4. OPTICAL PROPERTIES OF CHITOSAN IN AQUEOUS SOLUTION

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1. Introduction

Chitosan is a substance that has recently found many applications in industry, agriculture and medical sciences [1]. This natural polymer is characterised by optical chirality, and the specific rotation $[\alpha]^T_\lambda$, degree of deacetylation (DD) and mean molecular weight M_v are the most important parameters describing it. The circular dichroism (CD) spectroscopy can be used for determination of DD [2] and has been increasingly often used for characterisation of the physicochemical properties of chitosan and its derivatives [3]. Optical rotatory dispersion (ORD) brings less information than CD but it is cheaper and possible to use outside the absorption bands (for polysaccharides in the far-ultraviolet) so it may be very useful for description of the chiral properties of polymers. For characterisation of chiroptical properties of substances often the values of $[\alpha]^T_D$ for the yellow sodium line is given. The properties of chitin depend on the origin of the substance [4] so the properties of chitosan from different sources can also be different. However, to the best of our knowledge, no systematic ORD studies of chitosan in solution have been published. The values of specific rotation $[\alpha]_D$ of chitosan are quoted in few papers only, however, there is no consistency in the value of $[\alpha]_D$ e.g.$\{[\alpha]_D{}^{25}+30, (c,?), 2\%$ AcOH$\}$[5], $\{[\alpha]_D{}^{20}+250, (c,0.5), 2\%$ AcOH$\}$[6].

Possible application of chitosan films in optoelectronics [7] has stimulated the interest in optical properties of this substances, and very recently refractive index dispersion of spin cast chitosan film have been reported [8]. A systematic study of the optical properties of chitosan from different sources and their correlation with other parameters is of importance in material engineering for production of materials of target properties.

The aim of the paper was to determine the coefficients describing the optical phenomena such as refraction of light, ORD and Faraday Effect (magnetic analogue of ORD-

MORD) of chitosan in aqueous solution and to establish possible relations between these coefficients and DD. It will be shown that ORD may also provide us with information about characteristic concentration of chitosan in solutions.

2. Experimental

The chitosan samples used in the present study are characterised in Table 1, which gives the manufacturer, M_V and DD. DD was established by the IR method [9]. In our study a 1% acetic acid aqueous solution was used as a solvent. Chitosan aqueous solutions in the range of chitosan concentration from c = 0.005 g/dL to 3 g/dL (w/v) were prepared and after incubation of several hours the optical measurements were performed.

The pH values of all solutions were measured using an electronic pH-meter CP-401 (Elmetron) with an ERH-13-6 electrode at room temperature (RT) of about 22 ˚C. The results are presented in Figure 1 indicating that in all solutions the protonation of NH_2 group should be complete.

Refractive index n dispersion in the range of 350-650 nm was measured at a temperature stabilized to 24 ˚C using a Pulfrich refractometer – PR2 (Zeiss) equipped with a thermostat. For three different solutions temperature measurements were also made in the range of 20 - 50 ˚C for λ = 546 nm.

ORD was measured at RT using a Polamat A (Zeiss) polarimeter for several wavelengths in the range of 366 to 578 nm. The specific rotation $[\alpha]_\lambda$ is regarded as positive (+) or dextrotatory when the plane of polarization of incident light is rotated clockwise for light coming towards the observer.

The Faraday rotation measurements were performed also at RT with a lab-made "null position" spectropolarimeter [10] in the spectral range 400 - 600 nm. Magnetic field of the intensity of 1.09 T was produced by an electromagnet and its intensity during measurements was monitored with a hallotron teslameter RX-21b (Resonance Technology). For the sake of comparison similar optical measurements were performed for D-glucosamine hydrochloride (GHCl), D-glucosamine sulfate (GSO$_4$) and N-acetyl-glucosamine (NGAc) at a concentration c = 1 g/dL in a 1% acetic acid aqueous solution.

