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Research Article

PHYSICS

Determination of Chitosan Scattering Parameters by Using Multi Angle Static Laser Light Scattering(MASLLS) .

A.R.Ghazy[1], M.Shaheen[1], El-Refaie Kenawy[2] and F.El-Mekawy[1].

1: Laser Laboratory, Physics department, Faculty of sciences, Tanta University, Tanta, Egypt.

2:Polymer Research group, Chemistry Department, Faculty of Sciences, Tanta University, Tanta, Egypt.

Abstract: Measurement of polymer characteristics by using the static laser light scattering experiment needs a precise determination of its refractive index increment, dn/dc. Through the dependence of Rayleigh scattering coefficient on the square value of the refractive index increment$(dn/dc)^2$. The average molecular weight of the investigated polymer is determined using a home built of Mach-Zehender Interferometer(MZI) which has been constructed to measure the refractive index, n, of Chitosan at different concentration ranging from 0.002 to 0.008 gm/L. The measurements are carried out through the dependence of fringe numbers on the laser incidence angle of polymer's solutions. The used laser beam is a He-Ne laser with 543 nm wavelength and 2mw in power (from Meridith). The obtained value of dn/dc is 0.198011 L/gm . A static laser light scattering(SLLS) experiment is constructed in our lab to measure the angular distribution of the scattered laser light intensity. From which the Rayleigh ratio R_θ, molecular weight M_W, second virial coefficient A_2 and the square radius of gyration $<R_G^2>$ are calculated through what is called as Zimm plot method .

Key words: *Refractive index increment, Mach-Zehender interferometer, Static laser light scattering, Molecular weight, Second virial coefficient, and Radius of gyration.*

Introduction:

Chitosan is an important derivative of chitin, is the second most important natural polymer in the world after cellulose. The two polymers have unique chemical, physicochemical and biological properties. Chitin is produced in nature by a number of living organisms such as shrimps, crabs and lobster (the main commercial sources of chitin are crab and shrimp shells) and is also an ingredient of cell walls in fungi and yeast[1–3]. Chitin does not dissolve in water and common organic solvents but dissolves in specific solvents such as N,N-dimethylacetamide, hexafluoroacetone or hexafluoro-2-propanol. When the degree of deacetylation reaches about 50% (depending on the origin of the polymer), chitin becomes soluble in aqueous acidic media and is then called chitosan. Chitosan is widely used in different applications as solutions, gels, or films and fibers. It has important applications in photography, cosmetics, ophthalmology, environmental and biomedical engineering, pharmaceutical industry, nanotechnology, agriculture and many others [4,5]. Due to this wide range of applications, it becomes important to characterize the physicochemical properties of chitosan. . Light scattering is a potential method for characterizing the structure of polymers and nanoparticles in solution[6].In this study, we constructed a multi-angle static laser light scattering experiment based on Mach-Zehnder interferometer to determine the molecular weight and scattering parameters of chitosan

When a polymer is dissolved in its solution it becomes a colloidal solution[7]. A colloidal solution, sometimes known as a colloidal suspension, is a solution in which a material is evenly suspended in a liquid. In other words, a colloid is a microscopically small substance that is equally dispersed throughout another material. When light path through the colloidal solution the molecules of the solution absorbs the incident light and vibrate with the same frequency of that light because of this vibration the molecules re-emits the light with the same frequency in all direction, this process is called light scattering[8]. There are two types of light scattering independing on the conditions of the solution. When the pressure of the solution is the atmospheric pressure and the temperature is low and constant, this is called static light scattering, if the pressure and temperature is high and is variable, this is called dynamic light scattering.

Static laser light scattering is one of the important techniques and most accurate one to determine the molecular weight, mean radius of gyration and the second virial coefficient of the polymer solution[9]. Debye equation linked between the angular distribution of the intensity of scattered light and the molecular weight of the scattering medium. The angular distribution of the scattered light is the source of valuable information of physical properties and characteristics of scattering medium.

