

Studying the Effect of Ethanol and Operating Temperature on Purification of Lactulose Syrup Containing Lactose

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Abstract—Lactulose is a synthetic disaccharide which has remarkable applications in food and pharmaceutical fields. Lactulose is not found in nature and it is produced by isomerization reaction of lactose in an alkaline environment. It should be noted that this reaction has a very low yield since significant amount of lactose stays unreacted in the system. Basically, purification of lactulose is difficult and costly. Previous studies have revealed that solubility of lactose and lactulose are significantly different in ethanol. Considering the fact that solubility is also affected by temperature itself, we investigated the effect of ethanol and temperature on separation process of lactose from the syrup containing lactose and lactulose. For this purpose, a saturated solution containing lactulose and lactose was made at three different temperatures; 25°C (room temperature), 31°C, and 37°C first. Five samples containing 2g saturated solution was taken and then 2g, 3g, 4g, 5g, and 6g ethanol separately was added to the sampling tubes. Sampling tubes were kept at respective temperatures afterward. The concentration of lactose and lactulose after separation process measured and analyzed by High Performance Liquid Chromatography (HPLC). Results showed that ethanol has such a greater impact than operating temperature on purification process. Also, it was observed that the maximum rate of separation occurred at initial amount of added ethanol.

Keywords—Ethanol, lactose, lactulose syrup, purification.

I. INTRODUCTION

LACTULOSE which is chemically known as 4-O- β -D-galactopyranosyl-D-glucose is a synthetic disaccharide that has various applications in pharmaceutical and food fields. Basically, lactulose consists of two monosaccharide; galactose and fructose. Lactulose as a %50 w/w syrup is prescribed for chronic constipation treatment [1]-[3]. For patients whom are suffering portal systemic encephalopathy (PSE), prescription of lactulose syrup improves their life quality and their emotional behavior [4], [5]. Lactulose is non-caloric and sweet sugar which its sweetness can be reached as 62% of sucrose [6]. Hence, lactulose can widely be used in confectionary industry if it is produced economically. Lactulose can prevent the growth of harmful bacteria by stimulating of proliferation of bifidobacteria in infants. Hence, lactulose is a very good ingredient to add to the milk and commercial infants formulas [7]-[9].

Lactulose is mainly synthesized by isomerization reaction of lactose in presence of alkaline catalyst. It was produced by

Montgomery and Hudson by using calcium hydroxide as the catalyst in 1930 for the first time [10]. Thereafter, varieties of catalysts have been utilized for this reaction. Although the conversion of the reaction depends on catalytic system, this reaction has a very low yield. Plenty of side products and significant amount of un-reacted lactose composes the major part of the reaction product. On the other hand most of these side products are produced by degradation of lactulose which decrease the yield of reaction [11].

Lactulose is commercially found in the market as syrup nowadays. Although the majority portion of syrup contains lactulose, significant amount of lactose in syrup makes it undesirable for those applications which dietary restrictions of lactose should be considered [12]-[14]. In the past decades, researchers have provided different methods in order to purifying lactulose. For instance, pressurized liquid extraction (PLE), supercritical fluid extraction (SFE) and solid-phase extraction (SPE) are those methods which are based on dynamic extraction. Montañés, Fornari et al. (2007), purified lactulose up to 95% by applying SEF treatment using supercritical CO₂ [15]. Ruiz-Matute, Sanz et al. (2007), successfully purified lactulose up to 86.6% by using PLE method [16]. Several Methods also have been provided to enhance lactulose concentration in its respective syrup [17], [18]. Recent researches have investigated the solubility of monosaccharides and disaccharides in different alcohols [19]. Significant difference between the solubility of lactulose and lactose is one of the important keys to increase the efficiency of lactulose purification. Hence, in this study we attempted to conduct a research to investigate the effect of ethanol and operation temperature on purification process of lactulose.

