

A STUDY ON THE KINETICS OF ALKALINE ISOMERIZATION OF LACTOSE TO LACTULOSE USING THE AUTOMATIC POLARIMETER

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ABSTRACT

The kinetics of alkaline isomerization of lactose to lactulose in the presence of boric acid has been investigated using the property of rotating the plane of polarized light at different initial concentrations of lactose. The rate constant and the reaction rate have been calculated and recorded. The data revealed that the rate of reaction was influenced greatly by concentration of lactose while the rate constant was not affected. The order of reaction has been recognized by different ways. The kinetics data show a first order reaction with respect to lactose. On the basis of the experimental findings, a suitable mechanism has been proposed.

Key words: Alkaline isomerization, Chemical kinetics, Lactulose, Optical rotation.

INTRODUCTION

Lactulose (4-O-B-D- galactopyranosyl-D-fructose) is a synthetic disaccharide (chemical formula $C_{12}H_{22}O_{11}$ and molecular mass 342,3), highly valued as a pharmaceutical with world-wide markets. It is normally prepared by chemical isomerization of lactose in basic solution. For the first time lactulose was produced by Montgomery & Hudson 1930. The process produced low yield of lactulose, which was so difficult to refine. Further attempts have been developed for the preparation of lactulose from lactose in high yields by several different methods (Hicks & Parrish, 1980; Hicks *et al* 1984 and Kozempel *et al* 1999). Other interest also has focused on the separation

and purification of lactulose from the isomerized mixtures (Hicks *et al* 1984; Hicks *et al* 1986).

Hicks *et al* (1984) described a method for preparation of lactulose from sweet cheese whey ultrafiltrate. An amount of boric acid that was equimolar to the lactose content was added to the ultrafiltrate. The pH was adjusted to 11 with either triethylamine or sodium hydroxide, and then the solution was heated to 70°C for 3 hrs. The resulting yield of lactulose exceeding 80% based on starting lactose content. In spite of this widespread success in lactulose preparation, the problems of separation and purification still prevail. However, little attention has been directed towards the kinetics of the alkaline isomerization of lactose to lactulose

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(Dendene *et al* 1994 and Kozempel & Kurantz, 1994).

Polarimetric methods have been used extensively as one of the most convenient methods to estimate the optical rotation of many optically active substances; especially sugars such as lactose and lactulose (Montgomery & Hudson, 1930 and Mizota *et al* 1987). But, no literature cited their use for studying the kinetics of the alkaline isomerization of lactose to lactulose. Kinetics of such reaction can be monitored in time by observing the change of the optical rotation of the reaction mixture.

This prompted the author to perform the present work, which constitutes an investigation of the kinetics and mechanism of this reaction using the automatic polarimeter. The unique feature of this instrument, which makes it superior than other normal visual polarimeters and saccharimeters is that it gives the changes in optical rotation continuously through a digital display with an indication of the direction of rotation in time. A plus sign (+) on the display indicates dextro rotation and a minus sign (-) indicates levo rotation. Thus, this instrument may be ideally suited for following the progress of this reaction.

MATERIAL AND METHODS

Reagent grade α -lactose monohydrate (Sigma Chemical Co., St. Louis, MO, U.S.A) was dissolved in double distilled water to prepare 5 and 10% (w/v) lactose solutions. An equimolar quantity of boric acid was added to lactose solution of 5% lactose in a 250 ml round bottom flask. The pH of the solution was adjusted to 11 with NaOH 4 N. The flask

was then heated in a thermostatic water bath to 70°C. The changes in optical rotation were measured using the Rudolph Research Analytical Automatic Polarimeter (Autopol II-Wavelength 589 nm-Serial No.6562, U.S.A). To minimize the high noise level and drift in the measurements that occur when measuring the changes in optical rotation with variable temperature, the jacketed sample polarimeter tube (type 31J) was used. Once the desired temperature of the solution is reached, the jacketed polarimeter tube thermostated at the same temperature was immediately filled with the solution sample. Maintaining a constant temperature using a temperature controlled circulating water bath started the reaction time. Kinetic measurements was followed by recording the angle of rotation every 5 minutes intervals for the first hour, then every 10 minutes for the second hour, and every 15 minutes thereafter until a constant rotation is attained. The same reaction kinetics procedure was repeated at the same temperature for 10 % lactose solution. Duplicate experiments were carried out for each kinetic run. Kinetics data were statistically analyzed to fitting using the MSTAT computer program.