Table 1. *Some physicochemical properties and sources of the chitosan studied; a – IR method, b – differential UV method.*

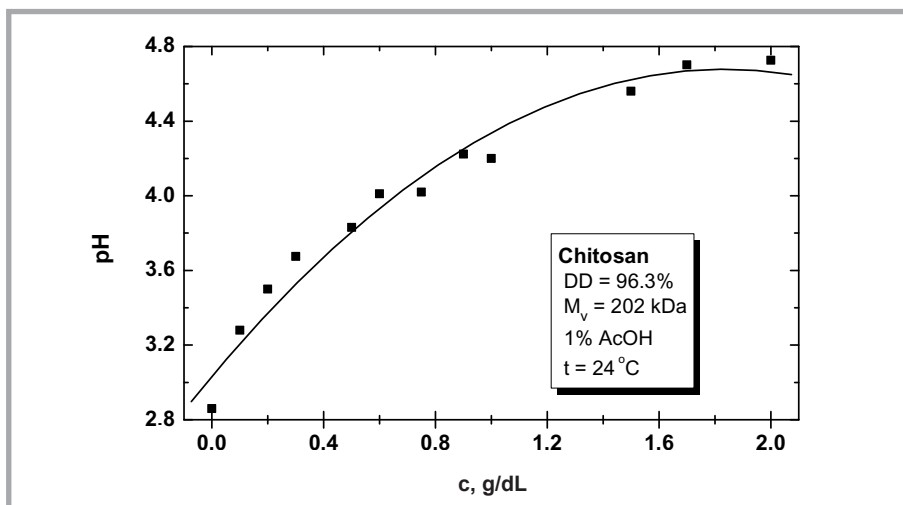| Substance	Symbol	Source (manufacturer)	DD^a, %	M_V, kDa
chitosan	A1	shrimp (Pandalus AS, Norway)	65.6	530
	A2	shrimp (Chemopol Co., India)	70.0	59
	A3	krill (Sea Fisheries Institute, Gdynia, Poland)	78.0	700
	A4	shrimp (Tech-Food Trading Ltd,	96.3^b	202

Figure 1. *Variation of pH changes as a function of concentration of chitosan in 1% acetic acid aqueous solution.*

3. Results and discussion

3.1. Refractive index

The refractive index dispersion of chitosan is shown in Figure 2 for all solutions studied. The value of n monotonically decreases with increasing wavelength. As expected, the shape of the dispersion curve is typical of saccharides having optical chromophores absorbing in the far-ultraviolet region. With increasing concentration of chitosan in solution, the refractive index increases linearly without any deviation from the additivity law (see Figure 3). These results allow us to calculate the refractive index increment $-\Delta n/\Delta c$ for all of the light wavelengths studied, its dispersion is shown in Figure 4. This dispersion of the refractive index increment is not strong but it should be taken into account when the molecular weight is calculated by the light scattering method (LSM) for different wavelengths in both experiments.

Temperature dependencies of the refractive index for the three solutions (see Table 2) in the range of 20 - 50 °C do not show any sign of anomalous changes and can be described by the equation:

$$n_1^t = at^2 + bt + n_1^o \tag{1}$$

where: a, b – coefficient, t – temperature in °C and n_1^o, n_1^t are the refractive index at temperature 0 °C and t respectively. The best fit parameters are collected in Table 2.