II. EXPERIMENTAL PROCEDURE

A. Materials

Lactulose syrup (51.2% w/w) was obtained from Milei GmbH which the exact properties are given in Table I. Lactose manufactured by Riedel-de Haën was used in laboratory. The standard lactulose which was used in this experiment was analytical grade purchased from Fluka. Acetonitrile manufactured by Merck was used as a solvent in HPLC column. Deionized water was also used as a solvent in HPLC column purified with Millipore Device. Ethanol with a minimum purity

of 99.9%vol purchased by Merk made in Germany.

B. Sample Preparation

To simulate the ratio of lactulose and lactose in our experiments in comparison to the synthetic sample in an alkaline environment [11] the following procedure was used to prepare three samples respect to three different temperatures:

Three grams of lactulose syrup was diluted by 25.6 grams of distilled water and mixed with the excess quantity of lactose until saturated solution was obtained. To approach the solubility, the mixtures were stirred in shaker incubator for a day at 25 °C, 31°C, and 37°C respectively. The mixtures were filtered; then 2grams of saturated solution was sampled and then 0, 2,3,4,5 and 6 grams of ethanol were separately added to the sample tubes (containing 2g saturated mixture) respectively to get following proportions of ethanol: syrup (0:2, 2:2, 3:2, 4:2, 5:2, and 6:2). To making sure that the samples are in thermodynamic equilibrium, sample tubes were held in their respective temperature for another 24 hrs. After the equilibrium was achieved, the white precipitate was filtered and the pure solution was analyzed using high performance liquid chromatography (HPLC).

TABLE I LACTULOSE SYRUP PROPERTIES	
Item	Analytical Data
PH	4.8
Lactulose	51.2 % W/W 68.9 % W/V
Galactose	13.1
Lactose	7.0
Epilactose	4.8
Fructose	<1.0
Tagatose	0.7

C. HPLC Analysis

Before running the HPLC analysis, to ensure the absence of suspended particles, the sample was passed through the 0.2 μ m filter. Analysis of sugars in the samples was done by the High Performance Liquid Chromatography (HPLC) system [20], [21]. Samples using a loop addition volume 20 μ l were entered into the system. Passing solvents, added sample into the HPLC column been spread and any sugar separately with the time delay was removed from the system. The solvents used for HPLC analysis were a mixture of acetonitrile and water with the ratio of 75 to 25 which entered two separate pumps with the general rate of 1.2 ml/min. Applied HPLC column was Carbohydrate high performance 4 μ m 4.6 \times 250 mm manufactured by Waters Co. The output value of different sugars at different times was obtained by refractive index detector manufactured by Jasco Co.

III. RESULTS AND DISCUSSION

HPLC analysis helped us to have a quantitative and qualitative study on the purification process. Figs. 1 (a) and (b) depict the HPLC chromatogram of the system at 37 °C.

As we expected just two peaks respect to lactulose and lactose were seen in HPLC chromatogram since the syrup

mainly contained two sugars. The other sugars such as fructose, tagatose and etc. which were available in lactulose syrup were not detectible by HPLC because of their very low concentrations. Compare to the previous studies [22] and HPLC calibration, the first peak at retention time (tR) ~ 18 min belongs to lactulose and the second one at tR ~ 20 min belongs to lactose. As Figs. 1 (a) and (b) show, the lactulose peak height at ethanol: syrup (0:2) ratio is almost similar to the ethanol: syrup (6:2) ratio while, the lactose peak height was dramatically reduced. It reveals that the majority of precipitate which separated from the system was lactose such that the lactose as impurity considerably removed from sytup by addition of ethanol. Concentration of lactulose and lactose after separation treatment at three different temperatures were measured and are given in Table II.

