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EFFECT OF IMPURITIES ON THE GROWTH OF LACTOSE CRYSTALS IN MODEL AND WHEY ULTRAFILTRATION PERMEATE SYSTEMS

BY

ARUN BHARGAVA ©

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE**

IN

FOOD PROCESSING

DEPARTMENT OF **AGRICULTURAL, FOOD AND NUTRITIONAL SCIENCE**
EDMONTON, ALBERTA

SPRING, 1995

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled **EFFECT OF IMPURITIES ON THE GROWTH OF LACTOSE CRYSTALS IN MODEL AND WHEY ULTRAFILTRATION PERMEATE SYSTEMS** submitted by **ARUN BHARGAVA** in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE** in **FOOD PROCESSING.**

Dr. P. Jelen

Dr. R. Segado

Dr. J. [illegible] Ryan

Date: January 30, 1995.

To my loving father, **Mr. Brijeshwar Prasad Bhargava** and
mother, **Mrs. Rama Laxmi Bhargava**

for their loving support and understanding

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ABSTRACT

The main objective of this lactose crystallization study was to investigate the effects of various salts ($CaCl_2$, Ca Lactate, LiCl, $MgSO_4$ and K_2HPO_4) added at low concentrations (1, 2, 3 and 5 g/100 g water) to model lactose solutions on the growth rate and morphology of individual lactose crystals. A new method was developed and applied to determine the lactose solubility in model lactose and permeate solutions. The growth rate of individual lactose crystals in solutions of reconstituted freeze dried sweet or acid whey ultrafiltration (UF) permeates and in model lactose solutions containing riboflavin was also studied.

Single lactose crystals with truncated and untruncated tips were grown under moving conditions for 24 h at 30°C in solutions containing 33.25 g anhydrous lactose/100 g water with or without salt addition. Increasing the concentration of $CaCl_2$ resulted in a maximum growth rate (% weight increase) at 2 g salt/100 g water, while an increase in the concentration of Ca Lactate, $MgSO_4$ and LiCl increased the growth rate at all concentrations studied. The maximum growth rate observed at 5 g of LiCl salt was 40.75% as compared with 8.87% in pure lactose solution. Increasing the concentration of K_2HPO_4 decreased the lactose growth rate. Similar results were obtained for both truncated and untruncated crystals. Lactose solubility in a pure lactose solution without any added salt was found to be 24.44 g anhydrous lactose/100 g water. Addition of salts to the model lactose solutions changed the lactose solubility or the supersaturation of the solutions. Addition of LiCl (5 g/100 g water) led to a maximum decrease in the lactose solubility, while K_2HPO_4 (5 g/100 g water) resulted in a maximum increase in the lactose solubility amongst the studied salts. Some of the added salts altered the morphology of lactose crystals; crystals grown in LiCl increased preferentially in length while those grown in $MgSO_4$ increased preferentially in width. Negligible amounts of Ca^{++}, Mg^{++} and K^{+} were found to be present in the lactose crystals grown in specific salt solutions ($CaCl_2$, Ca Lactate, $MgSO_4$ and K_2HPO_4, respectively). Riboflavin present at 10, 50 or 100 ppm in the model lactose solution did not affect the crystal growth rate and

negligible amounts of riboflavin were entrained in the crystal matrix at all the three concentrations.

Considering that growth occurred only at one face (bottom) of the lactose crystal, the crystallization velocity of lactose crystal grown in model lactose solution (33.25 g anhydrous lactose/100 g water) at 30°C was calculated as 342±58 mg/m^2/min. This calculated crystallization velocity of a lactose crystal is comparable to that of a sucrose crystal.

The growth rates of individual lactose crystals in permeate solutions were lower than those in model lactose solutions with or without the addition of specific salts. Lactose crystals grown in the permeate solutions (with lactose concentrations of 27.75 and 33.25 g anhydrous lactose/100 g water) for 18 h at 30°C had growth rates between 1.0-2.2% and 4.8-6.0%, respectively. The solubility of lactose in the permeate solutions was found to be between 25.00 and 25.67 g anhydrous lactose/100 g water.

CHAPTER 1

INTRODUCTION AND RESEARCH OBJECTIVES

1.1 Introduction

Crystallization is a commonly used technique for industrial purification and separation of solid material from solution (Grootscholten et al., 1982). Crystallization of sucrose is one of the oldest crystallization activities. In 500 AD Persians collected syrups which were boiled to make a purified form of crystalline sucrose (Bennett, 1969). The same general procedure was used until the nineteenth century when a French inventor developed a vacuum pan. The use of vacuum pans was a boon to the sucrose industry as it allowed the crystallization process to occur at a much lower temperature, and thus the discoloration of syrups was reduced. Development of the centrifugal separator in 1837 permitted the sugar industry to purify the product to a degree not previously achieved. Most of the crystalline sucrose was produced from cane and sugar beet by the batch process until the 1960's when continuous crystallizers were developed. The continuous crystallizer is capable of producing sucrose both from beet and cane sugar (Bennett, 1969). Studies on the different scientific aspects of sucrose crystallization started in the early twentieth century. A series of detailed works on sucrose crystallization were followed by the classic work of Kucharenko in 1928 (as cited by Jelen, 1972) on the crystallization velocity of sucrose.

Crystallization of lactose does not go back to prehistoric times; the first record of lactose isolation from milk was published by Bartolettus in 1633 (Jelen, 1972). During the eighteenth century lactose became a commercial commodity, used mainly in medicine (Holsinger, 1988). Unlike sucrose crystallization, there is no continuous crystallization process for lactose to date.

Conventionally, lactose has been recovered from whey, however, currently most of the lactose is recovered from whey UF permeates. In India and some other countries, use of whey as a raw material for lactose crystallization may lead to disposal of a large portion of proteins, salts and some lactose residue creating environmental problems and also unnecessary wastage of valuable whey nutrients. The use of whey as a raw material also results in the entrainment of impurities such as proteins, salts and riboflavin in the mass of the crystals, reducing the yield and purity of lactose crystals.

Recent uses of membrane processes in the dairy industry has generated large volumes of UF permeates. The permeate represents approximately 90-95% of the original whey volume, with lactose as the major solute present in it. With the removal of whey proteins from the whey, the potential for use of permeates in food and feed is greatly reduced. Therefore, ultrafiltered permeates are ideal raw material for manufacture of lactose because here protein does not interfere with the crystallization process or recovery steps. Recently, more and more lactose manufacturers have been recovering lactose from permeate due to the economic reasons (removal of whey proteins, process efficiency) and market demands (need for quality and product improvement) (van den Bos, 1987). In spite of this fact not much information on lactose recovery from whey UF permeates is yet publicly available; in particular, the effects of various whey permeate components on the growth of lactose crystals are not well documented.

During the last decade limited research on lactose crystallization has been carried out, and only about five studies (Visser, 1984; Modler and Lefkovitch, 1986; Smart, 1988; Smart and Smith, 1991; Guu and Zall, 1991) appeared in the literature. Traditionally, researchers have used several different techniques to study the growth of lactose crystals.

1. Microscopic measurement of the linear growth of crystal faces (van Kreveld and Michaels, 1965; Michaels and van Kreveld, 1966).

The advantage of this method is that the growth rate of different faces of the crystal can be measured separately and can be related to crystal structure. However, it is difficult to determine the effect of various factors on the growth rate of lactose crystals. In addition, the method is difficult as it involves precise measurement of the crystal faces.

2. Mass seeding experiments (Haase and Nickerson, 1966; Tweig and Nickerson, 1968; Smart, 1988; Smart and Smith, 1991; Guu and Zall, 1991).

This is the most often used method for the lactose crystal yield study. In this method the amount of soluble lactose in the solution is measured over time to determine the amount of lactose crystallized. This method is rapid and simple to carry out. However, it is very hard to separate the influence of various factors on crystal growth from the effects on the nucleation of the crystal. Thus, the results obtained using this method are only relative and can be affected by the amount of additional nucleation in the solution. Also, rapid change in the supersaturation may affect crystal morphology.

3. Single crystal growth experiments (Jelen and Coulter, 1973a, 1973b; Bhargava et al., 1994).

The advantages of this method are that the supersaturation of the solution can be considered constant at the isothermic conditions because of the large mass of solute to mass of crystal ratio. While it is relatively easy to determine crystal growth rate using this technique, the method is time consuming.

4. Mathematical modelling (Thurlby, 1976; Thurlby and Sitnai, 1976).

Mathematical equations have been derived to correlate the growth rate of lactose crystals with data from literature, e.g. by Hudson, 1908 (as cited by Thurlby and Sitnai, 1976). The simulations obtained are theoretical and for the most part may or may not agree with the practical results.

1.2 Research objectives

The aim of this work was to generate new information on the factors influencing the recovery of lactose from whey UF permeates. The main objective was to study the growth of individual lactose crystals in model lactose solutions (with or without the addition of specific salts or other compounds) and in sweet and acid whey UF permeates. In particular, specific objectives of the study were to elucidate:

1. The effect of minerals on the growth pattern and growth rate of lactose crystals and on the solubility of lactose in model lactose solutions.
2. The effect of riboflavin on the growth rate of individual lactose crystals grown in model lactose solutions.
3. The effect of combined impurities in real whey UF permeate systems on the growth of individual lactose crystals.

CHAPTER 2

REVIEW OF LITERATURE

2.1 Introduction to lactose - the milk sugar

2.1.1 Occurrence

Lactose, or milk sugar, occurs exclusively in mammalian milk (Nickerson, 1978b). The amount of lactose in cows' milk ranges from 3.23 to 5.77% (Sommer, 1952), with an average content of 4.8%. Previously, lactose as an industrial commodity or a laboratory reagent was obtained almost exclusively from whey, the by-product of the cheese, quarg and casein industry; however most of the lactose in United States is currently obtained from whey UF permeates (Jelen, University of Alberta, Personal Comm.).

2.1.2 Isomers of lactose

Lactose is a disaccharide consisting of D-galactose and D-glucose linked by β-(1-4)-galactosidic linkage. Lactose occurs in two isomeric forms, i.e., alpha (α) and beta (β) lactose, which differ in the steric configuration of the -H and -OH around C-1 of the glucose molecule (Fig. 2-1). In solutions, α- and β-forms are present in equilibrium which changes with temperature, with higher temperatures favouring the 'β' form. On dissolving lactose in water, the optical rotation will change until $[\alpha]D_{20} = +55.3°$ when the two forms achieve equilibrium (Harper, 1992). This equilibrium of is equivalent to the ratio of $\alpha:\beta = 38:62$ (Michaels and van Kreveld, 1966).

2.1.3 Morphology of the lactose crystal

As described by Hunziker and Nissen (1927), Herrington (1934a), Leviton (1943), and van Kreveld and Michaels (1965), α-lactose monohydrate crystals occur in different shapes, depending on the conditions of crystallization. The principal factor determining the crystallization behavior is the precipitation pressure or the supersaturation of the solution (ratio of actual concentration to solubility). At higher supersaturation only prisms form; as supersaturation decreases, the form changes to diamond-shaped plates, then to pyramids and tomahawks (Holsinger, 1988). In dairy products lactose crystals occur in pyramid form (Fig. 2-2). An α-lactose crystal can change its shape from the basic pyramid shape due to the difference in the relative growth rates of various faces of the crystals (Herrington, 1934a; van Kreveld and Michaels, 1965). Table 2-1 lists the different types of the α-lactose crystals formed due to relative growth in a particular axis (Fig. 2-2). Under usual situations (below 93.5°C) in a lactose crystallization process only the α-lactose crystals are formed. However, when lactose crystallization takes place at temperatures above 93.5°C, crystals of β-lactose are formed. When crystallized out of water, the usual form of crystal is an uneven-sided diamond, while it forms curved needle-like prisms when crystallized from alcohol (Holsinger, 1988). In literature, very little information on the shape of β-lactose crystal is documented.

2.1.4 Basic properties of lactose

Lactose is distinguished from other common sugars, i.e., sucrose, fructose, maltose etc., by its low sweetening power and very low solubility (Pritzwald-Stegmann, 1986). The physical and chemical properties of lactose were discussed in detail by Jenness & Patton (1959), Nickerson (1974, 1978a, 1979), Walstra and Jenness (1984), Morrissey (1985), Pritzwald-Stegmann (1986) and others.

The α- and β-forms of lactose differ in properties such as solubility, specific optical rotation, sweetness and crystallization behavior (Table 2-2). The α-lactose monohydrate

($C_{12}.H_{22}.O_{11}.H_2O$) crystallizes out at temperatures below 93.5°C. It has a specific optical rotation of $[\alpha]D_{20} = +89.4°$ and a melting point of 202°C. The textural defect in ice cream, condensed milk or processed cheese spread termed "sandy" is due to the presence of α-lactose monohydrate crystals above 30 microns in size (Hunziker, 1934; Nickerson, 1954; Walstra and Jenness, 1984).

Heating α-lactose monohydrate to approximately 120°C under partial vacuum converts it to anhydrous α-lactose. Lactose glass, or amorphous lactose, is obtained when a supersaturated lactose solution is dried rapidly so that crystallization cannot take place (Holsinger, 1988). The other form of lactose, i.e., the β-lactose, can be precipitated by careful addition of alcohol to the solution. The β-lactose shows a specific rotation of $[\alpha]D_{20} = +35.0°$ and a melting point of 252°C (Pritzwald-Stegmann, 1986).

2.1.5 World production of lactose

In international terms the production of lactose has been increasing continuously since 1940. The exact amount of world lactose production is very difficult to assess accurately and it is not even reported by the Food and Agriculture Organization (Sienkiewicz and Riedel, 1990). According to Booij (1985), the quantity of lactose produced on a worldwide scale (excluding Eastern Europe) in 1984 amounted to approximately 280,000 tonnes (Table 2-3). The main lactose producing countries of the world are Ireland, Holland, Australia and New Zealand (Jelen, University of Alberta, Personal Comm.). In other parts of world, especially in India a big demand for lactose had always existed, and, until 1992, all the lactose was imported. A large portion of unutilized whey is being disposed off by the existing cheese industry in the country. But recently, new lactose manufacturing industries in India are coming up to meet the expanding demand of lactose and to utilize the whey.

2.1.6 Nutritional significance of lactose

In the gut of lactose tolerant individuals or young infants, lactose may be hydrolysed by the enzyme β-galactosidase (lactase) to glucose and galactose, which are then absorbed by the body. Alternatively, lactose may be degraded to lactic acid by the microorganisms in the intestine resulting in a putrefactive germ-suppressing environment due to high acidity (Pritzwald-Stegmann, 1986). The slight acidic condition (pH>5.5) in the lower small intestine and the colon may promote the growth of *Lactobacillus bifidus* (Holsinger, 1988).

Consumption of milk with high lactose content provides conditions for good calcium absorption due to the formation of acid pH in the digestive tract which increases the solubility and availability of calcium (Pritzwald-Stegmann, 1986). Lactose also aids in the absorption of phosphorus (Nickerson, 1978b), magnesium and other essential trace elements (Renner, 1983). The use of lactose in preparing infant milk formulas from cows' milk by increasing the lactose content is well known. The galactose moiety of the lactose molecule is purportedly essential for normal mental development in infants. Lactose is recommended in the diets for diabetics (Goller, 1974). With diabetics, the increase in blood sugar level by lactose consumption is only 74 mg/100 mL as compared with an increase of 146 mg/100 mL on glucose consumption (Pritzwald-Stegmann, 1986). Thus diabetics can safely consume lactose in quantities of 35-50 g/day spread throughout the day (Pritzwald-Stegmann, 1986).

Lactose has become a subject of interest to public and to health professionals with the finding that about 70% of the world's population has low levels of lactase activity in the intestine (Holsinger, 1988). Absence of lactase in the body leads to fermentation of lactose in lower gut along with symptoms of diarrhea. A detailed study on the improvement of lactose absorption from dairy foods by lactose intolerant individuals was carried out by Shah (1991).

2.2 Solutions and lactose solubility

A solution is a spontaneously forming homogeneous mixture of two or more substances (with at least one solvent and/or solute). When a solid is dissolved in an excess of liquid it is termed solute, while the liquid is termed solvent. The solubility of lactose in water depends on the temperature, i.e., solutions of the same degree of saturation at different temperatures contain different amounts of lactose dissolved in the same amount of water (Table 2-4).

On addition of α-lactose monohydrate to an excess of water, with continuous agitation, a definite amount dissolves rapidly (line AB in Fig. 2-3); this amount is known as initial solubility. The initial solubility (e.g. 7 g of α-lactose monohydrate per 100 g water at 15°C) is the true solubility of the α-form (Nickerson, 1974).

With an increase in time, more lactose can be dissolved in the same amount of water due to mutarotation of α- and β-forms (Holsinger, 1988). As some of the α-lactose is converted to β-lactose, the solution becomes undersaturated with respect to α-, and more α-lactose monohydrate dissolves. This process continues until a final equilibrium is reached between the α- and β-forms of lactose and no more α-lactose monohydrate can be dissolved (e.g. at 15°C, 17 g of lactose will dissolve in 100 g water). This is the final solubility of lactose at that particular temperature (line CD in Fig. 2-3). Hence, in a saturated solution the amount of lactose present is equal to at least the final solubility value; while, the undersaturated solution contains less lactose that the final solubility value (Fig. 2-3). Neither nucleation nor crystal growth is possible in an undersaturated solution. The final solubility of lactose at different temperatures and that at 30°C according to different authors is given in Tables 2-4 [illegible] is a lack of agreement among the different authors on the exact values of final so[illegible]ose at particular temperatures.

To calculate the solubility of lactose at temperatures below 93.5°C, an equation has been proposed by Morrissey (1985), which does not always agree with the experimental results:

solubility of lactose = $(2.64-0.0027T)\times$(solubility of α-lactose)

where, T = Temperature (°C)

Further addition of α-lactose monohydrate to the lactose solution, along with heating, results in a supersaturated solution. Supersaturation can be expressed as grams of lactose or anhydrous lactose per 100 g water above the saturation value (i.e., "absolute supersaturation") or as a ratio of the amount present to the solubility value ("relative supersaturation") (Jelen, 1972).

2.3 Supersaturated solution

A supersaturated solution can be achieved from an undersaturated solution in three possible ways:

1. When an undersaturated solution is cooled without the loss of solvent;
2. When a solvent is removed from an undersaturated solution by evaporation; or
3. By a combination of cooling and evaporation. This is the usual method employed to achieve supersaturation.

Oswald in 1897 (as cited by Mullin, 1972) introduced the term supersaturation and extended it to "metastable" and "labile" regions. The crystallization from a solution begins in a supersaturated solution where the crystallizing substance is in a labile or a metastable state. The metastable state occurs as a result of either an increase in solids or a decrease in the temperature of solution. In this type of solution nucleation can only take place in the presence of nuclei or a mechanical shock; otherwise, spontaneous crystallization is impossible. The labile region is found at higher levels of supersaturation as a result of further increase in the total solids content or a further decrease in the temperature. This region is unstable and will cause spontaneous crystallization without the addition of seeding material or mechanical shock. Clear-cut distinction between the metastable and labile regions is very difficult and it may be more appropriate to consider this as a narrow band/zone rather than a sharp line. The width of the zone can be influenced by the presence of impurities in the crystallizing system (Mullin, 1972).

2.4 Mechanism of crystallization

In general, crystallization involves nucleation, and crystal growth (Mullin, 1972; Smart, 1988; Smart and Smith, 1991).

2.4.1 Nucleation

Nucleation is an important step in crystal development and involves the activation of small, unstable particles of solute to form a new stable phase prior to growth of crystal (Holsinger, 1988). The size of the critical nucleus required to induce crystallization is in the order of 100 molecules or a diameter of about 100Å (van Hook, 1961). The phenomenon of nucleation can be classified as either primary or secondary nucleation. The mechanism of nucleation has been reviewed by a number of researchers (Mullin, 1972; Nyvlt, 1978; de Jong, 1979; Grootscholten et al., 1982).

Primary nucleation may occur spontaneously or it may be induced artificially by a foreign particle; these two cases are often referred to as homogeneous and heterogeneous nucleation, respectively. Primary homogeneous nucleation occurs in pure, crystal-free and supersaturated solutions, while, primary heterogeneous nucleation is triggered by foreign particles (other than the material to be crystallized) such as dust particles (0.005-10 μm) or by the walls of the crystallizer. In solutions in which crystals are already present, the solution nucleates much more readily and this is known as secondary nucleation.

As nucleation proceeds, the supersaturation of the solution drops below a certain limit, but not below the supersaturation value; and the solution is no longer labile. In such a situation, no new nuclei will form; however, the existing crystals will continue to grow until the solution surrounding them becomes stable. Thus, during the crystallization process without concurrent evaporation of the solvent, the solute to solvent ratio of the solution will change

2.4.2 Crystal growth

After the formation of a nucleus, the growth of a crystal depends on the rate of solute transfer to the crystal surface and the rate of subsequent orientation of solute molecules at the crystal surface. Lactose crystal growth begins with the adsorption of lactose molecules from the solution on to the surface of nuclei. On crystallization of lactose below 93.5° C, the α-form will crystallize out first and some β-form will convert to α-form, thus maintaining the equilibrium. Hence, the crystal yield and the crystallization rate involve conversion of soluble β-lactose to soluble α-lactose and the conversion of soluble α-lactose to α-lactose hydrate crystals (Zadow, 1984).