Equation (1) was used for the calculation of the refractive index increment for several temperatures from the range 20 - 50 °C. The temperature dependence of the refractive index increment is linear and stable in the range of the error. It
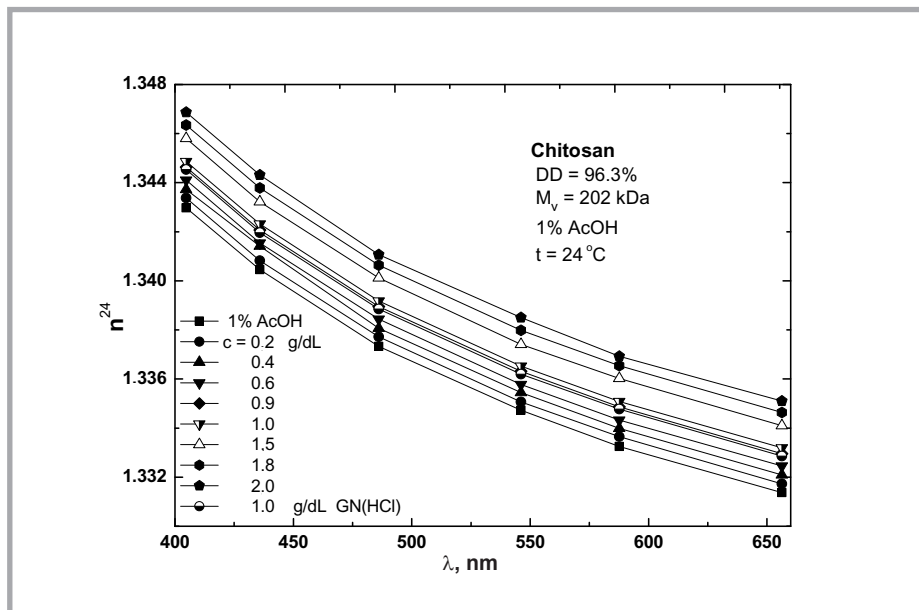
Figure 2. *Refractive index n²⁴ dispersion of chitosan of different concentrations in aqueous solution (DD = 96.3%).*
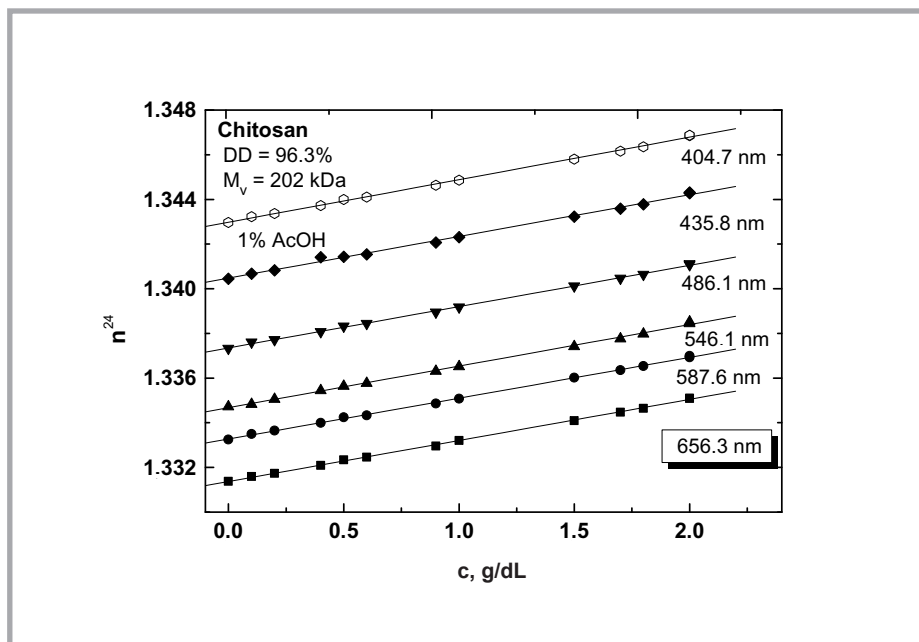

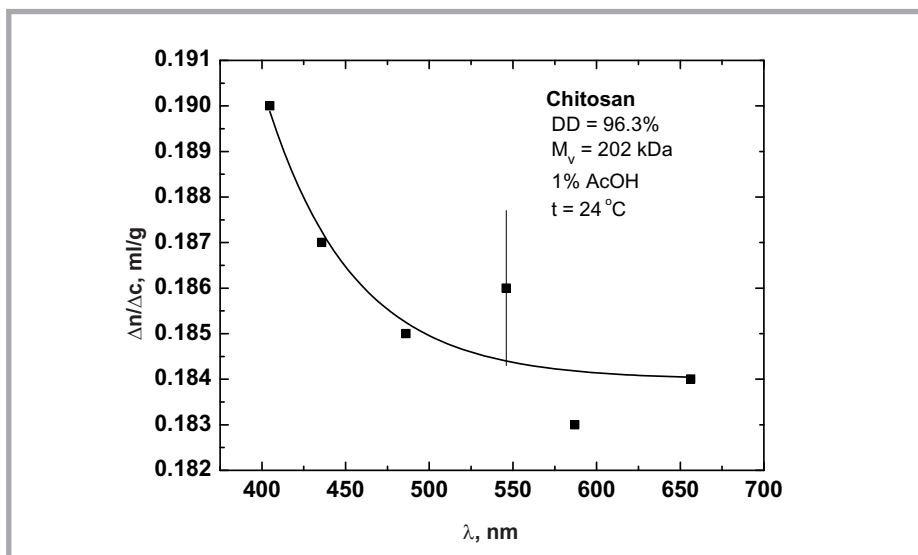**Figure 3.** *Refractive index of chitosan in aqueous solution as a function of concentration.*

Figure 4. *The dispersion of refractive index increment of chitosan in aqueous solution.*

