

CHARACTERISATION OF GAMMA-IRRADIATED NUCLEOHISTONE BY SPECTROPHOTOMETRY

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ABSTRACT

Nucleohistone (DNH) shows two absorption maxima, Y-peak and X-peak. In ECA, in the unirradiated and irradiated states, their intensities increase with increase in concentration. The position of X-peak remains unaltered throughout the concentration and dose range. For a particular concentration of DNH in the unirradiated and irradiated states the values of Y-peak increase with increasing molarity. Moreover, both in the unirradiated and irradiated states there is a continuous red-shift in the Y-peak with an increase in molarity of sodium chloride. The sharper change in the Y-peak reveals close dynamic interaction between the DNH and the solvent system. Electronic transitions responsible for Y-peak are not the same transitions that are responsible for X-peak.

INTRODUCTION

NUCLEOHISTONE (DNH) has been characterised by melting profile studies¹, electron microscopy², circular dichroism³, microelectrophoresis⁴ etc. Recently DNA has been characterised from its absorption in the X-peak and Y-peak by various physico-chemical parameters⁵. The importance of X-peak and Y-peak in the case of DNA in solution and their behavioural differences under different solution parameters have been reported exhaustively⁶⁻¹⁰. The present investigation has been undertaken to characterise DNH from X-peak and Y-peak covering three variables namely concentration of DNH, dose of gamma irradiation and molarity of solvent.

MATERIALS AND METHODS

Calf-thymus DNH obtained from Calbiochem (Lot No. 100790) was used without further purification. DNH was dissolved in ECA and sodium chloride of different molarities ranging from 0.002 to 0.20 M. The pH of the solutions was 7.24 ± 0.10 for DNH in ECA and 6.3 ± 0.1 for DNH in different molarities of sodium chloride. The UV spectra of all the solutions (irradiated or unirradiated) were taken in the wavelength range of 190 to 300 nm by a Carl Zeiss spectrophotometer PMQ II in which optical absorbance could be measured accurately upto 0.005 units.

Gamma-Cell 220 supplied by the Atomic Energy of Canada was used as source of γ -irradiation. The dose rate (6.915 Gy/min) was ascertained by Fricke dosimetry. Equal volumes of DNH solutions were taken in test tubes of the same diameter. These experimental solutions were subjected to different doses of γ -irradiation upto 500 Gy approximately. The solutions after irradiation were kept at 0°C for 10 min to avoid renaturation. The irradiated samples were then allowed to attain room temperature at which absorbance was measured against the respective blanks (different solvents).

RESULTS AND DISCUSSION

In the case of unirradiated DNH in ECA, as the concentration of DNH increases from 15 $\mu\text{g/ml}$ to 75 $\mu\text{g/ml}$, the O.D. values of the X-peak and Y-peak increase continuously. The Y/X ratio is highest for lowest concentration and decreases with increase in concentration. (figure 1, table I). This has been observed by other workers also in the case of DNA⁵.

In table 1 the O.D. values of Y-peak, X-peak and their corresponding absorption maxima along with Y/X ratios have been shown for DNH in ECA in the unirradiated and irradiated states for different concentrations. Figure 2 shows the nature of typical curves obtained for DNH in ECA for a dose of 97.9 Gy. Similar curves are obtained for higher doses upto 489.93 Gy. It is evident from table 1 and also from figure 2 that irrespective of dose with increase in concentration of DNH the O.D. values of X-peak and Y-peak increase. For irradiated DNH the Y/X ratio remains fairly

Abbreviations used: DNA—deoxyribonucleic acid; DNH—deoxyribonucleohistone; ECA— 10^{-3} M EDTA, 10^{-3} M sodium cacodylate, pH 8.

constant. Moreover, both for unirradiated or irradiated DNH there is a red shift in the Y-peak with an increase in the concentration of DNH. This has also