Therefore, a great work have been done to develop the apparatus and photometers, by using the laser beam. The measurements of the scattered light have been more accurate because of the monochromatic and coherence of the beam.

The angular distribution of scattered light intensities is handled by Debye equation to determine the molecular weight and size information. light scattering have limitation range from 10 Da to 100000 KDa in the molecular weight and size from 10 nm to 1000 nm. In addition of measuring how much the interaction is done (second virial coefficient)[10,11].

Debye equation shows the important of measuring refractive index increment in the way of measuring scattering parameter. Therefore, we have construct a Mach-Zehender interferometer to take much better measurements of the refractive index at the same wavelength of the scattering beam.

2. Theoretical

Rayleigh scattering theory :

2.1. For particles diameters less than λ/20:

Debye equation linked between intensity of light scattering, refractive index increment, molecular weight, second virial coefficient and radius of gyration[9].

$$\frac{KC}{R_\theta} = \frac{1}{M_W} + 2A_2C + 3A_3C^3 \quad (1)$$

Where :

$$K = \frac{2\pi^2 n_0^2}{N_A \lambda_0^4}\left(\frac{dn}{dc}\right)^2 (1 + cos^2\theta) \quad (2)$$

$$R_\theta = \frac{I_\theta r^2}{I_0 V} \quad (3)$$

n_0 is refractive index of solvent;

d*n*/dc is refractive index increment;

λ_0 is wave length of light in vacuum;

N_A is Avogadro's number;

C is concentration;

A_2 is second virial coefficient;

A_3 is third virial coefficient;

V is scattering volume;

r is the distance from the scattering volume to observer;

and I_0 is the incident light intensity.

By measuring scattering intensity at single angle θ for different concentrations and plot the results value of *KC/Rθ* as a function of C, one can measure the molecular weight according to to eq. (4).

$$\lim_{c\to 0}\left(\frac{KC}{R_\theta}\right) = \frac{1}{M_W} \quad (4)$$

The second virial coefficient as the slope of the line *KC/Rθ* vs C.

2.2. for particles diameter larger than λ/20:

The scattered light from particles have diameters larger than λ/20 reaches the observer with different phase and interfere, which results in a decrease in its intensity. Unlike for small particles, large particles the intensity of scattered light depends on the angle of the observation. The factor by which the scattering intensity I_θ is reduced by interference at an angle θ is called the scattering function $P(\theta)$, which combine the effect of chain size and conformation on the angular dependence of scattered light intensity[10,12]. While, the term $P(\theta)$ describes the angular variation of light scattering at a constant concentration but the form $P^{-1}(\theta)$ is dependent upon the size and the shape of the scattering particles (for small particles$P^{-1}(\theta) = 1$) .

$$P^{-1}(\theta) = 1 + q^2 < R_G^2 > /3 + \ldots\ldots\ldots\ldots \quad (5)$$

where

$$q = \frac{4\pi}{\lambda}\sin\left(\frac{\theta}{2}\right) \quad (6)$$

Then

$$P^{-1}(\theta) = 1 + \frac{16\pi^2}{3\lambda^2} < R_G^2 > \sin^2\left(\frac{\theta}{2}\right) \quad (7)$$

Where q is called scattering wave number; $<R_G>$ is the mean square radius of gyration.

Then the equation that describes the light scattering from polymer solution is:

$$\frac{KC}{R_\theta} = \frac{1}{M_W}\left[1 + \left(\frac{16\pi^2}{3\lambda^2}\right) < R_G^2 > sin^2\left(\frac{\theta}{2}\right)\right] + 2A_2C \quad (8)$$

In terms of the characteristic dimension D of the scattering particle:

1- For rods $D^2 = 12 <R_G^2>$

2- For spheres $D^2 = 5 <R_G^2>$

3- For flexible coil $D^2 = 6 <R_G^2>$

Where D is the length of the rod, the radius of the sphere and the root mean square end-to-end distance of the coil[10,13].