The growth of lactose crystals is affected by a number of factors such as supersaturation, temperature, viscosity, pH and presence of impurities (Hunziker and Nissen, 1926, 1927; Whittier 1944; Simpson et al., 1948; Cerbulis, 1973; Nickerson and Lim, 1974; Brinkman, 1976; Majd and Nickerson, 1976; Olano et al., 1977a, 1977b; McCommins et al., 1980; Quickert and Bernhard, 1982; Modler and Lefkovitch, 1986). The details of the kinetics of lactose crystallization have been studied and/or reviewed by several authors (van Kreveld and Michaels, 1965; Tweig and Nickerson, 1968; van Kreveld, 1969; Jelen and Coulter, 1973a, Nickerson and Moore, 1974a, 1974b; Thurlby, 1976; Thurlby and Sitnai, 1976; Visser, 1982; Griffiths et al., 1982).

The growth rate of a lactose crystal is directly proportional to the supersaturation of the solution. The crystallization process is affected by the temperature in two opposing ways. An increase in the temperature reduces the growth rate of lactose crystals because of an increase in the lactose solubility; on the other hand, it results in an increase in the growth rate because of an increase in the kinetic activity, accelerated diffusion and reduced viscosity (Jelen, 1972). The presence of impurities in the solution has a negative effect on the crystallization velocity and purity of the crystals. In most cases the crystallization velocity is reduced with an increase in the concentration of impurities. It has been noted by Jelen (1972) that in case of sucrose crystallization, the crystallization velocity is about 50% of that of pure sucrose solution at 95% purity, while it is about 11% at a purity level of 80% (on dry matter basis).

Impurities in the solution also affect the morphology of the crystals. It has been concluded by Davey (1976, 1979) that foreign substances in the crystallizing systems alter the growth kinetics of certain crystallographic faces due to adsorption in the crystalline system. A mechanism of how additives determine the morphology of the crystal has yet to be understood fully (Larson, 1982).

2.4.3 Theories of crystal growth

A number of theories regarding the growth of crystals in solutions have been discussed (Buckley, 1951; Strickland-Constable, 1968; Mullin 1972; Bennema, 1976; Visser, 1982). The two major theories of crystal growth are the 'Birth and Spread theory' and the 'Spiral growth theory'.

A model was designed by Kossel in 1934 (as cited by Mullin, 1972) to demonstrate the birth and growth theory. As shown in Fig. 2-4, a crystal surface is considered to be planar with three major binding sites available for incorporation of a solute molecule. The growth units that might build up the crystal are represented by cubes (D). The steps (B) are the rough surfaces on the flat faces (A) of the crystal that contains kinks (C), where the growth units are most easily integrated (Fig. 2-4). Face A is energetically unfavourable and a molecule adsorbed here will most likely either migrate or desorb. Surface migration will take the molecule, to a step (site B) or a kink (site C), both of which are energetically favourable.

Kink C is more favourable than the step B due to increased binding. A growth unit is transferred from the bulk of a supersaturated solution to the kink site; the kink moves along the step and the completion of the layer gives a smooth face on the crystal surface. Further, surface nucleation could also create a fresh step. A crystal should grow fastest when all surfaces of the crystal are covered with kinks. However, the number of kinks would not remain at its maximum value for any length of time. It has been observed that broken or surface-eroded crystals grow at a much faster rate initially during which time the surface rapidly heals (van Kreveld and Michaels, 1965; Jelen, 1972) and later the crystal grows at a much slower rate.

It was later observed that many crystal faces grew faster at a relatively lower level of supersaturation, which renders the Kossel model unreasonable for the growth of crystals at lower supersaturation levels (Mullin, 1972). Hence, to explain the above phenomenon, another model known as spiral growth theory was proposed by Frank in 1949 (as cited by Visser, 1982). According to this model the growth of the crystal takes place in a layer-by-layer fashion without formation of any imperfection in the crystal pattern. Most crystals contain fault or dislocation on the crystal surface that cause the formation of a step. Fig. 2-5 explains how a screw dislocation can cause a step that is self-perpetuating at a constant velocity.

2.5 Influence of impurities on growth and morphology of lactose crystals

The presence of impurities has an important bearing on lactose crystallization rates and crystal morphology. From an industrial viewpoint, solutions containing lactose alone are rather unrealistic. Most industrial crystallizaton is carried out using whey or whey UF permeate, which contain significant quantities of minerals, especially sodium, calcium, magnesium and other impurities such as lactate, citrate and riboflavin. To understand the impact of minerals on lactose crystallization, one first needs to consider the effects of salts on the structure of bulk water.

2.5.1 Effect of salts on structure of water

Von Hippel and Schleich (1969) have presented an extensive review on the effect of various salts on the structure of water. Many possible mechanisms on the interaction of ions with water have been discussed in great detail. A general conclusion is that some ions lead to perturbation (structure breaking), while some others lead to reorganization (structure making) of the water structure. The epitome of various mechanisms (of how the ions/salts act on water) is that ions of high charge density (Li^+, Ca^{2+}, Ba^{2+}, Sr^{2+}) orient the water structure and possibly reduce the total amount of free water molecules in the solution due to change in the state of water (from free water molecules to "bound" water). Conversely, ions of low charge density (Cl^-, Br^-, I^-) lead to breaking of the water structure because the charge density is too small to electrostatically organize the water effectively. This would probably lead to an increase in the total amount of free water molecules in the solution due to change in water from bound to free state.

2.5.2 Effect of impurities on nucleation and on the growth rate of lactose crystals.

The influence of impurities on the crystallization of lactose has been studied by a number of researchers (Michaels and van Kreveld, 1966; van Kreveld, 1969; Jelen and Coulter, 1973b; Nickerson and Moore, 1974b; Smart, 1988; Smart and Smith, 1991; Guu and Zall, 1991). The presence of impurities affects both the nucleation and the growth process of crystals (Botsaris, 1982). Impurities either increase or decrease the nucleation rate, thus making the mechanism complicated and giving rise to a need for sound mechanistic explanation (Davey, 1982). According to Khamskii (1979) and Mullin (1979) the presence of an impurity changes the kinetics of the crystallization process due to changes in the solubility of the substance to be crystallized. This change in the solubility results in a change of supersaturation of the solution, thereby affecting both the nucleation and growth rate. Nickerson and Moore (1972) and Whittier (1944) showed that lactose solubility decreased in the presence of sucrose. Another study (Solstad, 1965) concluded that some carbohydrates change the solubility of sucrose. Herrington (1934c) reported that some calcium salts (chloride, bromide, nitrate) increase the solubility of lactose. It has been argued that the adsorption of impurities is one of the key mechanisms in crystal growth retardation.

Because riboflavin is naturally present in whey, its possible effect on the lactose crystallization process is of particular importance (van Kreveld, 1969; Smart, 1988; Smart and Smith, 1991). The color of lactose crystallized from whey is usually slightly yellow due to adsorption of riboflavin into or onto the lactose crystal. The adsorption of riboflavin on the lactose crystals depends upon the concentration of riboflavin in the solution, on the degree of supersaturation of lactose and on the temperature of the solution (Leviton, 1943; Michaels and van Kreveld, 1966). Increasing the temperature results in reduced riboflavin adsorption, while lower levels of supersaturation increase riboflavin adsorption (Holsinger, 1988). Michaels and van Kreveld (1966) reported that riboflavin alters the shape of lactose crystals but, offered no possible mechanism for the observed phenomenon.

Growth of lactose crystals in presence of various salts as additives was studied by a number of researchers (Michaels and van Kreveld, 1966; Jelen, 1972; Smart, 1988; Guu and Zall, 1991; Smart and Smith, 1991; Bhargava et al., 1994). It is well established that salts either increase or decrease the growth rate of lactose crystals. Different mechanisms have been suggested for the alteration in the growth rates. It is believed that some impurities may incorporate into the lactose crystal thereby inhibiting the growth rate or alternatively, increase the growth rate due to an increase in the weight of lactose crystal by the incorporated salt.

Many researchers have reported an improvement in lactose recovery by addition of salts and other compounds. The addition of NaCl (Polyanskii et al., 1977 as cited by MacBean, 1979), mixtures of $Ca(OH)_2$, NH_4Cl and NaOH (Olano et al., 1977a), mixtures of $CaCl_2$, NaOH with or without organic solvents (Olano et al., 1977b), $MgCl_2$ with or without NaOH (Kwon et al., 1981) and alcohol (Majd & Nickerson, 1976) increase the lactose recovery rate.

The presence of impurities during the crystallization process may have some beneficial effects in the crystallization industry. Some of the impurities alter the crystal shape and size resulting in increased crystal recovery. Table 2-6 illustrates the importance of crystal shape and size at various processing steps in an industry. An increase in the total cost of crystallization and problems with disposing of the mother liquor containing the additives are some negative aspects of the use of additives in a crystallization process.

2.5.3 Effect of impurities on the growth morphology of the crystal

Presence of impurities sometimes alters the shape of the crystals (Buckley, 1951). The change in crystal morphology can be observed using either optical or electron microscope or with the naked eye if the crystals are large enough.

Jelen and Coulter (1973b) showed how the morphology of lactose crystals is changed by the presence of salts. The effect of salts on crystal morphology is not unique for lactose. Smythe (1971) reported the effects of salts on sucrose crystallization. Similarly, Gómez (1979) reported the growth habit modifications of sucrose crystals by inorganic impurities present in molasses. Modification of either a lactose crystal or a sucrose crystal with increasing concentration of the other sugar was reported by Nickerson and Patel (1972) and Hunziker and Nissen (1934), who showed that lactose crystals become shorter and broader as sucrose concentration is increased in sweetened condensed milk.

2.6 Industrial production of lactose

Industrially, lactose is produced in four general grades of increasing purity (Wittenberg, 1978), as illustrated in the Table 2-7. With an increase in the purity of the lactose crystals there is a decrease in level of impurities, i.e., protein (from 1.0% to 0.01%), ash, fat content and riboflavin, when lactose is recovered from whey.

The principal raw material for the industrial production of lactose is whey or whey UF permeate, the by-products of the cheese and casein industries. Crystallization of lactose from concentrated whey has been the principal method of lactose recovery. Recently, most of the lactose industries have been shifting their attention to the manufacture of lactose from whey UF permeates. The production of lactose from whey UF permeate ensures easier process, higher yield and high purity of lactose in comparison to when produced from whey (Muller, 1979).

2.6.1 Production of lactose from whey

The crystallization of lactose from whey involves the concentration of whey by evaporation, crystallization, separation, refining, drying and milling (Hobman, 1984). The basic procedures used in lactose crystallization from whey have been reviewed in detail by Bell et al., (1928), Weisberg (1954), Nickerson (1970), Webb (1970), Sienkiewicz and Riedel, (1990) and Keller (1982). The yield and purity of the lactose crystals are affected by the presence of whey proteins and minerals in whey. Heat coagulation (Marshall, 1982), and UF (Matthews, 1979) of whey are efficient techniques to recover and remove whey proteins from whey; while, the suitable methods for removal of minerals are chemical precipitation, demineralization or membrane separation.

Modler and Lefkovitch (1986) investigated the effects of casein and whey proteins on the yield and quality of lactose crystals. According to them, sweet whey should preferably be used over acid whey as a raw material for lactose crystallization. The use of sweet whey ensures good quality and a high yield of lactose (Modler and Lefkovitch, 1986). Acid whey contains high levels of minerals (Nickerson, 1975) which interfere with the crystallization process, reduce the crystal quality and increase the associated washing treatment cost (Smart and Smith, 1991). Table 2-8 indicates the disadvantages associated with the use of acid whey as a starting material for crystalline lactose production.

Concentration of acid whey for subsequent crystallization leads to precipitation of calcium complex salts such as phosphate and citrate (Muller, 1979; Nickerson, 1979; Pederson, 1980). Due to the inverse temperature-solubility characteristics of calcium phosphate (Brule et al., 1978; Summers and Okos, 1982), the calcium salts might contaminate the crystals and it may be difficult to remove the adsorbed salts by washing with water (Nickerson, 1979). Additionally, these salts might also lead to fouling and scaling of heat exchanger surfaces.

To increase the yield and improve the quality of lactose obtained from acid whey, the best alternative would be to reduce the amount of calcium prior to evaporation (Hull, 1957; Wallace, 1958a, 1958b) using different techniques, including ion exchange (McGlasson and Boyd, 1951; Kavanagh, 1975; Goldman and Short, 1977) and electrodialysis (Kavanagh, 1975; Short and Doughty, 1977; Short, 1978; Higgins and Short, 1980; Houldsworth, 1980; Jönsson and Olson, 1981). Since ion exchange and electrodialysis processes create waste disposal problems and require substantial amount of capital, their industrial use is rare. The other alternative to reduce the level of calcium is by the addition of a food grade calcium chelating agent (e.g. sodium hexametaphosphate) to form insoluble complexes that may be removed prior to crystallization (Evans and Young, 1982; Evans et al., 1982). This step increases the lactose quality and yield.

Several modifications of the method for producing commercial lactose from whey have been suggested by various authors. British Patent No. 1 430 490 (as cited by Harju, 1990) describes a method of lactose recovery by chromatographic separation of whey. However, this method has limitations regarding the purity of lactose and handling of large volumes of whey. This method has problems with particular wheys (i.e., Edam cheese whey) containing relatively high amounts of calcium. High calcium contents coupled with low pH cause precipitation of calcium salts in the chromatographic separation columns resulting in a greater need of regeneration.

In a recent patent, Harju and Heikkilä (1990) demonstrated a method for recovery of high yields of lactose from whey. In this process, a major part of lactose is crystallized first from concentrated whey and the crystals separated. The mother liquor is purified by heating (60-70°C) at pH of approximately 5.8 to 7.0 and then filtering out any precipitates. The filtered mother liquor is then subjected to chromatographic fractionation. This separation results in recovery of at least three fractions, i.e., a protein fraction which may contain salts, an intermediate fraction and a lactose fraction which is subjected to further crystallization.

In another method invented by Francis and Pollard, (1973) the time of crystallization cycle and formation of de novo crystals is greatly reduced.

2.6.2 Production of lactose from UF permeate

Due to economic reasons there will be increased industrial use in the future of processes suitable to separate proteins from whey, e.g. using UF procedures. After removal of proteins from whey, the ultrafiltered permeate is an ideal raw material for lactose crystallization. In the absence of whey proteins, the permeate can be concentrated to higher total solids content, which would result in an increased lactose crystal yield (Brinkman, 1976). Other advantages include shorter crystallization times and a higher purity of lactose crystals (van den Bos, 1987). Also, there have been attempts to recover crude lactose from UF permeates by demineralization using ion exchange chromatography followed by roller drying (Kavanagh, 1975). A higher yield of lactose from permeates could be obtained by a combined process consisting of continuous crystallization at a moderate temperature followed by low temperature batch crystallization (Short, 1978).

A method to produce high yield of relatively pure lactose and a separate salt product was described by Pederson (1980). Whey permeate was obtained by UF and concentrated to a total solids content of 40 - 45%. The permeate was held at 82 - 93° C for 30 - 90 minutes, leading to precipitation of calcium and citrate salts without substantial precipitation of phosphate salts. The precipitate was removed and further concentration of permeate led to lactose crystallization. Crystallized lactose was removed from the permeate and the remaining solution spray dried to recover the remaining lactose to be used in food or feed. Gjersvig (1987) described a one step crystallization process to isolate United States Pharmaceutical (USP) grade lactose with a yield of 45 - 65%.

A simple and economical method to produce USP quality lactose from acid or sweet whey permeate was developed by Evans and Young (1982). In this method, an effective amount of a food grade chelating agent was added to the permeate and the lactose crystallized at 10-24° C. In a subsequent patent, a method considering the current requirements for lactose pH imposed by the United States government and other countries was developed (Evans et al., 1982).

2.6.3 Other methods of lactose recovery

In the sugar beet industry, the Steffen process (Hartmann, 1974) has been used for sucrose recovery from molasses by precipitation using alkaline earth metals. Several other researchers (Cerbulis, 1973; Kwon et al., 1981 and Quickert et al., 1982) have tried to apply the Steffen process principle for the recovery of lactose from cheese whey. This process could be applied effectively because reducing sugars such as lactose are more sensitive to alkaline reagents than non-reducing sugars. Cerbulis (1973) reported that up to 81% of the lactose could be precipitated out using this method. The precipitation was carried out at a low temperature (3-5°C) to avoid breakdown of lactose. At ambient temperature (25°C), the lactose yield was 30% lower than that obtained at 3-5°C (Cerbulis, 1973).

As discussed earlier, alcohol reduces the solubility of lactose, accelerating lactose crystallization and influencing the crystal morphology (Majd and Nickerson, 1976). Thus, alcohol might be used advantageously in lactose recovery (Leviton, 1949; Leviton and Leighton, 1938). It may be possible to produce lactose with different functional properties using alcohol in the crystallization process (Majd and Nickerson, 1976; Olano and Rios, 1978). When alcohol is added to a solution containing lactose, the lactose crystallizes out after a few minutes. The composition of lactose varies with the amount of alcohol added. Only α-monohydrate crystals are precipitated at low concentrations of alcohol, while at higher concentrations, β-lactose is also included in the precipitate (Holsinger, 1988). Ethanol and/or methanol have been used to extract lactose from skim milk or whey powders (Hoff et al., 1987).

2.7 Uses of lactose

Lactose has wide number of uses described in great detail by Weisberg (1954), Nickerson (1975, 1978b), Parrish (1976), Holsinger (1978), Short (1978), Booij (1985) and Zadow (1984, 1986). According to Booij (1985), approximately 50% of the world production was utilized by the food industry, 25% by the pharmaceutical and chemical industry and the remainder was used for other applications.

Lactose is used in the food industry in baby foods, pastries, soups, sauces, sauce binders, instant drinks, spice mixes, meat products etc., (Harper, 1992). The use of lactose in confectionery products offers advantages as an aroma enhancer, reduced sweetener and a low cost replacer for solids non fat. The addition of lactose to high sucrose solutions delays the crystallization of sucrose thus offering considerable advantage in the coating of sweets and dragees. In particular, it improves the body, texture, chewiness and shelf life of caramels and fudges (Webb and Whittier, 1970). The ability of lactose to prevent the color and flavor loss has resulted in its use in products such as sachet wafers and seasonings. Lactose is also used in baked goods because of its ability to increase the browning of crust (often highly desirable) and the fact that it is not fermented by bakers' yeast. Lactose enhances the sensory quality of beer because lactose is not fermented by brewers' yeast (Holsinger, 1988; Harper, 1992).

The low hygroscopicity, low reactivity, neutral taste and good flowing property of lactose make it an excellent base material as a bulking agent in the pharmaceutical industry (Pritzwald-Stegmann, 1986). Lactose can be used also in the chemical industry for the production of lactulose, lactitol and lactobionic acid (Booij, 1985). Lactose as an additive to the culture solution for fermentation such as antibiotic production, offers advantages due to its delayed decomposition. Endicott et al., (1973) developed a low bulk-density sweetener having a sweetness of equal volume of sucrose. The product contained about 97-99.25% lactose and 0.75-3% saccharine. Miller et al., (1993) and Chambers et al., (1985) have invented procedures for incorporation of lactose to animal feed.

The current industrial practice to crystallize out lactose from whey and whey UF permeate is based on very incomplete data on the behaviour of lactose in crystallization processes. The quality and yield of lactose varies from batch to batch (Keller, 1982). There are various factors that influence the lactose crystallization process. One of the major factor is presence of impurities like whey salts and riboflavin in the raw material. Virtually no published information is available on the effect of impurities on crystallization of lactose in whey UF permeates. Hence, a study of the lactose crystal growth in model and actual systems was considered necessary to allow a better understanding of the effect of impurities on the crystal growth process.

Table 2-1. Modification of lactose crystal due to variation of growth in particular axes

Crystal morphology	a-axis	b-axis	c-axis
Tomahawk	++	+++	0 to ±
Prism	++	++	0 to +
Diamond	++	+	0 to +
Pyramid	+	+++	+
Triangle	+	+++	±
Triangular plate	±	+++	±
Trapezoidal plate	±	+++	+

(Adapted from van Kreveld and Michaels, 1965).

(Expressed in the increasing scale: 0, ±, +, ++, +++, where 0 indicates no growth and +++ indicates maximum growth)

Table 2-2. Physical properties of α- and β- lactose

Properties	α-lactose monohydrate	β-lactose anhydride
Density (g/L)	1.54	1.59
Melting point (°C)	~202 (Disintegration)	~252 (Disintegration)
Heat of combustion (J/g)	16106	15465
Specific heat (J/kgK)	1251	1193
Heat of solution (J/g)	-50.24	-9.62
Molecular weight (Da)	360.10	342
Initial solubility (g/100 g)	7.4	55

(Adapted from Harper, 1992)

Table 2-3. Estimated world production of lactose in 1984 (in tonnes).

