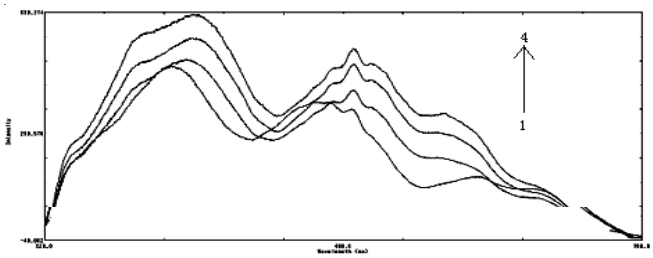

Fig. 12. Resonance light scatting spectra of DNA, leucine and chrysophanol; $C_{chrysophanol}$ 1-4: 2×10^{-7} mol L^{-1}; $C_{leucine}$ 2-6: 5×10^{-5} mol L^{-1}; C_{DNA} 1-4: 0, 7.56×10^{-8}, 2.268×10^{-7}, 3.78×10^{-7} mol L^{-1}

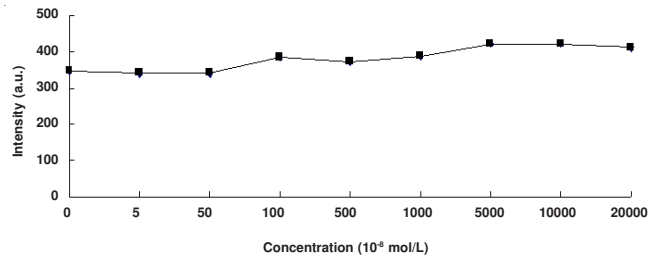

Fig. 13. Resonance scattering intensity of different concentration of valine on the system of chrysophanol-DNA

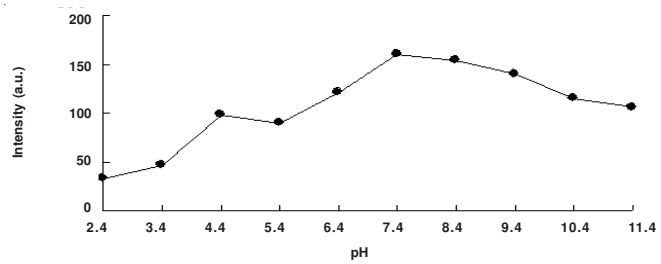

Fig. 14. Resonance scattering intensity of different pH on the system of chrysophanol-DNA

7.4 is 0.53 (Fig. 1). The saturation value binding with DNA of pH 3.5 is 0.09 (Fig. 15).This is because DNA have positive charge at pH 3.5. The phosphate anion of DNA molecules can not combine with H^+ while acid medium increase. Thus chrysophanol is not close to the DNA by electrostatic and then the amount of chrysophanol interaction with DNA reduces. The saturation value binding with DNA is lower. The saturation value binding DNA of pH 8.2 is 0.26 (Fig. 16). DNA have negative charge at this time and DNA double helix gradually melting into a single-stranded DNA with alkaline increase[21]. Thus the binding site of chrysophanol interaction with DNA reduced and the saturation value binding with DNA is lower.

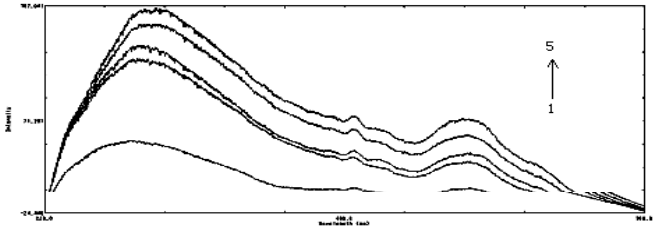

Fig. 15. Resonance light scatting spectra of DNA, pH = 3.5 and chrysophanol; $C_{chrysophanol}$ 1-5: 2×10^{-7} mol L^{-1}; C_{DNA} 1-5: 0, 1.436×10^{-6}, 1.814×10^{-6}, 2.19×10^{-6}, 2.57×10^{-6} mol L^{-1}

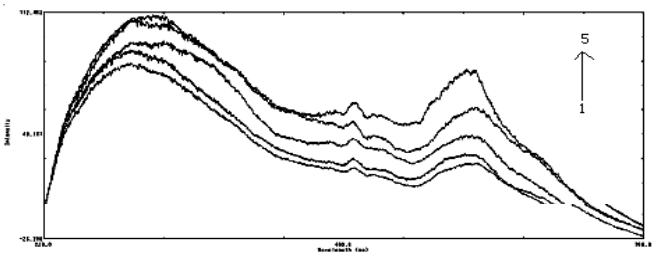

Fig. 16. Resonance light scatting spectra of DNA, pH = 8.2 and chrysophanol; $C_{chrysophanol}$ 1-5: 2×10^{-7} mol L^{-1}; C_{DNA} 1-5: 0, 4.536×10^{-7}, 5.292×10^{-7}, 6.804×10^{-7}, 7.56×10^{-7} mol L^{-1}