Table 2. *The coefficients from equation 1.*

| Coefficient	chitosan c, g/dL			GN (HCl) c = 1 [g/dL]
	0.1	0.8	2.5	
a [10^{-6}]	-1.44413	-1.54939	-1.56120	-1.78138
b [10^{-5}]	-2.83920	-2.19620	-2.2186	-1.01037
n_{546}^{o}	1.33665	1.33764	1. 34068	1.33784
R	0.99999	0.99987	0.99991	0.99877

means that different temperatures of measurements of the refractive index incre-ment and LS do not bring additional errors in the $\overline{M}_V$ calculation. The value of the refractive index increment of chitosan is in very good relation with literature data and is higher than those of obtained for nonionic polymer such as dextran and carrageenan being a weak polyanion (see Table 3). The values of the refrac-tive index increment may help us in classification of the new polymer studied. Figure 5 shows the refractive index of chitosan in an aqueous solution (c = 1g/dL) as a function of the degree of deacetylation. For all light wavelengths used the value of n very slightly increases with increasing of DD.

3.2. ORD

ORD of chitosan aqueous solution for several concentrations are presented in Figure 6. For the sake of comparison, the same figure also shows the ORD of a glucosamine so-lution. All the substances studied are characterised by plain rotatory dispersion curves. For glucosamine compounds the rotatory dispersion in the visible region is positive. It

Table 3. *Refractive index increment of some polyglucans (λ = 632.8 nm); w – week, s – strong.*

Substance	Sign/ionic strength	Solvent	Δn/Δc, ml/g	Reference
ι-carrageenan	-/s	H_2O	0.127	[11]
κ-carrageenan	-/w	H_2O	0.148	[11]
dextran	0	0.9% NaCl	0.148	[12]
chitosan	+/s	0.2 M AcOH 0.15 M AcONH$_4$	0.185	[13]
chitosan	+/s	0.18 M AcOH	0.184	autors
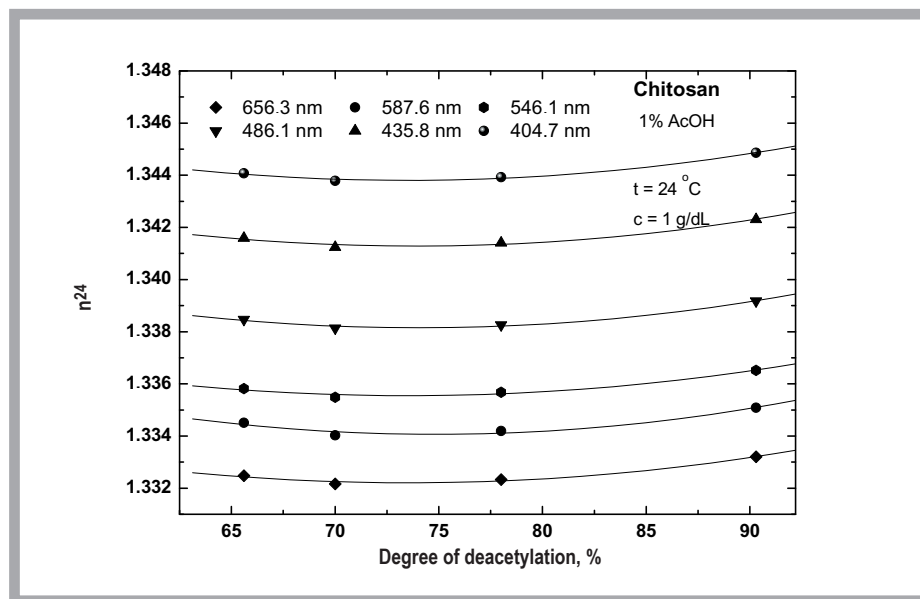
Figure 5. *Refractive index n^{24} of chitosan in aqueous solution as a function of the degree of deacetylation (c = 1 g/dL).*

means that the longest wavelength Cotton effect contribution to the total ORD should be positive in contradiction to the negative Cotton effect contribution observed for chitosan. Both observations are in good correlation with CD measurements of chitosan solution [2], chitin and glucosamine films [14].

ORD measurements of chitosan with different DD were made (c = 1 g/dL). The specific rotation [α]$_\lambda$ as a function of DD is presented in Figure 7. Qualitative agreement with CD results of Domard [2] is apparent.