Geographical region	Estimated Production (tonnes)
Europe	190,000
USA and Canada	70,000
Rest of the world	20,000
TOTAL	280,000

(Adapted from Booij, 1985).

Table 2-4. Solubility of lactose at different temperatures

Temperature (°C)	Solubility (g anhydrous/100 g water
30	24.0
50	44.0
60	59.2
70	77.8

(Adapted from Jelen, 1973a).

Table 2-5. Lactose Solubility at 30°C

Reference	Lactose Solubility g anhydrous lactose/100 g water
Francis et al., 1973	25.0
Jelen, 1972	24.0
Whittier, 1944*	23.2
Rozanov, 1952*	24.8

(* Adapted from Jelen, 1972).

Table 2-6. Importance of crystal size and shape as influenced by additives/impurities in a general crystallization process

Processing stage	Influence of crystal size and shape on process efficiency
Filtration and Washing	For efficient recovery, altered crystal size and shape are of vital importance in the filtration and washing processes.
Handling of slurries and pastes	1. The rheological characteristic of product is a function of crystal size and shape. 2. Viscosity of mother liquor can be altered.
Milling, tableting and packaging	Ease and economics related to the crystal size and shape, i.e. handling of bigger crystals is easier.
Scale formation in plants and heat exchangers	1. May be reduced by additives which lower the nucleation rate. 2. Possible reduction in the salt (calcium phosphate) and protein precipitates in milk process pipe lines in dairy industry.

(Modified from: Davey, 1982).

Table 2-7. Industrial grades of lactose

Grades	% Purity
Fermentation	98.00
Crude	~ 98.40
Edible	> 99.00
Pharmaceutical	> 99.85

(Adapted from Wittenberg, 1978).

Table 2-8. Disadvantages of using acid whey for lactose manufacture

Characteristic of acid whey	Effect on lactose manufacture
Low pH (4.4)	May cause severe corrosion of evaporator
High titratable acidity (0.5% as Lactic acid)	
High mineral content (0.71%)	Precipitation of calcium phosphate complexes during concentration may foul heat exchanger surface and contaminate crystals
High calcium concentration (1.24 g/kg)	
High phosphate concentration (1.99 g/kg)	
High lactate concentration (0.64%)	May interfere with lactose crystallization

(Adapted from Hobman, 1984).

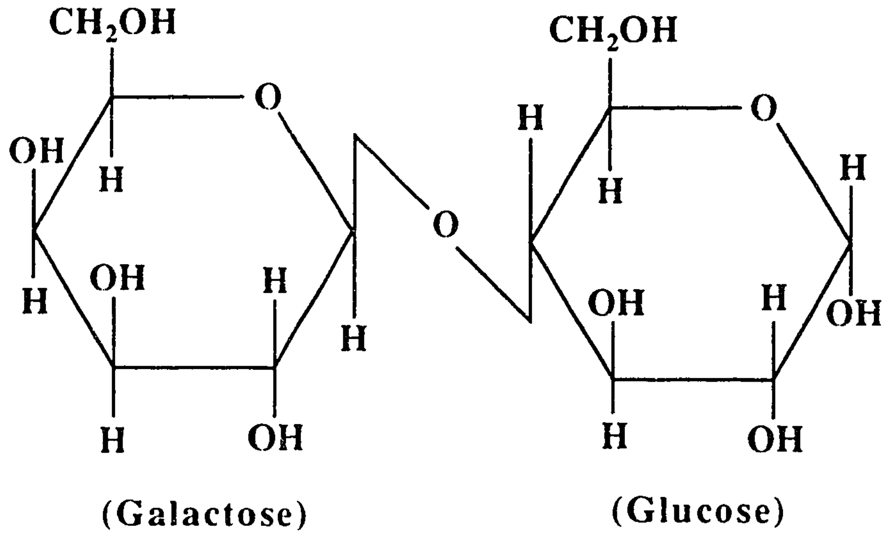

Figure 2-1. Structural formula of lactose

(4-O-β-D-galactopyranosyl-D-glucopyranose).

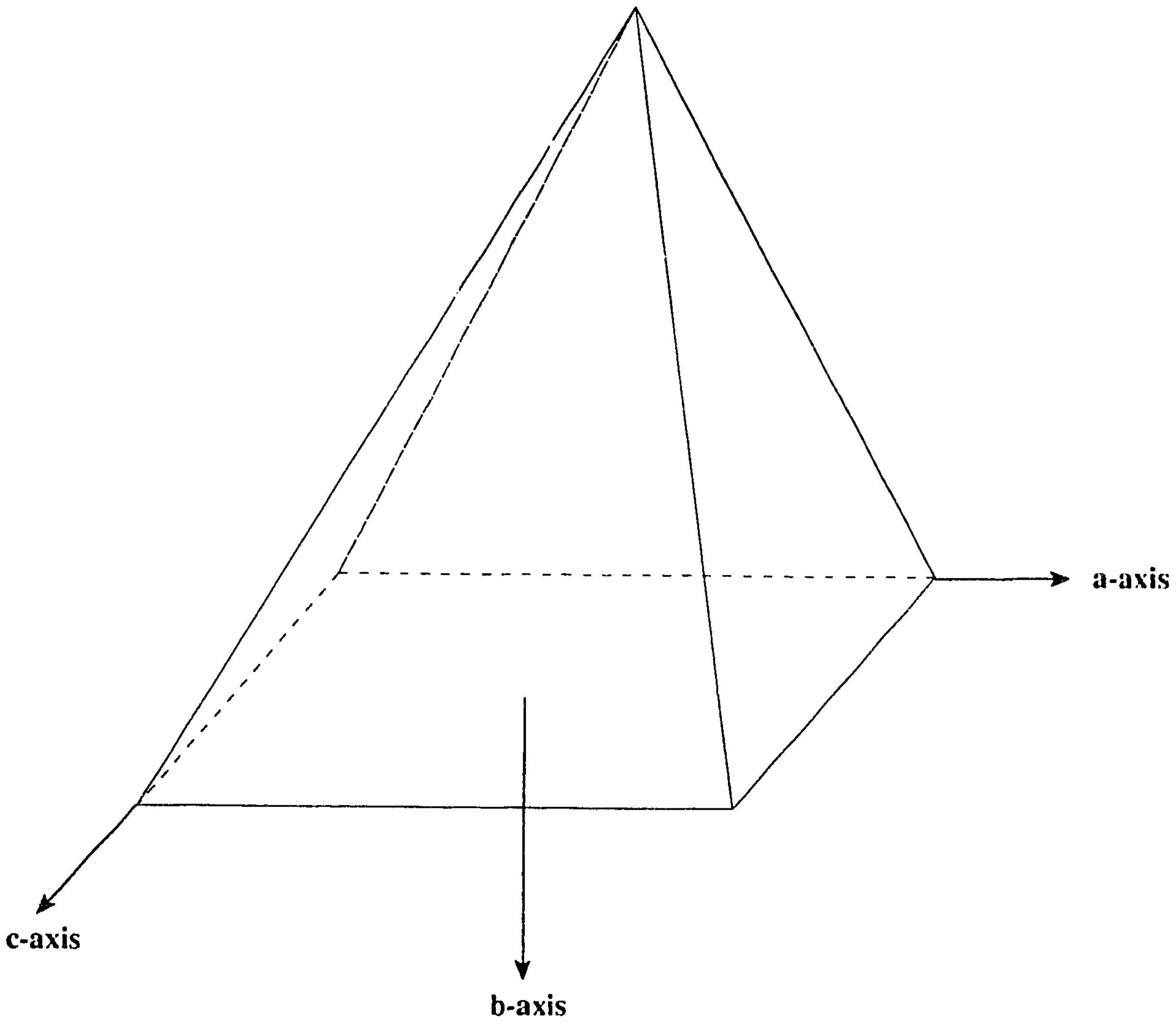

Figure 2-2. Pyramid-shaped crystal of α-lactose monohydrate.

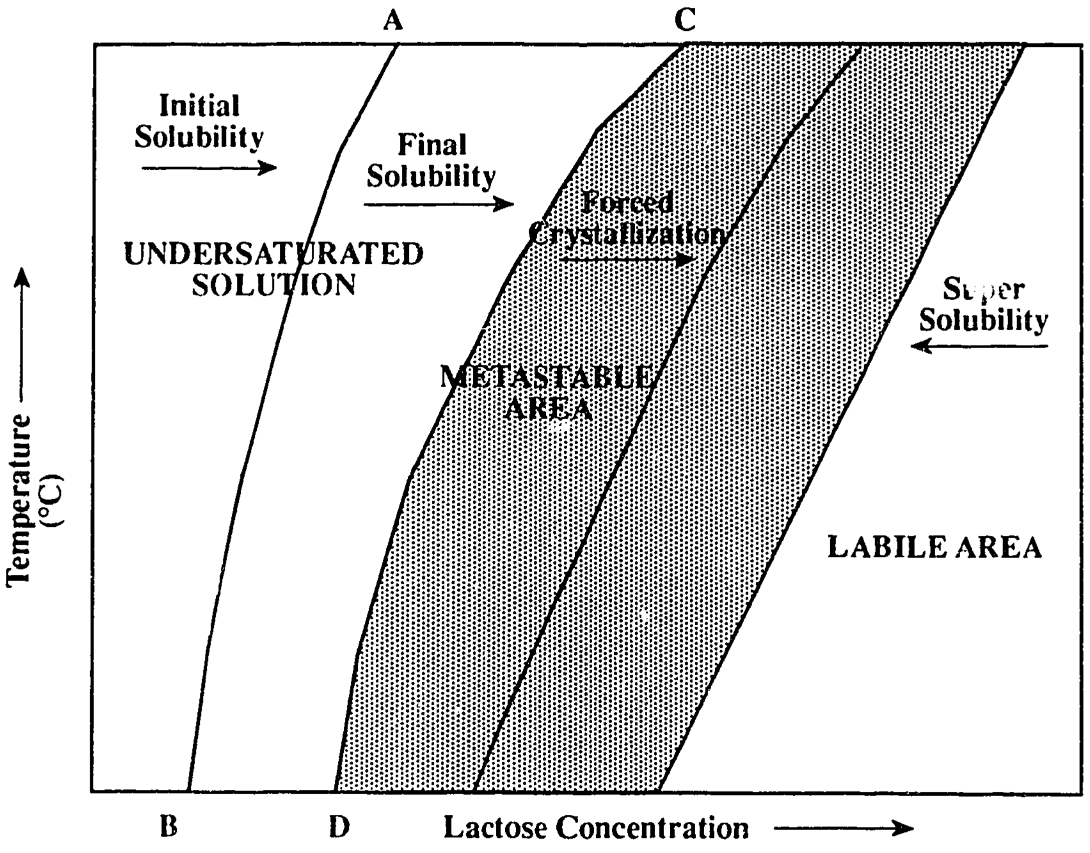

Figure 2-3. Lactose solubility diagram.

(Modified from Holsinger, 1988).

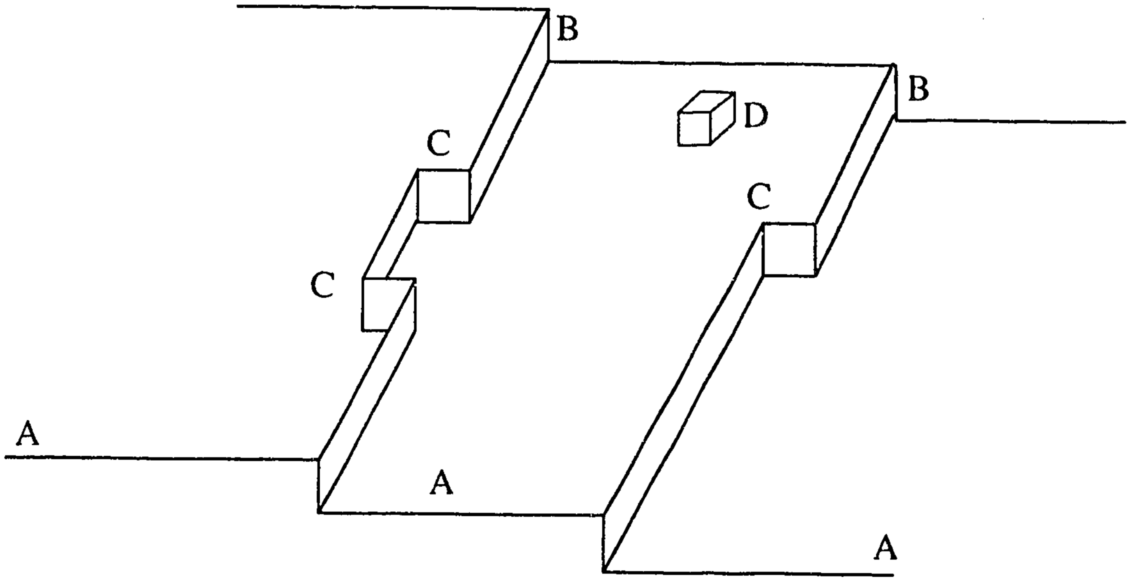

Figure 2-4.Crystal binding sites.

(Adapted from Mullin, 1972).

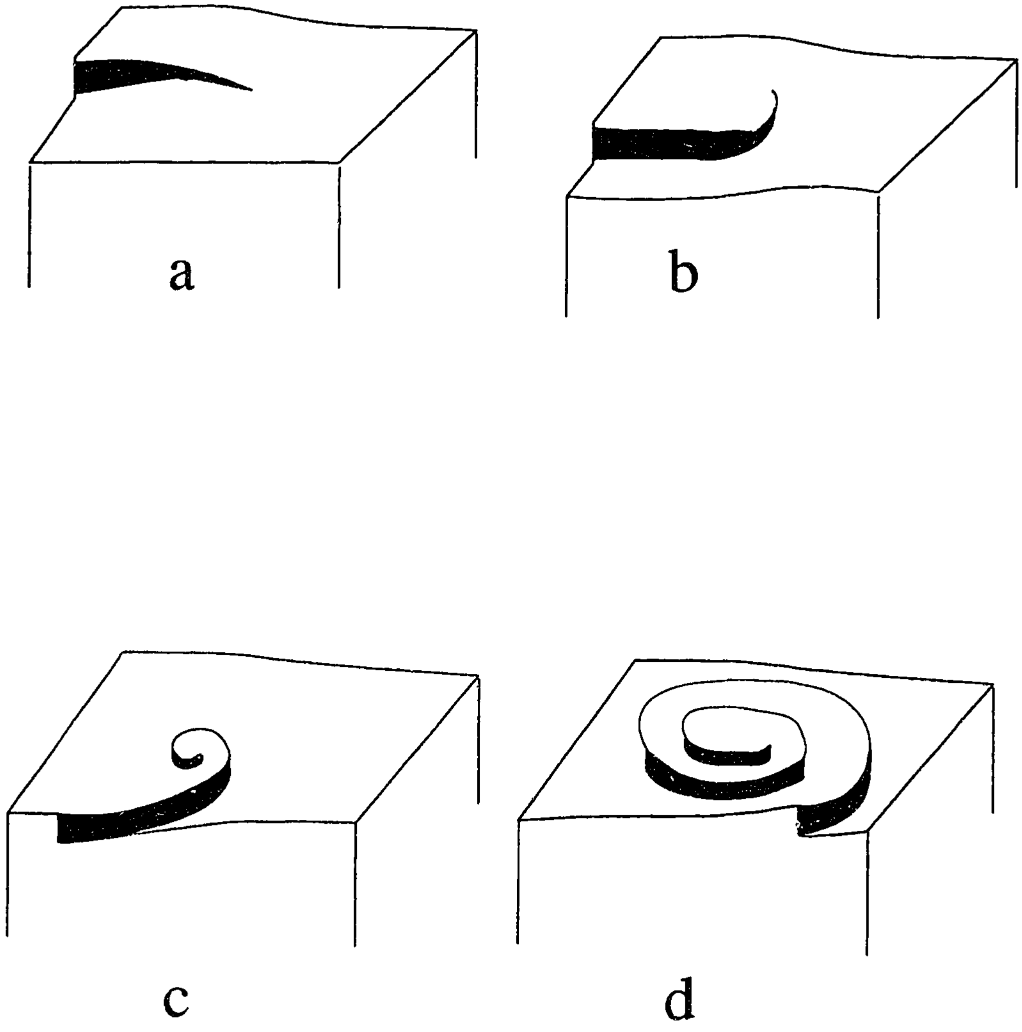

Figure 2-5. Steps involved during the development of a spiral growth.
(Adapted from Visser, 1982).

CHAPTER 3

MATERIALS AND METHODOLOGY

3.1 Materials

Lactose powder used for the study was analytical grade α-lactose monohydrate (>99% purity, Product information pamphlet), obtained from Fisher Scientific (Fair Lawn, NJ). The salts used were laboratory grade calcium chloride dihydrate and calcium lactate obtained from Fisher Scientific, Fair Lawn, NJ, potassium phosphate dibasic anhydrous (Amachem, Portland, OR), lithium chloride anhydrous (Sigma, St. Louis, Mo) and magnesium sulfate (Anachemia Ltd., Montreal). The absolute alcohol was purchased from Fisher Scientific (Nepean, Ontario). Powdered rennet (No. R-3376) and riboflavin were supplied by the Sigma Chemical Co., St. Louis, Mo., and lactic acid (85%) was obtained from Fisher Scientific (Fair Lawn, NJ). Milli-Q water was used for preparation of all the solutions. Pasteurized, bulk skim milk obtained from a local dairy was used to prepare UF permeates.

3.2 Preparation of lactose seed crystals

A highly supersaturated lactose solution was prepared by dissolving 50 g of lactose powder in 100 g of milli-Q water. The solution was kept in a hot water bath at 90°C with continuous manual stirring, until all the lactose was fully dissolved. The solution was allowed to stand in a 500 mL Erlenmeyer flask at room temperature (20°C) for one week until sufficient crystals of desired size were formed.

Single crystals were then transferred to 50 mL Erlenmeyer flasks containing 25 mL of slightly supersaturated lactose solutions (27.75 g anhydrous lactose/100 g water). This lower supersaturation resulted in no additional nucleation in the solution thus allowing more time for individual lactose crystals to gain weight in uniform conditions of virtually constant supersaturation. The crystals were grown for a considerable time (approx. 2 weeks) until they reached a weight of 0.04 ± 0.01 g.

3.3 Model lactose solutions

Different solubility values of lactose at 30°C have been reported by various authors (Table 2-5) with an approximate value of 24 g anhydrous lactose/100 g water. In this study, 35 g lactose powder/100 g water corresponding to 33.25 g anhydrous lactose/100 g water was used. This supersaturation was high enough to support measurable growth of the added individual lactose crystals with no development of false grains in the solution, even after 24 h. This was the main reason for using this supersaturation in all the growth related experiments in model lactose solution.

Specific amounts of salts (1, 2, 3 or 5 g/100 g water) were added to the model lactose solutions containing 33.25 g of anhydrous lactose/100 g water. The resulting solutions were kept in a water bath at 90°C with continuous manual stirring until all the lactose and the salt were fully dissolved. Particular care was taken that the solution became absolutely clear to prevent the formation of undesirable nucleation during the growth period. Smart (1988) reported that in some cases the additives remain partially undissolved before commencement of the mass crystallization experiment. The presence of undissolved additives in the solution could have led to unreliable results due to the formation of additional nucleation. The Erlenmeyer flasks containing 25 mL aliquots of the model lactose solutions were transferred to a 30±0.1°C temperature-controlled chamber for temperature equilibration. When the temperature of the solutions stabilized at 30°C, individual lactose crystals were added to the solutions.

3.3.1 Crystal growth experiments

Healed and pre-weighed lactose crystals (0.03-0.05 g) weighed to five decimals were added to Erlenmeyer flasks at the rate of one crystal per flask. Addition of more than one crystal per flask induced additional nucleation during the 24 h period as reported earlier by van Kreveld and Michaels (1965). The mouths of the Erlenmeyer flasks were covered with 'Parafilm' and the flasks were mounted on an angled plate, rotating at the rate of 10 rpm. The rotating plate was mounted on top of a multipurpose rotator that accommodated ten flasks. The moving condition of the crystals in the Erlenmeyer flasks allowed uniform crystal growth. The Erlenmeyer flasks used for the growth experiments were indented at one side (Plate 3-1) by heating it with a glass blowing torch to form a ledge on the inside of the flask. The ledge ensured uniform growth of crystals by keeping them tumbling inside the flask throughout the time period. After 24 h the crystals were removed from the Erlenmeyer flasks and dipped 2-3 times into absolute alcohol to remove excess lactose solution from the surface; the crystals were air dried and then weighed to calculate the weight increase.

