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Carbohydrate Research 263 (1994) 163–166

CARBOHYDRATE RESEARCH

Note

Solubility of lactulose trihydrate

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Received 12 January 1994; accepted in revised form 29 April 1994

Keywords: Lactulose trihydrate, solubility of

Lactulose (4-*O*-β-D-galactopyranosyl-D-fructose) was synthesized by Montgomery and Hudson [1] in 1930. Its physiological significance as a growth-promoting factor for *Bifidobacterium* (Bifidus Factor) was discovered by Petuely [2] in 1957. *Bifidobacterium* is a typically beneficial genus of the intestinal microflora and has useful functions for the host. Lactulose is now widely used as a Bifidus Factor in food, and as a remedy for the treatment of hepatic encephalopathy and chronic constipation in many countries of the world.

Lactulose was crystallized first from alcohol solution as the anhydride [1]. In 1992, Jeffrey et al. [3] examined lactulose trihydrate that had been crystallized from aqueous solution. The crystal structure and NMR analysis of lactulose trihydrate revealed that it was comprised only of β-furanose, whereas lactulose anhydride showed the presence of β-furanose, α-furanose, and β-pyranose in the ratio of 75:10:15 [4]. Because lactulose trihydrate has been reported only recently, its physicochemical properties are not known in detail. The solubility of lactulose in water at 30, 60, and 90°C was reported by Oosten [5] and it showed a high solubility of above 76% w/w. However, information concerning the solubility profile of lactulose in aqueous solution at temperatures below 30°C is not available. Since pharmaceutical products consisting of lactulose solutions with high lactulose concentrations are marketed worldwide, it is worthwhile to obtain further solubility information. This study examines the solubility of the crystal form of lactulose in aqueous solution at temperatures below 30°C.

1. Experimental

Preparation of lactulose trihydrate for solubility measurements.—Lactulose trihydrate was prepared by seeding a 70% w/w aqueous solution of lactulose (Merck, Darmstadt)

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SSDI 0008-6215(94)00154-8

with crystalline lactulose (anhydride, Merck, Darmstadt) at 5°C for 21 days. Lactulose crystals for solubility measurements were obtained by seeding MLS-50 (Morinaga Lactulose Syrup, Morinaga Milk Industry Co., Ltd., Japan) with lactulose trihydrate crystals at 5°C for 14 days, then a 70% brix aqueous solution of the separated lactulose crystals was recrystallized at 5°C for 120 h. Analysis of the crystals by HPLC, as described in U.S. Pharmacopoeia XXII (Supplement 6), gave a single peak.

Preparation of lactulose anhydride standard for brix measurement.—Lactulose crystals for solubility measurements were recrystallized again and the isolated crystals were vacuum-dried under reduced pressure at 18°C for 15 h. The dried lactulose crystals were dehydrated by heating them gradually from 40 to 70°C under a reduced pressure of 30 torr. The water content of the dried lactulose was measured by the Karl–Fischer method.

Solubility measurement.—The lactulose crystals for solubility measurements were dissolved in water at concentrations of 59.2, 61.6, 65.2, and 74.4% brix for testing at temperatures of 5, 10, 20, and 30°C, respectively. Portions of the solutions (20 mL) were poured into 50-mL screw-capped tubes and seeded with lactulose anhydride (Merck, Darmstadt) or lactulose trihydrate crystals. The quantity of seeding crystals was 0.5% of the solution.

Suspensions of lactulose crystals and water were mixed at calculated lactulose concentrations of 54.2, 55.8, 61.9, and 70.5% w/w for incubation at temperatures of 5, 10, 20, and 30°C, respectively, and 20-mL portions of each suspension were poured into 50-mL screw-capped tubes. A glass ball was placed into each screw-capped tube for agitation. These tubes were then placed in a water bath adjusted to the prescribed temperature and shaken until the brix of supernatant liquid became constant. After this equilibration, the crystallized solution was centrifuged at 10 000*g* for 30 min. Brix of the supernatant liquid was measured using a refractometer and the concentration of lactulose was calculated using the factor obtained from the standard.

Identification of crystal form.—The separated lactulose crystals were dried at room temperature under reduced pressure for 15 h. Their water contents and melting points were measured by the Karl–Fischer method and a differential scanning calorimeter (DSC), respectively.

Factor for conversion of brix to lactulose concentration.—The lactulose anhydride standard for brix measurement was dissolved in water at 11 different solid concentrations between 44 and 71% w/w and brix was measured at 20°C.

2. Results and discussion

Factors for conversion of brix to lactulose concentration calculated at different lactulose concentrations were almost the same within the concentration range of 44–71% w/w. The average value was 0.972 (% w/w)/brix(%) at 20°C and we adopted this value as our conversion factor. It resembled the value of 0.970 reported by Reijke for a concentration range of 55–75% w/w [6].

