TEMPERATURE AND METAL IONS INFLUENCE ON OPTICAL PROPERTIES OF CHITOSAN IN AQUEOUS SOLUTION

Marceli Koralewski, K. Henryka Bodek[1], Tomasz Wachowski

Institute of Physics, A. Mickiewicz University,
ul. Umultowska 85, 61-614 Poznań,. Poland

[1] *Department of Pharmacy and Applied Pharmacy Medical University of Łódź,*
ul. Muszyskiego 1, 90-151 Łódź, Poland

1. Introduction

Chitosan is a linear polysaccharide of β (1→4) linked D-glucosamine which has recently obtained wide application as multifunctional biopolymer [1, 2]. Structure of chitosan molecules in solid state was studied mostly through X-ray diffraction [3] and NMR [4]. It was proved that chitosan chain in crystal state assumes an extended or relaxed two-fold helix conformation in anhydrous and hydrated polyphorms respectively. Chitosan in aqueous solution under different physicochemical conditions are predicted to appear in several different conformation i.e.: rod-shaped, stiff or random coil or even in compact sphere.

It is well known that chiroptical spectroscopies provided a sensitive probe of conformational changes of molecules and polymers. For some polysaccharides, e.g. carrageenan or xanthan, random coil to helix conformational transition under influence of temperature or K^+ ion were established by optical rotation measurements [5]. Domard [6] discussed circular dichroism (CD), optical rotation, mostly known for the sodium D line, was described recently for several wavelengths [7] but there are no information on temperature and metal ions influence on chiroptical properties of chitosan.

The aim of the paper was to determine the effect of temperature and biologically important metal ions on the refractive and chiroptical properties of chitosan in aqueous solution.

2. Experimental

The measurements were performed for chitosan from shrimp characterized by molecular weight $M_v \approx 202$ kDa and degree of deacetylation (DD) DD $\approx 96,3$ %. DD was established by the IR method [8]. In the reported study a 1% acetic acid aqueous solutions was used as a solvent. Chitosan aqueous solutions with chitosan concentration c = 0.2 g/dl or 0.3 g/dl

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(w/v) were prepared with different amount of salt (c_i) in the range of 0 up to 1 mol. Chloride salt of sodium, potassium and calcium were used. After incubation at least one day the optical measurements were performed.

Refractive index and optical rotatory dispersion (ORD) was measured using a thermostatically controlled Pulfrich Refractometer – PR2 (Zeiss) and a Polamat A (Zeiss) polarimeter respectively. Optical rotation was measured at wavelengths of 366, 406, 436, 546 and 578.9 nm using cells of pathlength from 0.1 to 0.5 dm to keep absorbance below 1.2. Temperature dependence of optical rotation was measured at 578.9 nm using jacketed cell of 2 dm. Temperature attachment with a thermostat MK 70 (Zeiss) were used in both set up. The uncertainty in the refractive index and optical rotation was ± 0.00001 units and ± 0.003° respectively, at a temperature control better than ± 0.1 °C. Specific rotation $[\alpha]^t_\lambda$ was calculated using standard relation $[\alpha]^t_\lambda = 100\ \alpha/(c{\cdot}l)$ where: α is in degree, c in g/dl and l in dm.

3. Results and discussion

3.1. Refractive index

The refractive index dispersion is shown in Figure 1 for pure and salt added chitosan aqueous solutions. The value of n monotonically decreases with increasing wavelength. The shape of the dispersion curve is typical of saccharides having optical chromophors absorbing in the far-ultraviolet region and may be well described by Sellmeier equation:

$$n^2 - 1 = S_0\lambda^2/(\lambda^2 - \lambda^2_0) \tag{1}$$

where: S_0 is constant for given λ_0 characteristic for particular substance, λ_0 is characteristic wavelength related with true or some effective absorption maximum (in fitting procedure λ_0 is usually taken in [μm] so this define dimension of S_0; similar apply to equation 3). The coefficients of equation 1 for some solutions studied are given in Table 1. Free metal ion polarizabilites α_i (atomic units were used) [9] and refractive index are also collected in Table 1. The values of λ_0 is nearly equal for pure and ion-treated chitosan solutions and suggest that contributed electronic transitions of metal ion do not change the character and shape of dispersion curve in spectral range studied.

