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ในสารละลายอาหารที่ให้ทางหลอดเลือดดำสำหรับทารก

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วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาเภสัชศาสตรมหาบัณฑิต
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SOLUBILITY OF CALCIUM GLUCONATE AND SODIUM GLYCEROPHOSPHATE IN INFANT PARENTERAL NUTRITION SOLUTIONS

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A Thesis Submitted in Partial Fulfillment of the Requirements
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Department of Food and Pharmaceutical Chemistry
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ณัฐพร เถาลัดดา : การละลายของแคลเซียมกลูโคเนตและโซเดียมกลีเซอโรฟอสเฟตในสารละลายอาหารที่ให้ทางหลอดเลือดดำสำหรับทารก (SOLUBILITY OF CALCIUM GLUCONATE AND SODIUM GLYCEROPHOSPHATE IN INFANT PARENTERAL NUTRITION SOLUTIONS) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: อ. ภญ. ดร.ทิพวรรณ ศิริเทียรทอง, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: ผศ. พญ.อัญชลี ลิ้มรังสิกุล, 181 หน้า.

การเกิดตะกอนของแคลเซียมและฟอสเฟตเป็นปัญหาสำคัญที่พบในสารละลายอาหารที่ให้ทางหลอดเลือดดำ ปัญหาดังกล่าวมักเกิดกับผู้ป่วยที่มีความต้องการแร่ธาตุแคลเซียมและฟอสฟอรัสปริมาณสูงโดยเฉพาะผู้ป่วยทารก การศึกษานี้มีวัตถุประสงค์เพื่อศึกษาผลของความเข้มข้นของกรดอะมิโน แคลเซียมกลูโคเนต โซเดียมกลีเซอโรฟอสเฟต และสภาวะการเก็บรักษาต่อการละลายของแคลเซียมกลูโคเนตและโซเดียมกลีเซอโรฟอสเฟตในสารละลายอาหารที่ให้ทางหลอดเลือดดำสำหรับทารก โดยสูตรสารละลายอาหารที่ใช้ในการทดสอบนี้ประกอบด้วยเด็กซ์โทรสร้อยละ 10 แมกนีเซียม 2.025 มิลลิโมลต่อลิตร โซเดียมคลอไรด์ 51.3 มิลลิโมลต่อลิตร กรดอะมิโนร้อยละ 1.5, 2, 2.5 แคลเซียมความเข้มข้นตั้งแต่ 0 ถึง 100 มิลลิโมลต่อลิตร ฟอสเฟตความเข้มข้นตั้งแต่ 0 ถึง 150 มิลลิโมลต่อลิตร วิเคราะห์การเกิดตะกอนโดยการมองด้วยตาเปล่า วัดค่าความเป็นกรดด่าง ค่าความขุ่นของสารละลาย และการส่องด้วยกล้องจุลทรรศน์ ที่สภาวะการเก็บรักษา 4 สภาวะ ได้แก่ อุณหภูมิห้อง เป็นเวลา 30 นาที อุณหภูมิห้อง เป็นเวลา 1 วัน อุณหภูมิ 4 องศาเซลเซียส เป็นเวลา 1 วัน และอุณหภูมิ 4 องศาเซลเซียส เป็นเวลา 7 วัน พบว่าสารละลายอาหารที่ให้ทางหลอดเลือดดำที่ใช้กรดอะมิโนความเข้มข้นร้อยละ 2.5 ไม่พบตะกอนเกิดขึ้นในทุกความเข้มข้นของแคลเซียมและฟอสเฟตที่ทดสอบ ในขณะที่การใช้กรดอะมิโนความเข้มข้นร้อยละ 1.5 และ 2 มีการตกตะกอนเกิดขึ้น แต่เป็นช่วงความเข้มข้นของแคลเซียมและฟอสเฟตที่สูงกว่าการใช้จริงในทางคลินิก หลังจากนั้นนำผลที่ได้มาสร้างเป็นข้อมูลการละลายของแคลเซียมกลูโคเนตกับโซเดียมกลีเซอโรฟอสเฟตในสารละลายกรดอะมิโนความเข้มข้นต่าง ๆ ที่สภาวะการเก็บรักษาต่าง ๆ และนำสูตรสารละลายอาหารสำหรับทารกที่มีการสั่งใช้จริงในช่วงเดือนกรกฎาคมถึงธันวาคม พ.ศ. 2559 มาทดสอบการละลาย พบว่าข้อมูลการละลายที่ได้จากการศึกษาสามารถใช้เป็นข้อมูลประกอบการพิจารณาสั่งใช้และการเตรียมสารอาหารที่ให้ทางหลอดเลือดดำสำหรับทารกได้

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NATTAPORN THOWLADDA: SOLUBILITY OF CALCIUM GLUCONATE AND SODIUM GLYCEROPHOSPHATE IN INFANT PARENTERAL NUTRITION SOLUTIONS. ADVISOR: TIPPAWAN SIRITIENTONG, Ph.D., CO-ADVISOR: ASST. PROF. ANCHALEE LIMRUNGSIKUL, M.D., 181 pp.