The specific rotation measurement may give us simple and fast check out of DD of chitosan with not bad accuracy but in the range below 90%. Above this range the sen-
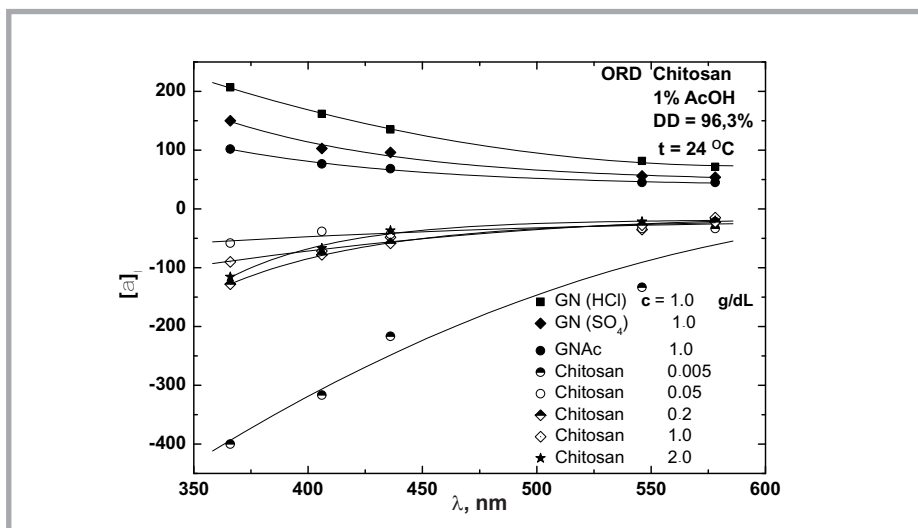
Figure 6. *ORD of D-glucosamine and chitosan aqueous solution for several concentration at t = 24 ˚C.*

sitivity of ORD in the visible region seems to be too weak for reliable determination of deacetylation degree. However, for quantitative analysis of DD by ORD, a relation between $[\alpha]_{\lambda}$ and molecular weight should be also determined.

Results of specific rotation dependence on the concentration of chitosan are presented in Figure 8. It is well known that the chitosan molecules form a flexible polymer chain. In crystals the chitosan molecules are arranged in a helical structure [15] but in solution Gaussian coil or wormlike chain conformations are taken into consideration [16]. In much diluted solutions the value of $[\alpha]_{\lambda}$ is five times higher than in those of high concentrations. In highly diluted solutions the phenomenon of the chitosan chains overlapping does not take place and the flexible chains assume the natural helicoidal form it means there are good condition for high optical activity. We hope that the study of the effect of ionic strength, molar mass and DD on the specific rotation $[\alpha]_{\lambda}$ of diluted chitosan solutions will permit making distinctions between the conformations discussed.

In the intermediate range of dilutions i.e. (0.05 ÷ 0.5) g/dL, the behaviour of $[\alpha]_{\lambda}$ suggest that the overlapping of chitosan molecules starts at about 0.05 g/dL. The value of c = 0.05 g/dL is in agreement with the critical overlap concentration c* given in [17]. The local maximum value of $[\alpha]_{\lambda}$ (dependence on concentration) was observed for the chitosan concentration of 0.2 g/dL which quite well corresponds to the critical aggregation concentration (cac) observed by the fluorescent method in the chitosan solution in 0.3 M AcOH [17]. For high concentrations of chitosan (0.5 ÷ 3) g/dL a very broad maximum value of $[\alpha]_{\lambda}$ as a function of chitosan concentration appears at about 2 g/dL. Increasing concentration
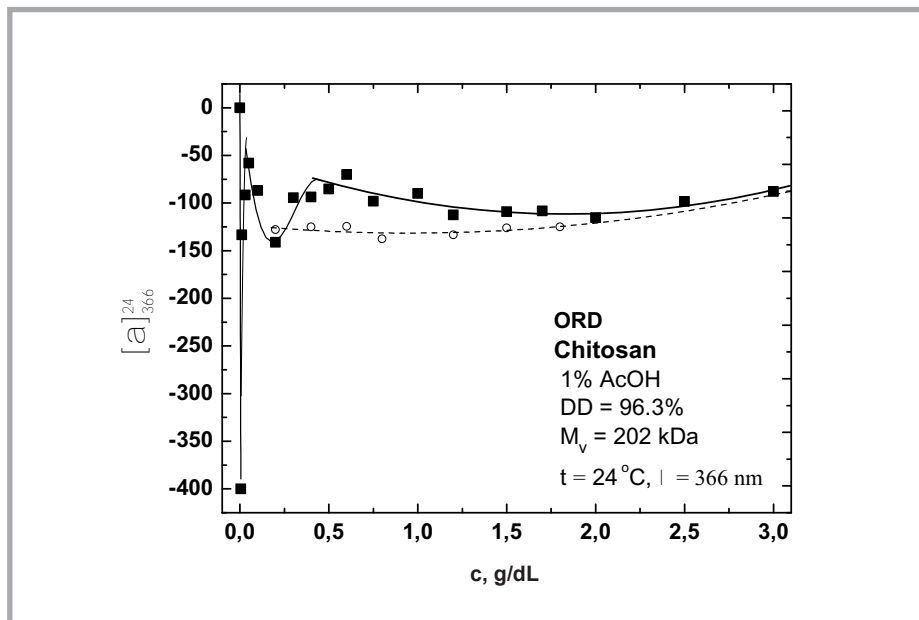
Figure 7. *Specific rotation [α]24366 of chitosan aqueous solution as a function of concentration.*