Influence of sodium chloride on the system of chrysophanol-DNA: Chrysophanol (2×10^{-7} mol L^{-1}) and DNA (3.78×10^{-7} mol L^{-1}) are fixed and resonance light scattering in the 468 nm changes (Fig. 17). Sodium chloride solution affect the stability of the system of chrysophanol and DNA. It shows that chrysophanol interaction with DNA is partly based anion. Sodium chloride in low concentration make DNA structure constraint. The hydrophobicity of DNA double helix groove increase and DNA contract to form super-helical structure. It has resulted in the accumulation of surface of nucleic acids and resonance light scattering signals has enhanced when the concentration of sodium chloride is 5×10^{-7} mol L^{-1}, DNA molecular structure contract and reach the limit. The accumulation of surface of nucleic acids reach maximum and show a maximum intensity of resonance light scattering signals. DNA molecules is surrounded by a excess of sodium chloride when the sodium chloride concentration exceed 5×10^{-7} mol L^{-1}. Therefore part of the DNA double helix reduces the hydrophobicity of the groove. It also indirectly illustrates the binding mode of chrysophanol and DNA. Small molecules are inserted into the DNA base pairs between adjacent in the plug-in combination and small molecular probes are combined with the groove of DNA double helix in groove binding. Changes in environmental conditions are not sensitive because of the shielding effect of the base while the plug-in combination compared with groove binding. The interaction of chrysophanol and DNA is mainly the way of plug-in combination when the sodium chloride concentration is 5×10^{-8} mol L^{-1} to 1×10^{-6} mol L^{-1}.Chrysophanol may be combined DNA in between the major groove and minor groove when the sodium chloride concentration is 5×10^{-9} to 5×10^{-8} mol L^{-1} and 1×10^{-6} to 2×10^{-3} mol L^{-1}. Therefore, changes in environmental of salt concentration have little effect on resonance light scattering signals. The saturation value binding DNA is 1.89 when the concentration of sodium chloride is 5×10^{-7} mol L^{-1} (Fig. 18).

Influence of glucose on the system of chrysophanol-DNA: As the body's fluid environment contains large amounts

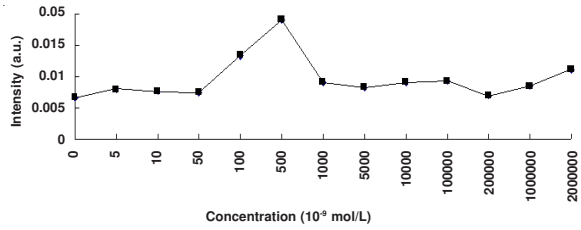

Fig. 17. Resonance scattering intensity of different concentration of sodium chloride on the system of chrysophanol-DNA

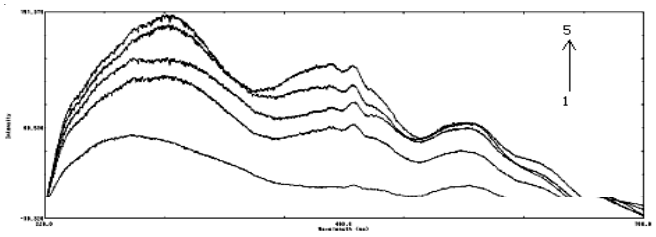

Fig. 18. Resonance light scatting spectra of DNA, sodium chloride and chrysophanol; $C_{chrysophanol}$ 1-5: 2×10^{-7} mol L^{-1}; $C_{sodium\ chloride}$ 1-5: 2×10^{-7} mol L^{-1}; C_{DNA} 1-5: 0, 3.024×10^{-8}, 0, 7.56×10^{-8}, 9.072×10^{-8}, 1.058×10^{-7} mol L^{-1}

of glucose, we study the effect of glucose on drugs, which is particularly important. Chrysophanol (2×10^{-7} mol L^{-1}) and DNA (3.78×10^{-7} mol L^{-1}) are fixed and resonance light scattering in the 468 nm changes (Fig. 19). Glucose affects chrysophanol-DNA system and resonance light scattering signal of them is reduced. The saturation value binding DNA is 0.34 when the concentration of glucose is 5×10^{-9} mol L^{-1} (Fig. 20). The interaction of glucose and the small groove of DNA is the way of electrostatic or hydrogen. DNA double helix has become tight and part of the DNA close to chrysophanol is affected. So the saturation value binding DNA is lower.