Precipitation of calcium and phosphate in parenteral nutrition (PN) solutions remains a significant problem in patients who need high amounts of calcium and phosphorus especially infant patients. The aim of this study were to investigate the effects of concentrations of amino acids, calcium gluconate, sodium glycerophosphate (NaGP), and storage conditions on solubility of calcium gluconate and NaGP in infant PN solutions. The tested PN solution consisted of 10% dextrose, 2.025 mM/L of magnesium, 51.3 mM/L of sodium chloride, 1.5, 2, and 2.5% amino acid, 0-100 mM/L of calcium and 0-150 mM/L of phosphate. Visual inspection, pH measurement, turbidity test and microscopic particle count test were examined. Each admixture was tested according to 4 following storage conditions; room temperature for 30 minutes, room temperature for 1 day, 4 ºC for 1 day and 4 ºC for 7 days. Samples containing 2.5% of amino acid showed no precipitation at all concentrations of calcium gluconate and NaGP used in this study. On the other hand, samples containing 1.5% and 2% of amino acid showed precipitation in the PN solutions at concentrations of calcium gluconate and NaGP higher than those concentrations used in clinical practice. All results from solubility tests were applied to construct solubility data of calcium gluconate and NaGP. Solubility data were applied by infant PN solution orders during July to December in 2016. Solubility data from this study can be used for prescription and preparation of infant PN solutions.

Department:	Food and Pharmaceutical Chemistry	Student's Signature	.....
		Advisor's Signature	.....
Field of Study:	Food Chemistry and Medical Nutrition	Co-Advisor's Signature	.....
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LIST OF ABBREVIATIONS

ASHP Guidelines	American Society of Health-System Pharmacists Guidelines
ASPEN	the American Association of Enteral and Parenteral Nutrition
ATP	adenosine triphosphate
Ca	calcium
ECF	extracellular fluid
EN	enteral nutrition
et al.	et alibi, and others
FA	fatty acid
FDA	The US Food and Drug Administration
g	gram
GI	gastrointestinal
G1P	glucose-1-phosphate
H_2O_2	hydrogen peroxide
ICF	intracellular fluid
JPEN	Journal of Parenteral and Enteral Nutrition
kcal	kilocalories

kg	kilogram
L	liter
mg	milligram
mg/mL	milligram per milliliter
ml	mililiter
mM	millimole
mosm/L	milliosmole per liter
NaGP	sodium glycerophosphate
NF-κB	the nuclear factor kappa-B
NTU	nephelometric turbidity units
P	phosphate
PN	parenteral nutrition
PTH	parathyroid hormone
S.D.	standard deviation
The ESPGHAN/ESPEN guidelines	The European Society of Infant Gastroenterology, Hepatology and Nutrition and the European Society for Clinical Nutrition and Metabolism
TNF	tumor necrosis factor
TPN	total parenteral nutrition
VLBW	very low birth weight

%	percentage
°C	Degree Celsius
μm	micrometer

CHAPTER I
INTRODUCTION

1.1 Background and Rationale

Precipitation of calcium and phosphate in parenteral nutrition (PN) solutions remains a significant problem. Precipitates of dibasic calcium phosphate that particle sizes appear greater than 4 μm are potentially embolic (Joy et al., 2010). In 1994, the US Food and Drug Administration (FDA) issued a safety alert of death, respiratory distress caused by precipitate of dibasic calcium phosphate during PN infusion. In the clinical setting, several case reports have been described precipitation of dibasic calcium phosphate as a cause of central venous catheter occlusion and subacute interstitial pneumonitis secondary to pulmonary calcium phosphate deposition (Koletzko, Goulet, Hunt, Krohn, & Shamir, 2005; Parikh, Dumas, Silvestri, Bistrian, & Driscoll, 2005; Ronchera-Oms, Jimenez, & Peidro, 1995). This problem occurs in patients who need high amounts of calcium and phosphorus especially patients with hypocalcaemia, hypophosphatemia and preterm infants.

Preterm infants need high amount of calcium and phosphorus during their first year of life or until they reach approximately 10-12 kg in weight for enhancing bone mineralization and preventing abnormal growth (Marks & Crill, 2004). The European Society of Infant Gastroenterology, Hepatology and Nutrition and the European Society for Clinical Nutrition and Metabolism (The ESPGHAN/ESPEN)

recommends that growing newborn infants should receive 1.3–3.0 mM calcium/kg/day and 1.0–2.3 mM phosphorus/kg/day, with a calcium-to-phosphorus (Ca:P) ratio (mole/mole) in the range of 1.3–1.7 (Koletzko et al., 2005; Ribeiro et al., 2009). Unfortunately, these requirements are seldom met because of the limitation in prescribing calcium and phosphate. The previous study reported that the prescriptions of calcium and phosphate were 0.6±0.4 mM/kg/day and 0.7±0.4 mM/kg/day for low birth weight infants, respectively. (Bouchoud, Fonzo-Christe, Sadeghipour, & Bonnabry, 2010). Therefore it is important to increase calcium and phosphate amounts in PN prescription for neonates. However, practical solubility factors relevant to the safe administration of calcium and phosphate are concerned (Marks & Crill, 2004).