As shown in Figure 2, refractive index of chitosan with added salt increases with increasing of ion concentration, c_i, most strongly with $Ca^{2+} > K^+ \geq Na^+$ in the order given (see also Table 1). The refractive index increases linearly up to concentration of salt equal around 0.5 mol. Above this concentration very small nonlinearities especially noticeable for K^+ ion

Table 1. *Refractive index n^{24}_{546}, refractive index increment $\Delta n/\Delta c$, Sellmeier equation coefficients (λ_o and S_o) for pure and ion-treated chitosan and free ion polarizability α_i (t = 24 °C); e) chitosan concentration c = 0.3 g/dL; f) chitosan concentration c = 0.2 g/dL; g) ref. [9]; h) - [ml/g] ref. [7].*

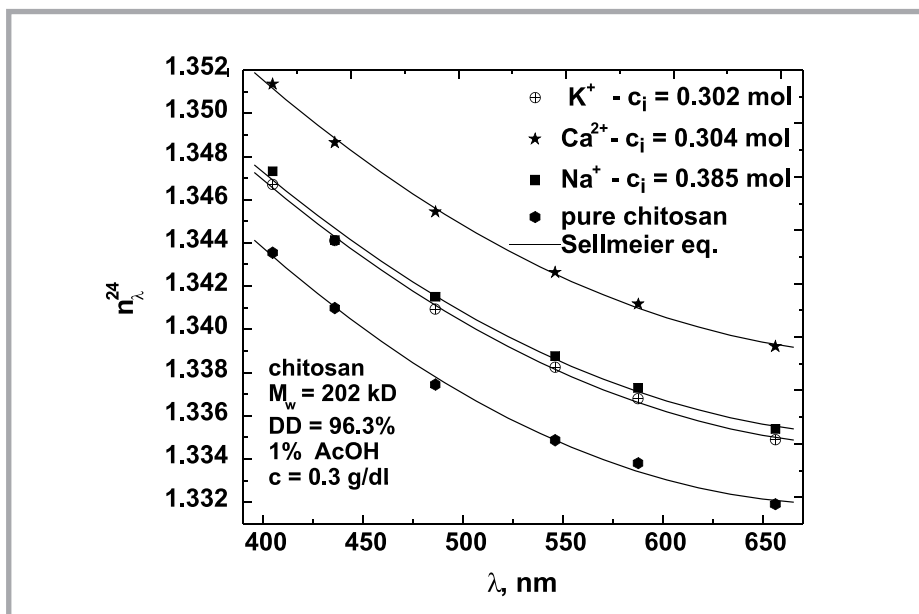| Ion	c [e)], mol	n^{24}_{546}	S_o	λ_o, nm	α_i [g)], au	$\Delta n/\Delta c$, mol^{-1}
Na$^+$	0.385	1.33878	0.7639	105.1	1.15	0.0100
K$^+$	0.302	1.33824	0.7624	105.0	5.82	0.0098
Ca^{2+}	0.304[f)]	1.34264	0.7733	105.8	3.47	0.0247
pure	0	1,33526	0,7548	104,8	-	0.184[h)]

