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Effect of Intercalation of the Antitumour Antibiotics—Daunomycin and Nogalamycin—on the Dielectric Relaxation of DNA

M SINHA (BERA)*, A SEN and N N DAS GUPTA
Department of Physics, Electron Microscope Centre, University College of Science, 92, Acharya Prafulla Chandra Road, Calcutta 700009

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A comparative study was made on the effect of two antitumour antibiotics, daunomycin and nogalamycin, on the dielectric behaviour of native DNA. For both the drugs, the nature of ϵ'' vs ϵ' plots changed from symmetric Cole-Cole to asymmetric Davidson-Cole type. The maximum asymmetry was induced at the same level of drug-binding (D/P=0.25) in both cases. As a result of intercalation of drug molecules the relaxation time (τ) of the DNA molecule increased gradually with D/P at the first instance and then attained a constant value. With daunomycin, τ_{max} was 3.5×10^{-4} sec. while for nogalamycin, the value was 4×10^{-4} sec. The corresponding length extensions of the double helix in the two cases were 43% and 53% respectively.

Key Words: **Intercalation, Daunomycin, Nogalamycin, Dielectric, Relaxation**

Introduction

A series of dielectric studies were made on the interaction of acridine dyes with DNA (Goswami et al. 1973, Goswami & Das Gupta 1974, Goswami 1977). That the phenomenon of intercalation of the dye molecules into the base pairs has a prominent effect on the dielectric parameters was further established from the interaction of a nonintercalative drug berenil (Sinha et al. 1978). In the present paper, some comparative dielectric measurements have been made on the binding of two antitumour antibiotics—nogalamycin and daunomycin. Previous other physical studies (Waring 1970, Das et al. 1974, Sinha et al. 1977, Philips et al. 1978) support a partial intercalative model for both the drugs. But there are large variations in the amount of unwinding of the double helix and consequent length extensions due to intercalation measured by different workers (Waring 1970, Freifelder 1971, Das Gupta et al. 1973, Sinha et al. 1977). The present dielectric study is specially an attempt to throw some light on this aspect.

**Present address*: Bethune College, Calcutta

Materials and Methods

Sodium salt of calf thymus DNA (Type D 1501, Sigma Chemicals, USA) having the approximate molecular weight 3×10^6 (obtained from viscometric study) was used in the present study. The drugs daunomycin and nogalamycin were obtained as gifts from Dr B Camerino, Farmitalia, Italy and Dr P F Wiley, Upjohn, USA. Their concentrations were estimated from absorbances at 480 and 475 nm respectively. The method of preparation of drug-DNA complexes, the details of dielectric measurements and determination of dielectric parameters from the measured data have been described earlier (Goswami et al. 1973).

Results and Discussion

Figure 1 (A and B) showed the dielectric dispersion patterns for the two drug-DNA complexes at different levels of binding. The decrement in the value of ϵ_o was greater in case of nogalamycin than in case of daunomycin indicating that nogalamycin is more effective in neutralizing the DNA phosphate. This was clearly shown by plotting $\triangle\epsilon/\triangle\epsilon_o$, the ratio of dielectric increments in presence ($\triangle\epsilon$) and absence ($\triangle\epsilon_o$) of drug, against D/P in figure 3A. The slope of the curve was same over whole range of D/P for both the drugs. In case of acridine dyes, (behaviour of proflavine has been shown by dotted curve in the figure) slope increased at higher values of D/P (Goswami & Das Gupta 1974). This was due to stacking affinity of these dyes. For the present drugs, this effect was absent. From previous studies, both these drugs were found to exist as monomers in aqueous solutions (Kersten 1971).

Figure 2 (A and B) showed the dielectric loss (ϵ'') vs dielectric constant (ϵ') plots for the drug-bound DNAs. These plots revealed that the curves changed from symmetric Cole-Cole type (Cole & Cole 1941) to the skewed Davidson-Cole type (Davidson & Cole 1951) as a result of drug-binding just like the acridine dyes (Goswami & Das Gupta 1974). But with non-intercalative drug berenil, the curves retained their Cole- Cole type behaviour (Sinha et al. 1978). The skewness i.e. asymmetry in the ϵ'' vs ϵ' plot was a characteristic of intercalative binding and is measured by the Davidson-Cole parameter (β). These are given in table 1.