Several factors affecting the solubility of calcium and phosphate in PN solutions, include order of mixing, concentration of amino acid, pH of PN solution, storage duration and temperature, salt forms of calcium and phosphate, concentrations of calcium and phosphate, and other additives added to the solutions (Bouchoud et al., 2010; Migaki, Melhart, Dewar, & Huston, 2012; Ribeiro et al., 2009). Previous studies reported that these factors affected solubility of calcium and dipotassium phosphate for PN solutions containing various concentrations of amino acids. (Mo-suwan, Puetpaiboon, & Apiromrak, 1997; Newton & Driscoll, 2008). Equations, ratios, graphs, and guidance statements have been developed to assist practitioners to avoid calcium and phosphate precipitation in PN solutions (MacKay &

Anderson, 2015). In case of high risk of precipitation, either calcium or phosphate concentration needs to be reduced to decrease risk of calcium phosphate precipitation which leads to insufficient mineral administration to the patient. Separate infusions of calcium and phosphate solutions are alternative methods to achieve requirement of the infant patient but they resulted in hyperphosphatemia and hypocalcemia during phosphate infusion and hypercalcemia and hypophosphatemia during calcium infusion (Marks & Crill, 2004; Pereira-da-Silva et al., 2003). To avoid this mineral imbalance, calcium phosphate admixture is still required. Therefore, there is a challenge to find the resolution of calcium phosphate precipitation and patient's requirement (Newton & Driscoll, 2008).

Currently, there is a new parenteral phosphate product available in the market named sodium glycerophosphate (NaGP). NaGP is an organic phosphate; a phosphate group covalently bonded to glycerol, claimed to increase solubility compared to inorganic phosphate even in a solution containing high concentration of calcium or high pH value. (Ronchera-Oms et al., 1995). Therefore, PN would be comfortably prepared and precipitation of calcium phosphate can be avoided even high concentration of mineral contents (Costello, Powell, & Williams, 1995). Previous studies used NaGP corresponding to calcium gluconate with amino acids solutions (TrophAmine®). Concentrations of NaGP at 50 mM/L and concentrations of calcium gluconate at 25 mM/L showed no precipitation in PN solutions (MacKay & Anderson, 2015). NaGP may be a good choice of phosphate source for patients to meet the

requirements of phosphate instead of dipotassium phosphate. Although use of NaGP in PN preparation gradually expands, there is still no conclusive evidence about maximum concentrations of calcium gluconate and NaGP with no precipitation in PN solutions. In Thailand, Aminoven infant® is the most common amino acid solution prescribed for neonates. The effects of the storage conditions and Aminoven infant® on the calcium and NaGP compatibility in infant PN solutions were also evaluated.

1.2 Objective of the study

To investigate the effects of various concentrations of amino acids, calcium gluconate, sodium glycerophosphate, and storage conditions on solubility of calcium gluconate and sodium glycerophosphate in infant PN solutions.

1.3 Scopes

1.3.1 PN solutions in this study selected from Ramathibodi Hospital infant PN solutions orders during June to August in 2015 and solubility data were applied according to practical infant PN orders at Ramathibodi Hospital infant PN solutions orders during July to December in 2016. Infants were defined as patients who aged less than 1 year old.

1.3.2 Experimental laboratory since July in 2016 to February in 2017 at the Department of Food and Pharmaceutical Sciences, Chulalongkorn University.

1.4 Benefits

1.4.1 This study showed factors involved in solubility of calcium and NaGP in parenteral solutions.

1.4.2 This study showed maximum concentration of calcium gluconate and NaGP in infant PN solutions according to different concentrations of Aminoven infant® and storage conditions.

CHAPTER II
LITERATURE REVIEW

2.1 Infant parenteral nutrition

Parenteral nutrition (PN) is the intravenous delivery of nutrients, including fluid, carbohydrates, proteins, lipids, electrolytes, trace elements and vitamins (Mizock & Troglia, 1997). PN plays an important role when severe gastrointestinal (GI) disorders occur and enteral nutrition (EN) has failed. Common PN indications in infants include bone marrow transplant, severe respiratory disease, cancer, cardiac failure, congenital anomalies of the GI tract, extensive burns, severe generalized peritonitis, shock stage, severe abdominal distention, prolonged diarrhea, necrotizing enterocolitis, GI obstruction, severe inflammatory bowel disease, small bowel ischemia, short bowel syndrome and preterm infant (Kleinman, 2004; รังสรรค์ ภูรยานนทชัย, 2549). PN should be initiated within the first day of birth in very low birth weight (VLBW) infants and within 5-7 days in infant patients who are unable to meet their nutrient requirements with oral intake or EN (Phillips, 2004). Early aggressive PN in preterm infant results in good neurodevelopmental outcomes and improve nutritional status.

2.2 Infant requirements during PN

2.2.1 Energy

To determine the energy requirements of the patients is the first step of PN formulation. Energy requirements of infants are based on age, gender, stage of growth and comorbidities. Energy requirement in infant population varies 90 to 120 kcal/kg/day by age (Table 1). Infants normally need more energy than adults due to their growth and development. In acute status or stress condition, energy requirement may be excessive; however lower calories target may be set for some critically ill patients until hemodynamically stable status (Phillips, 2004). Common distributions of energy sources are from protein 8-12%, carbohydrate 40-60% and lipid 25-35% of total energy requirement (พิภพ จิรภิญโญ, 2538).

Table 1 Estimated energy needs modified from (A.S.P.E.N Board of Directors, 2002; Koletzko et al., 2005)

Age (year)	Energy requirements (kcal/kg/day)
Preterm (gestational age < 37 weeks)	110-120
0-1	90-120
1-7	75-90
7-12	60-75
12-18	30-60
> 18	25-30

2.2.2 Fluid

Water is the most abundant component of the human body, contributing 80% of the weight of the preterm infant, 70% of the weight of the full term infant, and approximately 60% of the body weight of an adult. Fluid requirement can be calculated by Holliday-Segar formula (Table 2) (Baker, Baker, & Davis, 2007).