Figure 1. *Refractive index dispersion n^{24}_λ of pure and salt added chitosan in aqueous solution, solid line describe Sellmeier equation (Equation 1).*
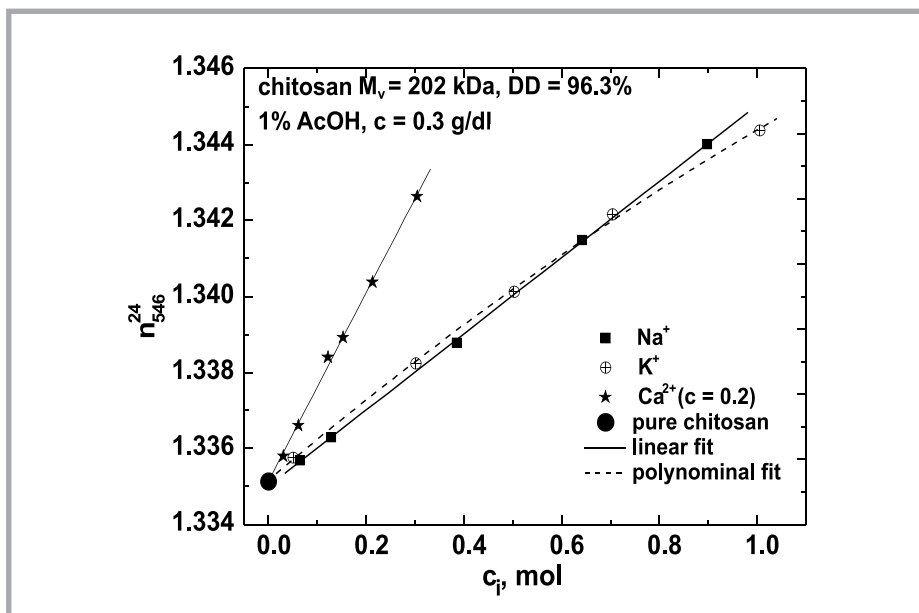

Figure 2. *Refractive index n^{24}_{546} of chitosan in aqueous solution as a function of metal ion (salt) concentration c_i.*

may be observed. Taking into account linear character of this dependence, refractive index increment for particular ion was calculated and is collected in Table 1. The valency of the ion plays major role in comparison to the value of polarizability of free ion. However for given valency changes of refractive index of ion-treated chitosan seem to be in correlation with the value of free ion polarizabilites (see Table 1).

Temperature dependencies of the refractive index of ion-treated chitosan in the range of 20 - 50 °C do not show any sign of anomalous changes (see Figure 3) and can be easily described by the equation:

$$n^t_\lambda = at^2 + bt + n^o_\lambda, \qquad (2)$$

where: a, b – coefficient, t – temperature in °C and n^o_λ, n^t_λ are the refractive index at temperature 0 °C and t in °C at given light wavelength λ respectively. The best fit parameters are collected in Table 2.

Such dependence without anomalous changes does not suggest conformational transition of polymer chain under metal ions influence in studied temperature range and at first approximation reflects mainly temperature dependence of n for pure water.

3.2. ORD

ORD of chitosan aqueous solutions for some concentrations of different salt added are presented in Figure 4. For the sake of comparison, the same figure also shows results of two different series of the ORD of a pure chitosan solution (two solution of equal concentration
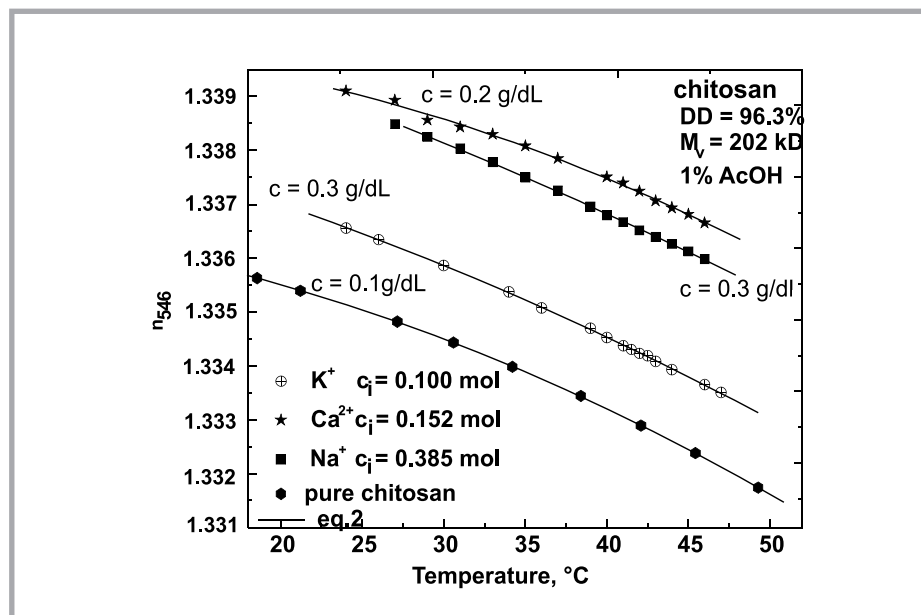