RESULTS AND DISCUSSION

Kinetic data of alkaline isomerization of lactose to lactulose for 5 and 10 % lactose solutions are given in Table (1). The second and fifth columns of this table contain the values of optical rotation for the two experiments corresponding to the time intervals. It could be noted that, the angle of rotation was proportional to the initial concentration of lactose in each experiment. The sign of the optical rotation is changed from plus to minus during

Table 1. Kinetic data of alkaline isomerization of lactose to lactulose for 5 and 10 % lactose solutions at 70°C

Time (min)	5 % lactose solution			10 % lactose solution		
	$[\alpha]$	$-dc/dt$	$\frac{1}{t} \ln \frac{(\alpha_0 - \alpha_\infty)}{(\alpha_t - \alpha_\infty)}$	$[\alpha]$	$-dc/dt$	$\frac{1}{t} \ln \frac{(\alpha_0 - \alpha_\infty)}{(\alpha_t - \alpha_\infty)}$
000	(+) 2.34	-----	-----	(+) 4.70	-----	-----
005	(+) 1.83	0.102	0.0359	(+) 3.69	0.202	0.0358
010	(+) 1.41	0.084	0.0357	(+) 2.85	0.168	0.0356
015	(+) 1.07	0.068	0.0351	(+) 2.18	0.134	0.0350
020	(+) 0.78	0.058	0.0350	(+) 1.60	0.116	0.0349
025	(+) 0.54	0.048	0.0348	(+) 1.12	0.096	0.0347
030	(+) 0.35	0.038	0.0342	(+) 0.73	0.078	0.0344
035	(+) 0.18	0.034	0.0341	(+) 0.38	0.070	0.0344
040	(+) 0.03	0.030	0.0342	(+) 0.10	0.056	0.0342
045	(-) 0.10	0.026	0.0344	(-) 0.15	0.050	0.0343
050	(-) 0.21	0.022	0.0346	(-) 0.37	0.044	0.0345
055	(-) 0.30	0.018	0.0347	(-) 0.54	0.034	0.0344
060	(-) 0.37	0.014	0.0346	(-) 0.69	0.030	0.0345
070	(-) 0.48	0.011	0.0343	(-) 0.91	0.022	0.0343
080	(-) 0.56	0.008	0.0343	(-) 1.08	0.017	0.0345
090	(-) 0.62	0.006	0.0344	(-) 1.19	0.011	0.0344
100	(-) 0.66	0.004	0.0343	(-) 1.27	0.008	0.0343
110	(-) 0.69	0.003	0.0345	(-) 1.33	0.006	0.0344
120	(-) 0.71	0.002	0.0344	(-) 1.37	0.004	0.0344
135	(-) 0.73	0.001	0.0344	(-) 1.41	0.003	0.0343
150	(-) 0.75	0.001	0.0382	(-) 1.45	0.003	0.0382
165	(-) 0.76	0.001	-----	(-) 1.47	0.001	-----
180	-----	-----	-----	-----	-----	-----

the course of reaction. This could be explained on the basis that; in alkaline solutions lactose isomerizes to lactulose and multiple side by-products essentially galactose (Corbett & Kenner, 1953; Montgomery, 1962; Parrish *et al* 1980 and Kozempel *et al* 1997).

Both of lactose and galactose are dextrorotatory, whereas lactulose is levorotatory. Since the optical rotation at any time of reaction is due to the combined effect of these sugars, thus, as the reaction proceeds, the reaction mixture becomes more levorotatory.

A plot of the changes in optical rotation versus time yields a smooth curve (Fig. 1). It is clearly seen from this figure that the rate of change in the optical rotation (which should be proportional to the initial concentration of lactose) is decreasing with time. The curve is much steeper at the beginning and as time progresses, the curve approaches a horizontal line. Thus, this figure evidently shows that the rate of change in the optical rotation is dependent on how much starting lactose is left.