Table 2 Calculation of daily fluid requirement using Holliday-Segar formula (Baker et al., 2007)

Body weight (kg)	**Amount of fluid per day (mL)**
0-10	100 mL/kg
11-20	1,000 mL + 50 mL/kg for every kg > 10 kg
>20	1,500 mL + 20 mL/kg for every kg > 20 kg

Fluid requirement varies with age, disease and environment. LBW infant and preterm infant needs fluid intake increment between 60-95 mL/kg/day in the first 2-3 days. Fluid restriction is suggested in patients with congestive heart failure. Other environmental factors given to infants, such as ventilators, phototherapy and radiant warmers need fluid 120 mL/kg/day for 1 week after birth then 150-160 mL/kg/day (Baker et al., 2007). Fluid balance is normally monitored by urine output and urine specific gravity. Urine output is commonly in the range of 1-3 mL/kg/hour or 24-72 mL/kg/day and urine specific gravity is approximate 1.008-1.012. High urine specific gravity indicates to dehydration status (พิภพ จิรภิญโญ, 2538).

2.2.3 Carbohydrate

Carbohydrate is a major source of energy in PN which comprise 40-60% of the caloric intake. An intravenous carbohydrate is dextrose monohydrate containing caloric yield to 3.4 kcal/g (Koletzko et al., 2005). Intravenous dextrose solution is a mixture of dextrose and water or normal saline. Types of available commercial product include 5% dextrose in water (D-5-W), dextrose in lactated ringer solution (D-5-LR), 5% dextrose in normal saline (D-5-S), 5% dextrose with 0.45% sodium chloride (D-5-N/2), 5% dextrose with 0.3% sodium chloride (D-5-N/3), 5% dextrose with 0.18% sodium chloride (D-5-N/5), 10% dextrose in water (D-10-W), 10% dextrose in normal saline (D-10-S), 10% dextrose with 0.45% sodium chloride (D-10-N/2), 10% dextrose with 0.3% sodium chloride (D-10-N/3), 10% dextrose with 0.18% sodium chloride (D-10-N/5) and 50% dextrose in water (D-50-W).

Cautions about intravenous dextrose solution administration include hyperosmolality, hyperglycemia, hepatic steatosis and lung disease. Dextrose provides the osmolality in the PN solution. Peripheral PN concentrations with more than 12.5% glucose (more than 625 mOsm/L) increase the risk of phlebitis due to high osmolality. Glucose concentration higher than 12.5% must be administered via a central vein (Baker et al., 2007).

A complication during dextrose infusion has been linked to the development of hyperglycemia, especially during the first few days of PN therapy. PN might cause

hyperglycemia in patients with no history of diabetes mellitus (Gosmanov & Umpierrez, 2013; Lee, Koh, & Park, 2011). Insulin therapy together with dextrose infusion may be considered in some cases to control blood glucose level and enhance total calories intake. Glucose infusion rates at 5-12 and 2-5 mg/kg/minute are recommended in neonates and adolescents, respectively (Baker et al., 2007).

PN is associated with hepatic biochemical and morphologic changes. Suggested causes of PN associated cholestasis include excessive glucose infusion rate, fatty acid deficiency, and enterically derived hepatotoxins escaping atrophied bowel (Koletzko et al., 2005; Sax, Talamini, Brackett, & Fischer, 1986). The dextrose infusion causes an increase of carbon dioxide production and creates a metabolic demand for oxygen that usually cannot be met. Therefore, specific formula of PN for patient with lung diseases should compose of lipid for energy source and limit glucose in order to lower the respiratory quotient (Rose, 1992).

2.2.4 Protein

Protein accretion rates in the fetus at 24-25 weeks, 27-28 weeks, and 30-32 weeks of gestation are estimated to be 4.0, 3.6, and 3.3 g/kg/day, respectively. A range of protein requirement according to age is between 0.8 and 4.0 g/kg/day as shown in Table 3 (Phillips, 2004). Protein requirement decreases as infant grow up and a teen needs protein as much as an adult does. Receiving protein less than 2 g/kg/day can cause growth retardation and edema in an infant. On the other hand, receiving protein more than 4 g/kg/day can cause depression, acidosis, high urea and

ammonia which damage nervous system (พิภพ จิรภิญโญ, 2538). A preterm infant needs high amount of amino acids, especially tyrosine, taurine, histidine and cysteine. Cysteine is a conditionally essential amino acid for neonates to increase calcium and phosphate solubility in PN solutions (Baker et al., 2007).

Table 3 Estimated protein requirement modified from (A.S.P.E.N Board of Directors, 2002)

Age (year)	**Protein requirements (g/kg/day)**
Preterm	3-4
0-1	2-3
1-10	1-1.2
11-18	0.8-1.5

2.2.5 Lipid

An intravenous lipid is provided in PN as a lipid emulsion which provides energy and essential fatty acids (FAs). Lipid emulsion has low osmolality and contains the highest energy density among the energy yield nutrients. Parenteral FAs should be initiated in the first days of life in VLBW infants because VLBW infants are at higher risk of essential FAs deficiency compare to full term infants. Essential FAs deficiency can be prevented by starting an infusion of FAs at a rate of 1-2 g/kg/day. Intravenous lipid emulsion is not recommended to mix with dextrose and amino acids due to turbidity of the PN solution and detection error. Recommended dosing for intravenous lipid is shown in Table 4 (Baker et al., 2007).