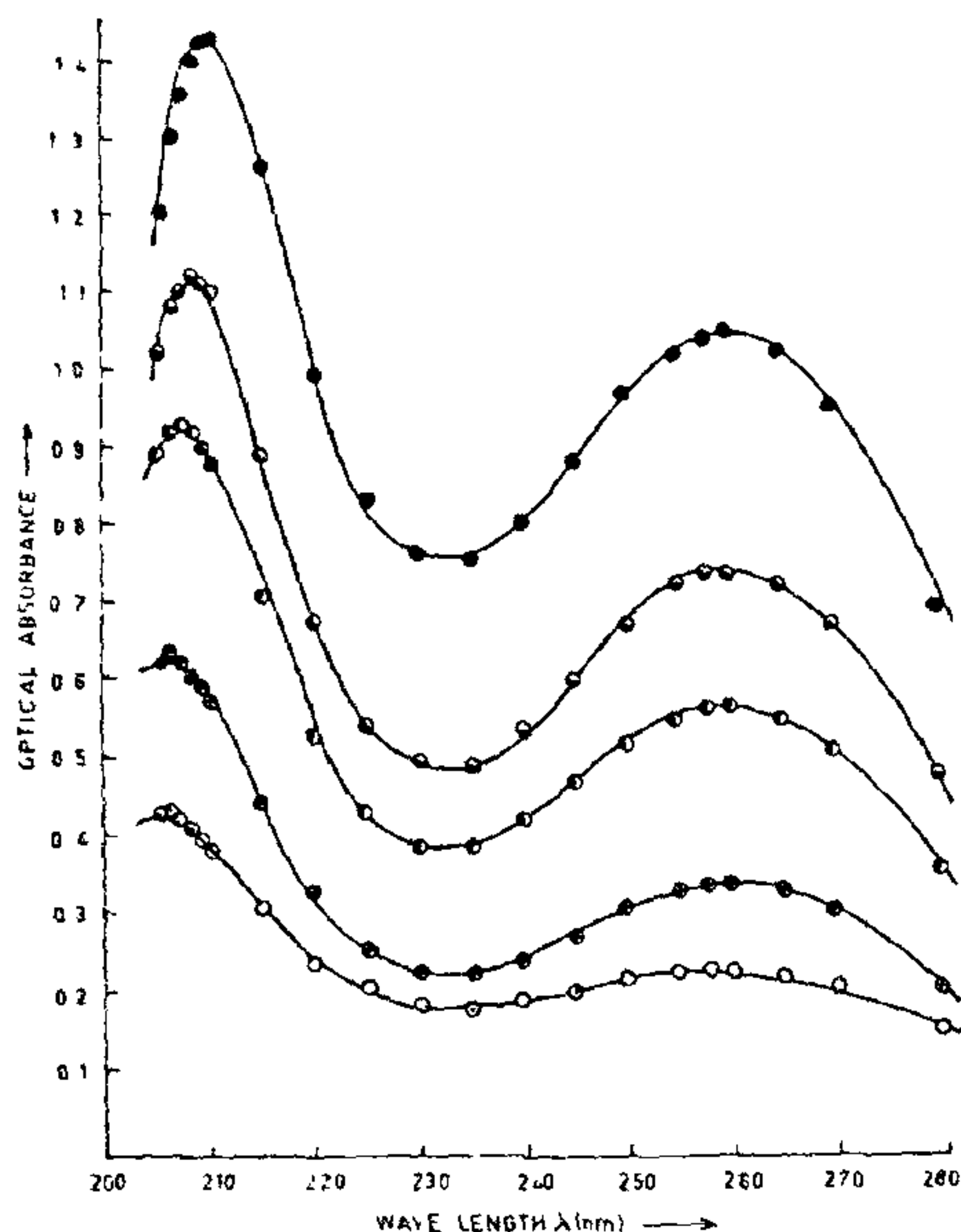


Figure 1. UV spectra of unirradiated nucleohistone in ECA solvent. Nucleohistone concentration—
○—15 μg/ml; ⊗—30 μg/ml; ●—45 μg/ml; ●—60 μg/ml; ●—75 μg/ml.

been observed in DNA⁵. It appears that the DNH configuration is highly susceptible to environmental changes which is reflected in terms of modified electronic transitions in far UV region. Whether for the unirradiated or irradiated samples the increase in the value of X-peak, with per unit concentration increase is less compared to that of Y-peak with per unit concentration increase. This sharper change in the Y-peak compared to the X-peak has been attributed to strong $\pi \rightarrow \pi^*$ transition in far UV region for DNA⁵. For X-peak it is either $n \rightarrow \sigma^*$ or $n \rightarrow \pi^*$ transition which is responsible¹¹.