Polymer molecules are treated as a flexible coil, then

$$\frac{KC}{R_\theta} = \frac{1}{M_W}\left[1 + \left(\frac{8\pi^2}{9\lambda^2}\right) D^2 sin^2\left(\frac{\theta}{2}\right)\right] + 2A_2C \quad (9)$$

This equation contains a linear dependence of the factor KC/R_θ on the concentration **C** (when θ is constant) and on $sin^2\left(\frac{\theta}{2}\right)$ (when **C** is constant).

3. Experimental

3.1. Sample Preparation

Two grams of chitosan is dissolved in 100mL of 1.0% acitic acid, this stock is used to prepare the investigated concentrations (0.002, 0.004, 0.006, 0.008 gm/L).

Chitosan is from Across Co., the acetic acid is from Tedia Co. and the deionized water is from Fisher Co.

The samples were filtered by the usual conventional cellulose acetate membrane filters with 0.45 µm pore size in order to remove dust and multichain aggregation.

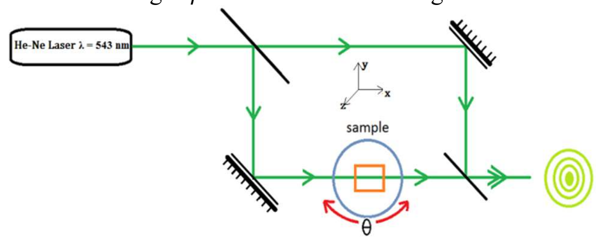
3.2. Determination of refractive index increment

Measurements of refractive index of chitosan dissolved in acetic acid as a function of concentration from 0.002 to 0.008 gm/L by using MZI shown in Fig(1)

The refractive index can be measured accurately by using Snell's law of refraction[14,15]:

$$n = \frac{(t-N\lambda)(1-cos\varphi)+N^2\lambda^2/2t}{t(1-cos\varphi)-N\lambda} \quad (10)$$

Where t is the thickness of the sample, φ is the angle of the incident laser beam, n is the refractive index of the sample, N is the number of fringes corresponding to angle φ and λ is laser wavelength.



Fig(1). The optical structure of mach-zehender interferometer.

The polymer solution is contained in a quarts rectangular cell with 10 mm width the cell is fixed on a rotating table of a spectrometer which enable us to change the angle accurately up to 1 second of arc. The empty cell is first used to measure the number of fringes N_1 at used angels then we fill the cell with the solution then determine the number of fringes N_2 at the same angels then we calculate $N = N_2-N_1$ which is the number of fringes from solution only.

Equation (10) is made suitable for graphical representation[16]:

$$\frac{1}{sin^2\left(\frac{\varphi}{2}\right)} = \frac{2t(n-1)}{n\lambda}\frac{1}{N} + \frac{2}{n} \quad (11)$$

We can calculate refractive index n by plot a relation between $\frac{1}{sin^2\left(\frac{\varphi}{2}\right)}$ and $\frac{1}{N}$, the slope is equal to $\frac{2t(n-1)}{n\lambda}$. (the value of n from the slope is more accurate than the value from the intercept).

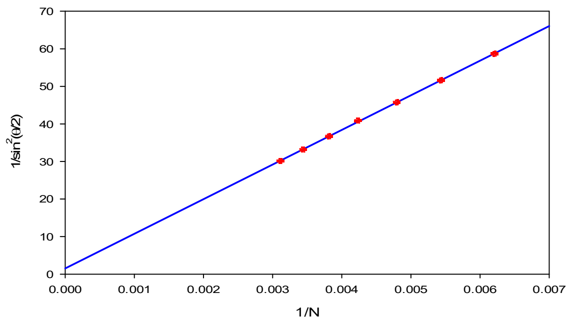
The concentration dependence was obtained by performing the increments on the different polymer concentrations. The refractive index increment dn/dc of polymer is determined and also the value of Δn/c against c is plotted to determine the value of $\lim_{c\to 0}(\frac{\Delta n}{\Delta c})$.