Cautions about intravenous lipid emulsion administration include patients with atherosclerotic diseases, coagulation defects, acute pancreatitis, acute respiratory distress syndrome and thrombocytopenia. Intravenous lipid emulsion contains essential FAs (linolenic acid; omega-3 FA, linoleic acid; omega-6 FA). A healthy lipid emulsion contains a balance of omega-3 and omega-6 FA. Omega-3 FA reduces inflammation, and some omega-6 FA tends to promote inflammation. Therefore, there is a concern in prescribing intravenous lipid in infectious patients.

Table 4 Recommended dose of intravenous lipid (g/kg/day)
Modified from (Walker, Watkins, & Duggan, 2003)

Age	**Starting**	**Daily Dose Increase**	**Maximum Dose**
Preterm	0.5-1.0	1.0	3.5
0-6 months	1.0-1.5	1.0-1.5	3.5
6-12 months	1.0-1.5	1.0-1.5	3.0
1-10 years	1.0	1.0-1.5	3.0
11-18 years	1.0	1.0	2.0-3.0

2.2.6 Electrolytes

Total body water is divided in to 2 compartments: intracellular fluid (ICF) and extracellular fluid (ECF). ICF contains potassium, magnesium, phosphorus and sulfates. ECF contains sodium, chloride, bicarbonate, with small amounts of

potassium, calcium, magnesium, phosphorus and sulfates. The daily intravenous electrolytes requirement is shown in Table 5 (Baker et al., 2007).

Table 5 Recommended daily intravenous intake of selected electrolytes modified from (Baker et al., 2007; Goday & Mehta, 2015; Walker et al., 2003)

Age (year)	Electrolytes requirement (mM/kg/day)					
	Sodium	Potassium	Chloride	Magnesium	Calcium	Phosphorus
Preterm	2.0-5.0	2.0-4.0	2.0-3.0	0.15-0.25	1.6-2.5	1.0-2.0
0-1	1.5-4.3	1.4-3.1	1.1-3.4	0.105	1.6-2.0	1.0-1.5
1-10	2.0	2.0	2.0	0.125	1.5	1.0
11-18	30.4	20.5	56.4	0.125-0.25	1.25-1.5	0.5-1.0

2.2.6.1 Sodium

Sodium is the major cation of ECF. Most of the body's sodium is located in blood and fluid around the cells. Sodium helps the body keep a normal fluid balance and plays a key role in normal nerve and muscle function (Lewis, 2017). It is provided in PN as a chloride, acetate, or phosphate salt. The normal sodium requirement is 1.5-30.4 mM/kg/day. Infants with congestive heart failure, acute renal failure, or chronic diuretic therapy need closely monitoring of sodium intake (Baker et al., 2007).

2.2.6.2 Potassium

Potassium is the most abundant cation in ICF. The intracellular concentration of potassium is 150-160 mM/L and the extracellular concentration is 3-5 mM/L.

Potassium is essential in cell function including control of cell volume and acid-base balance, isotonicity, DNA and protein synthesis, maintenance of the electrical property of cell membranes. Potassium is provided in PN as a chloride, acetate, or phosphate salt. The normal potassium requirement is 1.4-20.5 mM/kg/day (Baker et al., 2007).

2.2.6.3 Chloride

Chloride is the anion of ECF. It plays a role in maintenance of fluid balance, acid-base status, and preservation of electrical neutrality. Chloride is provided in PN as a sodium or potassium salt. The normal chloride requirement is 1.1-56.4 mM/kg/day (Baker et al., 2007).

2.2.6.4 Magnesium

Magnesium is the second most abundant cation in ICF. Infants have about 0.8 g of magnesium, with 80% being accreted in the last trimester of pregnancy. It plays an important role in muscle contractility, DNA synthesis, protein synthesis, carbohydrate metabolism, and enzyme function. It also provides appropriate body energy metabolism and electrolyte balances. Most of the magnesium in the body is found in bones and ICF. A tiny amount of magnesium normally presents in blood. Magnesium is provided in PN as a sulfate salt. The normal magnesium requirement is 0.105-0.250 mM/kg/day (Walker et al., 2003).

2.2.6.5 Calcium

Most calcium is found in the skeleton (99%), the remainder is in the teeth, soft tissue, and ECF. Extracellular calcium exists in 3 forms: protein bound form (40-45%), active free form (40-45%), complex form as sulfate or phosphate salts (8-10%). At birth, calcium accounts for 0.9% of body weight. Calcium availability is regulated by parathyroid hormone (PTH), vitamin D, and calcitonin. It plays a role in neuromuscular activity, contractility of the heart and smooth muscle, coagulation, and bone metabolism. During rapid growth, infants need net calcium retention of 3.75-5.00 mM of calcium/day, whereas in adults, the net balance is zero. Calcium is provided in PN as a gluconate or chloride salt. The normal calcium requirement is 1.3-3.0 mM/kg/day (Baker et al., 2007).

2.2.6.6 Phosphorus

Phosphorus is the major anion in ICF. Approximately 85% of body phosphorus is in the skeleton and teeth, 14% is in soft tissue, and the other is in ECF. It plays an important role in protein phosphorylation, nucleotide and phospholipid metabolism, and adenosine triphosphate (ATP) formation. Plasma membranes require phosphorus as a component of phospholipids. Phosphorus is provided in PN as a potassium or sodium salt. The normal phosphorus requirement is 1.0-2.3 mM/kg/day (Baker et al., 2007).