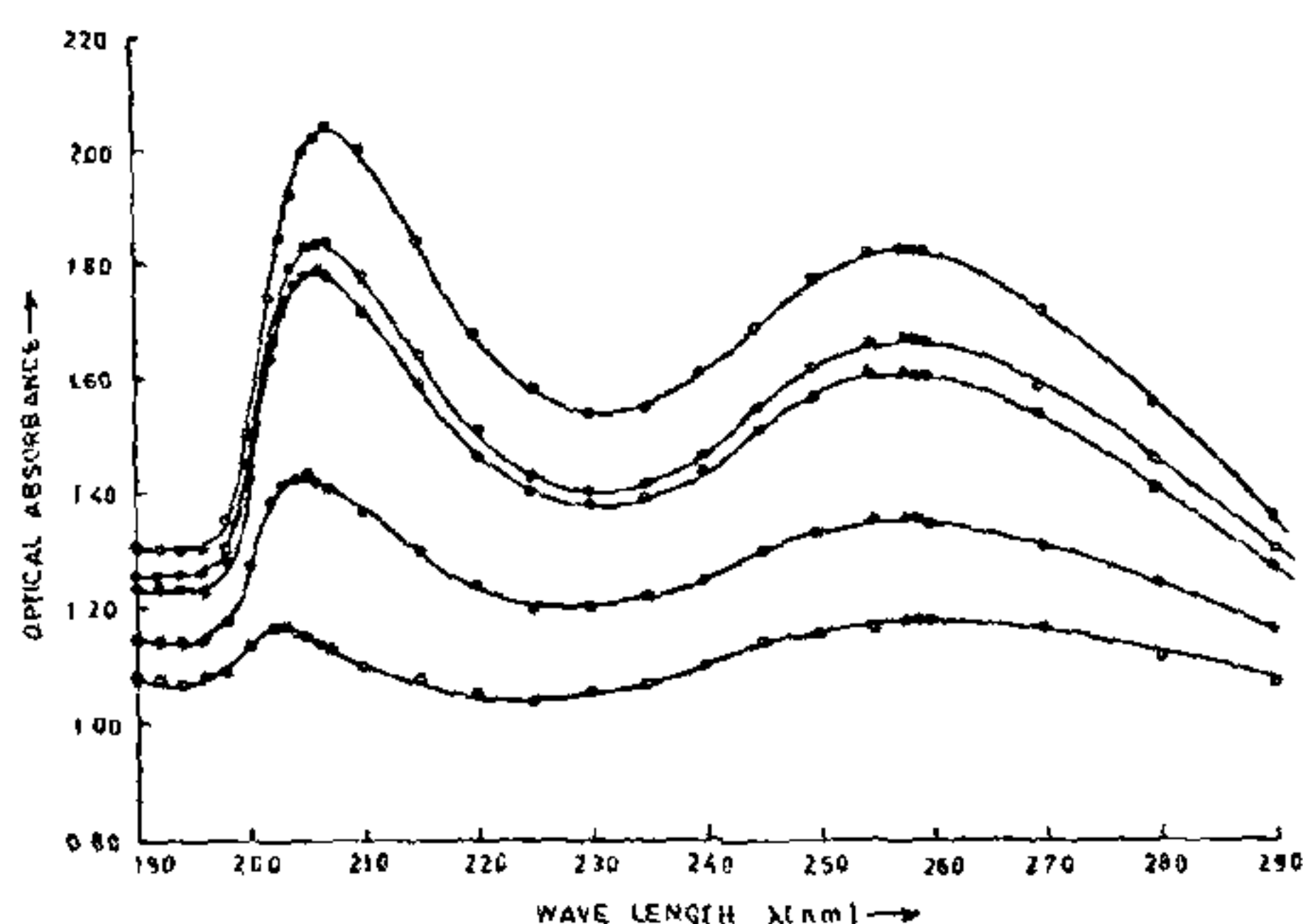


Figure 2. UV spectra of nucleohistone in ECA irradiated to 97.90 Gy. Nucleohistone concentration—
○—15 μg/ml; ●—30 μg/ml; ●—45 μg/ml; ●—60 μg/ml; ●—75 μg/ml.

Table 1 Absorption parameters of nucleohistone in ECA for different concentrations in the unirradiated and irradiated states.