The crystals described above were not discarded but used in further experiments. The crystals were washed with milli-Q water to roll back the increased weight and then healed in a supersaturated lactose solution (27.75 g anhydrous lactose/100 g water) under rotating condition for 24 h. Healing is an important step and was used to insure uniformity of the results.

All the above experiments were repeated with the truncated crystals to investigate the growth pattern of broken crystals. The crystals were truncated by breaking off their tips by micro manipulation. This resulted in some damage to the side faces of the crystal as well; therefore, to ensure uniform results, the truncated crystals were healed before the commencement of growth studies.

All the experiments were repeated at least three times with at least four replicate crystals grown in each case.

3.4 Growth of lactose crystals in model lactose solutions containing riboflavin

Model lactose solutions containing 10, 50 or 100 ppm riboflavin were prepared. Small lactose crystals (0.05-0.1g) were grown at 30°C for 24 h as described in the previous section. Precaution was taken that all glassware used in this experiment were wrapped with aluminum foil to prevent any destruction of riboflavin by light. After weighing the crystals, individual crystals were dissolved in 10 mL milli-Q water and the solution analyzed for riboflavin at wavelengths of 374 and 446 nm on an 8452A Diode Array Spectrophotometer (Hewlett Packard, Germany). The lactose crystals were not partially but, fully dissolved in 10 mL of milli-Q water to minimize any exposure of the solution to light. All experiments were repeated at least twice with at least three replicate crystals grown in each case.

3.5 Growth of crystals for mineral detection experiment

Crystals of very small size (0.001 to 0.002 g) were selected from the crystal mass (Section 3.1) and weighed accurately to 0.01 mg. The crystals were grown in model lactose solution containing 5 g salt/100 g water and 33.25 g anhydrous lactose/100 g water. After 96 h, the crystals were washed in absolute alcohol by dipping 2-3 times to remove any sticking impurities. The crystals were air dried and weighed. To be more precise in calculating the amounts of individual minerals in the crystal mass, the crystals were dissolved partially in 5 mL milli-Q water until the weight of the crystals had been reduced to their initial weights (weights of crystals before growth in model lactose solutions). The solutions were analyzed for the specific minerals by atomic absorption spectrophotometry, as discussed in Section 3.9.7. The undissolved portion of the crystal was air dried and weighed to calculate the actual weight of dissolved crystal. This procedure gave more precise results as compared to dissolving the whole crystal in water, which would otherwise have included the initial weight of crystal and made the results less reliable.

3.6 Calculation of crystallization velocity and measurement of the angles of the base of the lactose crystal

Good, clean, pyramid-shaped crystals with a smooth-surfaced base were selected for calculating crystallization velocity of lactose crystals and the angles of the base of the crystal. Most of the crystals had the base in the form of a perfect rhombus, making the calculations easier and more reliable. The crystals were weighed (0.10 to 0.30 g) and measured for the sides, height and the two diagonals of the base using a Vernier calliper (Mitutoyo, Japan). The crystals were then grown in model lactose solution containing 33.25 g anhydrous lactose/100 g water at 30°C. After 62 h, the crystal was taken out of the solution and the length of the sides, height and the two diagonals of the base were measured. Crystallization velocity (mg/m^2/min) was calculated assuming that all the growth took place at the base of the crystal only. Changes in the angle of the rhombus were calculated trigonometrically.

3.7 Solubility experiments

The objective of solubility experiments was to find a point where there was no increase or decrease in the weight of experimental single lactose crystals. Pre-weighed crystals were grown at 30°C for 18 h in model lactose solutions containing lactose concentrations in the range of 20-30 g lactose anhydrous/100 g water. The salt content in the model lactose solution was kept fixed (5 g/100 g water) for calcium lactate, potassium phosphate, lithium chloride, and magnesium sulfate. For calcium chloride, solubility was determined for 1, 2 and 5 g salt/100 g water concentrations. In the case of whey permeates, pre-weighed crystals were grown in permeate solutions containing lactose concentrations in the range of 20-35 g lactose anhydrous/100 g water for 18 h at 30°C.

Crystals grown in model lactose solutions and in permeates were removed after 18 h and dipped 2-3 times in absolute alcohol to remove excess lactose solution or any other impurities from the surface. The crystals were air dried and weighed to an accuracy of 0.01 mg on a Mettler AE 163 weighing balance (Mettler Instrumente, Ag., Zürich, Switzerland) to calculate the change in weight. Determination of lactose solubility point in the solutions was carried out by interpolation between the two values around the solubility point.

3.8 Preparation of permeate powders

3.8.1 Preparation of sweet whey permeate powder

Skim milk obtained in bulk from a commercial source was used as raw material for preparation of whey UF permeates. The skim milk (20 kg) was taken in a pilot-plant steam sterilized cheese making vat. The temperature of the skim milk (pH 6.7) in the cheese vat was maintained at 30±1 °C. Powdered rennet (Sigma Chemical Co., St. Louis, Mo), mixed with distilled water in the ratio of 1:4 (w/w), was added to milk (1 g/L milk) with constant agitation to ensure uniform mixing. The milk was allowed to stand at 30°C; after 45 minutes the curd was cut and the temperature raised to 50°C. The curd was cooked at this temperature for 30 minutes and filtered by passing through a combination of cheese cloth and wire mesh. The whey was ultrafiltered at 15° C through a DDS unit (de Danske Sukkerfabrikker, Nakshov, Denmark) model no. 20-0.36 LAB with polysulphone membranes (GR6P, molecular weight cut off 25,000 Da) and the permeate collected.

3.8.2 Preparation of acid whey permeate powder

The temperature of the milk in the steam sterilized vat was maintained at 50°C. Lactic acid (85%), diluted 1:4 (v/v) with distilled water, was added to milk with continuous stirring until the pH of the milk reached 4.6. At this pH value the casein portion of the milk separated out as curd leaving the whey which was collected. Two different ultrafiltration units, i.e., the

DDS unit described above and a Carbosep unit (SFEC Carbosep, Bollene, France) model no. S 7 with zirconium oxide membranes (molecular weight cut off, 20,000 Da) were used to ultrafilter acid whey. The acid whey UF permeates prepared by using different type of membranes, i.e., polysulphone and zirconium oxide, resulted in different concentrations of riboflavin and minerals. This is due to the differences in the pore size of the membranes used and due to the partial adsorption of riboflavin by carbon in the zirconium oxide membranes (Dunford and Jelen, 1991).

Both sweet and acid whey permeates were freeze dried in a Virtis freeze drier (Virtis C Inc., Gardiner, NY) and analyzed for their composition. The reason for the use of dried permeates was to insure that the reconstituted permeates contained the same lactose/water supersaturation, a very important criterion for the crystal growth study experiments.

3.9 Preparation of solutions and growth experiments in permeates

The method of preparation of permeate solutions differed from that for model lactose solution in respect to the quantity of permeate powder added to water. From the percentage of lactose present in the permeate powders (Table 4-9), exact quantity of powders containing 35 and 30 g lactose were calculated and dissolved in 100 g of milli-Q water at 90°C. There was no problem in dissolving the permeates unlike in the work of Smart (1988), where a concentration of 35 g anhydrous lactose/100 g water was not attainable due to gelling/ viscosity problem when using commercial whey and whey permeate powders.

Precipitation of salts in both acid whey samples occurred, and these had to be filtered out before addition of individual lactose crystals. The solutions were kept at 30°C for 18 h to ensure that most of the precipitation took place. The solutions were warmed to 50°C and quickly filtered through a Whatman No. 1 filter paper to obtain a clear filtrate. Aliquots of solutions (15 mL) in 50 mL Erlenmeyer flasks were placed in a 30±1°C temperature-controlled chamber to equilibrate the solution to 30°C. Pre-weighed lactose crystals were grown for 18 h at 30°C in acid (DDS and Carbosep) and sweet whey permeates according to the procedure described earlier (Section 3.2.1). All experiments were repeated at least three times with at least four replicate crystals grown in each case.

3.10 Analytical procedures

3.10.1 pH

The pH of the solutions was measured using a Corning 150 pH meter (Corning Science Products, Essex, England).

3.10.2 Moisture content

The moisture content of the dry permeate powders and the salt precipitates was determined using a microwave moisture analyzer (CEM Corp., Indian Trail, NC). A sample aliquot was distributed evenly on a filter paper and dried for 5 minutes. The result was displayed directly as % moisture.

3.10.3 Protein

Protein determination (Total Nitrogen × 6.25) of all the samples (permeates and the salt precipitate obtained during crystal growth study in permeate) was carried out by a Leco FP-428 nitrogen determination system (Leco Corp., St. Joseph, MI) using the standard procedures as available in the Department of Agricultural, Food and Nutritional Science, University of Alberta. The principle of protein determination is described in company literature (anonymous, undated).

3.10.4 Lactose

The lactose content of the permeates was determined directly by HPLC using maltose as an internal standard. The reconstituted permeate sample was first passed through 45μm millipore membranes (Millipore Corp., Bedford, MA) and then through a Sep-Pak C_{18} cartridge (Waters Associates, Inc., Milford, MA) before injecting into the HPLC unit (Shimadzu Corp., Kyoto, Japan). The system was operated at a column temperature of 65°C and a flow rate of 0.7mL/min, using 0.009 N H_2SO_4 as the mobile phase. A Shimadzu RID-6A refractive index detector (Shimadzu Corp., Kyoto, Japan) was used to measure the lactose. An Aminex HPX-87H cation exchange column with a cation H^+ Microguard Precolumn (Bio-Rad laboratories, Mississauga, ON) was used for separation.

Indirect determination of lactose was also carried out by difference by quantitative analysis of other major components in the permeate powder and subtracting them from 100. The differences in results obtained by the two methods were relatively small. The values reported (Table 4-9) are the average values of both results.

3.10.5 Lactate

Lactate in the permeate powders was determined using an HPLC. A standard solution was prepared using calcium lactate (10 ppb concentration). Both a Shimadzu RID-6A refractive index detector (Shimadzu Corp., Kyoto, Japan) and a Toso-Haas TSK-6040 UV-VIS detector (Hadley Tekscience, Oakville, ON) set at 220 nm were used to measure the lactate content of samples.

3.10.6 Ash

Ash was determined in all permeate powders and in the precipitates from reconstituted permeate solutions. A sample of permeate powder or precipitate was weighed accurately in a pre-weighed and dried porcelain crucible. The porcelain crucible was placed in a muffle furnace (Thermolyne Corporation, Dubuque, Iowa) at 550°C. After 18 h the dish was cooled in a desiccator and weighed again to calculate the ash content (AOAC, 1990).

3.10.7 Mineral detection by atomic absorption spectrophotometry

Standards containing 4 ppm calcium, 2 ppm potassium and 0.5 ppm magnesium were prepared in milli-Q water using calcium chloride, potassium phosphate and magnesium sulfate, respectively.

The solutions containing the dissolved crystals were analyzed for the presence of respective minerals on a 4000 atomic absorption spectrophotometer (Perkin-Elmer, Norwalk, Conn.). The readings were taken at 422.7 nm, 766.5 nm, 285.2 nm wavelengths for calcium, potassium and magnesium, respectively. A nitrous oxide-acetylene flame was used for the detection of calcium and magnesium, while an air-acetylene flame was used for the detection of potassium.

3.11 Statistical analyses

The data for growth rates of lactose crystals in model solutions were analyzed statistically for test of significance using UNIQUE sum of squares with the help of the SPSS 6.1 statistical package (SPSS Inc., Chicago, IL). However, the data for the lactose solubility experiments and for the growth rates of lactose crystals in permeate solutions were analyzed with the help of an Excel 5.0 package (Microsoft Excel, Microsoft Corp., Redmond, WA).

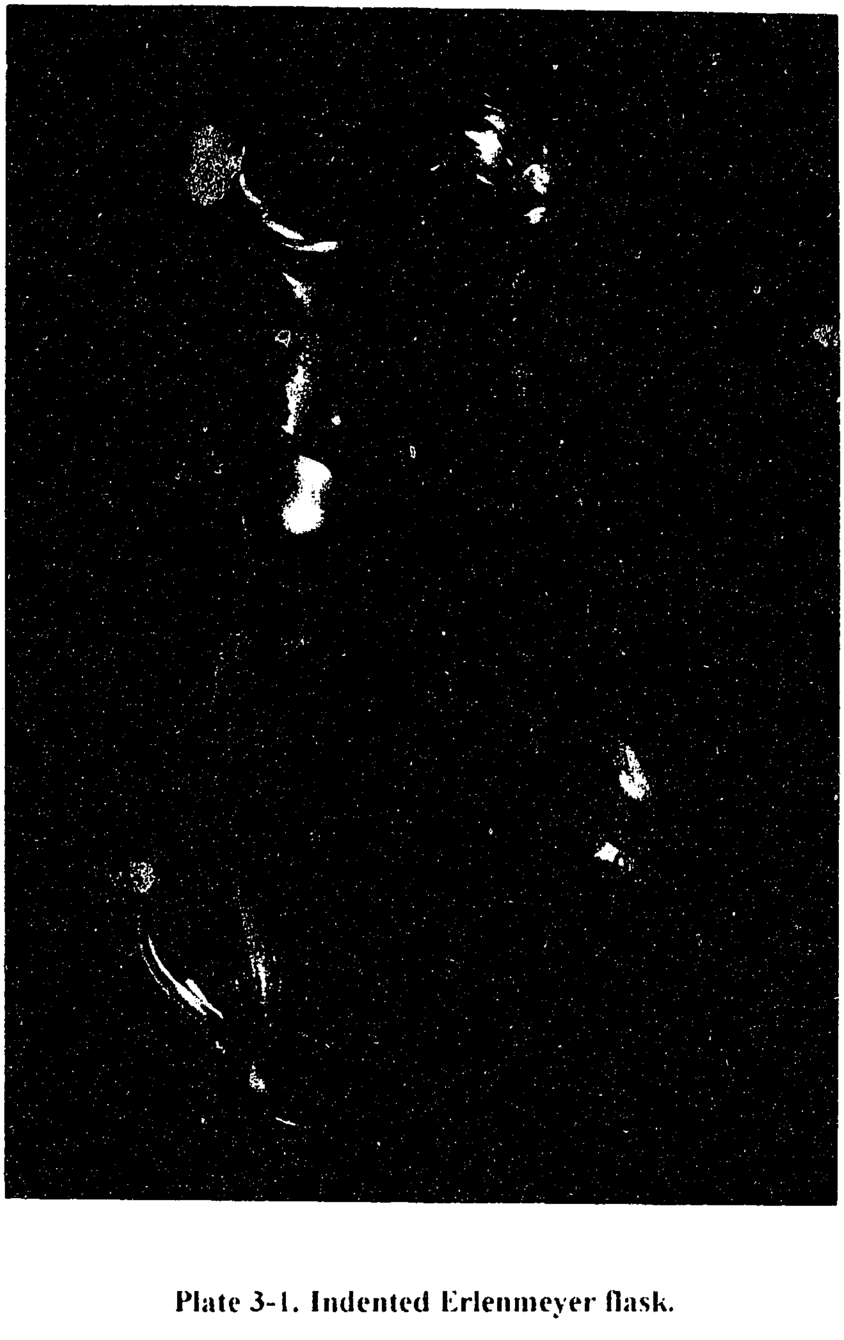

Plate 3-1. Indented Erlenmeyer flask.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Observations on the growth of lactose crystals in pure lactose solutions

This study was based on the experiments at low concentration (33.25 g anhydrous lactose/100 g water) as the effects of additives are more apparent at low supersaturation (van Kreveld, 1969) with slow rates of crystallization (Nickerson and Moore, 1974b). In addition, the supersaturation used the present study resulted in sufficient measurable crystal growth without formation of any false grains in the solution, even after 24 h. The development of false grains or additional nucleation had to be taken into account as it might have influenced the growth experiment in two ways. First, it would have resulted in a decrease in the supersaturation of the solution, thus affecting the growth rate of lactose crystals. Visser (1982) suggested that the relative reduction in the supersaturation of solution by the formation of false grains would be no more than 1% in most unfavourable case. According to his assumption this slight change (1%) in the supersaturation (equals to 0.22 - 0.27 g anhydrous lactose/100 g water in our experiments; Figs. 4-3 to 4-10) of the solution would not result in any noticeable change in the growth rate of lactose crystals. However, we found that even a small difference (0.25 g anhydrous lactose/100 g water) in the lactose/water concentration produced a wide aberration in the growth rate of lactose crystals (Figs. 4-3 to 4-10). Secondly, the sticking of false grains (additional nucleation) on the individual crystal would have resulted in damage to the crystal, which would directly influence the growth rates.

During our preliminary experiments, it was observed that presence of more than one crystal per Erlenmeyer flask resulted in an additional nucleation. With an increase of the number of crystals per flask, a proportionate increase in additional nucleation was observed.

Van Kreveld and Michaels (1965), had earlier observed that more than one crystal per flask induced additional nucleation. This observed phenomenon might be due to an increase in the collision of lactose molecules in solution, created by the forces of attraction and repulsion between the [illegible] molecules. Therefore, in further experiments only one crystal was added per flask; this resulted in no additional nucleation. Presumably, at the studied supersaturation all the lactose molecules in the solution were attracted only towards the added single crystal.

For the growth studies both truncated and untruncated lactose crystals were used in separate experiments (Section 3.3 1). The purpose of using truncated crystals was to confirm that the broken tip of the crystals would not grow back. To achieve consistent results, all the truncated and the untruncated crystals were healed prior to the growth experiments. When the two pieces of the truncated lactose crystal were grown separately in model lactose solutions, it was observed that the truncated part of the crystal never grew the tip back; however, the growth of the broken tip resulted in a new lactose crystal. Hence, it was confirmed as suggested by van Kreveld and Michaels (1965) that the original nucleus of a lactose crystal is present at the tip of the crystal and growth of the lactose crystal is in one direction, i.e., from top to bottom, with most of the growth taking place at the base of the crystal in comparison to other faces. The crystallization process of lactose is slow as most of the growth takes place only at one face of the crystal (i.e., the base) and this seems to be the main reason why industrial crystallization of lactose is much slower process (Nickerson, 1970; Jelen, 1973a; Visser, 1980), compared to sucrose which exhibits multidirectional growth.

To correct the literature about the slow growth of lactose crystals it became important to determine the crystallization velocity of lactose in pure solution. The mean crystallization velocity of the nine selected lactose crystals grown at 30°C in pure lactose solutions (33.25 g anhydrous lactose/ 100 g water) was found to be 342 mg/m^2/min (Table 4-1). In this work, only the area of the base was considered in calculating the crystallization velocity. The results reported here are four to five times higher than those reported earlier by Jelen (1972) and Thurlby (1976). While calculating the crystallization velocity Jelen (1972) and Thurlby (1976) took into consideration all five main faces of the crystal, assuming that growth on all faces of the crystal occurs at the same rate. Therefore, this error resulted in much higher surface area measurements or a much lower calculated crystallization velocity. However, it must be assumed that negligible growth takes place on the sides with most of the growth taking place at the base of the crystal as discussed above.

Michaels and van Kreveld (1966) and van Kreveld (1969) suggested that the low crystallization rate of α-lactose in comparison to that of sucrose is due to the retarding effect of β-lactose present in the solution. Haase and Nickerson (1966) and Tweig and Nickerson (1968) reported that mutarotation, which produced β- lactose, reduced the crystallization rate only when crystallization was exceptionally fast. The results of the present study show that the mean crystallization velocity of a lactose crystal (342 ± 58 mg/m^2/min) is very similar to that of a sucrose crystal (255 mg/m^2/min) at approximately the same absolute supersaturation (10 g of excess solute/100 g water, above the solubility point at 30°C) (Hirschmuller, 1953). Although the crystallization velocities of lactose and sucrose appear to be very similar when compared at same absolute supersaturation (as defined in Section 2.2), the rate of crystallization of sucrose is much higher as compared to that of lactose. This is true because sucrose crystal growth occurs simultaneously on at least two and possibly on all six faces of the crystal, while lactose crystal growth occurs only at the base of the crystal. Thus, due to the different mechanics of crystal growth for sucrose and lactose, the lactose crystal growth is much slower than that of sucrose.

To obtain uniform results in all the growth experiments, particular care was taken to ensure that all the lactose crystals were healed (Section 3.2.1) in lactose solution of low concentration (27.75 g anhydrous/100 g water) prior to the growth experiments, as lactose crystals washed with milli-Q water showed higher initial growth rates in agreement with the earlier reports (van Kreveld and Michaels, 1965; Jelen and Coulter, 1973a; Nickerson and Moore, 1974b). It can be hypothesised that, when a lactose crystal is washed in water, all the five faces of the crystal dissolve at a certain rate depending upon its surface area. This partial dissolution of lactose crystal results in loss of lactose molecules from the surface of the crystal mass. The mechanism of lactose crystal dissolution is different from its growth, where most of the growth takes place at only one face, i.e., base, of the crystal. Therefore, even slight variation in the surface area of the lactose crystals resulted in big deviations in the crystal dissolution rates with respect to the growth rates (Figs. 4.3 - 4.10). When a lactose crystal previously washed with water is added to a supersaturated solution it quickly incorporates the lost lactose molecules resulting in an increased initial growth rate. Once the crystal is fully recuperated the growth proceeds at a normal rate, with most of the growth taking place at the base of the crystal only. It was observed in our experiments (Figs. 4.3 - 4.10) that at the same difference of lactose/water concentration, the rate of lactose crystal dissolution in an undersaturated solution was much faster as compared to the growth rate in a supersaturated solution.