Fig(2) shows the determination of refractive index from the relation between $1/sin^2\left(\frac{\theta}{2}\right)$ and 1/N for c = 0.002gm/mL.

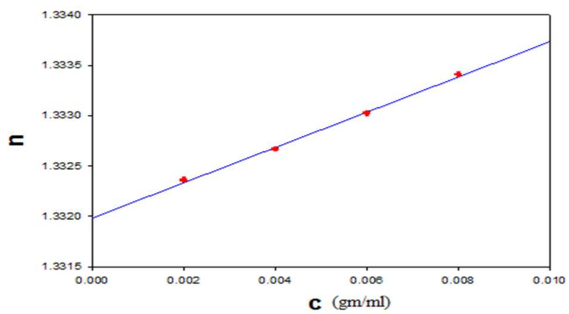
The value of refractive index is calculated for nine different concentrations of chitosan as shown in Fig(3).

The relation between Δn and c is shown in Fig(4).

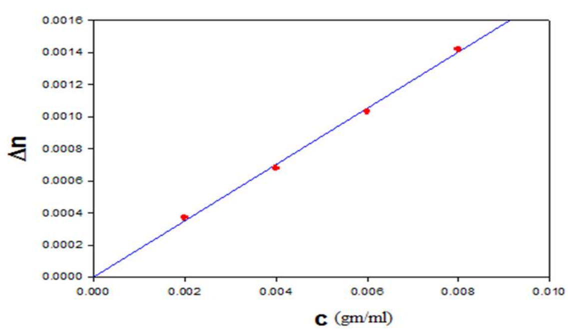
The value of dn/dc is calculated as $\lim_{c\to 0}\frac{\Delta n}{\Delta c}$ as shown in Fig(5).



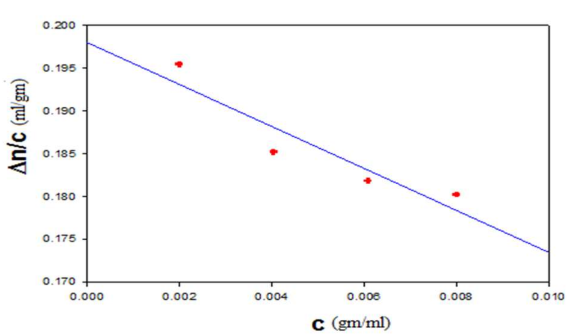
Fig(2). The relation between $1/sin^2\left(\frac{\theta}{2}\right)$ and 1/N for a chitosan c=0.002gm/ml.



Fig(3). Relation between the refractive index n and concentration c for chitosan.



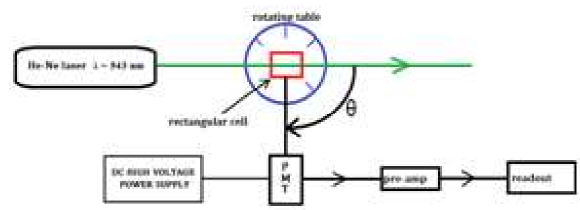
Fig(4). Plot of Δn versus c .



Fig(5). Relation between Δn/c versus c.

3.3. Measurements of scattering parameters

Measurements were performed at room temperature (21^o) using an experiment shown in Fig 6, the sample is contained in a rectangular cell fixed on a spectrometer and a photomultiplier tube is fixed on the arm of the spectrometer which is able to rotate around the cell to measure the intensity of scattered light at different angels. The measurements was calibrated at angle 90^0 using toluene[17].



Fig(6).

3.4. Methods of calculation

In order to determine the molecular weight according to Eq. (8), one has to carry out angular distribution light-scattering measurements at different concentrations from 40^o to 140^o for chitosan dissolved in acetic acid. The results obtained are plotted graphically as KC/R_θ vs c and KC/R_θ vs $sin^2\left(\frac{\theta}{2}\right)$.