	Conc. of DNH (μg/ml)														
	15			30			45			60			75		
Unirradiated irradiated (Gy)	O.D. value of Y peak	O.D. value of X peak	Y X	O.D. value of Y peak	O.D. value of X peak	Y X	O.D. value of Y peak	O.D. value of X peak	Y X	O.D. value of Y peak	O.D. value of X peak	Y X	O.D. value of Y peak	O.D. value of X peak	Y X
Unirradiated	0.43	0.23	1.89	0.63	0.34	1.85	0.93	0.57	1.65	1.12	0.74	1.51	1.43	1.04	1.38
97.90	1.17	1.18	0.99	1.44	1.36	1.06	1.79	1.61	1.11	1.84	1.67	1.10	2.04	1.83	1.11
195.81	1.18	1.18	1.00	1.45	1.37	1.06	1.62	1.50	1.08	1.80	1.65	1.09	2.01	1.86	1.08
293.72	1.08	1.20	0.98	1.42	1.88	1.03	1.48	1.50	0.99	1.57	1.68	0.93	1.82	1.83	0.99
391.62	1.11	1.17	0.95	1.46	1.38	1.06	1.62	1.51	1.07	1.80	1.67	1.08	1.95	1.84	1.08
489.93	1.07	1.17	0.91	1.39	1.85	1.03	1.62	1.52	1.07	1.78	1.65	1.08	2.04	1.86	1.10

It will be observed from figures 1 and 2 that the position of the X-peak remains unaltered for unirradiated or irradiated DNH even if there is a change in concentration. Moreover, it will be observed from table 1 that the values of X-peak or Y-peak are not a function of dose. It has also been observed in the case of DNA⁵ that the position of X-peak and Y-peak remains unaltered upto a dose of 1000 Gy.