The supernatant liquid of the crystallized lactulose solutions, which originated from dissolved solutions seeded with lactulose trihydrate or lactulose anhydride, and the suspensions showed the same final brix level after crystallization periods of 17, 20, 20, and 20 days at 4.5 ± 0.2, 10.1 ± 0.2, 20.0 ± 1.0, and 30.2 ± 0.1°C (average ± maximum deviation),

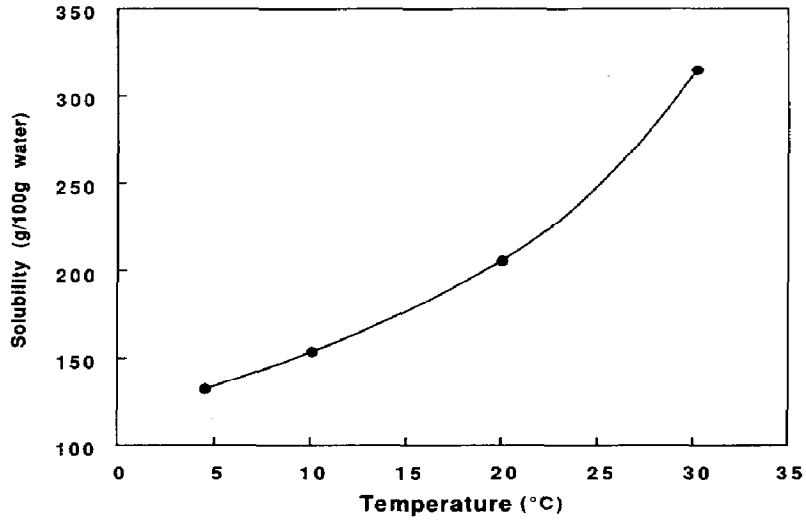

Fig. 1. Solubility of lactulose trihydrate in water.

respectively. The calculated lactulose concentrations of the supernatant liquid were 49.3 ± 0.3, 52.4 ± 0.5, 58.1 ± 0.04, and 65.6 ± 0.1% w/w (average $\pm$ standard deviation) at the above temperatures, respectively. The crystals isolated from those solutions had a water content of 13.0–14.0% w/w and melting points of 69.0–71.5°C. These data suggested that lactulose trihydrate was selectively crystallized from the lactulose solutions at temperatures below 30°C even if they were seeded with lactulose anhydride. Therefore, lactulose trihydrate shows lower solubility than lactulose anhydride at temperatures below 30°C. The solubility of lactulose trihydrate in water, as shown in Fig. 1, was 134, 154, 206, and 312 g in 100 g water at 5, 10, 20, and 30°C, respectively.

The solubility of lactulose in water was reported by Oosten [5] to be 76.4, 81.0, and over 86.0% w/w at 30, 60, and 90°C, respectively. In our study, the solubility of lactulose in water at 30°C was 65.4% w/w, whereas Oosten [5] observed a solubility of 76.4% w/w. The solubility of 76.4% w/w was obtained by placing lactulose anhydride in contact with water for 10 days and calculated from its optical rotation and refractive index. The value of 65.4% was calculated from the brix of the supernatant compared to the standard brix of lactulose anhydride. Crystalline lactulose anhydride and trihydrate show mutarotation upon dissolving in water and, when equilibrated, show the same values of optical rotation [3] and brix per unit of lactulose anhydride. This discrepancy concerning the solubility of lactulose does not originate from differences in the calculation methods, but rather is based on differences of observed data. Our experimental results, shown in Fig. 2, indicated that more time is required to reach the equilibrated condition of solubility when lactulose anhydride is employed for seeding than when lactulose trihydrate is used under these temperature conditions. Therefore, Oosten's value of 76.4% w/w observed at high lactulose concentration might represent the solubility of lactulose anhydride or the solubility of lactulose on the way from anhydride to trihydrate.

In general, pharmaceutical lactulose solutions now marketed are comprised of about 50% w/w lactulose and 32% w/w water. The lactulose concentration in water is calculated to

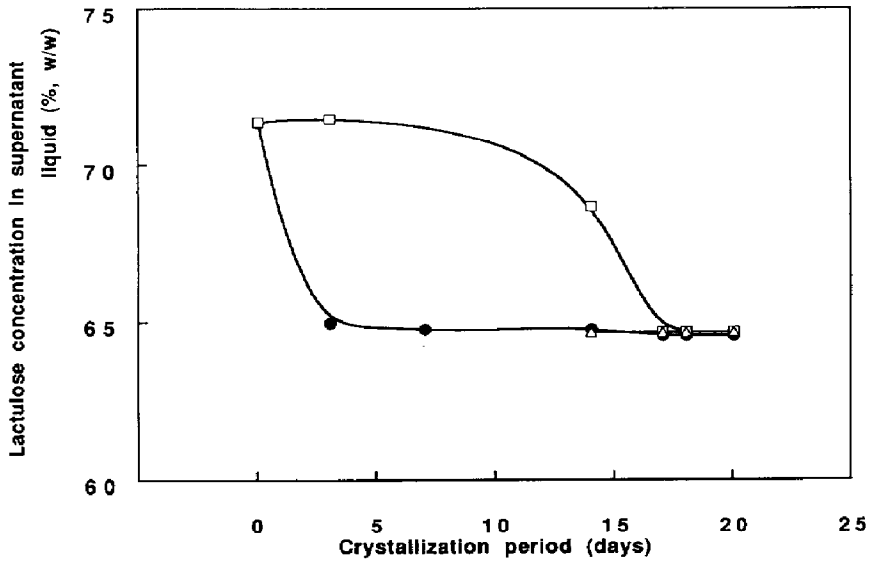

Fig. 2. Changes in lactulose concentration in the supernatant liquid during crystallization at 30°C seeded with: ●, lactulose trihydrate; □, lactulose anhydride; △, suspension of lactulose trihydrate.

be 61% w/w, that is, 240 g lactulose trihydrate in 100 g water. This value exceeds the solubility of lactulose at temperatures below 24°C, as demonstrated in this study. Therefore, when the commercial lactulose solution is seeded with lactulose crystals and kept at low temperature, it may show crystallization of lactulose.

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