Figure 3. *Temperature dependence of the refractive index n_{546} of pure and salt added chitosan in aqueous solution.*

Table 2. *The value of coefficients in equation 2 for pure and ion-treated chitosan (λ = 546nm); k) chitosan concentration c = 0.3 g/dL; j) chitosan concentration c = 0.2 g/dL.*

Coefficient	ion (c_i [mol])[k]			pure chitosan, g/dL (c=0.1)
	Na+ (0.385)	K+ (0.100)	Ca2+ [j] (0.152)	
a [10^{-6}]	-0.70752	-0.95288	-2.23798	-1.44413
b [10^{-5}]	-8.1929	-6.6227	-5.1058	-2.8392
n^{24}_{546}	1.34123	1.33871	1,33904	1.33665
R	0.99955	0.99975	0.99574	0.99999

was prepared). Some scattering of the value $[\alpha]^{24}_\lambda$ for particular wavelength from expected smooth dispersion curve may be noticed (see Figure 4). The ORD of all substances studied hardly but still may be characterised by plain rotatory dispersion curves which is described by Drude equation:

$$[\alpha]_\lambda = K_o/(\lambda^2 - \lambda^2_o) \qquad (3)$$

where: K_o is the molecular rotation constant for given λ_o characteristic for particular substance, and λ_o is the characteristic wavelength related with true or some effective absorption maximum similarly like in equation 1 (see Table 3). It needs to be notice that independly of the ion for several solution scattering of $[\alpha]^{24}_\lambda$ do not allow reasonable Drude fit. The scattering or some variation of the value $[\alpha]^{24}_\lambda$ suggest that under physicochemical condition used in this study supermolecular structure is probably not stable. Such situation may be realised throughout dynamic changes in segment –segment repulsion related with instability
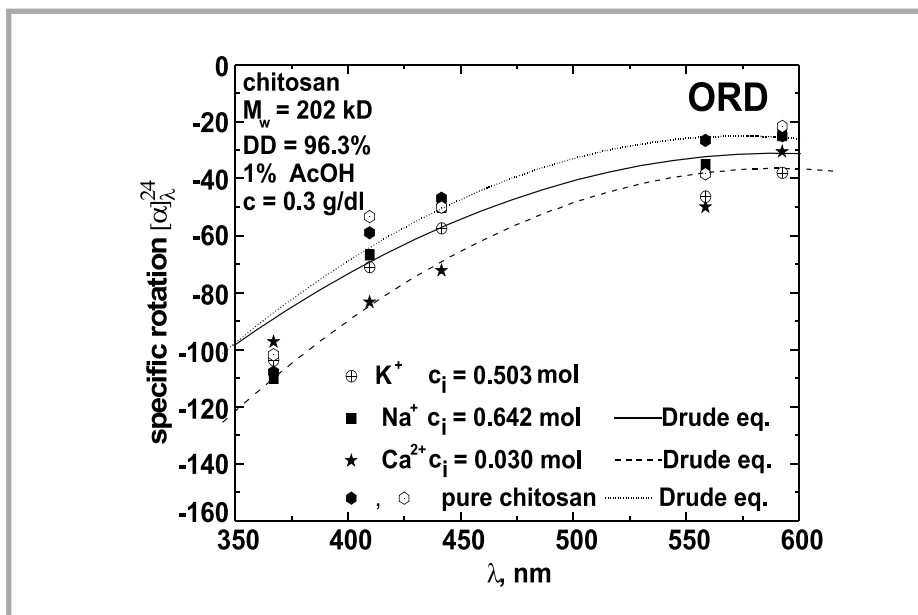