In both cases KC/R_θ vs c at constant θ and KC/R_θ. vs $sin^2\left(\frac{\theta}{2}\right)$ at constant c, the measurements yield a series of straight lines which can be extrapolated to the ordinate and therefore permit determination of KC/R_θ at c 0, and at θ 0. Therefore in Eq. (8), in one case the term $2A_2$ c =0, and in the other case the term $\left(\frac{16\pi^2}{3\lambda^2}\right) < R_G^2 > sin^2\left(\frac{\theta}{2}\right)$ =0.

One obtains at c 0: →

$$\frac{KC}{R_\theta} = \frac{1}{M_W}\left[1 + \left(\frac{16\pi^2}{3\lambda^2}\right) < R_G^2 > sin^2\left(\frac{\theta}{2}\right)\right] \qquad (12)$$

For $sin^2\left(\frac{\theta}{2}\right)$ 0: →

$$\frac{KC}{R_\theta} = \frac{1}{M_W} + 2A_2C \qquad (13)$$

If we plot the extrapolation values for ($\lim_{c\to o} {KC}/{R_\theta}$) at θ_1, θ_2, θ_3, θ_4, θ_5 and θ_6 vs the $sin^2\left(\frac{\theta}{2}\right)$ values corresponding to those θ values we obtain a line according

to Eq. (12). In a similar manner, we can obtain by extrapolation the KC/R_θ values for the concentration c_1, c_2, c_3 and c_4 for $sin^2\left(\frac{\theta}{2}\right)$. If these extrapolation values are plotted as a function of concentration c, in a similar manner, we obtain the straight line corresponding to Eq. (13). Both straight lines yield $1/M_w$ as the intercept at the ordinate [12,18]. From the slope of the straight line at c=0 we can obtain the value

$$\frac{1}{M_W}\left(\frac{16\pi^2}{3\lambda^2}\right) < R_G^2 > sin^2\left(\frac{\theta}{2}\right)$$

and from this we calculate the $< R_G^2 >$ (the radius of gyration). The tangent of the slope of the KC/R_θ, vs c, line $sin^2\left(\frac{\theta}{2}\right)$ is equal to $2A_2$ (A_2, the second virial coefficient).

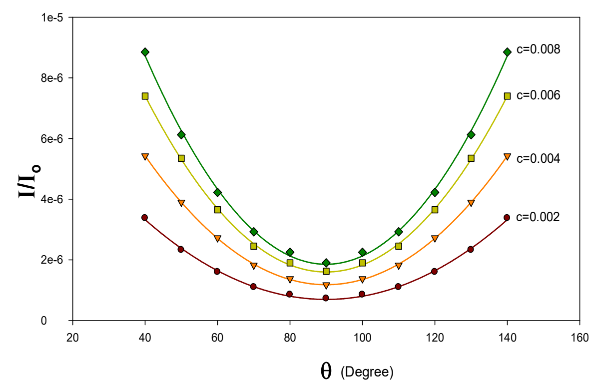
3.5. Zimm plot construction

By using this method of plotting (introduced by Zimm) we can obtain from light scattering measurements at different angles θ and different concentration c, in addition to molecular weight, the radius of gyration and the second virial coefficient A_2.

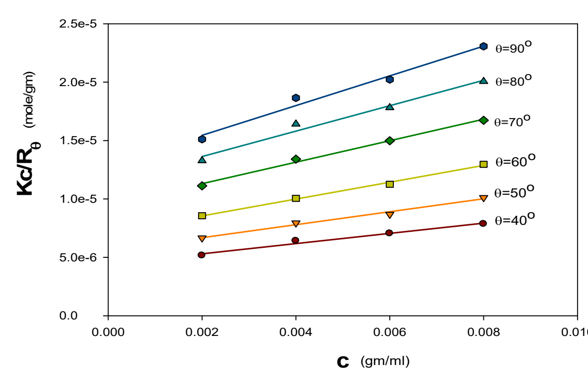
In general, the extrapolation according to the above is not carried out separately, but in general in a single diagram by plotting KC/R_θ, vs $sin^2\left(\frac{\theta}{2}\right)$ $+kC$ choosing the value of k, which will make the concentration term comparable with $sin^2\left(\frac{\theta}{2}\right)$. It is better to extrapolate the lines of constant angle to zero concentration before extrapolating lines of constant concentration to zero angle because of the uncertainty of the lower angles. This produces a grid-line graph, the two limiting lines of which $KC/R_{\theta\ c \to 0}$ and $KC/R_{\theta\theta \to 0}$ on the extrapolation should meet at the same intercept and this a criterion for the validity of Eq. (8).