The fetus accumulates 3.00-3.75 mM/kg/day of calcium and 2.25-2.75 mM/kg/day of phosphorus in bone, teeth and soft tissue during the third trimester, a

period of rapid skeletal mineralization (Marks & Crill, 2004). Therefore, inadequate calcium supplementation in preterm infants results in rickets and bone fractures. Preterm infants require higher calcium and phosphorus intake than full term infants, older children, and adults (Table 5) (Walker et al., 2003). In PN, the concern is how to deliver adequate amounts of calcium and phosphorus without precipitation.

2.2.7 Trace elements

Available parenteral trace element products in Thailand are Addamel N® and Peditrace®. Addamel N®, adult preparation of trace element, contains zinc, copper, chromium, manganese, selenium, iodine, molybdenum, iron, and fluoride. Peditrace®, infant preparation of trace element, contains zinc, copper, manganese, selenium, iodine, and fluoride.

Zinc is essential for growth. It is involved in chromosome replication, regulation of genetic translation, stabilization of ribosome and membranes, and components of enzymes. A recommended intravenous PN infusion of zinc is 100 µg/kg/day. Zinc requirement may be much greater in patients who continue to have excessive excretion of zinc and also in preterm infants, 300 µg/kg/day of zinc should be administered (Baker et al., 2007).

Copper is important in many enzymatic systems, mainly oxidases, hydroxylases, and superoxide dismutases. It is necessary for formation of melanin

and cross-linking of elastin and collagen. A recommended intravenous infusion of copper is 20 µg/kg/day, with a maximum up to 0.3 mg/day (Baker et al., 2007).

Chromium acts as a part of a glucose tolerance factor and involvs in insulin activation. In chromium deficiency-related hyperglycemia, a therapeutic trail of intravenous chromium over several days might be beneficial. However, excessive amount of chromium has been found to accumulate in the liver of patients who has been on long term PN. A recommended intravenous infusion of chromium is 0.2 µg/kg/day (Goday & Mehta, 2015).

Manganese is a cofactor for the enzyme pyruvate carboxylase and a part of the mitochondrial form of superoxide dismutase. It play a significant role in antioxidant protection and energy metabolism. A recommended intravenous intake of manganese during PN is 1 µg/kg/day (Baker et al., 2007).

Selenium is a part of glutathione peroxidase, which catalyzes the reduction of hydrogen peroxide (H_2O_2) to water (H_2O). Selenium, in the form of selenoproteins, plays a pivotal role in the antioxidant defence system of the cell. The nuclear factor kappa-B (NF-κB) signaling pathway has been associated with enhanced inflammatory response and its activation has been significantly correlated with interleukin-6 and tumor necrosis factor (TNF)-alpha production. Selenium may inhibit the activation of NF-κB by modulating selenoprotein genes expression. Increased selenium level by increasing selenoprotein biosynthesis leading to suppressed C-reactive protein production thereby attenuates the inflammatory process. Selenium level drops

during the first weeks of life. Selenium-dependent enzyme activity in tissue and body fluids may be a better measure of selenium status than serum selenium in the neonatal period. A recommended intravenous intake of selenium during PN is 2 µg/kg/day (Goday & Mehta, 2015).

Iodine plays a major role in thyroid function. Because of the importance of the thyroid hormones, iodine is essential for the development of brain, muscle, heart, pituitary gland, and kidneys. A recommended intravenous intake of iodine during PN is 1 µg/kg/day (Baker et al., 2007).

Molybdenum is a cofactor of 3 enzymes including sulfite oxidase, xanthine oxidase, and aldehyde oxidase. These enzymes are important for the metabolism of sulfur amino acids and heterocyclic compounds. Therefore, in long term PN without molybdenum, an amino acid imbalance which is correctible by intravenous administration of molybdenate may occur. A recommended intravenous intake of molybdenum during PN is 0.25 µg/kg/day.

Iron is an essential component of heme proteins such as hemoglobin, myoglobin, and cytochrome P450. Iron deficiency is the most common cause of anemia in children. Preterm infants are at particular risk for iron deficiency because iron is transferred to the fetus during the last trimester (Baker et al., 2007). A recommended intravenous intake of iron depends on age. Preterm infants requires 2-4 mg/kg/day of iron whereas children 0-1 year, 1-3 years, 4-8 years and 9-13 years

require 1 mg/kg/day, 7 mg/day, 10 mg/day and 8 mg/day of iron, respectively (Food and Nutrition Board of the Institute of & Medicine., 2000).

2.2.8 Vitamins

Vitamins have been classified according to hydrophilicity as lipid soluble (A, E, K, and D) or water soluble (ascorbic acid, thiamin, riboflavin, niacin, pantothenic acid, pyridoxine, biotin, folate, cyanocobalamin). Vitamins are available in many trade names. The uses of vitamins vary with age of patients. Infants with chronic fat malabsorption require high intake of fat soluble vitamins (Baker et al., 2007). Recommended intake of parenteral vitamins are shown in Table 6 (Walker et al., 2003).