When individual lactose crystals were grown in pure lactose solutions (control) the crystals did not seem to change their shape. It is also interesting to note that the morphology of the base of the crystal did not change when grown in the control solution. The crystals that were flat at the base remained flat while those that had an irregular base maintained the same features (Plates 4-1 and 4-2). The lactose crystals used in the experiment had a rhombus-shaped base. The angles of the rhombus (Fig. 4-1) both before and after growth for 62 h did not change appreciably (Table 4-2). However, it is interesting that in most cases there was a very slight increase in angles 1 and 3 and decrease in angle 2 and 4, but, the variations are too slight and may be considered as an experimental error. Measurement of the flat part of the broken tip of the individual lactose crystals before and after the growth revealed no change (data not shown); if any growth occurred it was too little to measure.

4.2 Effect of salts on the growth rate of lactose crystals

The presence of added salts either increased or decreased the growth rate of lactose crystals (Fig. 4-2). This agrees with earlier observations made by other researchers that the weight of lactose crystals may increase, decrease or remain unchanged depending on the nature and concentration of the additive (Michaels and van Kreveld, 1966; Jelen and Coulter, 1973b; Smart, 1988; Smart and Smith, 1991; Guu and Zall, 1991). The results showed good reproducibility and good linearity with time both for truncated and untruncated crystals. The similarity of results for the truncated and untruncated crystals further supported the unidirectional growth hypothesis. Results from truncated and untruncated crystals were combined for analyzing the results.

Without the addition of any salt, an average increase of 8.87% in weight occurred when the individual lactose crystals were grown at 30°C for 24 h in model lactose solutions (Fig. 4-2). This result is slightly lower than 10.77% as recalculated from the data of Jelen and Coulter (1972), who reported weight increase in terms of (g) per h, while the present results are in terms of percentage. The method of determining the growth rate in terms of weight increase (g) per h does seems to be less accurate as crystals of larger surface area would result in a higher growth rate compared to the ones with comparatively smaller surface area. Thus, there is a possibility of variation in the results amongst the crystals of different surface areas grown under the same conditions. Reporting the growth rate in terms of percentage took into consideration the limitation of slight variation in the surface area of the crystals.

With the addition of salts to the model lactose solutions the pH of the solutions changed from the initial pH of 4.7 (Table 4-3). Herrington (1934b), Tweig and Nickerson (1968), Smart (1988), Smart and Smith (1991) considered that pH is an important factor in lactose crystallization, the effect being due to the acceleration of lactose mutarotation by high acid or alkali conditions (Nickerson and Moore, 1974b). In this study, the change in the pH was more or less independent of the amounts of salts added to the model lactose solutions. However, addition of Ca Lactate and K_2HPO_4 increased the pH towards the basic side at all the studied salt/water concentrations (Table 4-3). It could be concluded that the pH of the solutions was not an important factor in alteration of the lactose crystal growth rates.

The results of these experiments showed that, among the salts studied, LiCl lead to the greatest increase in the growth rate of lactose crystals (Fig. 4-2). As the concentration of LiCl increased from 1 g to 5 g/100 g water, a consistent increase in the growth rate from 14.56% to 40.75% was observed. Although LiCl is not present in milk, it was used in our experiments as Li^- was reported to reorganize the structure of water due to its high charge density (von Hippel and Schleich, 1969) and consequently it might affect the growth rate of the lactose crystal.

Similarly, as the concentration of $MgSO_4$ was increased from 1 g to 5 g/100 g water, a consistent increase in the growth rate from 15.56% to 28.87% was observed (Fig. 4-2). These results contradict those reported by Smart (1988), who in a mass seeding experiment observed no significant effect of $MgSO_4$ on the growth rate of lactose crystals. Also, as the concentration of Ca lactate increased from 1 g to 5 g/100 g water, a linear increase in the growth rate from 9.66% to 20.41% (Fig. 4-2) was observed. These results are consistent with those results of Smart (1988) and Smart and Smith (1991) who showed that there is an increase in the growth rate of lactose crystals despite the form of lactate present. In contrast Jelen and Coulter (1973b) and Nickerson and Moore (1974b) found that lactic acid inhibited lactose crystallization and Michaels and van Kreveld (1966) reported little effect of lactic acid on crystallization of lactose.

The addition of $CaCl_2$ resulted in a growth rate pattern quite different to the one observed with addition of other salts used in this work. The addition of 1 g to 2 g $CaCl_2$/100 g water increased the growth rate from 11.78% to 21.40%. With further increase up to 5 g/100 g water there was a decrease in the growth rate to 14.84% (Fig. 4-2). The growth rate pattern of our experiment agrees with that of Jelen and Coulter (1973b). However, it was not possible to compare the results directly as the temperature of study used by Jelen and Coulter (1973b) was 50°C, while, the present experiment was based on crystal growth at 30°C. The difference in the growth temperatures resulted in different growth rate with higher temperature favouring higher growth rate and vice versa. Smart (1988), reported no detectable effects of $CaCl_2$ in their mass seeding experiment when present at the rate of 1 and 10 g/ 100 g solution. It is important to note that Smart (1988) studied the effect of $CaCl_2$ at only two salt concentrations (1 and 10 g/100 g solution) and missed out the whole range between the two values. With the confirmation of the results of Jelen and Coulter (1973b), it seems that in the growth measurement studies, the different techniques used (i.e., mass seeding experiment and single crystal growth) might give different results. Additionally, low supersaturation (Jelen and Coulter, 1973b) or high supersaturation (Smart, 1988) might also be a criterion.

K_2HPO_4 was the only salt that decreased the growth rate consistently from 10.84% to 4.31%, with increasing salt concentration from 1 g to 5 g/100 g water (Fig. 4-2). The growth rate of lactose crystal in the solution containing 1 g salt/100 g water was slightly higher than that in the control solution. As the level of salt was increased to 3 g and 5 g salt/100 g water, the growth rate declined below the control values. The pH of the lactose solutions containing different amounts of added K_2HPO_4 (1,2,3,5 g/100 g water) were virtually the same but different from the control (Table 4-3). Thus, it seems that pH was not an important factor in deciding the growth rate of lactose crystals at different concentrations of K_2HPO_4. Jelen and Coulter (1973) have earlier observed a slight increase in the growth rate of lactose crystal at low concentration of KCl; with further increase in the salt concentration they reported a reduction in the growth rate. Similarly, Smart (1988) observed a reduction in the growth rate of lactose crystals with an increase in the concentration of KCl, and their data (Jelen and Coulter, 1973; Smart, 1988) are comparable to the results of van Hook (1959), who reported KCl as a growth retarding agent in sucrose crystallization. Gómez (1979) had reported a negative effect of K^+ on the nuclei formation and on the growth of sucrose crystal.

As shown in Fig. 4-2, the differences in the growth rates of the lactose crystals were significant ($p < 0.001$) for each salt solution in comparison to the control. Significant differences were also found within the same salt solution at different salt concentrations

(Table 4-4), confirming that the salt concentration is a significant variable determining the growth rate of lactose crystals. It can be concluded that different salts behave differently in determining the growth rate of lactose crystals and it cannot be generalized that a particular type of salt group would either increase or decrease the growth rate. Table 4-5 shows the standard deviations for all lactose crystals grown in model lactose solutions with or without the addition of salts, indicating reasonably good reproducibility of the results obtained.

It can be hypothesised that the altered growth rates of the lactose crystals in model lactose solutions with added salts might be either due to the incorporation of salt in the lactose crystal mass or due to the effect of salt on the substrate (water). Therefore, the reasons for altered growth rates were investigated further.

4.2.1 Adsorption of minerals in the lactose crystal matrix

For a general crystallization process, Botsaris (1982) has classified the impurity adsorption models into two groups; those in which the impurities are not adsorbed into the crystal mass and the ones where impurities are incorporated into the crystal mass. Negligible amounts of Ca^{++}, Mg^{++} and K^+ were found to be present in the crystal matrix when grown in model lactose solutions containing specific salts ($CaCl_2$, Ca Lactate, $MgSO_4$ and K_2HPO_4) at a concentration of 5 g salt/100 g water (Table 4-6). The results are in agreement with those of Jelen and Coulter (1973) who found no measurable amount of Ca^{++} in lactose crystals and concluded that the entire weight increase of lactose crystal consisted of pure lactose only. Smart and Smith (1991) obtained mixed results in their mass seeding experiments; they reported association of low levels of Li_L(+)-lactate, NaH_2PO_4, Na_2SO_4 and $CaCl_2$ with the mass of crystals when added individually to the model lactose solution, while high levels of calcium were found to be associated with the crystals when various salts were added in combination. They also reported very little incorporation of lactate and sulphate into lactose crystals, while phosphate was found to be associated to a certain extent. Visser (1980) reported that the acid impurity present in pharmaceutical grade lactose is incorporated in the crystal lattice and is not a surface contaminant.

The addition of some salts to the model lactose solution seemed to change the shape of the crystal. Individual lactose crystals increased preferentially in length when grown in model lactose solutions containing LiCl (Plate 4-3) while a preferential increase in the width or thickness of the crystals was observed in the presence of $MgSO_4$ (Plate 4-4). For other salts, minimal change in crystal shape was observed. Smart and Smith (1991) also observed major morphological changes in lactose crystals when grown in the presence of galactosyl lactose. The crystals changed to a more needle-like shape rather than maintaining the normal tomahawk shape of the lactose crystal. No photographs were presented by them to strengthen their claim.

4.2.2 Effect of salts on the solubility of lactose

Lactose solubility in a solution can be determined within a short period of time (~18 h) by the new method developed in this work. By using this new method quite precise results can be obtained and it is possible to accurately determine the solubility of lactose in a solution. Previously published methods (e.g. Hunziker and Nissen, 1926) were quite tedious and time consuming involving either the determination of the maximum amount of lactose which will dissolve in or crystallize out from solutions of varying graduated concentrations, or calculating the lactose solubility by determining the total solids in the filtered solutions or by the gravimetric Fehling's solution method. Mathematical equations have also been derived to calculate the solubility of lactose (Thurlby and Sitnai, 1976). The equations can be used to calculate the lactose solubility in the temperature range of 0-90°C.

It was observed that addition of salts to the model lactose solutions resulted in a change in the lactose solubility (Table 4-7). The estimated solubility of lactose at 30°C in pure lactose solution (without any salt) was found to be 24.44 g anhydrous lactose/100 g water.

The estimated solubility points were determined by interpolation between the two values (weight gain and weight loss) around the lactose solubility point (Fig. 4-3). The difference in % lactose supersaturation (from pure lactose solution) caused by addition of specific salts to the model lactose solutions of constant lactose/water composition were calculated by the following formula:

$$A = (B - C)$$

where,

A = Difference (i.e., increase or decrease) in % lactose supersaturation from control or from the nominal supersaturation of the solution..

B = Lactose supersaturation (%) in model lactose solution on addition of salt.

C = Lactose supersaturation (%) in pure lactose solution.

The addition of LiCl (5 g/100 g water) to the model lactose solution resulted in a maximum decrease in the lactose solubility among all the studied salts. The estimated lactose solubility was found to be 21.98 g anhydrous lactose/100 g water at 30° C (Table 4-7) resulting in a difference of 15.23% lactose supersaturation from control. It is well known (von Hippel and Schleich, 1969) that some ions, including Li^+, rearrange the structure of water resulting in a decrease in the total amount of free water molecules available for solutes to dissolve. According to this hypothesis, a reduction in total amount of free water molecules available would result in a decrease in lactose solubility. The growth profile of lactose crystals in the vicinity of lactose solubility point for a model solution with 5 g LiCl/100 g water, is illustrated in Fig. 4-4.

The addition of $MgSO_4$ (5 g/100 g water) or Ca Lactate (5 g/100 g water) also decreased the lactose solubility to 23.03 g and 23.86 g anhydrous lactose/100 g water, respectively, at 30°C. The difference in the % lactose supersaturation due to the addition of these salts was calculated as 8.33% for $MgSO_4$ and 3.31% for Ca Lactate. The growth profiles of lactose crystals in the vicinity of the solubility points for these salts are illustrated in Figs. 4-5 and 4-6.

The solubilities of lactose in solutions with the additions of $CaCl_2$ (1, 2, 5 g/100 g water) were found to be 24.55, 24.64 and 24.25 g anhydrous lactose/100 g water, respectively at 30°C. Here the results of lactose solubilities were not consistent with the growth rates and such discrepancies could not be explained except possibly by the relatively large experimental error. The growth profiles of lactose crystals around the solubility points are illustrated in Figs. 4-7, 4-8 and 4-9.

The addition of K_2HPO_4 to the model lactose solution increased the lactose solubility to the maximum extent among the studied salts, thus reducing the effective supersaturation of the solution (10.77%) (Table 4-7). The solubility of lactose with the addition of K_2HPO_4 (5 g/100 g water) was found to be 26.54 g anhydrous lactose/100 g water at 30°C (Table 4-7). The growth profile of lactose crystals around the solubility point is illustrated in Fig. 4-10.

All of the salts studied either increased of decreased lactose solubility values in the model lactose solutions. Lactose solubility values seemed to relate directly to the growth rates of the lactose crystals (except in case of $CaCl_2$ values). Generally, however, it can be said that changes in lactose solubility with the addition of salts could be one of the reasons for the increased or decreased growth rates observed due to changes in the supersaturation of the solutions. A change in the supersaturation of the solution would affect both the nucleation and growth of the crystal. Hence, it is very important to know the solubility of lactose in an experimental solution, particularly for mass seeding experiments (Smart, 1988; Smart and Smith, 1991; Guu and Zall, 1991) where the results would be affected to a larger extent due to the change in the supersaturation. In such experiments nucleation has an important bearing on the results as the yield is determined by the amount of lactose crystal produced in the solution. Nucleation in model lactose solutions containing different salts will take place at different lactose concentrations due to different supersaturation values.

"Why crystals of sodium thiosulphate grew much faster than the borax crystals at the same temperature and supersaturation" or "Why crystals of nickel sulphate grew at the same rate as that of ammonium alum crystals" could not be explained based on relevant equilibrium solubilities and the reasons remain obscure (Mullin, 1979). It has been assumed by many researchers (Jelen and Coulter, 1973; Guu and Zall, 1991) that the minerals present in whey probably influence the solubility of lactose in the supernatant liquor during crystallization, which may partially answer the question of different growth rates in the presence of impurities. However, the authors did not present any experimental evidence of change in the solubility values. Nickerson and Moore (1974a) reported that a decrease in the solubility of lactose in the presence of sucrose resulted in an increase in the initial supersaturation. An increase in the supersaturation of the solution led to an increase in the growth rate of lactose crystals.

Visser (1984) studied the influence of some inorganic salts of milk and whey (KCl, NaCl, $MgCl_2$ and $CaCl_2$) on the growth rate of the base of lactose crystals grown in pharmaceutical grade lactose solutions. He reported that the results were influenced by the increase or decrease in lactose solubility due to salts. However, no lactose solubility values were given for the respective salts, rendering the conclusion somewhat suspect. The data were also not supported by any experimental method to determine the lactose solubility in the solution. Visser (1984) also reported that the growth rate of the base of the lactose crystal increased with an increase in salt concentration for all the studied salts. However, he found that NaCl had a small and $MgCl_2$ had a large solubility depressing action on lactose, while $CaCl_2$ and KCl were reported to increase the lactose solubility to a smaller and a much higher extent, respectively. In light of the observed increase in the growth rate for all the above studied salts, it seems that all the salts only decreased the lactose solubility with none of the salts increasing the solubility.

In another study, Michaels and van Kreveld (1966) ruled out the possibility that the addition of alcohols to the system could depress the solubility thereby increasing the lactose crystal growth rate. However it appears that it was only due to change in the solubility of lactose that van Kreveld and Michaels, (1965) and Olano et al., (1977b) achieved higher yields of lactose by the addition of acetone or alcohols.

4.3 Effect of riboflavin on the growth rate of lactose crystals

Riboflavin had no measurable effect on the growth rate of individual lactose crystals when grown in model lactose solutions containing 10, 50 or 100 ppm riboflavin (Fig. 4-11). The results at 10 ppm riboflavin concentration agree closely with those of Smart (1988) and Michaels and van Kreveld (1966) who found no effect of riboflavin when added at a final concentration of 10 - 20 ppm. However, the results at 50 and 100 ppm in the present study are not in full agreement with those of van Kreveld (1969), who reported slight effect of riboflavin on the growth of lactose crystals. The method used by van Kreveld (1969) involved microscopic observation of the different faces of the crystal, while in this work weight increase method was used to determine any effect of impurities on the lactose crystal. This may partially account for the differences between the present study and those of van Kreveld (1969).

Negligible amounts of riboflavin were found to be present in the solutions containing dissolved lactose crystals (Appendix 1) as discussed earlier in Section 3.4. Previously, Smart and Smith (1991) reported negligible amounts (<1 ppm) of riboflavin entrapment in the crystal when present at levels of 20 ppm in the solution.

4.4 Growth of lactose crystals in whey permeates

Individual lactose crystals were grown in three different reconstituted whey UF permeates, i.e., sweet whey permeate (B) and acid whey permeate (D) prepared by using DDS unit and acid whey permeate (C) prepared by using Carbosep unit (Section 3.8). The composition of the dried permeate powders used for preparation of reconstituted permeates are given in Table 4-8. All the three permeate powders were prepared under different conditions and their compositional analyses shows wide variations in the levels of lactose and whey impurities. The different methods for manufacture of whey UF permeate were deliberately chosen so as to compare any possible effects of particular/combined whey impurities on the lactose crystal growth.

In the preliminary work, it was observed that there was precipitation of water-insoluble salts in both acid whey permeate solutions; the compositional analyses of the precipitates are given in Table 4-10. Growth of individual lactose crystals in acid whey permeate solutions without removal of the precipitate resulted in sticking of the precipitate on to the surface of the lactose crystals. The precipitate covered the crystal surface and could not be cleaned by simple methods, such as washing with water or alcohol, leading to unreliable results. The amount of precipitate that covered the crystal surface varied with individual crystals. The growth of lactose crystals in presence of precipitated salts resulted in very high increase in weight due to sticking of salts on the crystal surface (Plate 4-5). Hence, to standardize the growth rate of lactose crystals in acid whey permeate solutions it became necessary to filter out precipitates prior to the addition of individual lactose crystals. When lactose crystals were grown in precipitate free acid whey permeate solutions no further precipitation of salts was observed. Only the data obtained by growing the crystals in precipitate free acid whey permeate solutions were used for analysis.

No precipitation of salts was observed in the sweet whey permeate solution prior to the addition of individual lactose crystals. However, after the growth of lactose crystals in sweet whey permeate a slight suspension in the solution was observed. The suspension was filtered out and analysed (Table 4-10). Also, no problems with sticking of any suspensions was observed upon growth of lactose crystals in sweet whey permeate solution.

The mean growth rates of lactose crystals grown in the acid and sweet whey permeate solutions for 18 h were found to be in the range of 1.0 - 2.2% and 4.8 - 6.0% for samples with lactose concentrations of 27.75 and 33.25 g anhydrous lactose/100 g water, respectively (Fig. 4-12). Because of the large variability of the experimental data as indicated by large standard deviations the differences in the growth rates observed between the control and the three permeate samples were not statistically significant.

The large standard deviation in the results could be due to several factors beyond our control. It was very hard to determine the exact amount of lactose concentration in the dried permeate powders. Analysis of lactose in dried permeate powders using two different methods, i.e., HPLC and difference of quantitative analysis, showed slight difference, but, very close results (Table 4-9). Hence, the average of both values were considered for calculations in preparation of reconstituted permeate solutions.

Secondly, there was a wide variation in the amount of impurities [100-(%lactose + %moisture)] present in the three permeate solutions, i.e., 15.63%, 23.17% and 36.82% for sweet whey permeate (B), acid whey permeate (C) and acid whey permeate (D), respectively. Because the results were the same regardless of the impurity level (36.82% vs 15.63%), the lactose crystal growth does not seem to be related to these differences.

Thirdly, the removal of precipitated salt from both acid whey permeate solutions prior to addition of individual lactose crystals probably would have changed the impurity and possibly the supersaturation levels in the permeate solutions.