Figure 4. *The specific rotation $[\alpha]^{24}_\lambda$ dispersion of pure and salt added chitosan in aqueous solution, solid line described Drude equation (Equation 3).*

Table 3. *Specific rotation and Drude equation coefficients (K_o and λ_o) for pure and ion-treated chitosan ($t = 24$ °C); m) chitosan concentration $c = 0.3$ g/dL; n) chitosan concentration $c = 0.2$ g/dL.*

Ion	c_i [m], mol	$[\alpha]^{24}_{546}$	K_o	λ_o, nm
Na^{+}	0.642	-110	8.873	187
K^{+}	0.503	-104	-	-
Ca[2+ n)]	0.030	-97	10.256	205
pure	0	-108/-102	6.602/7.045	243/232

of protonation of ammonium group or/and with anomerization of portion of β-glycosidic bonds. Anomerization process was observed for chitin [10].

Results of specific rotation dependence on the concentration of metal salt added to aqueous solution of chitosan are presented in Figure 5. Addition of positively charged metal ions slightly influence the specific rotation of chitosan but very week maximum of $[\alpha]^{24}_{366}$ can be noticed around 0.15 mol for Ca^{2+} and around 0.35 mol for Na^+ and K^+, i.e. approximately double values for Ca^{2+} ion. Relation between maxima observed suggests that ionic radii of the ion is not as important as its valency i.e. its charge. However no drastic changes of $[\alpha]^{24}_{366}$ upon influence of different metal cation one may observe. Repulsion of positively charged segment of chitosan chain is amplified through repulsion of segment-metal cation/ solvent. The effective charge increases and the structure becomes more extended. This additional interaction change chitosan chain toward extended coil conformation, which
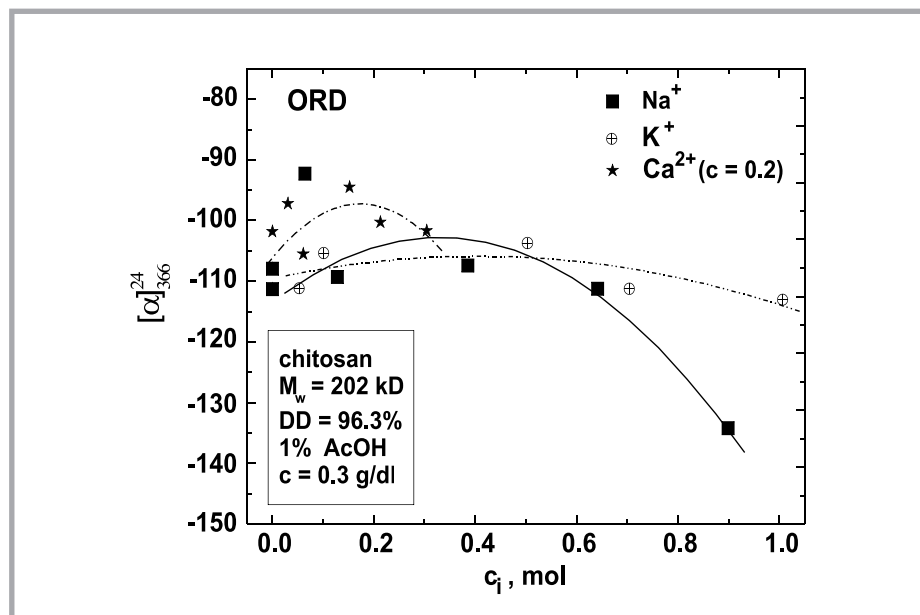
Figure 5. *The specific rotation $[\alpha]^{24}_{366}$ of chitosan in aqueous solution as a function of metal ion (salt) concentration c_i (solid lines are guide for eyes).*
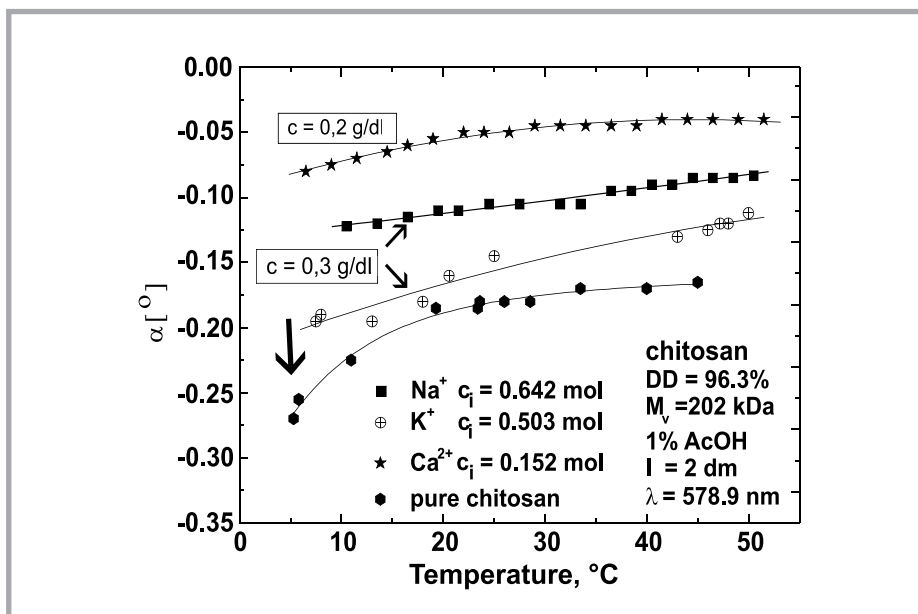
Figure 6. *Temperature dependence of the optical rotation angle α of pure and salt added chitosan in aqueous solution (solid lines are guide for eyes).*