Table 6 Dairy intakes of parenteral vitamins in infants and children modified from (Baker et al., 2007)

Vitamin	Preterm infant	Infant aged < 2.5 years	Children aged > 2.5 years < 11 years
A, retinol equivalent (μg)	500.00	280.00	700.00
E (mg)	2.80	2.80	7.00
K (μg)	80.00	80.00	200.00
D (IU)	160.00	160.00	400.00
Ascorbic acid (mg)	25.00	32.00	80.00
Thiamin (mg)	0.35	0.48	1.20
Riboflavin (mg)	0.15	0.56	1.40
Niacin (mg)	6.8 0	6.80	17.00
Pantothenic acid (mg)	2.00	2.00	5.00
Pyridoxine (mg)	0.18	0.40	1.00
Biotin (μg)	6.00	8.00	20.00
Folate (μg)	56.00	56.00	140.00
Cyanocobalamin (μg)	0.30	0.40	1.00

2.3 Factors influencing calcium phosphate solubility in PN solutions

High calcium and phosphate requirements for some patients might exceed the solubility in PN solutions, particularly when patients are fluid restricted or have several other intravenous fluid lines in place (Walker et al., 2003). The factors affecting the solubility of calcium and phosphate in PN solution, include order of mixing, concentration of amino acid, pH of PN solution, concentration of dextrose, storage duration and temperature, forms of calcium and phosphate salt, concentrations of calcium and phosphate, and other additives added to the solutions (Baker et al., 2007; Bouchoud et al., 2010; Migaki et al., 2012; Ribeiro et al., 2009).

Order of mixing: order of mixing is important. The sequence of compounding starts from amino acid solution, dextrose, phosphate, other additives, and calcium (figure 1). When adding calcium and phosphate to a PN solution, the phosphate should be added first, and the line should be flushed between adding of any potentially incompatible components and the calcium lastly added for decreasing risk of precipitation. The mixture is thoroughly mixed at every step of addition to ensure that all parts of the solution are homogeneous. Vitamin is added before administration (คณะแพทยศาสตร์ มหาวิทยาลัยขอนแก่น, 2543).

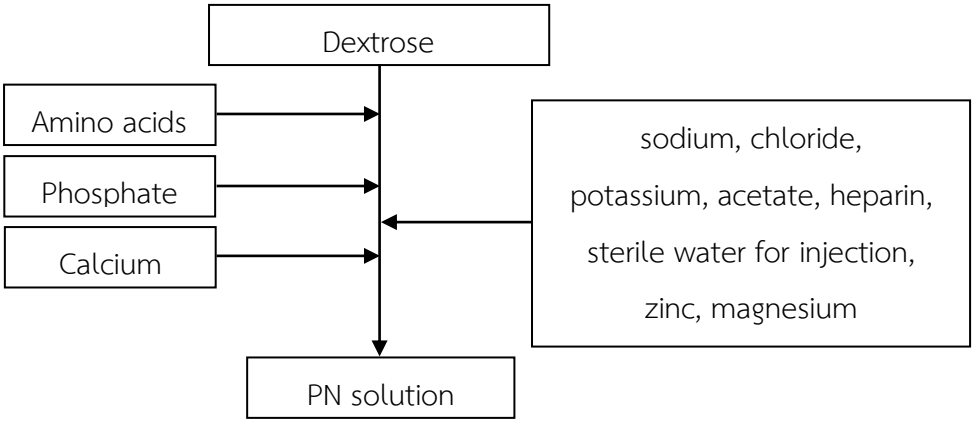


Figure 1 Order of mixing of PN solution

Concentration of amino acid: the concentration of amino acids affects the pH of solution (Marks & Crill, 2004; Migaki et al., 2012). Amino acid solution has buffering capacity and lowers the pH of PN admixture. The pKa for phosphoric acid is 2.15 for the first dissociation, 7.20 for the second dissociation and 12.35 for the third dissociation. These values are based on an ambient temperature of 25 °C, and are known as pKa1, pKa2, and pKa3, respectively. The pKa value indicates the acidity of a solution based on how the hydrogen ions of the acid dissociate when added to an aqueous solution. Since phosphoric acid, represented by the molecular formula H_3PO_4, has three hydrogen atoms, dissociates three times, into $H_2PO_4^-$, HPO_4^{2-}, and PO_4^{3-}. The pKa value is based on the Ka value, which is called the acid dissociation constant. When pH of admixture is lower than 7.20, the equilibrium of phosphate salts shift from dibasic (HPO_4^{2-}) into monobasic ($H_2PO_4^-$) forms which are able to form complex with calcium as shown in Figure 2

$H_3PO_4 \leftrightarrow H_2PO_4^- + H^+$; shifts to right when pH > 2.15 (1)

$H_2PO_4^- \leftrightarrow HPO_4^{2-} + H^+$; shifts to right when pH > 7.20 (2)

$HPO_4^{2-} \leftrightarrow PO_4^{3-} + H^+$; shifts to right when pH > 12.35 (3)

Figure 2 Dissociation equilibrium and pKa of phosphoric acid

(Newton & Driscoll, 2008)

The monobasic calcium phosphate (Ca $[H_2PO_4]_2$) is greater soluble than dibasic calcium phosphate ($CaHPO_4$) (18 and 0.3 mg/mL, respectively) (MacKay, Jackson, Eggert, Fitzgerald, & Cash, 2011; Newton & Driscoll, 2008). The addition of L-cysteine hydrochloride 40 mg/g of protein with a pH of approximately 1.5 further decreases the pH of PN solutions and increases the solubility of calcium and phosphate (Marks & Crill, 2004; Migaki et al., 2012).