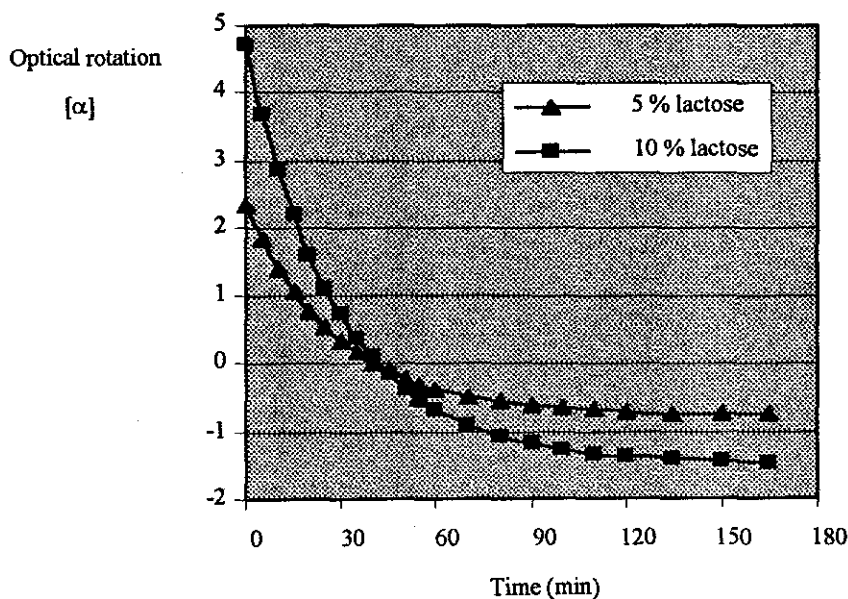


Fig. 1. Changes in optical rotation versus time of alkaline isomerization of lactose to lactulose for 5 and 10% lactose solution at 70°C

Determination of the rate constant of the reaction

The first step in developing a model is to determine the rate constant of the reaction. So, we may write

$$-dc/dt = kc \quad (1)$$

where: c is the concentration of lactose, t is the time and k is the rate constant.

The integrated form of the above equation is:

$$-kt = \ln \frac{c}{c_0} \quad (2)$$

Since the optical rotation $[\alpha]$ is a function of concentration, at each time t , we can write

$$c_0 = Q(\alpha_0 - \alpha_\infty) \quad (3)$$

and

$$c = Q(\alpha t - \alpha_\infty) \quad (4)$$

where: Q is a proportionality constant, α_0 is the optical rotation of lactose solution at zero time, αt is the optical rotation at time t , and α_∞ is the final rotation at completion.

The changes of the angle of rotation versus time can be used to obtain the rate constant for this reaction. So that equation (2) becomes:

$$kt = \ln \frac{(\alpha_0 - \alpha_\infty)}{(\alpha t - \alpha_\infty)} \quad (5)$$

This expression makes it possible to calculate the rate constant of the reaction from the optical rotation measurements.

The values of the rate constant (k) calculated from the optical rotation data for 5 and 10 % lactose solutions are presented respectively in the fourth and seventh column of (Table 1).

In addition, the values of $\ln [(\alpha_0 - \alpha_\infty) / (\alpha t - \alpha_\infty)]$ were plotted versus time. This yields a straight line (Figure 2, a and b), the slope being k . The linear equation was computerize calculated and displayed on the graph.

The data were conducted to the least square linear regression using MSTAT statistical program to fit the best values obtain the regression line slope and intercept. The results of the statistical analysis are presented in Table (2).

Inspection of these results, it could be observed that, The correlation coefficient values approach 1. This indicates that the data appeared to be statistically correlated. The positive correlation coefficient and slope values exhibit a positive relationship between the two pairs of coordinates. This is the pattern that defines as a positive statistical relation. The T-test value and the probability also revealed that the relationship between the two variables is significant. Because the graph in an xy-coordinate system is a straight line (linear regression), the regression line can be presented by the linear equation. The equation of the general form is:

$$y = bx + a \quad (6)$$

Substituting the values of intercept (a) and slope (b) into the general equation for a straight line, we obtain the regression equation that can be used to predict the y-value associated with a specified value of x.