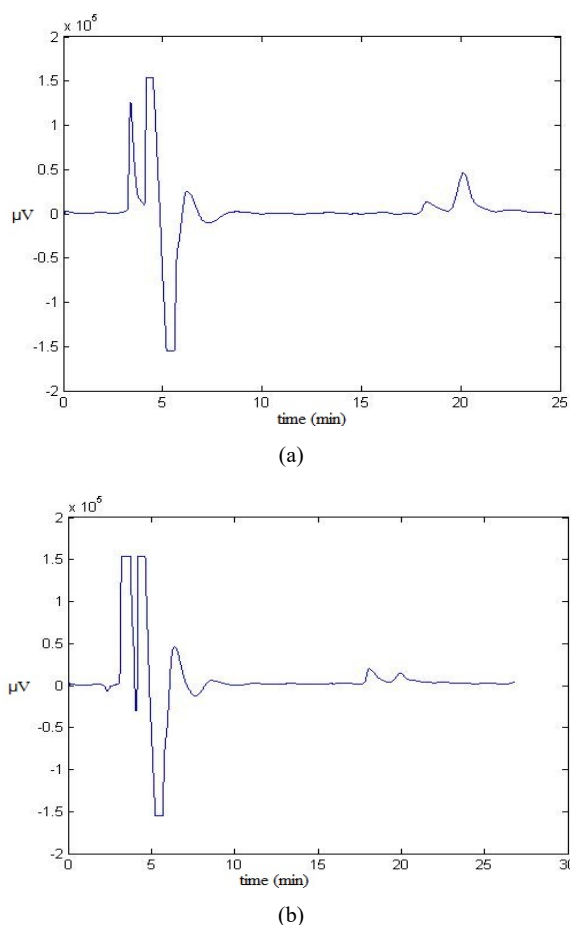


Fig. 1 (a) Ethanol: syrup (0:2) ratio at 37 °C (b) Ethanol: syrup (6:2) ratio at 37 °C

As Table II shows, addition of ethanol to the sugar mixture significantly changes the concentration of lactose while, lactulose almost stays unchanged. Also, it is seen in Table II, the rate of changes in the concentration of lactose was increased at higher temperatures.

Figs. 2-4, show the effect of ethanol on the system at 25°C, 31°C, and 37°C respectively.

TABLE II
SOLUBILITY OF LACTOSE AND LACTULOSE IN SYRUP*

Ethanol: syrup portion %w/w	25 °C		31 °C		37 °C	
	La ¹	Lo ²	La	Lo	La	Lo
0:2	5.70	20.30	4.17	21.41	3.85	23.00
2:2	5.42	12.05	3.97	10.34	3.64	7.53
3:2	5.40	9.43	3.77	7.24	3.55	5.06
4:2	5.30	6.95	3.75	6.10	3.54	3.65
5:2	5.27	5.70	3.74	5.37	3.52	3.16
6:2	5.20	4.95	3.74	4.34	3.52	3.00