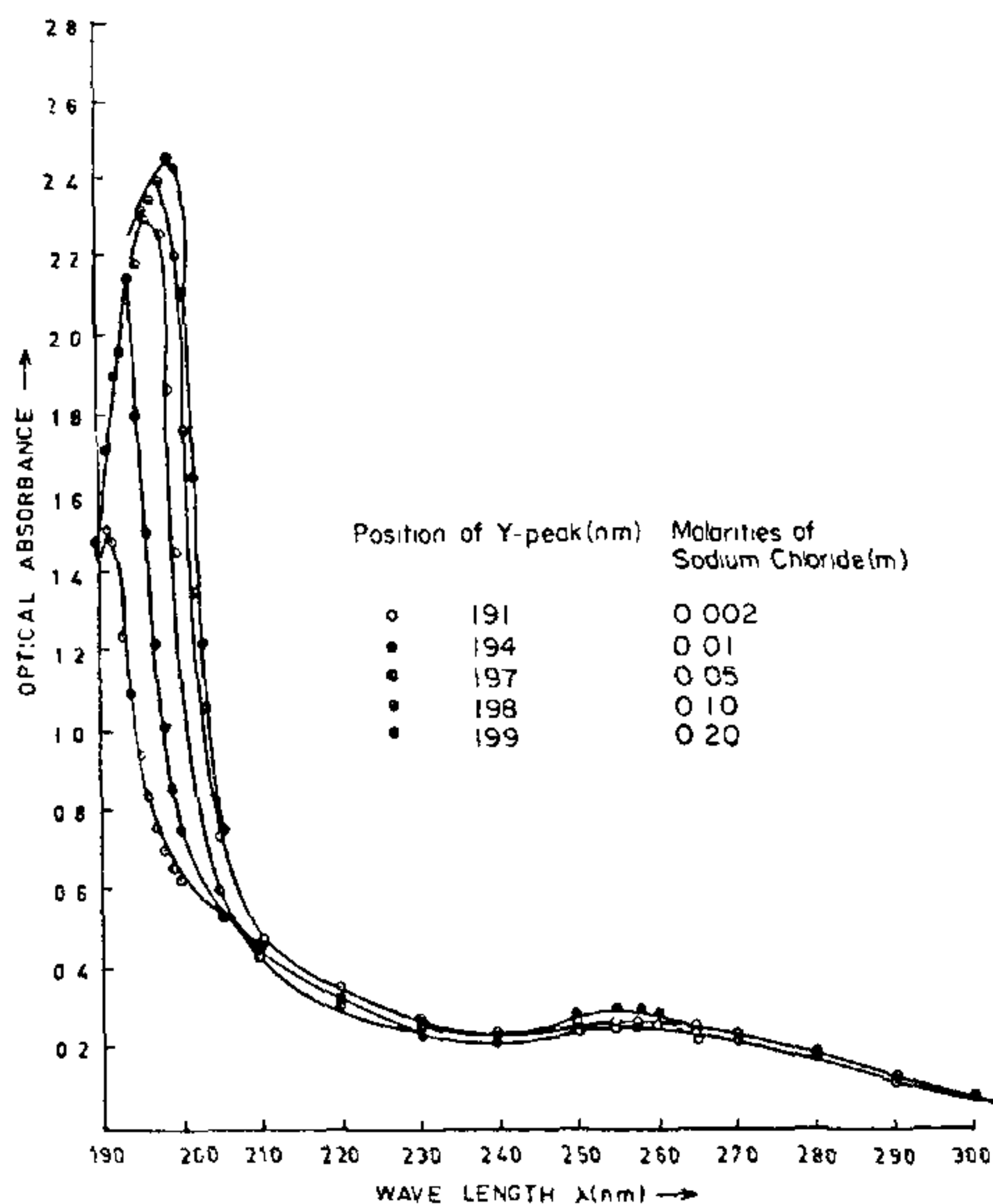


Figure 3. UV spectra of nucleohistone prepared in different molarities of sodium chloride (concentration 30 µg/ml).

It is known¹² that soluble DNH goes into solution easily at sodium chloride molarities below 0.02, whereas the gel fraction has a very low solubility at molarities above 0.001. The minimum solubility is at a molarity of 0.15. At this point there is a little more of protein in solution than DNA. So depending upon the solvent molarity the dissociation of DNH takes place and that is reflected in the X-peak and Y-peak.

It has been reported¹³ that the different behaviour shown by the two peaks can be explained if the chromophoric groups of the bases responsible for both the peaks could be divided into three distinct groups: (a) The electronic transitions which are exclusively seen at the Y-peak. (b) Transitions which are common to both X- and Y-peaks. (c) Transitions which are exclusively seen at the X-peak.

For a particular concentration of unirradiated DNH (figure 3, table 2) as the molarity of sodium chloride increases from 0.002 to 0.20, the Y-peak values increase and there is a continuous red shift in the Y-peak maximum from 191 to 199 nm. The occurrence of X-peak is not significant. It has been observed¹⁴ that the intensity of absorption for DNH in different molarities of sodium chloride is much more than bare sodium chloride at different molarities.

For irradiated DNH with a dose of 300.1 Gy (figure 4) under identical conditions, similar increase in the values of Y-peak with increasing molarity of sodium chloride is observed. In fact it can be seen from table 2 that the red shift in the Y-peak with increasing molarity of sodium chloride occurs for all doses upto 500.6 Gy. Thus it appears that the red shift in the absorption maximum of Y-peak is dependent on molarity of sodium chloride rather than the dose of irradiation. Similar red shift in the Y-peak maximum has also been observed with increase of ionic strength for DNA⁵.

Table 2 Wavelength of maximum absorption (λ_{max}) and optical absorption values of Y-peak for different molarities of sodium chloride for Nucleohistone (Conc. 30 µg/ml) in the unirradiated and irradiated states.

Molarities of sodium chloride (M)	Irradiated (Gy)											
	Unirradiated		99.9		199.8		300.1		399.7		500.6	
	λ_{max} (nm)	O.D.	λ_{max} (nm)	O.D.	λ_{max} (nm)	O.D.	λ_{max} (nm)	O.D.	λ_{max} (nm)	O.D.	λ_{max} (nm)	O.D.
0.002	191	1.51	191	1.52	191	1.49	191	1.09	191	1.54	191	1.52
0.01	194	2.15	194	1.98	194	1.90	194	1.77	194	2.05	194	2.09
0.05	197	2.35	197	2.38	197	2.25	197	2.10	197	2.43	197	2.31
0.10	198	2.40	199	2.40	199	2.37	199	2.18	199	2.45	199	2.40
0.20	199	2.45	200	2.45	200	2.35	200	2.26	200	2.50	200	2.42