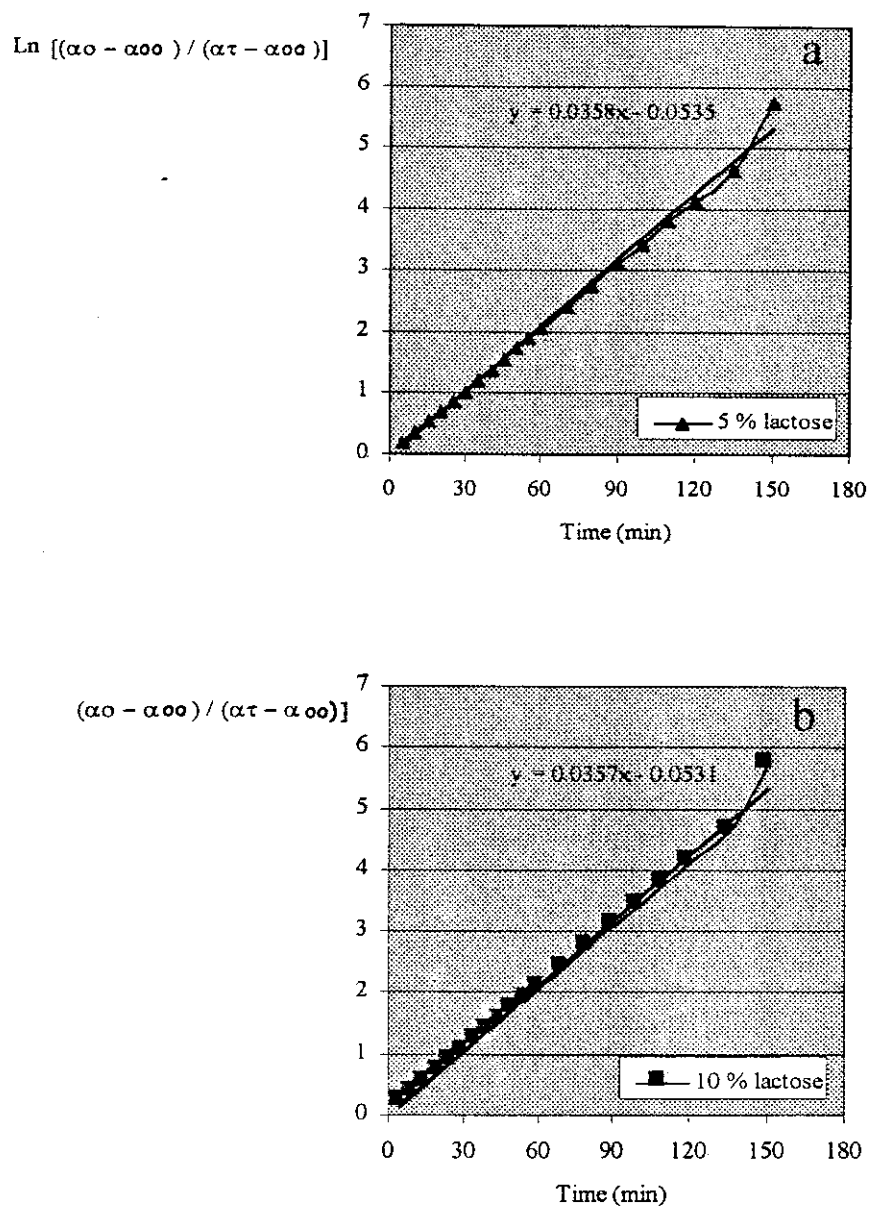


Fig.2. Plots of $\ln [(\alpha_0 - \alpha_{\infty}) / (\alpha_t - \alpha_{\infty})]$ versus time of alkaline isomerization of lactose to lactulose for 5 and 10 % lactose solution at 70 °C