This value of $KC/R_{\theta\ c \to 0, \theta \to 0}$ is equal to the weight average molecular weight.

- The measured values of the scattered laser light intensities I/I_o are plotted as a function of different scattering angles θ, to show the angular distribution, is shown in Fig. 7.
- The values for KC/R_θ at constant observation angles θ_1=40º, θ_2=50º, θ_3=60º, θ_4=70º, θ_5=80º and θ_6=90º were plotted as a function of the concentration, corresponding to those θ values lines as shown in Fig. 8. were obtained.
- In a similar manner, the values of the KC/R_θ at constant concentrations c_1=0.002, c_2=0.004, c_3=0.006 and c_4=0.008 g/ml, can be plotted as a function of $sin^2\left(\frac{\theta}{2}\right)$ as shown in Fig. 9.
- If these extrapolation values are plotted as a function of concentration, c, vs the $sin^2\left(\frac{\theta}{2}\right)$ (at c = 0) values corresponding to those θ values one obtains a straight line according to Eq. (12) as shown in Fig. 10.
- In a similar manner, KC/R_θ values (at $sin^2\left(\frac{\theta}{2}\right)$ =0) can be obtained by extrapolation of the KC/R_θ values vs the concentration, as shown in Fig. 11.
- From Figs. 10 and 11 the straight lines corresponding to Eqs. (12) and (13) were obtained . Both straight lines yield $1/M_w$ as the intercept on the ordinate. From the line at c = 0 we can obtain from the slope of the straight line the value of $< R_G^2 >$(mean radius of gyration). The tangent of the slope of the KC/R_θ, vs c, line ($sin^2\left(\frac{\theta}{2}\right)$ =0) is equal to $2A_2$.
- To construct a Zimm plot using four different concentrations, and six scattering angles, there are 24 data points, and the zero concentration line would consist of six points and the zero scattering angle line would consist of four points. Fig. 12 shows a Zimm plot for the chitosan solved in acetic acid, all results are tabulated in table 1.

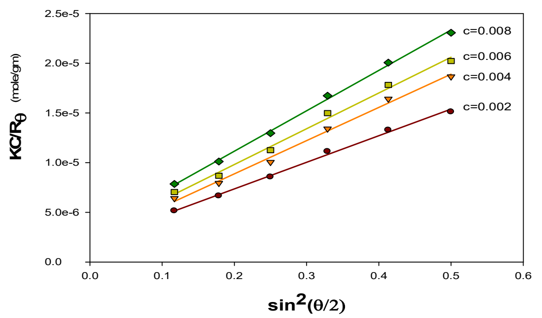


fig(7). The angular distribution of scattered light intensity for chitosan.

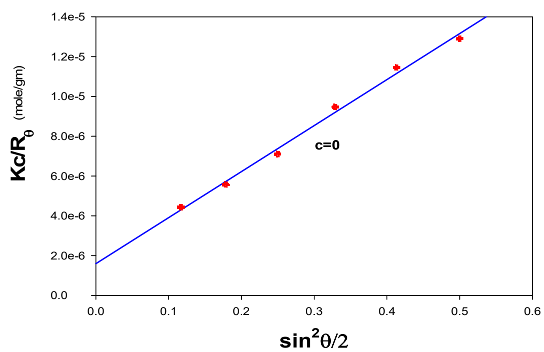


Fig(8). Kc/Rθ for different angels vs concentration.