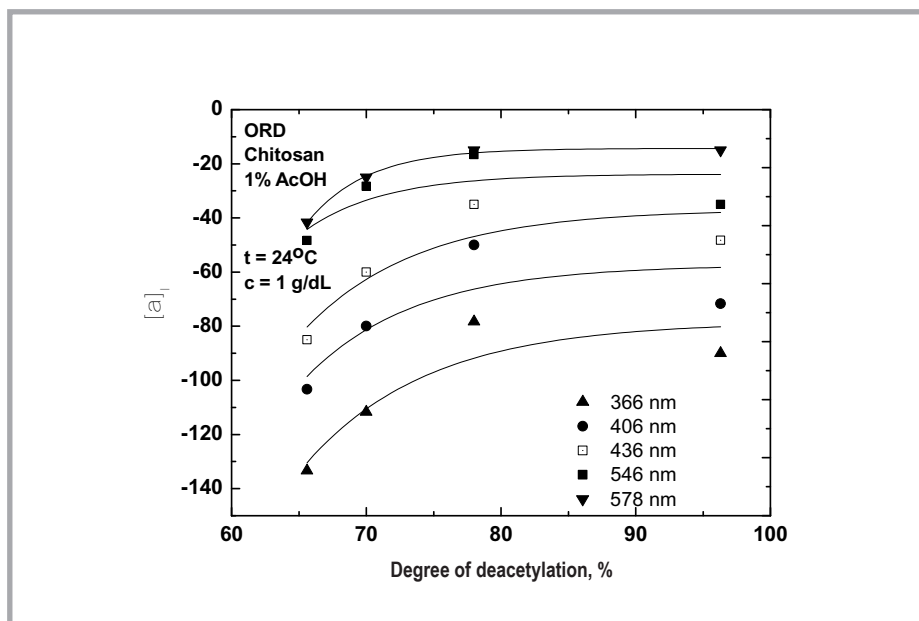**Figure 8.** *Specific rotation [α]24λ of chitosan aqueous solution as a function of the degree of deacetylation (c = 1 g/dL).*

Table 4. *Refractive index – n^{24}, specific rotation – $[\alpha]_{366}$ and the Verdet constant - V of chitosan in aqueous solution (c = 1 g/dL, t = 24 °C,); a - λ =546.1 nm; b - not optically active; c - λ = 410 nm; d – c = 0.8.*

substance		$n^{24 a}$	$[\alpha]_{366},$ o ml/gdm	$V^{c},$ o/Tm
Chitosan	A1	1.33582	- 133	-
	A2	1.33542	- 112	-
	A3	1.33568	- 78	-
	A4	1.33652	- 90	410 ^{d}
GN(HCl)		1.33619	207	426
GNAc		1.33561	102	406
1% AcOH		1.33472	0 ^{b}	280