The solubility of lactose in sweet whey permeate (B), acid whey permeate (C) and acid whey permeate (D) solutions was found to be 25.67, 25.00 and 25.09 g anhydrous lactose/100 g water, respectively. However, in case of the pure lactose solution the solubility of lactose was found to be 24.44 g anhydrous lactose/100 g water (Section 4.2.2). The differences in the lactose solubility could be because of the presence of different amounts of impurities in the permeate solutions. The growth profiles of the lactose crystals grown in permeate solutions around the lactose solubility point are illustrated in Figs. 4-13, 4-14 and 4-15.

It can be concluded that addition of salts to the model lactose solutions alter the lactose solubility or the supersaturation of the solutions. This information could be of particular importance for mass seeding experiments as even a slight change in the lactose solubility would affect the results greatly. Growth experiments with individual lactose crystals, particularly in both acid whey permeates, were complicated by the precipitation of salts, which covered the crystal like a scale formation. No visible scale formation was observed when the salts were filtered out prior to the addition of individual lactose crystals. Hence, it can be concluded that to increase the lactose crystallization efficiency, high levels of minerals and riboflavin present in the ultrafiltered permeate should be removed prior to crystallization. This would also ensure lactose crystals of relatively high purity.

Table 4-1 Crystallization velocity of lactose crystals

Crystal Number	**Crystallization Velocity (mg/m^2/min)**
1	322
2	381
3	412
4	359
5	391
6	231
7	319
8	381
9	285
Mean Velocity	~ 342
Standard Deviation (±)	~ 58

Table 4-2 Change in the angles* at the base of lactose crystals grown in model lactose solution

Crystal No.	Angle (°)							
	1		2		3		4	
	O	N	O	N	O	N	O	N
1	108.8	110.2	68.9	69.8	114.6	110.2	67.7	69.8
2	111.4	116.8	68.6	63.2	111.4	116.8	68.6	63.2
3	110.0	111.5	70.0	68.5	110.0	111.5	70.0	68.0
4	111.2	112.7	68.8	67.3	111.2	112.7	68.8	67.3
5	70.0	73.3	110.0	106.7	70.0	73.3	110.0	106.7
6	70.1	69.7	109.9	110.3	70.1	69.7	110.0	110.3
7	117.5	114.6	62.5	65.4	117.5	114.6	62.5	65.4
8	99.8	103.6	70.2	66.9	114.8	118.8	75.2	70.8
9	110.2	112.9	69.8	67.1	110.2	112.9	69.8	67.1

where, O= Angle before the growth of the lactose crystal.

N= Angle after the growth of the lactose crystal.

(*) For a definition of the angle, see Fig. 4-1.

Table 4-3 Influence of added salts on the pH values of model lactose solutions (*)

Salt	**Concentration of salt (g/100 g water)**			
	1	**2**	**3**	**5**
$CaCl_2$	4.3	4.4	4.5	4.8
$MgSO_4$	4.7	4.9	4.7	4.7
Ca Lactate	6.3	6.4	6.4	6.4
LiCl	4.3	4.3	4.3	4.2
K_2HPO_4	8.6	8.6	8.7	8.8

(*) The pH of the control solution without added salt was 4.7

Table 4-4 Analysis of variance on the growth rates of lactose crystals in the presence of added salts

Test of significance using UNIQUE sum of squares

Source of Variation	**Sum of Square**	**Degree of Freedom**	**Mean Square**	**F - value**	**Significance of 'F'**
Groups*	5990	5	1198	132	p < 0.001
Weight**	815	3	271	30	p < 0.001
Group by weight	2285	12	190	21	p < 0.001
Model	10206	20	510	56	p < 0.001

* Group represents the five salts and the control.

** Weight represents the weight of salts used (1 g, 2 g, 3 g and 5 g).

Table 4-5 Growth experiments with lactose crystals grown in model lactose solutions (with or without added salts).

Salt	Salt Concentration (g/100 g water)	Mean (% weight gain/24 h)	Standard Deviation (±) (% weight gain/24 h)
Control	-	8.87	3.24
Potassium Phosphate	1	10.84	2.16
	2	10.83	2.84
	3	7.77	0.93
	5	4.31	0.86
Calcium Chloride	1	11.78	2.22
	2	21.40	3.04
	3	21.00	3.41
	5	14.84	1.67
Calcium Lactate	1	9.66	1.23
	2	15.05	2.89
	3	17.34	4.1
	5	20.41	2.71
Magnesium Sulfate	1	15.56	4.62
	2	20.29	2.46
	3	23.31	2.45
	5	28.87	4.3
Lithium Chloride	1	14.58	1.8
	2	28.82	4.19
	3	31.47	3.84
	5	40.75	4.79

Table 4-6 Mineral entrapment (%) in lactose crystals grown in model lactose solutions containing specific salts (5 g/100 g water)

Mineral	**Salt**	**% weight***
Calcium	$CaCl_2$	0.0049
Calcium	Ca Lactate	0.0015
Magnesium	$MgSO_4$	0.0055
Potassium	K_2HPO_4	0.0705

* Relative to the weight increase of experimental lactose crystal (see Section 3.5 for full details)

Table 4.7 Effect of salts on lactose crystal growth rate, lactose solubility and supersaturation in model lactose solutions (with or without salts).

Lactose solution with	Salt conc. (g/100 g water)	% Mean weight gain	SD ± (%)	Estimated solubility point (g anhydrous lactose/ 100 g water)	Difference in % supersaturation (from control)*
Control (no salt)	-	8.87	3.25	24.44	-
Potassium phosphate	5	4.31	0.86	26.54	-10.77
Calcium chloride	1	11.78	2.22	24.55	-0.61
	2	21.40	3.04	24.67	-1.27
	5	14.84	1.67	24.25	+1.07
Calcium lactate	5	20.41	2.71	23.86	+3.31
Magnesium sulfate	5	28.87	4.30	23.03	+8.33
Lithium chloride	5	40.71	4.79	21.98	+15.23

(–): % Decrease in lactose supersaturation from control.

(+): % Increase in lactose supersaturation from control.

* The supersaturation of the control solution (33.25 g anhydrous lactose/100 g water) was 36.29%.

Table 4-8 Compositional analysis of dried permeate powders

Sample Description**	% Total Protein	% Moisture	% Ash	% Lactate	pH
B	4.11	0.95	11.74	0	6.7
C	3.49	1.18	11.47	8.83	5.0
D	5.64	0.76	18.99	13.81	4.7

Sample Description**	% Riboflavin	% Ca	% Mg	% Na
B	0.80	0.90	0.13	1.35
C	0.20	1.70	0.21	0.62
D	1.32	1.87	0.21	1.22

Sample Description**	% K	% Phosphorus	% Chloride
B	3.24	0.073	4.20
C	2.68	0.119	1.80
D	5.07	0.169	3.80

** where, Sample B = Sweet whey permeate; C = Acid whey permeate (Carbosep) and D= Acid whey permeate (DDS).

Table 4-9 Compositional analysis of dried permeate powders for lactose using different methods

Sample Description*	HPLC (% Lactose)	By Difference (% Lactose)**	Average (% Lactose)
B	83.70	83.15	83.42
C	76.30	75.0	75.65
D	64.10	60.74	62.42

* where, Sample B = Sweet whey permeate; C = Acid whey permeate (Carbosep) and D = Acid whey permeate (DDS).

** From Table 4-8.

Table 4-10 Compositional analysis of precipitate from permeate samples

Sample Description*	Amount of precipitate (**)	% Moisture	% Lactose (dmb)	% Total Protein (dmb)	% Ash (dmb)	pH	Unknown ***
B	16.49	2.4	18.26	2.0	19.2	6.5	58.14
C	29.04	4.3	22.86	2.0	16.0	5.0	54.84
D	28.03	5.4	26.03	5.7	24.9	5.0	37.97

* where, Sample B = Sweet whey permeate; C = Acid whey permeate (Carbosep)
and D = Acid whey permeate (DDS).

** % of dried permeate powder used for preparation of permeate solution.

*** Unknown component in the precipitate is probably citrate as per Pederson (1980).

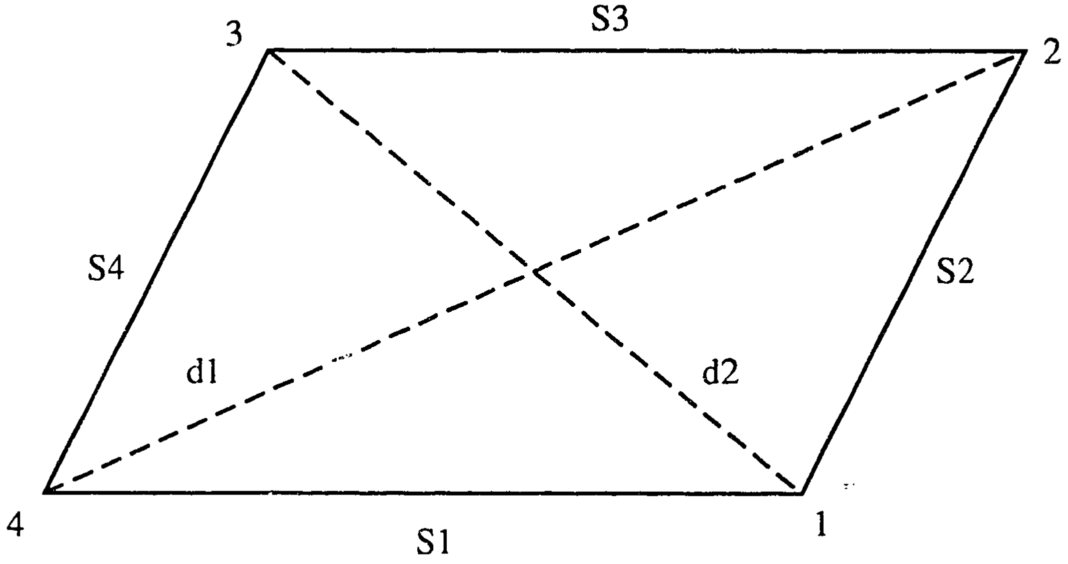

Fig. 4-1 Base of the lactose crystal

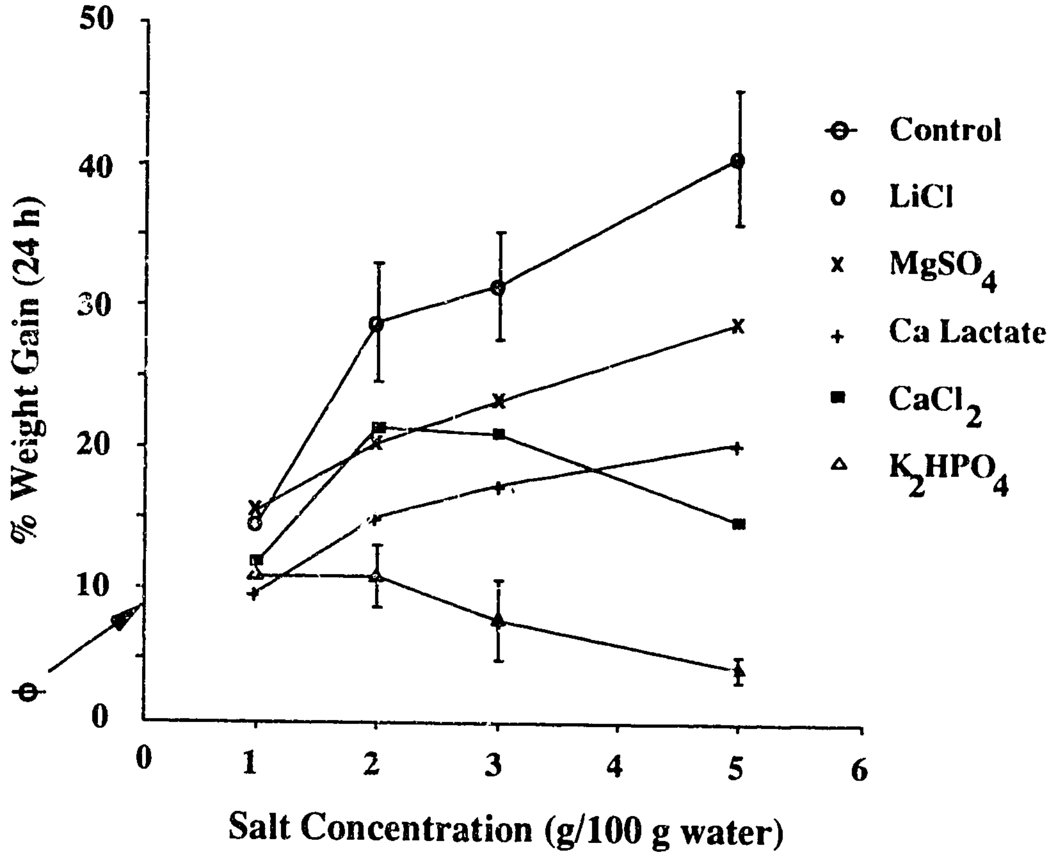

Fig. 4-2 Average growth rates of lactose crystals in model lactose solutions with or without the addition of salts. Error bars indicate the standard deviation. To maintain the clarity only some error bars are shown; for details refer to Table 4-5.

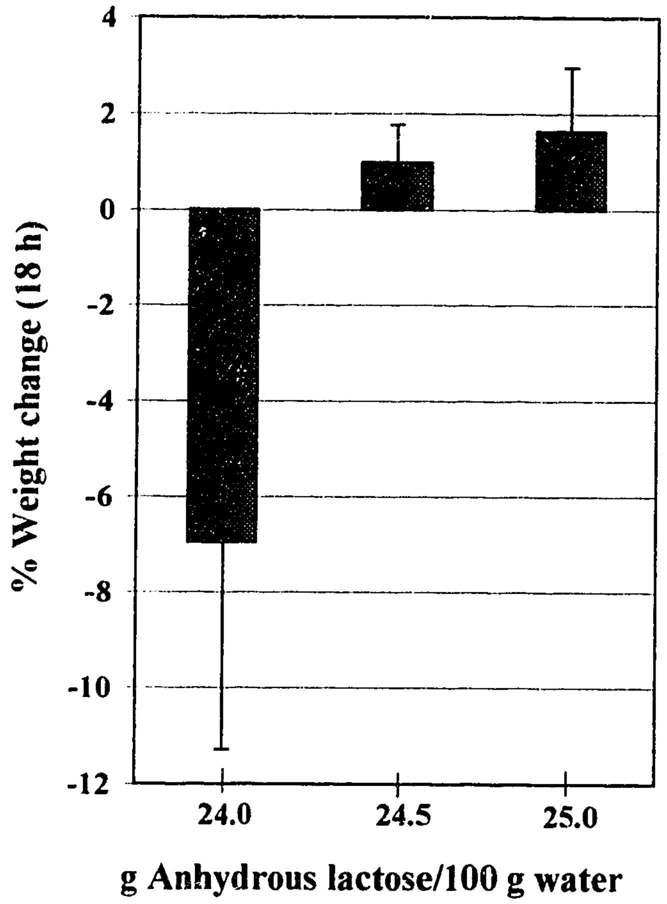

Fig. 4-3 Average growth rates of lactose crystals in control solutions (no added salts) containing different quantities of dissolved lactose. Error bars indicate the standard deviation.

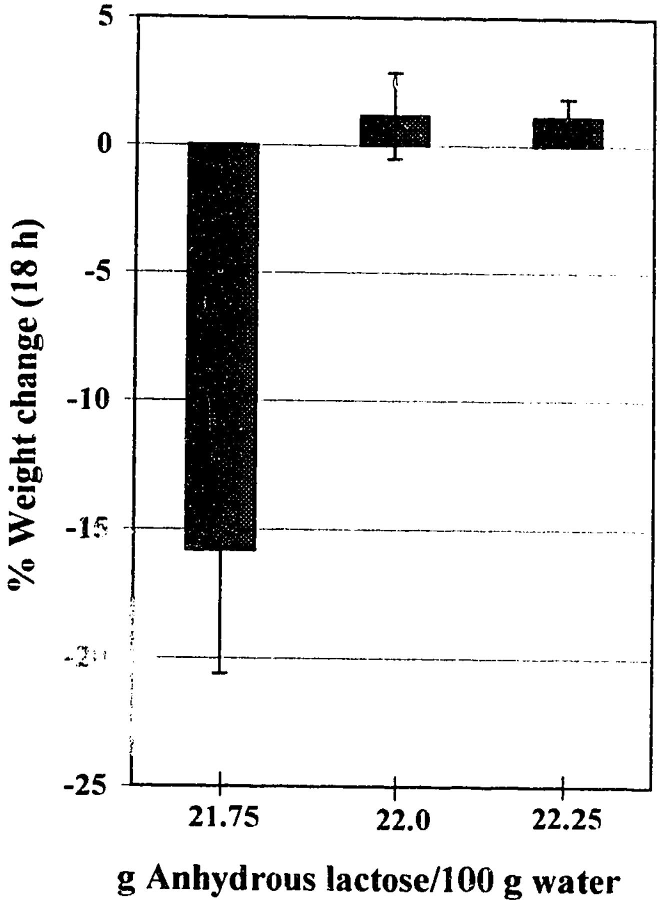

Fig. 4-4 Average growth rates of lactose crystals in lithium chloride solutions (5 g/100 g water) containing different quantities of dissolved lactose. Error bars indicate the standard deviation.

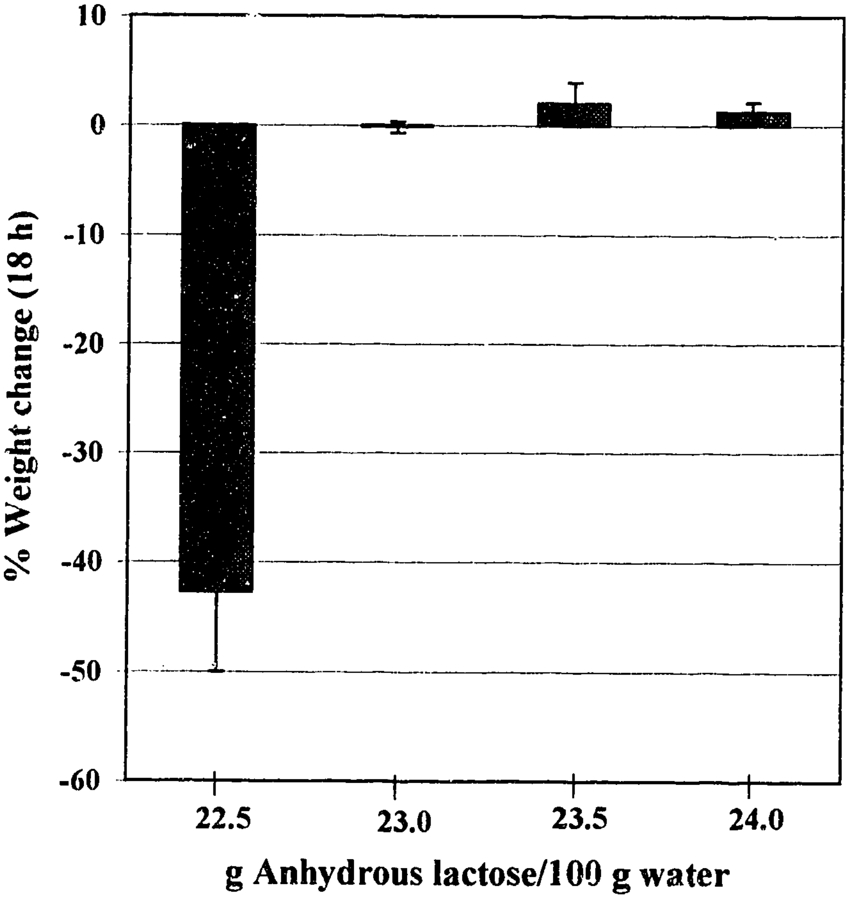

Fig. 4-5 Average growth rates of lactose crystals in magnesium sulfate solutions (5 g/100 g water) containing different quantities of dissolved lactose. Error bars indicate the standard deviation.