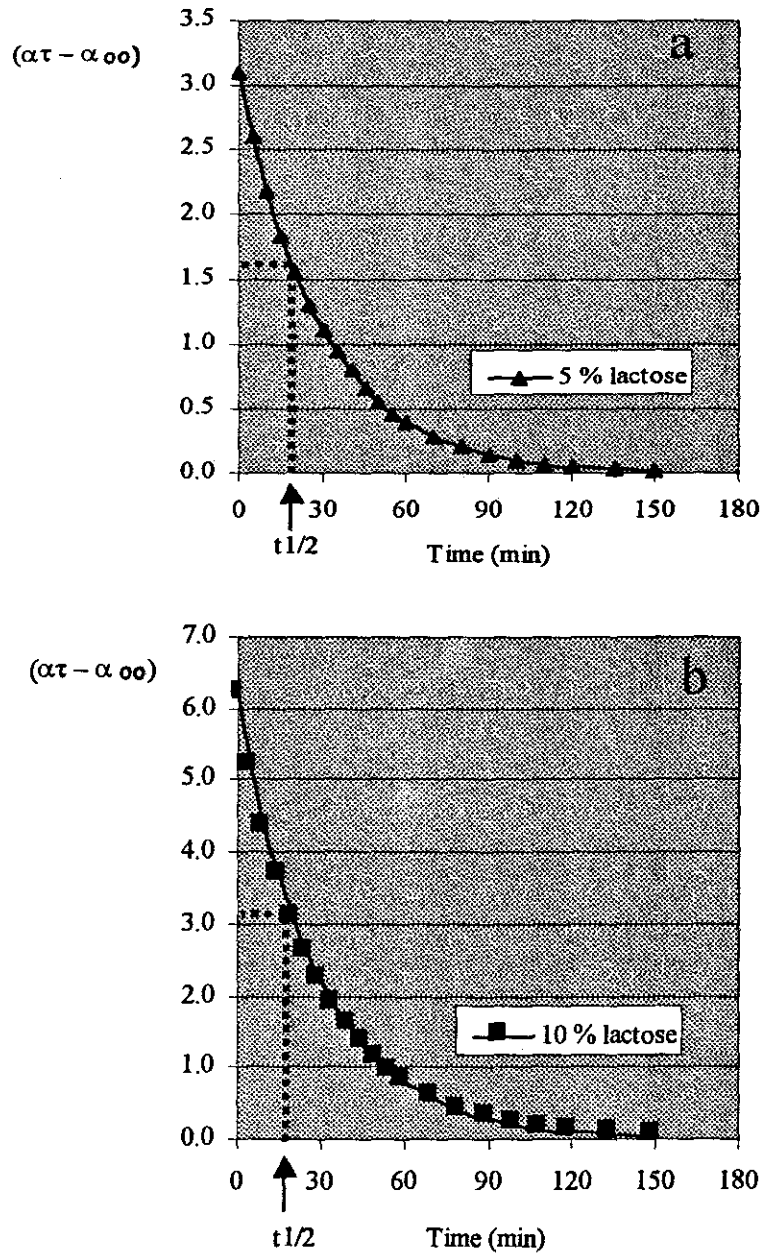


Fig.3. Plots of $(\alpha\tau - \alpha_{\infty})$ versus time of alkaline isomerization of lactose to lactulose for 5 and 10 % lactose solution at 70 °C

Now, It is obvious that the reaction of formation of the product, lactulose, proceeds at a faster rate than that of formation of the intermediate. This mechanism assumes that the first step, in which lactulose boric acid complex forms is the rate controlling step and the reaction may be described as a consecutive first step reversible reaction. Therefore, the rate constant obtained in the present study is considered as (k1). No need to determine the rate constant for the second reaction (k2) because its finding will have no effect on our investigation. From a practical viewpoint, only the first reaction that was needed a mathematical model.

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مجلة حوليات العلوم الزراعية ، كلية الزراعة ، جامعة عين شمس ، القاهرة ، ٤٧م ، ع(٢) ، ٧٧٩ - ٧٨٩ ، ٢٠٠٢

متابعة حركة تفاعل تحويل سكر اللاكتوز إلى لاكتيلوز باستخدام الأوتوماتيك بولاريميتير

[٤٩]

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في حساب ثابت التفاعل ومعدل التفاعل كما تم التعرف علي رتبة التفاعل. وقد أشارت النتائج إلى أن قيمة ثابت التفاعل كانت واحدة في كلا التركيزين بينما تضاعف معدل التفاعل بتضاعف تركيز المحلول. كما أثبتت النتائج أن هذا التفاعل هو تفاعل من الرتبة الأولى وأن المرحلة الأولى من هذا التفاعل و هي مرحلة تكوين معقد اللاكتيلوز مع حامض البوريك تسير بمعدل أبطأ من المرحلة الثانية والتي يحدث فيها انحلال سريع لهذا المعقد وينفرد اللاكتيلوز عند إضافة حامض. لذلك فإن المرحلة الأولى من هذا التفاعل تعتبر هي الأهم من الوجهة التطبيقية.

في هذا البحث تم تتبع سير هذا التفاعل عن طريق قياس التغير في الدوران الضوئي لمحلول سكر اللاكتوز أثناء التفاعل وذلك باستخدام جهاز الأوتوماتيك بولاريميتير. وقد أجريت الدراسة علي تركيزين مختلفين من محلول اللاكتوز هما ٥ ، ١٠ % (و/ ح) . حيث عومل كلا منهما بكمية مكافئة من حامض البوريك ثم اضيفت الصودا الكاوية حتي الوصول إلى (11 pH) وسخن المحلول علي ٧٠ درجة مئوية حتي نهاية التفاعل والذي استمر نحو ثلاث ساعات . حيث تم خلال هذه المدة تسجيل القياسات علي فترات زمنية متقاربة تتناسب وسرعة التفاعل في مراحل المختلفة. وقد استخدمت هذه القياسات

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