of chitosan to above 2 g/dL leads to a decrease in $[\alpha]_{\lambda}$. Such a behaviour of $[\alpha]_{\lambda}$ can be related to very strong overlapping of chitosan molecules and it means that about 2 g/dL formation of associations of chitosan polymer chains may takes place. Measurements of the specific rotation of chitosan aqueous solution after several days of preservation in a closed glass vessel at room temperature give $[\alpha]_{\lambda}$ as a function of chitosan concentration, shown in Figure 8 by a thin dotted line. In the concentration range c = (0.1 ÷ 2) g/dL, $[\alpha]_{\lambda}$ is nearly stable. It may be concluded from this observation that a strong overlapping appears in the whole concentration range i.e. 0.1 ÷ 2 g/dL.
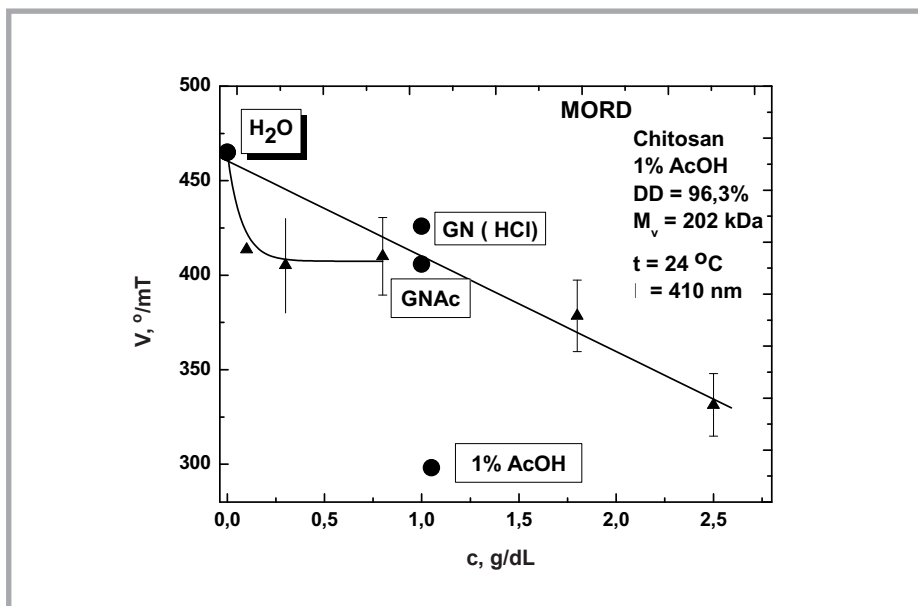

Figure 9. *The Verdet constant of chitosan aqueous solution as a function of chitosan concentration. The value of Verdet constant of water; 1% AcOH; GN(HCl) and GNAc (c = 1g/dL) are shown by black points (λ = 410 nm, t = 24 ºC).*

3.3. MORD

Dispersion of the Faraday effect has been measured for several concentrations of chitosan in the range c = 0 ÷ 2.5 g/dL as well as for the 1% acetic acid aqueous solution and D-glucosamine hydrochloride and N-acetyl-glucosamine. In the spectral range 400 ÷ 600 nm the Verdet constant is described by the positive plain dispersion curve. The values of the Verdet constant of the compounds studied are lower than that of water. The Verdet constant as function of chitosan concentration is shown in Figure 9. The same figure presents the value of the Verdet constant of water and 1% of AcOH, G(HCl) and GNAc aqueous solution. For low concentrations of chitosan, c < 0.5 g/dL the contribution of electronic transitions of the AcOH optical chromophores are dominant in the Faraday effect of the chitosan aqueous solution. Above that concentration the Verdet constant linearly increases with increasing chitosan concentration and its value is well correlated with the Verdet constant of GN and GNAc aqueous solution.

4. Conclusions

The results obtained allow drawing the following conclusions:
1. Dispersion of the refractive index increment is not strong but cannot be neglected.
2. Refractive index increment is stable in the temperature range 20 ÷ 50 °C.
3. Refractive index depends very weekly on the degree of deacetylation.
4. In the visible range of the spectrum the specific rotation – $[\alpha]_\lambda$ of chitosan is levorotatory and is described by a negative plain rotatory curve.
5. Specific rotation $[\alpha]_\lambda$ of chitosan depends on the degree of deacetylation in qualitative agreement with Domard [2] CD results.
6. The dependence of the specific rotation $[\alpha]_{366}$ of chitosan aqueous solution on chitosan concentration allows estimation of a characteristic concentration, being in good agreement with results obtained by other methods [17].
7. The dependence of the Verdet constant of chitosan aqueous solution on the chitosan concentration above 0.5 g/dL is linear. Below this concentration the optical chromophore of the solvent play a dominant role in the Faraday effect of chitosan aqueous solutions.

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