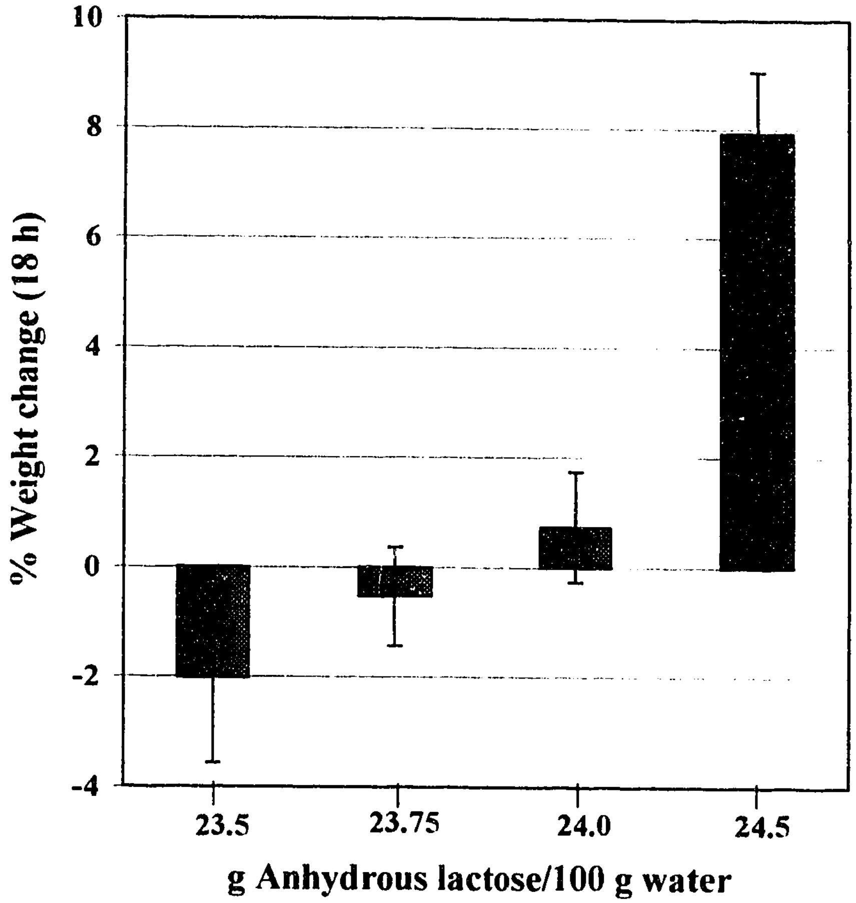

Fig. 4-6 Average growth rates of lactose crystals in calcium lactate solutions (5 g/100 g water) containing different quantities of dissolved lactose. Error bars indicate the standard deviation.

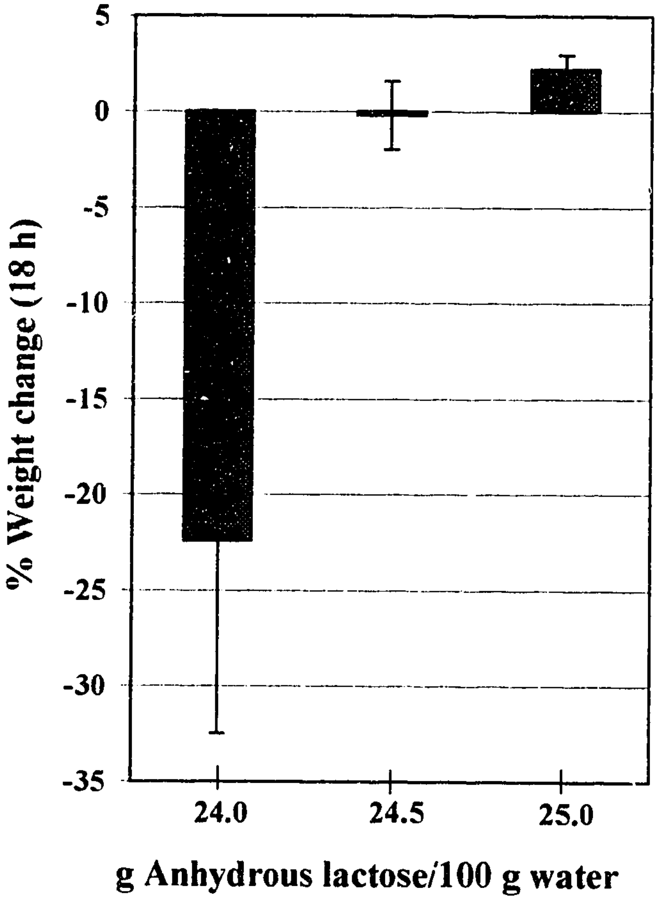

Fig. 4-7 Average growth rates of lactose crystals in calcium chloride solutions (1 g/100 g water) containing different quantities of dissolved lactose. Error bars indicate the standard deviation.

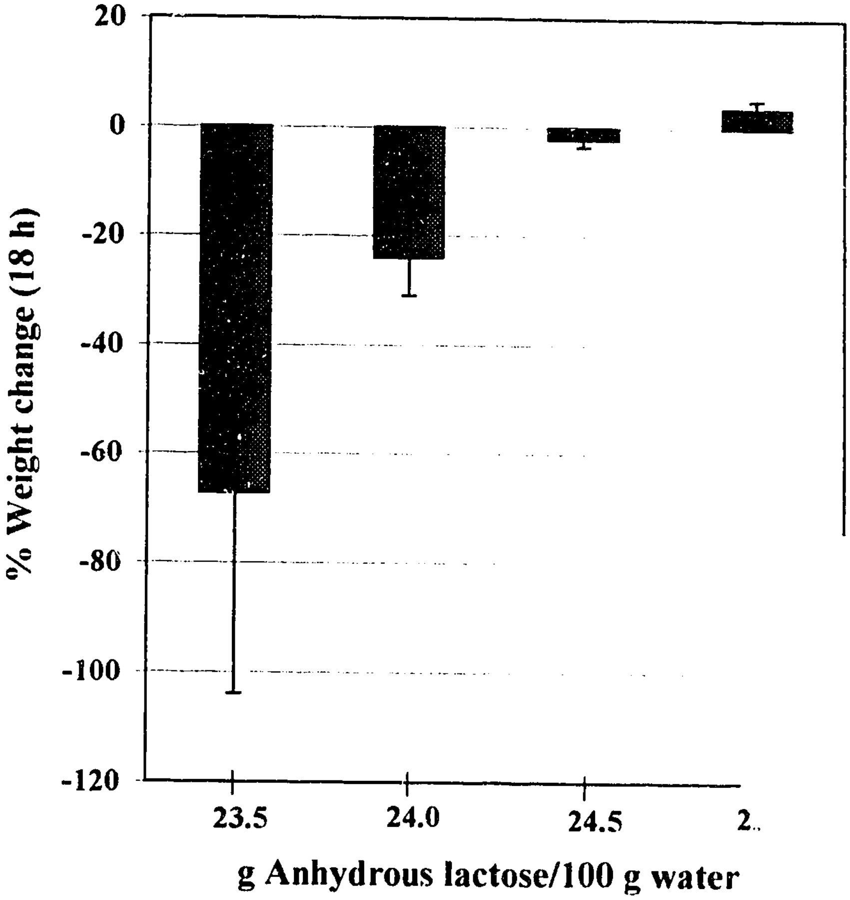

Fig. 4-8 Average growth rates of lactose crystals in calcium chloride solutions (2 g/100 g water) containing different quantities of dissolved lactose. Error bars indicate the standard deviation.

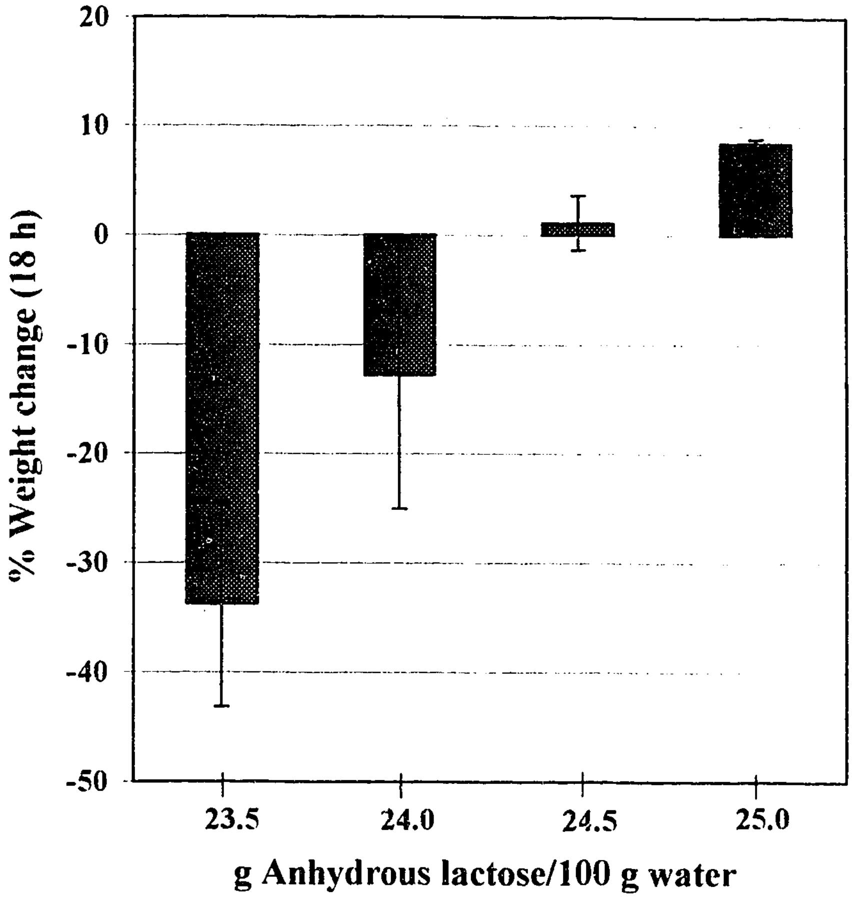

Fig. 4-9 Average growth rates of lactose crystals in calcium chloride solutions (5 g/100 g water) containing different quantities of dissolved lactose. Error bars indicate the standard deviation.

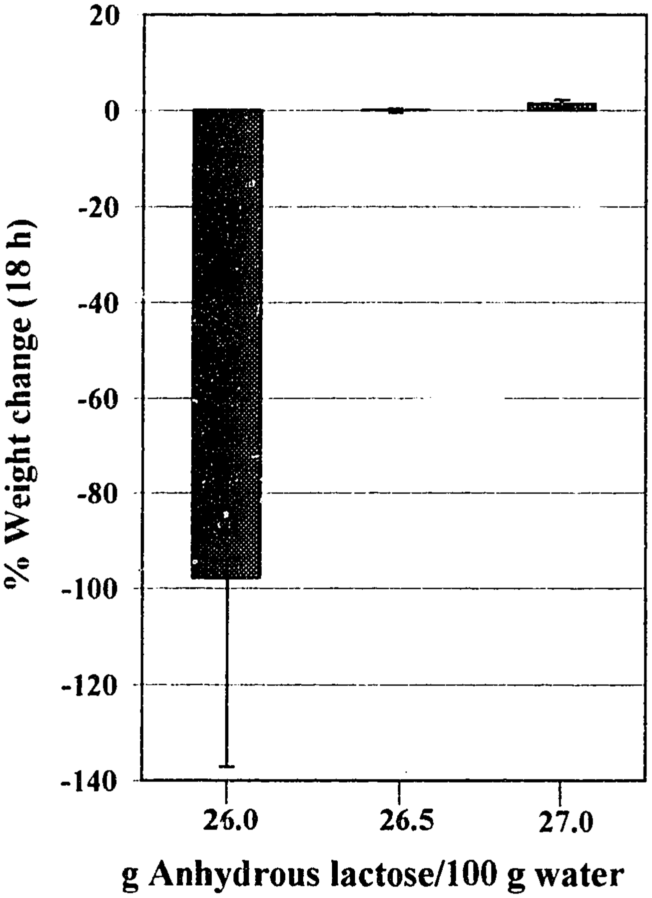

Fig. 4-10 **Average growth rates of lactose crystals in potassium phosphate solutions (5 g/100 g water) containing different quantities of dissolved lactose. Error bars indicate the standard deviation.**

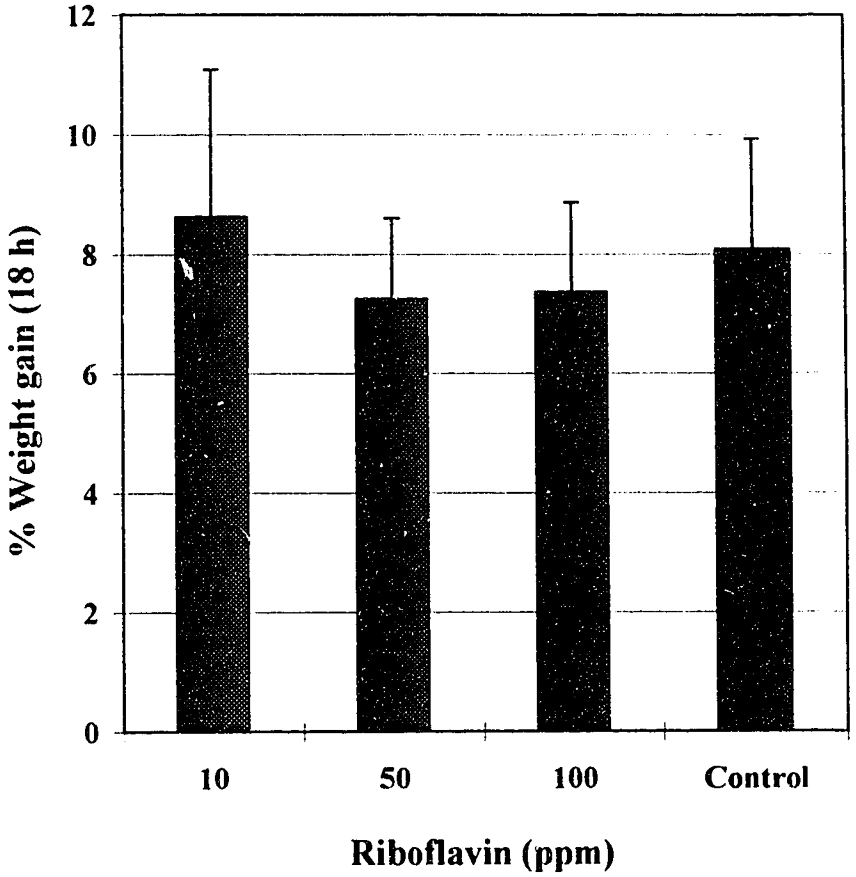

Fig. 4-11 Average growth rates of lactose crystals in model lactose solutions (33.25 g anhydrous lactose/100 g water) containing different amounts of riboflavin. Error bars indicate the standard deviation.

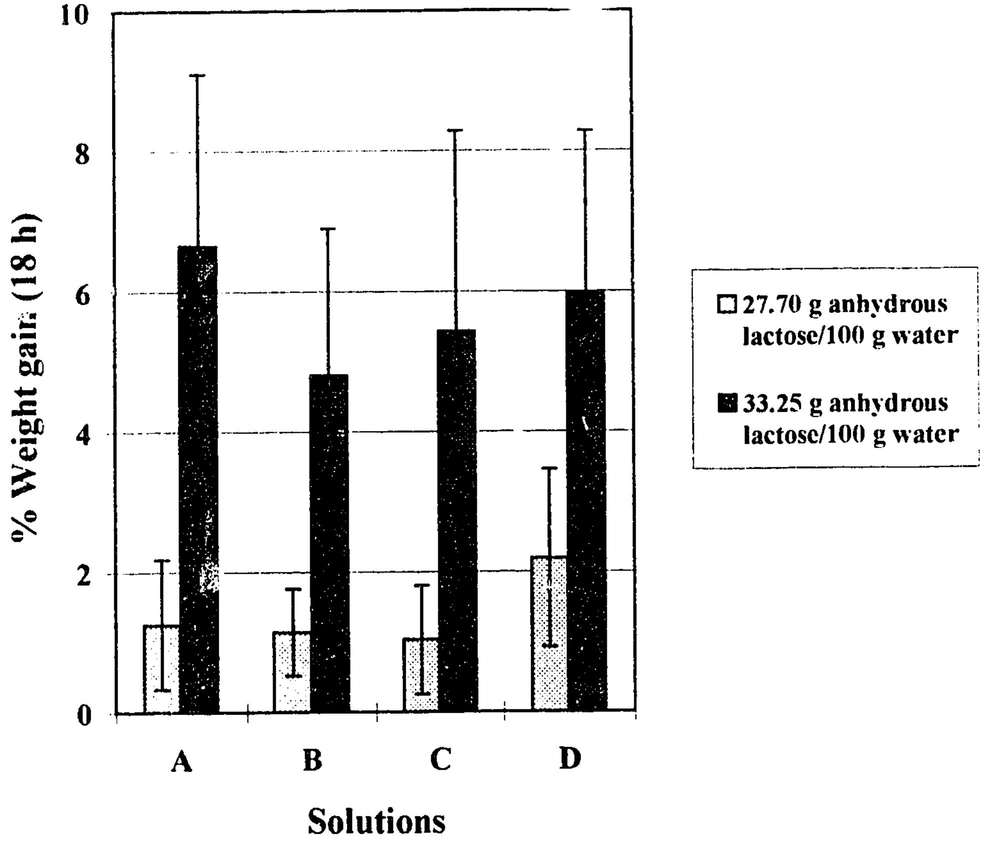

Fig. 4-12 Average growth rates of lactose crystals in A: control (pure lactose solution); B: sweet whey permeate, C: acid whey permeate (Carbosep) and D: acid whey permeate (DDS) solutions containing different quantities of dissolved lactose. Error bars indicate the standard deviation.

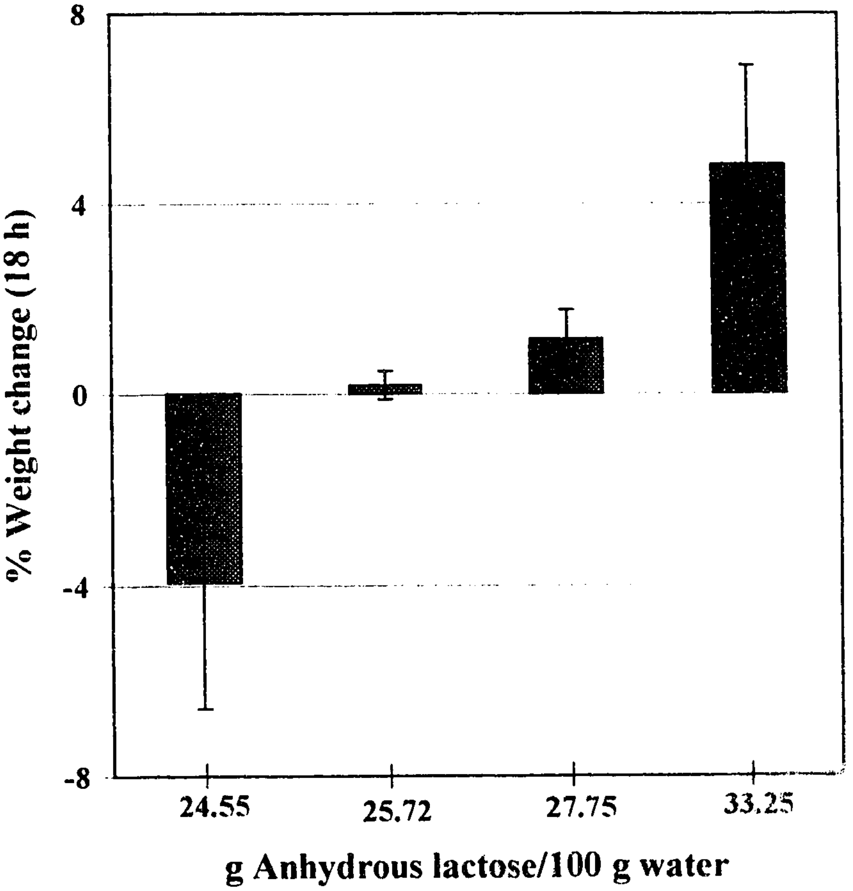

Fig. 4-13 Average growth rates of lactose crystals in sweet whey permeate solutions (B) containing different quantities of dissolved lactose. Error bars indicate the standard deviation.

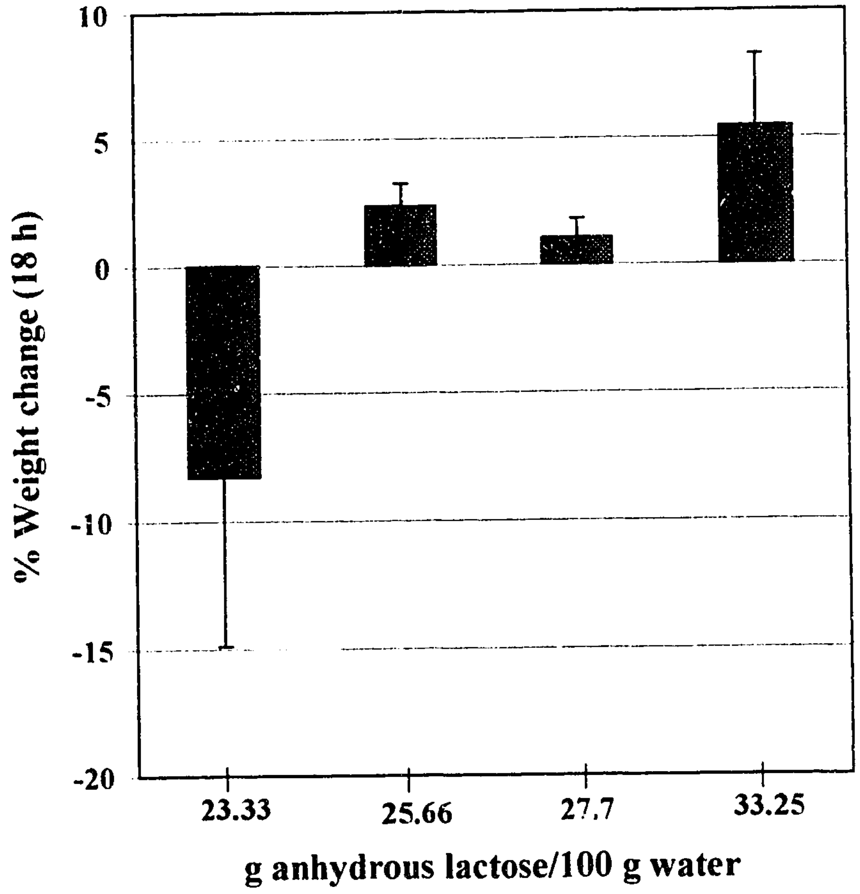

Fig. 4-14 Average growth rates of lactose crystals in acid whey permeate solutions (C) containing different quantities of dissolved lactose. Error bars indicate the standard deviation.