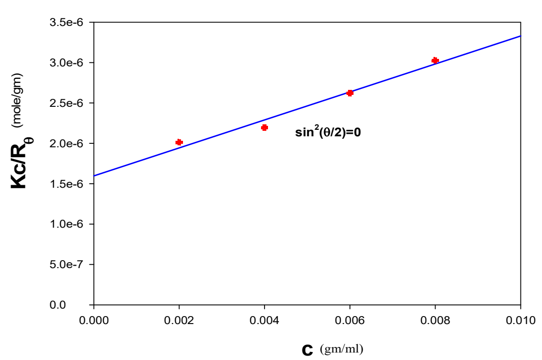
λ = 543 nm, T = 21°c	Measured value	Reference value
dn/dc	0.1980	0.162 – 0.252 [19]
M_w	626233.302 (gm/mole)	600000 – 800000 (gm/mole) (specification sheet)
A_2	$8.6649*10^{-5}$ (mol ml g^{-2})	4.5 – 11.6 (mol ml g^{-2}) [19]
R_G	34.746	



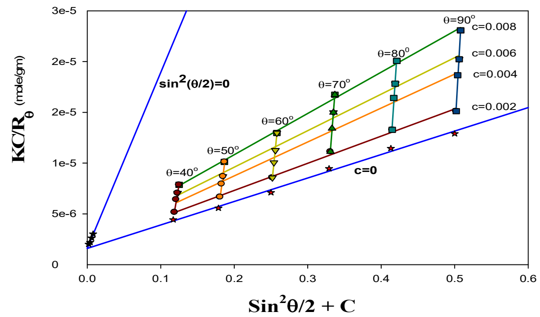
Fig(9). Kc/R_θ for different concentrations versus $\sin^2(\theta/2)$.



Fig(10). Kc/R_θ versus $\sin^2(\theta/2)$ at c=0.



Fig(11). Kc/R_θ versus concentration at $\sin^2(\theta/2)$=0.



Fig(12). The zimm plot for chitosan.

4. Conclusion:

1- We have constructed an experiment for measuring the angular distribution of light scattered intensity at scattering angels from (40° to 140°).

2- The angular distribution of scattered intensities of chitosan is dissolved in acetic acid at four different concentrations. By using this data in a Zimm plot we calculated the molecular weight of chitosan at acetic acid, which is in a good agreement with the values found in the specification sheet. Also important information is calculated using the same Zimm plot is the second virial coefficient A_2 for acetic acid and the radius of gyration of chitosan.

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تعيين معاملات الاستطاره للكيتوزان باستخدام اتطاره ضوء الليزر الساكنه متعدده الزوايا

احمد رياض غازي

قسم الفيزياء- كلية العلوم- جامعة طنطا

يعتبر بوليمر الكيتوزان من اهم البوليمرات وذلك لوجوده في الطبيعة بكثرة متمثلا في قشور الجمبري و الكبوريا وبعض الخنافس وبالاضافة الي استخداماته في العديد من التطبيقات العلميه و الطبيه. ونتيجة لهذه الاهمية قمنا بقياس معاملات الاستطاره الساكنه باستخدام شعاع ليزر. وتعتبر معاملات الاستطاره من اهم الخواص بالنسبه للبوليمر حيث انها تقيس الوزن الجزيئي و نصف قطر الدوران بالاضافه الي درجه الذوبان في المذيب المستخدم. لقد قمنا بدراسه استطاره رايلي علي الكيتوزان , وذلك عند اربع تركيزات مختلفه 0.002,0.004,0.006,0.008 في مذيب حمض الاسيتيك المخفف , وعند زوايا تتراوح بين 40 الي 140 درجه . حيث ان هذه القياسات تحتاج الي تعيين تغير معامل الانكسار بالنسبه للتركيز ,فقد قمنا بقياسه معامل الانكسار لهذه التركيزات باستخدام مقياس ماخ-تسندر للتداخل يدوي الصنع. وعند مقارنه النتائج المقاسه بالنتائج العالميه وجدنا ان هذه القياسات علي درجه عاليه جدا من الدقه.