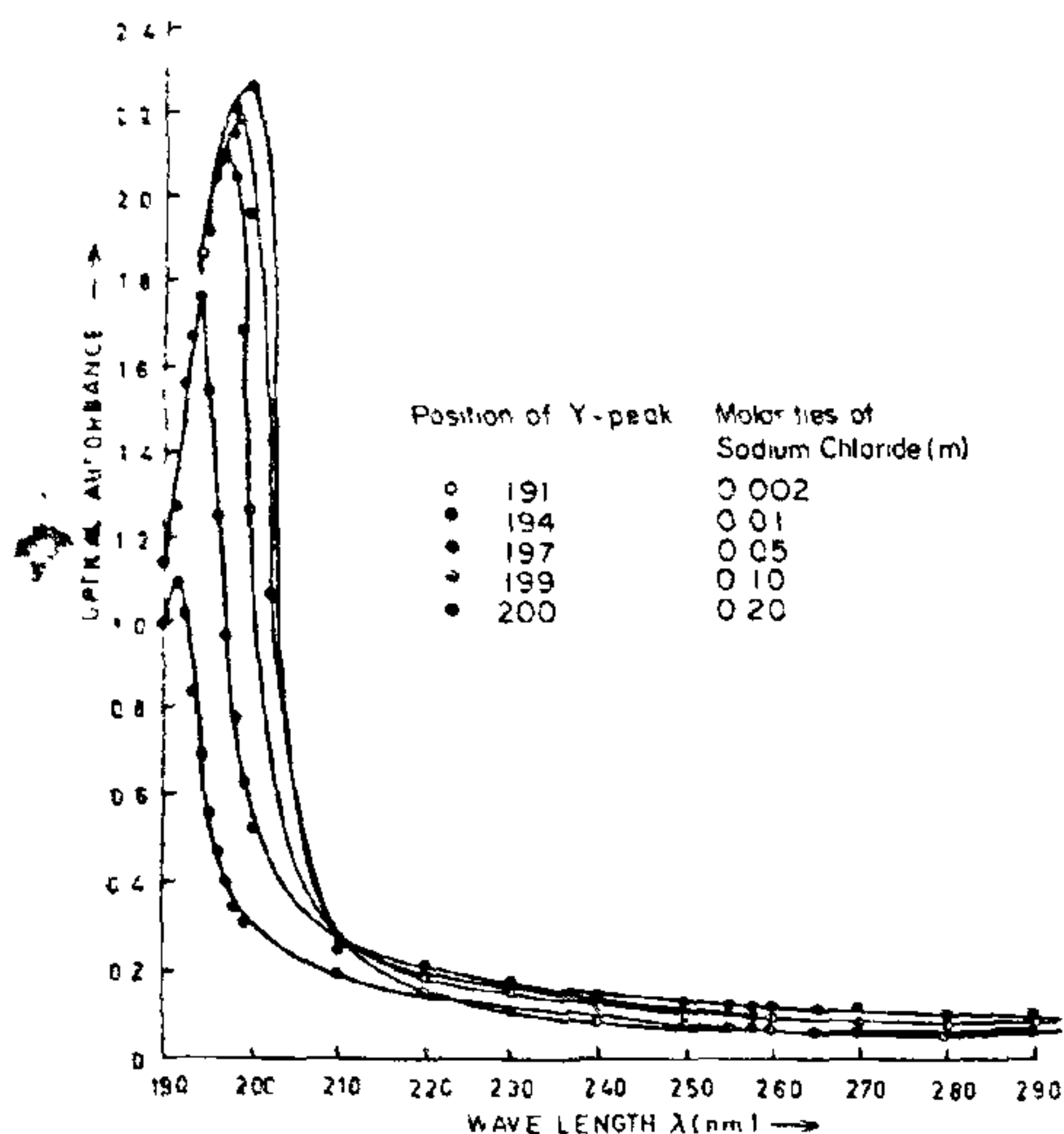


Figure 4. UV spectra of gamma-irradiated nucleohistone for different molarities of sodium chloride (concentration 30 $\mu\text{g/ml}$; Dose 300.1 Gy.)

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ANNOUNCEMENT

NATIONAL LEVEL SYMPOSIUM ON STRUCTURE-REACTIVITY CORRELATION BY KINETIC AND SPECTRAL STUDIES

A national level symposium on 'Structure-reactivity correlation by kinetic and spectral studies' sponsored by University Grants Commission, New Delhi is being organised at VHNSN college, Virudhunagar, Tamilnadu during 28-30 November 1984. The contributors are asked to send two copies of the paper and

a 500 word abstract by 15th September 1984 to the Head, Department of Chemistry, VHNSN College, Virudhunagar, 626 001. A scientific committee will select authors on the basis of their papers for oral presentation. The selected authors will be provided T.A and D.A according to UGC norms.