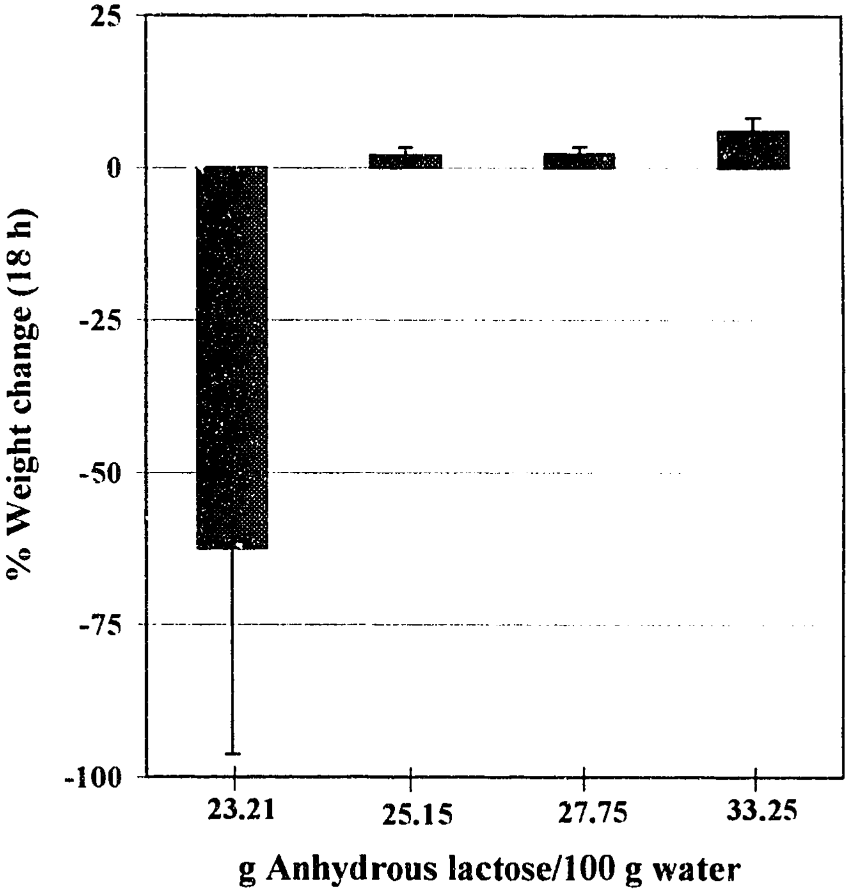

Fig. 4-15 Average growth rates of lactose crystals in acid whey permeate solutions (D) containing different quantities of dissolved lactose. Error bars indicate the standard deviation.

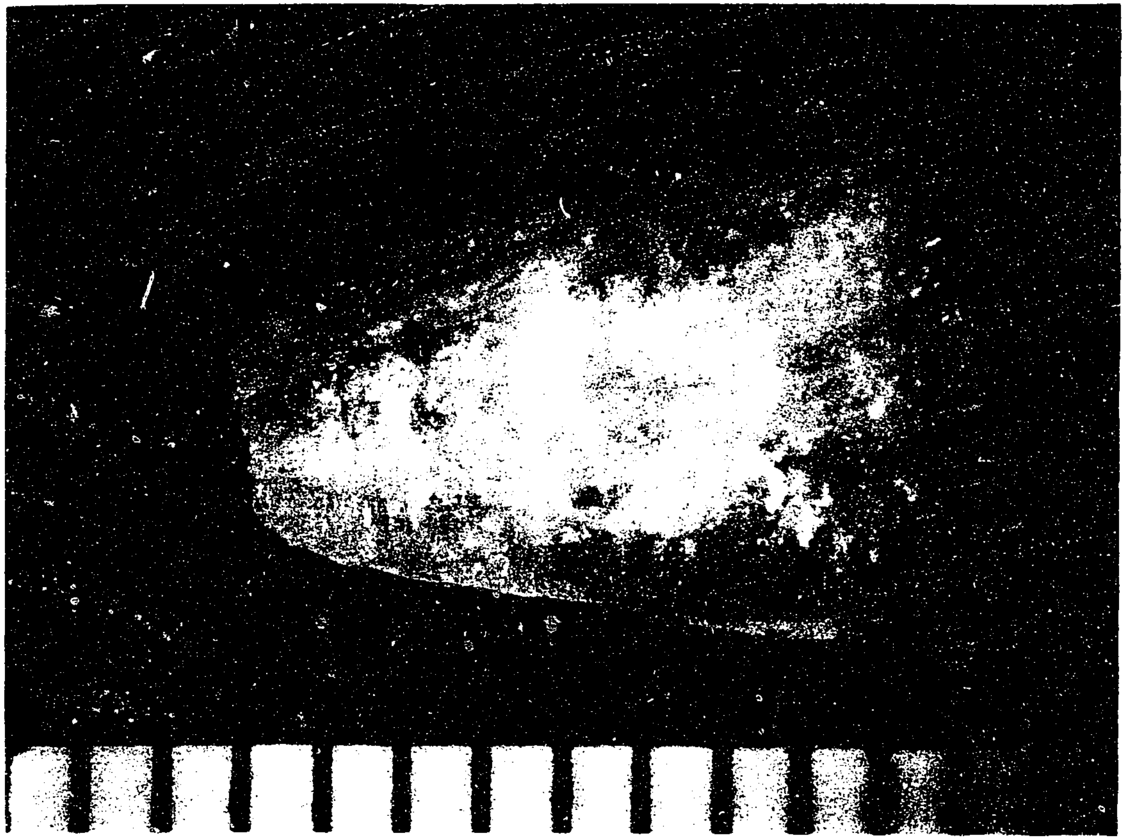

Plate 4-1. Lactose crystal with a flat base (Scale in mm).

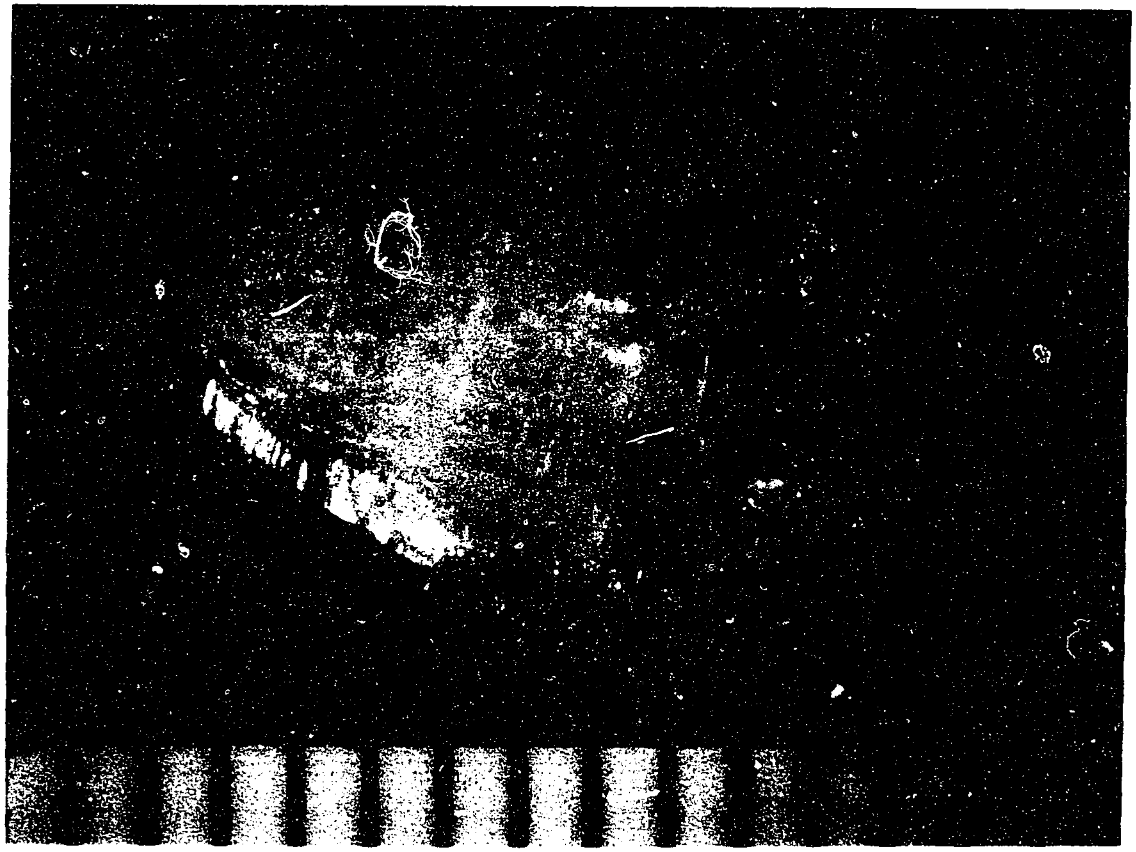

Plate 4-2. Lactose crystal with an irregular base (Scale in mm).

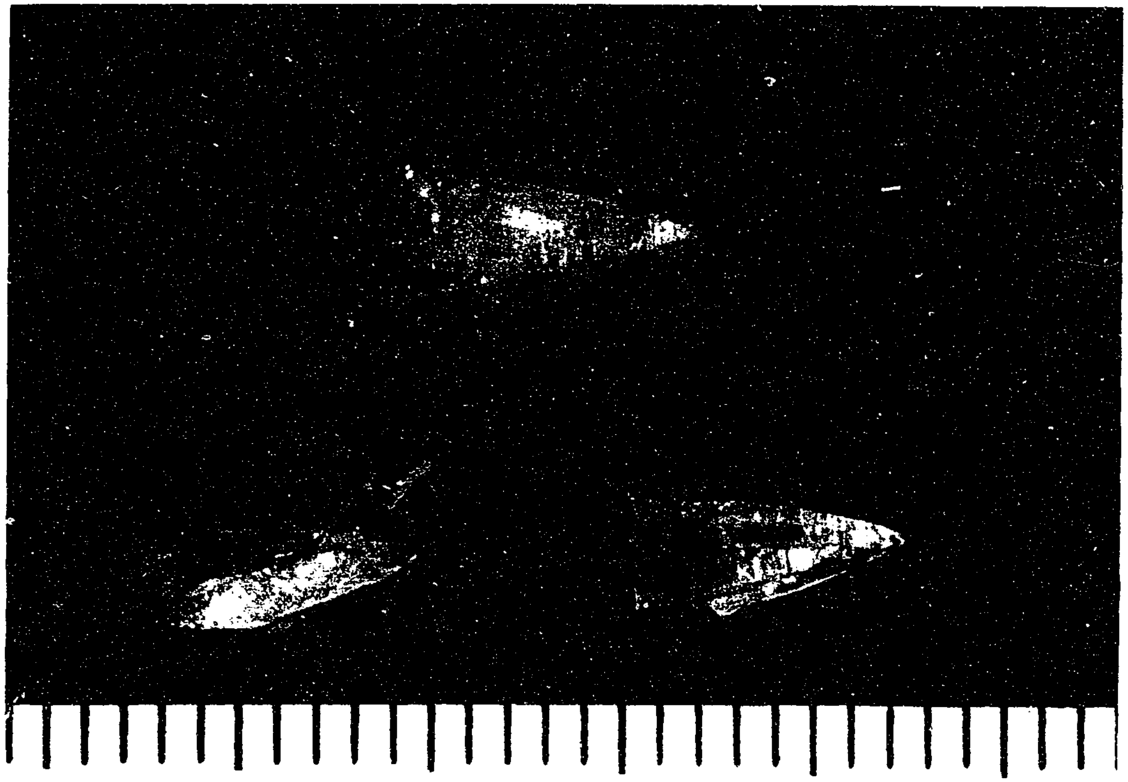

Plate 4-3. Lactose crystals grown in model lactose solutions containing LiCl (5 g/100 g water) (Scale in mm).

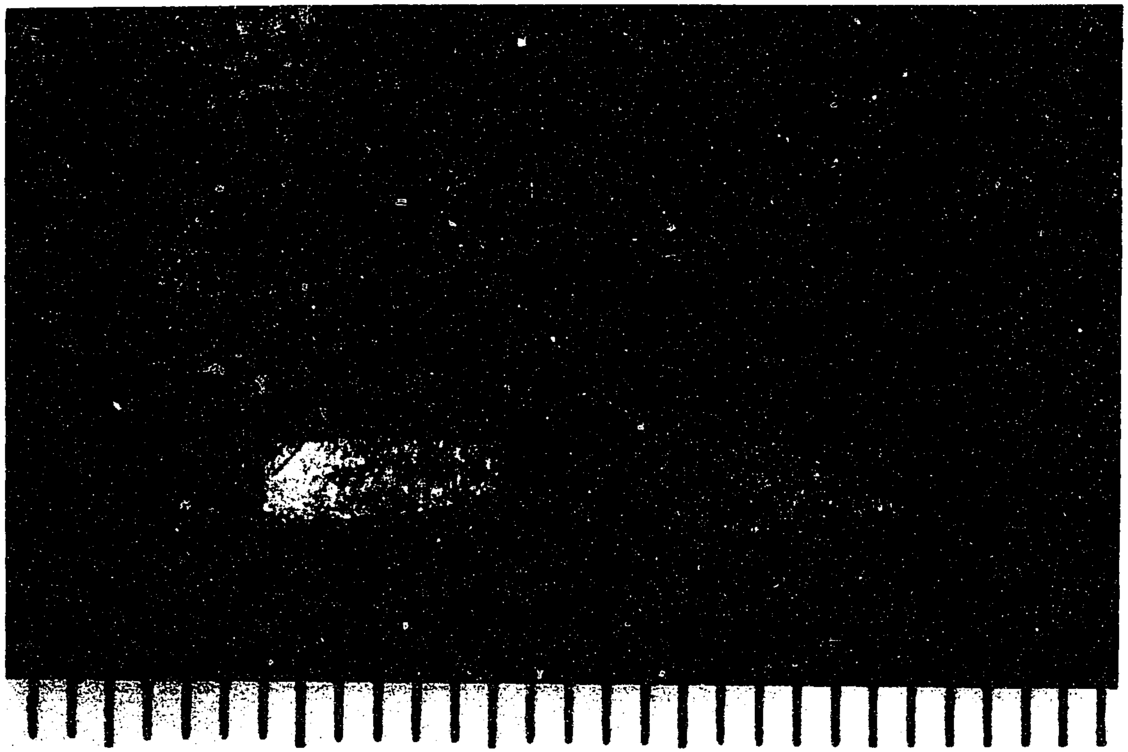

Plate 4-4. Lactose crystals grown in model lactose solutions containing $MgSO_4$ (5 g/100 g water) (Scale in mm).

Plate 4-5. Deposition of precipitate on crystals (A) grown in acid whey permeate solutions as compared with crystals (B) grown in lactose model solutions (Scale in mm).

CHAPTER 5

CONCLUSIONS AND FURTHER RESEARCH NEEDS

5.1 Conclusions

This work was primarily concerned with the investigation of lactose crystal growth rate, morphology and lactose solubility as affected by the various impurities found in whey UF permeates. Currently, an increasing number of lactose producing industries around the world are using ultrafiltered whey or skim milk permeates instead of whey as raw material for lactose production. It is likely that in the next few years most of the lactose around the world will be produced from ultrafiltered permeate.

The growth rate of lactose crystals was affected by addition of salts to the model lactose solutions. It was also observed that salts altered the lactose solubility or the supersaturation of the model solutions. As whey permeate contains high levels of salts, it is conceivable that these salts would change the supersaturation of lactose, thus affecting the nucleation and growth rate of lactose crystals. Therefore, a knowledge of the effect of the various salts could permit a better understanding of lactose crystallization in whey or UF permeates.

To summarize, in this study the following facts were experimentally established:

1. The addition of salts to model lactose solutions either increased or decreased the crystal growth rate, depending on the type and concentration of the salt. The maximum increase in growth rate was observed with the addition of LiCl, while the addition of K_2HPO_4 led to a decrease in the growth rate.

2. The addition of salts to the model lactose solutions changed the lactose solubility and thus the lactose supersaturation of the model solutions with constant lactose/water composition.
3. The crystallization velocity of lactose was found to be 342 ± 58 mg/m^2/min which was similar to that of a sucrose crystal (255 mg/m^2/min) at approximately the same absolute supersaturation (10 g of excess solute/100 g water, above the solubility point at 30°C). The reason why lactose crystallization is a slow process as compared to sucrose crystallization is because the growth of lactose crystals is unidirectional (from the base downwards), as opposed to sucrose which exhibits multidimensional crystal growth.
4. Negligible amounts of minerals were found to be entrained in the lactose crystals.
5. The morphology of the base of the lactose crystals grown in pure lactose solutions did not change. The crystals that were flat at the base remained flat while those that had an irregular base maintained the same features. However, the morphology of the crystals was modified in the presence of some specific salts in the model lactose solutions. Crystals grown in presence of LiCl increased in length, while those grown in presence of $MgSO_4$ increased in width.
6. Riboflavin had no measurable effect on the growth of individual lactose crystals grown in model lactose solutions containing 10, 50 or 100 ppm riboflavin.
7. Due to the large variability of the experimental data as indicated by large standard deviations, not much difference in the growth rates of lactose crystals was observed between the control and three whey UF permeates (one sweet and two acid whey permeates differing in composition). Growth of lactose crystals in acid whey permeate solutions without prior removal of water insoluble salt precipitates (from permeates) resulted in sticking of the precipitates on the lactose crystals.

5.2 Further Research Needs

Future experiments on lactose crystallization should include a study on the effect of riboflavin on crystal morphology. Additionally, the adsorption/absorption of riboflavin in the lactose crystal matrix must also be investigated in detail. The need for a continuous lactose crystallization process still exists. A continuous crystallization process could make the process faster, thereby reducing the cost of lactose production. It would be interesting to generate additional information on the combined effects of mixture of different salts (e.g. simulated milk ultrafiltrate) on growth of lactose crystals. Further information on combined effects of all the whey salts would help in designing a more efficient process for lactose recovery, and perhaps ultimately the development of a continuous process. It is particularly important to understand which specific food grade additives would increase the lactose yield. Also, information on impurities which affects the crystallization process negatively, especially minerals that should be removed prior to lactose crystallization is much desired. Minerals that decrease the recovery efficiency of lactose from whey can be selectively removed using suitable membrane processes such as nanofiltration. Studies on the pretreatment of whey or UF permeates prior to crystallization is of utmost importance to improve the process efficiency and the purity of lactose crystals. However, more importance should be given to the development of an efficient crystallization process that particularly uses permeate as a raw material source. The effect of salts on the growth morphology of lactose crystals grown in whey permeates must also be investigated. This information would be helpful in designing food industry applications, where the crystal shape and size might confer desirable or undesirable textural properties to the product. In epitome, a detailed understanding of the effects of specific salts on lactose crystallization must be determined to provide information on the crystallization of lactose in real systems, including whey or UF permeates. Such an understanding might enable improvements to be made in crystallization procedures and in the subsequent utilization of lactose. This study is an attempt in filling some of the gaps in current knowledge necessary for continuation of future research in the area of lactose crystallization.

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APPENDIX 1

Raw data of riboflavin absorption on a 8452A Diode Array Spectrophotometer

Riboflavin conc. (ppm)	**Wavelength**		**Weight of lactose crystal dissolved in water** (g)
	374 nm	**446nm**	
Standard Solutions			
2.55	0.07318	0.08485	-
5.10	0.14520	0.16910	-
10.19	0.28935	0.33789	-
50.95	1.32160	1.53610	-
Experimental Solutions			
Blank (water)	0.00003	0.00005	-
10	0.00442	0.00177	0.07090
10	0.00732	0.00723	0.07205
10	0.01149	0.01066	0.05424
50	0.01444	0.01291	0.06026
50	0.01232	0.01054	0.09157
50	0.01153	0.01427	0.06661
100	0.00716	0.00983	0.09460
100	0.01492	0.01706	0.05822
100	0.01551	0.01712	0.05762