Table 1 *Davidson-Cole parameters* (β) *for DNA-drug complexes*

D/P	DNA-Daunomycin	DNA-Nogalamycin
0.01	0.71	0.70
0.05	0.69	0.66
0.10	—	0.63
0.25	0.65	0.62
0.50	0.67	0.67
1.0	0.78	0.71
1.4	0.80	—

ϵ_o=Static Dielectric constant; ϵ'=Dielectric constant; ϵ''=Dielectric loss (or absorption); $\triangle\epsilon$=Dielectric increment of drug-DNA complex; $\triangle\epsilon_o$=Dielectric increment of native DNA (without drug); L_o=Average length of the DNA molecule as estimated from measurement of dielectric relaxation time (τ). (using Takashima's relation, $\tau \infty L^2$); $\triangle L$=Amount of length extension of the DNA molecule due to intercalation of drug chromophore between its base pairs. Detailed definition of the above dielectric parameters may be obtained from any text-book on dielectric properties of macromolecules, e.g., (1) *Dielectric Properties and Molecular Behaviour* ed. T M Sugden (London: Van Nostrand) (2) *Dielectrics*—J C Anderson, Chapman & Hall.

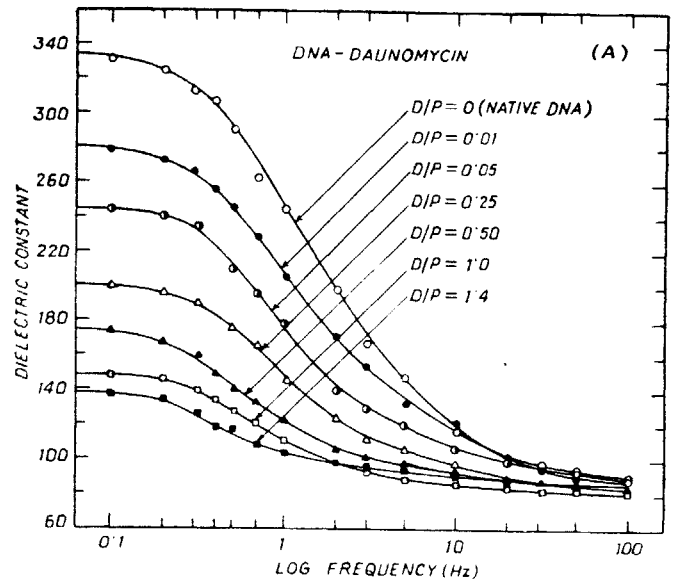

Figure 1 (A) Dielectric dispersion of DNA-daunomycin complexes at various states of binding. D and P denote molar concentration of the drug and DNA-phosphate. $P=1.5\times10^{-4}$M in each complex

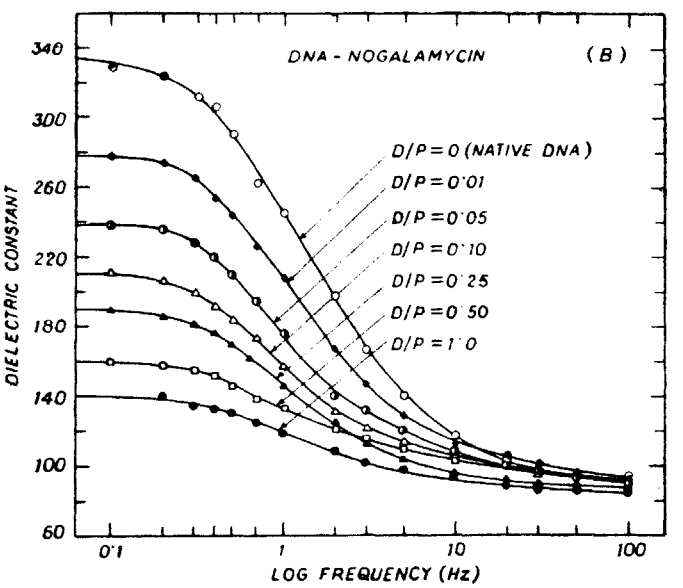

Figure 1 (B) Dielectric dispersion of DNA-nogalamycin complex at various states of binding. $P=1.5\times10^{-4}$M in each complex