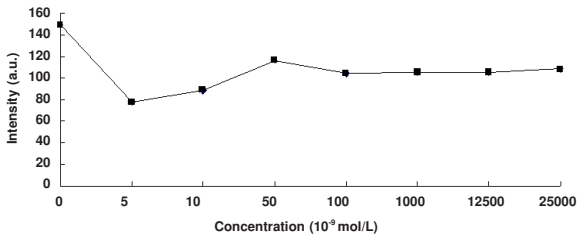

Fig. 19. Resonance scattering intensity of different the concentration of glucose on the system of chrysophanol-DNA

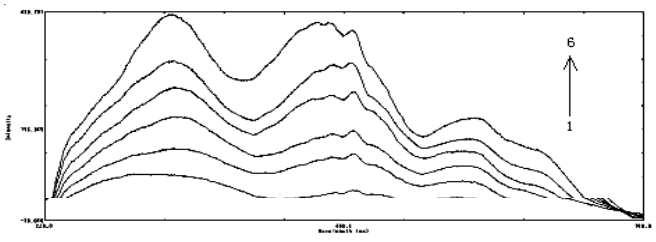

Fig. 20. Resonance light scatting spectra of DNA, glucose and chrysophanol; $C_{chrysophanol}$ 1-6: 2×10^{-7} mol L^{-1}; $C_{glucose}$ 1-6: 5×10^{-9} mol L^{-1}; C_{DNA} 1-6: 0, 7.56×10^{-8}, 0, 2.268×10^{-7}, 3.78×10^{-7}, 5.292×10^{-7}, 5.894×10^{-7} mol L^{-1}

Conclusion

We study the interaction of chrysophanol and DNA to reveal the toxic mechanism of chrysophanol by resonance light scattering spectrum. And the saturation value binding DNA is calculated from the resonance light scattering results to evaluate the ability of intercalating into DNA and the potential toxicity of anthraquinone molecule. The saturation value changes when environmental conditions or factors such as amino acids, sodium chloride, glucose and pH change (Table-2). The change rate of the saturation value binding with DNA of alkaline histidine and acidic aspartate is -66 % and +181 %, respectively. Non-polar amino acids has no effect on chrysophanol-DNA-pH 7.4 system. The change rate of saturation value binding DNA is +257 % when sodium chloride is added to the chrysophanol-DNA-pH 7.4 system. The change rate of saturation value binding DNA is -36 % when glucose is added to the chrysophanol-DNA-pH 7.4 system. The change rate of saturation value binding DNA is -83 % when the chrysophanol-DNA-pH 7.4 system is changed to the chrysophanol-DNA-pH 3.5 system. The change rate of saturation value binding DNA is -51 % when the chrysophanol-DNA-pH 7.4 system is changed to the chrysophanol-DNA-pH 8.1 system. The change rate of the saturation value binding DNA is -66 % when alkaline histidine is added to the chrysophanol-DNA-pH 7.4 system. The change rate of the saturation value binding DNA is +181 % when acidic aspartate is added to the chrysophanol-DNA-pH 7.4 system. The change rate of the saturation value binding DNA remain unchanged when non-polar amino acids is added to the chrysophanol-DNA-pH 7.4 system. The saturation value binding DNA can evaluate the potential toxicity of anthraquinone derivatives and its toxic effects in terms of external factors and conditions.

TABLE-2
CHANGE RATE OF THE SATURATION VALUE BINDING WITH DNA

Environmental conditions	AA	BB (%)
Chrysophanol-DNA-pH 7.4	0.53	–
Chrysophanol-DNA-leucine-pH 7.4	0.53	0
Chrysophanol-DNA-valine-pH 7.4	0.53	0
Chrysophanol-DNA-histidine-pH 7.4	0.18	-66
Chrysophanol-DNA-aspartate-pH 7.4	1.49	+181
Chrysophanol-DNA-tryptophan-pH 7.4	0.76	+30
Chrysophanol-DNA-sodium chloride-pH 7.4	1.89	+257
Chrysophanol-DNA-glucose-pH 7.4	0.34	-36
Chrysophanol-DNA-pH 3.5	0.09	-83
Chrysophanol-DNA-pH 8.2	0.26	-51

AA = Saturation value binding with DNA; BB = Change rate of the saturation value binding with DNA (with chrysophanol-DNA-pH 7.4 as a standard)

ACKNOWLEDGEMENTS

This study was supported by China Natural Science Foundation of Guangxi (2010GXNSFA013134).

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