* Solubility is given by %w/w ¹ La: Lactulose¹ Lo: Lactose

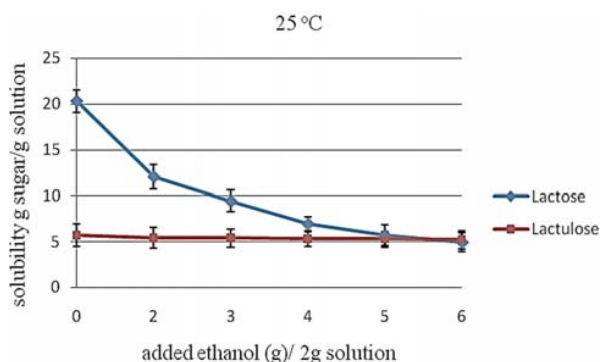


Fig. 2 Effect of added ethanol on the solubility of sugars in 25°C

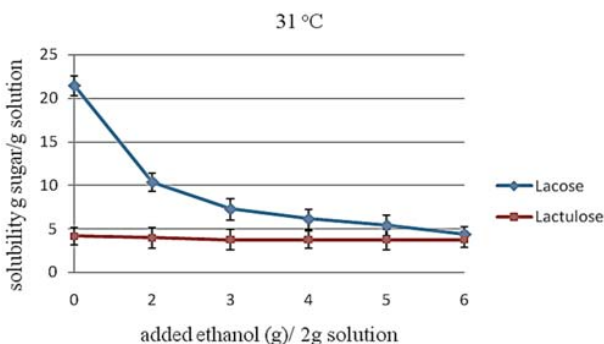


Fig. 3 Effect of added ethanol on the solubility of sugars in 31°C

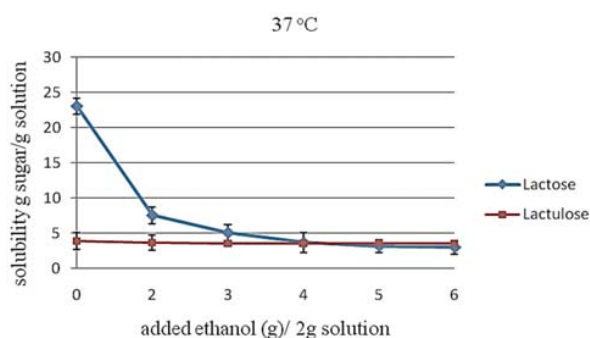


Fig. 4 Effect of added ethanol on the solubility of sugars in 37°C

At all three temperatures, lactulose was not affected by addition of ethanol. On the other hand, as ethanol was added to the system, lactose started to precipitate. As mentioned earlier, lactulose is much more soluble in ethanol compare to lactose.

Hence, when ethanol was added to the syrup, the system was shifted from aqueous phase to alcoholic phase. Since then, lactose which is less soluble in ethanol than lactulose precipitated in the solution. The point is that the initial amount of ethanol had the maximum effect on the separation of lactose from the syrup. After ethanol: syrup (4:2) ratio the rate of separation was very slow. It indicates that addition of more ethanol does not affect the purification process. Even this can be predicted that by addition of higher amounts of ethanol system completely shifts to alcoholic phase. Consequently, lactulose begins to participate as well which is not desirable for our purpose. Fig. 5 compares the effect of operating temperature on the separation process.

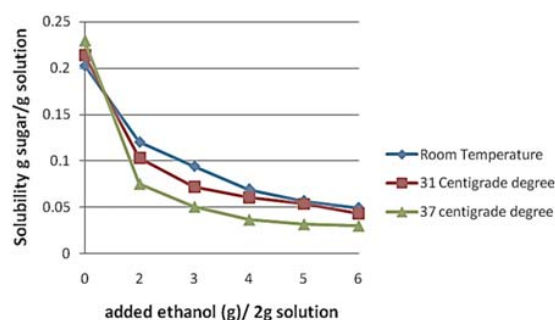


Fig. 5 Effect of temperature on the solubility of lactose

As it is seen, increasing the operating temperature results higher purification. It is because of the effect of temperature on the solubility of the sugars. It could be concluded that the solubility of lactulose was increased more than lactose with temperature. Also, as Fig. 5 shows, the concentration gradient (slope of the curves) at initial amount of ethanol at 37°C is higher than the other temperature. It means that the rate of separation of lactose was higher at the highest temperature. It should be noted, that operating at temperatures higher than 37°C increased the possibility of dissociation of lactulose and lactose to the other sugars. Table III summarizes the maximum percentage of lactose separation (as the impurity) from the syrup.

TABLE III
AMOUNT OF LACTOSE BEING REMOVED FROM THE SYSTEM

Temperature	25°C	31°C	37°C
Ethanol: syrup 2:2 ratio (%w/w)	40.6%	51.7%	67.2%
Ethanol: syrup 3:2 ratio (%w/w)	53.5%	66.2%	78.0%
Ethanol: syrup 4:2 ratio (%w/w)	65.7%	71.5%	84.1%
Ethanol: syrup 5:2 ratio (%w/w)	70.5%	74.9%	86.2%
Ethanol: syrup 6:2 ratio (%w/w)	75.6%	79.7%	86.9%

IV. CONCLUSION

Quantitative and qualitative effect of ethanol and operating temperature on purification of lactulose syrup containing lactose was investigated. Ethanol affected the system much higher than operating temperature. Increasing the addition of ethanol into the syrup resulted more lactose as impurity precipitated and left removed from the syrup. The maximum

rate of separation of lactose as impurity occurred at initial amount of ethanol. Increasing the temperature accelerated the rate of purification. Purification process in the operating temperature range of 31°C to 37°C, and proportion of ethanol: syrup from 4:2 to 6:2 gives the best results.

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