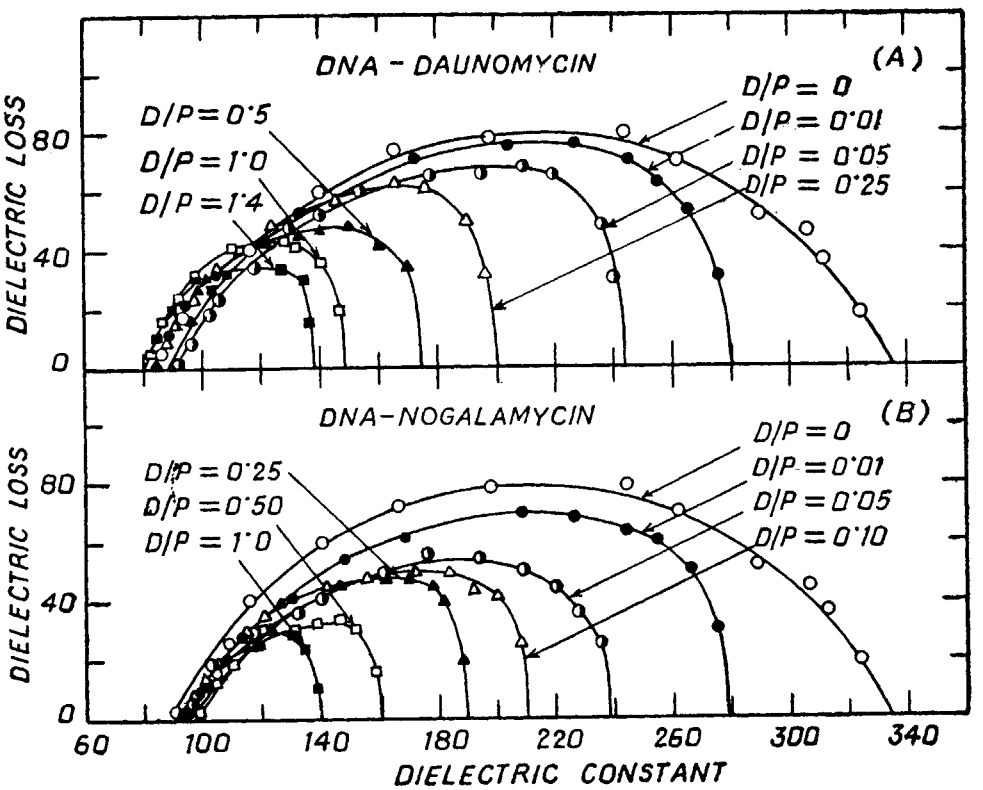

Figure 2 Dielectric loss (ϵ'') vs Dielectric constant (ϵ') plots $P=1.5\times10^{-4}$M in each complex

β values for both the drug-bound DNAs decreased progressively with increased amount of drugs and it reached a minimum value at D/P=0.25, i.e., maximum asymmetry was induced at this stage for both the drugs. This phenomenon was also observed in case of acridine dyes (Goswami & Das Gupta 1974) but the minimum value of β occurred at comparatively lower D/P values. Thus acridine dyes were more effective to induce asymmetry of the molecule than the drugs daunomycin and nogalamycin.