is manifested in changes of the value of $[\alpha]^{24}_{366}$.

The optical rotation angle α of pure chitosan decreases with decreasing temperature (see Figure 6) giving no evidence of coil-helix conformational phase transition in temperature range 278 ÷ 347 K. Although the shape of temperature of α changes suggest some ordering process to accure in chitosan chain toward more compact coil or even helix-like conformation. However temperature is approaching freezing point, which does not allow for final discussion of specific rotation changes with temperature. Solute with lower freezing point may put new light on this question. Under influence of metal cation (see Figure 6) temperature dependence of the optical rotation angle α changes the shape from slightly curvature line (pure chitosan) to nearly linear one. It means that in ion-treated chitosan solution, chitosan chain at lower temperature becomes less ordered in comparison to pure one, which is realised throughout electrostatic repulsion of metallic cation on charge of chitosan segment. So under influence of metallic cation the chain of chitosan changes toward more extended coil.

4. Conclusions

The results obtained allow drawing the following conclusions:
1. Dispersion of the refractive index of pure and salt added chitosan aqueous solution is described by Sellmeier equation.

2. Refractive index of pure and salt added chitosan aqueous solution dos not show anomalous changes in the temperature range 20 ÷ 50 °C.

3. Refractive index of chitosan aqueous solution depends linearly on the metal ion concentration in the order $Ca^{2+} > K^{+} \geq Na^{+}$.

4. In the visible range of the spectrum the specific rotation – $[\alpha]^{24}_{\lambda}$ of pure and salt added chitosan aqueous solution is laevorotatory but the dispersion curve is not very smooth and $[\alpha]^{24}_{\lambda}$ hardly may be described by Drude equation. Variations of $[\alpha]^{24}_{\lambda}$ suggest that chitosan chain conformation may be not stable.

5. Temperature dependence of the optical rotation angle α of pure and salt added chitosan aqueous solution do not show the order-disorder conformational transition, however such behaviour of chitosan chain was not excluded especially for not added salt chitosan.

6. In solute with metal cation chitosan chain shows slightly more extended conformation than in solute without that ions.

5. References

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