Relaxation times (τ) for the drug-bound DNAs were plotted as a function of D/P (figure 3B). τ was found to increase from its value of 1.7×10^{-4} seconds for native DNA with increased drug binding, reached a maximum and then became steady for both the drugs. No such increase in τ was observed for berenil-bound DNAs (Sinha et al. 1978). The gradual increase in τ was attributed to the increased length of the DNA molecule, brought about by intercalation of the drug molecules. Increase in τ was also observed in case of acridine dyes (Goswami & Das Gupta 1974) whereas an unimodal decrease in τ occurred in case of denatured DNA-dye complexes (Goswami 1977). Due to loss of double helical structure of the denatured DNAs, there was no question of intercalation between the base pairs in the later case. Relaxation time did not increase after D/P=0.5 for the present drug-DNA complexes. But $\Delta\epsilon/\Delta\epsilon_0$ decreased at the same

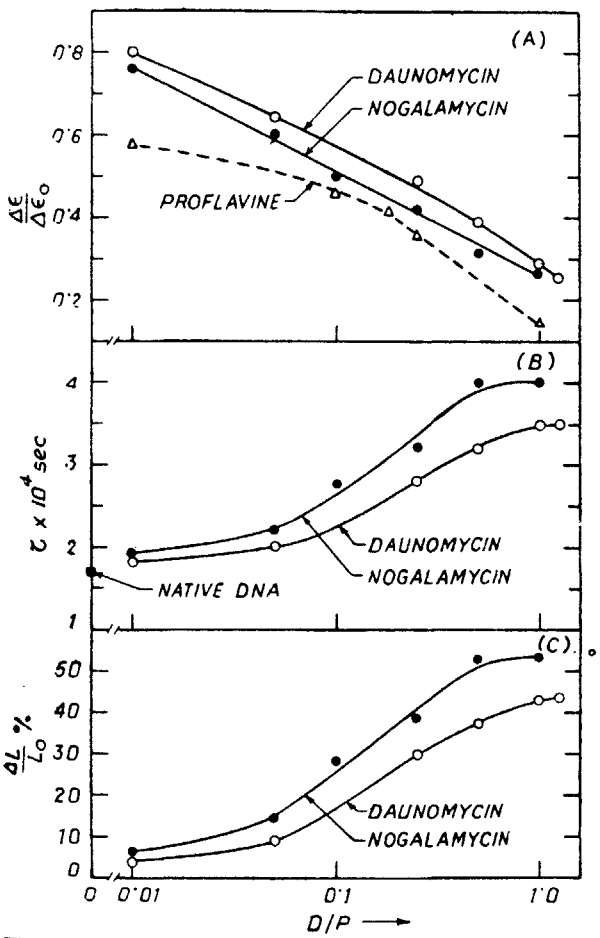

Figure 3 Variation of (A) Ratio of the dielectric increments ($\triangle\epsilon/\triangle\epsilon_o$) (B) Relaxation time ($\tau$) and (C) percentage length extension ($\triangle L/L_o \times 100\%$) of the drug-DNA complexes at various states of binding of the drugs daunomycin and nogalamycin. Dotted curve in A refers to the behaviour of DNA-proflavine complex

rate even after that value of D/P. In this region, only secondary binding (electrostatic) took place resulting in a decrease in static dielectric constant (ϵ_o) and also $\triangle\epsilon/\triangle\epsilon_o$. Evidence of secondary binding was also obtained from previous studies (Das et al. 1974, Sinha et al. 1977, Phillips et al. 1978).

Using Takashima's relation (1967) the amount of length extension was calculated (plotted in figure 3C). The maximum length extension was 53% in case of nogalamycin and 43% in case of daunomycin, i.e. nogalamycin was more effective in unwinding the double helix. From the sedimentation studies on the binding of intercalating drug to ϕ_x 174 RF DNA (Waring 1970) obtained higher value for the fraction intercalated in case of nogalamycin (68%) than in case of daunomycin (44%). Previous electron microscopic investigations on the intercalation of different acridine dyes showed about 27% length enhancements (Freifelder 1971, Das Gupta et al. 1973). Sinha et al. (1977) obtained the same order of length extension for DNA-nogalamycin complex. Recently, Butour et al. (1978) obtained a 50% length increment for DNA bound with ethidium bromide, which is in accordance with Lerman's prediction (1961). Although the present drugs are known to be partially intercalative (Waring 1970), they produced the same order of length extension i.e., unwinding of the double helix compared to the fully intercalative drug ethidium bromide. Whether the bigger size of the present drug chromophores is responsible for this, is a point to be investigated further.

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