Storage duration and temperature: storage condition affects stability of PN solutions. Long storage duration and high temperature enhance risks of precipitation in PN solutions. It is recommended that PN solution should be stored at 25 °C for 1 day and 4 °C for 7 days to avoid calcium phosphate precipitation. (Bouchoud et al., 2010; MacKay & Anderson, 2015; Pereira-da-Silva et al., 2003; Ronchera-Oms et al., 1995).

Concentrations of calcium and phosphate: high amounts of calcium and phosphate added to the solutions are risk of precipitation. Solubility curve for the specific amino acid brand and amino acid concentration have been developed by

specialist to avoid calcium and phosphate precipitation in PN solutions (Baker et al., 2007; MacKay & Anderson, 2015).

Salt forms of calcium and phosphate: the forms of calcium and phosphate salt affect stability of PN solution. The use of inorganic calcium salts (calcium chloride) and inorganic phosphate salts (sodium phosphate or potassium phosphate) result in higher risk of precipitation than the use of organic salts such as calcium gluconate and NaGP (Bouchoud et al., 2010; Huston et al., 2014; Pereira-da-Silva et al., 2003; Ronchera-Oms et al., 1995). Calcium chloride should not be used as the calcium source in parenteral admixture containing phosphate injections, due to high dissociation of calcium which results in $CaHPO_4$ precipitation (Newton & Driscoll, 2008).

Organic compounds are a class of complex molecules which characterized by their use of carbon as a molecule backbone. Molecules of organic compounds are interacting between atoms, by covalent bond. It is very stable and melting point or break up at 300 °C (สุภาวดี สินเส็ง, 2558). Organic phosphate was introduced on the pharmaceutical market as NaGP. NaGP is an intravenous phosphate supplement which FDA approved for the prophylaxis in intravenous nutrition to meet the requirement of phosphate in adult and infant patients. One milliliter of NaGP solution contains 1 mM of phosphate and 2 mM of sodium. NaGP was claimed to increase solubility in solution containing high concentration of calcium or high pH.

Previous studies used NaGP at the concentration of 50 mM/L corresponding to calcium gluconate at the concentration of 25 mM/L showed no precipitation (MacKay & Anderson, 2015). The used of 50 mM/L of organic phosphate showed no precipitation with either inorganic calcium or organic calcium (Bouchoud et al., 2010; Ronchera-Oms et al., 1995). Currently, there is no study about maximum concentration of NaGP and calcium gluconate without precipitation in PN solutions (Ribeiro et al., 2009).

Other additives added to the solutions: in practice, infant PN solutions contain other components including potassium chloride, potassium acetate, sodium acetate, zinc sulfate, magnesium sulfate, vitamins, and heparin. These additives added to the solutions in large volume may affect compatibility and stability of PN solutions (คณะแพทยศาสตร์ มหาวิทยาลัยขอนแก่น, 2543).

2.4 Compatibility and stability of calcium and phosphate in PN solutions

To meet the daily requirement of calcium and phosphorus in infants, amount of the minerals in PN could approach a critical concentration at which precipitation occurs. This is especially critical when fluid volume is restricted. Several studies were investigated to solve an incompatibility problem of calcium and phosphate in PN solution.

Previous studies have reported the incompatibility of inorganic calcium and inorganic phosphate. Migaki et al. (2012) studied the compatibility of PN solution

containing various amounts of amino acids (TrophAmine®), 5-12.5 mM/L of calcium chloride and 5-20 mM/L of sodium phosphate. PN solutions were stored at 37°C for 23–24 hours. Visual inspection was performed to evaluate for the presence of precipitation by three investigators. The study showed a maximum sodium phosphate concentration of 15 mM/L could be added to a solution containing 12.5 mM/L of calcium chloride without precipitation in the amino acid concentration ≥3 %. Huston et al. (2014) studied the compatibility of 2.5-15 mM/L of calcium chloride and 5-20 mM/L of potassium phosphate mixed with Trophamine® and Premasol®. PN solutions were incubated at 37 °C for 24 hours. Visual inspection and dynamic light scattering were done. In PN solutions containing 2.5–3% amino acids was compatible with 10 mM/L of calcium chloride and 7.5 mM/L of potassium phosphate. More studies are needed to determine the long-term effect of substituting calcium chloride for calcium gluconate in PN solutions.

Mo-suwan et al. (1997) reported the calcium and phosphate solubility curve for infant PN solutions containing Vaminolact®. The PN solutions containing various concentrations of amino acids, calcium gluconate and dipotassium phosphate were prepared. After 24 hours at room temperature, the samples were visually inspected for evidence of precipitates. The clear samples were checked under the microscope with polarizers at the power 40X for evidence of microcrystallization. Maximum compatible calcium and phosphate concentration is 1:1 of molar ratio in the PN samples at different levels of Vaminolact®.

Parikh et al. (2005) studied the compatibility of 15 mM/L of calcium gluconate and 5 mM/L of sodium phosphate mixed with 0.5-4% amino acids (TrophAmine®). All solutions contained 40 mg of cysteine hydrochloride per gram of amino acid. The number of particle counts per milliliter of PN solutions and pH measurement were performed within 1 hour and at 6, 24, and 30 hours after mixing at 23–27 °C. Precipitated compound was identified by polarized microscopy and infrared spectroscopy. The PN solutions with 0.5% amino acids, as well as the highest pH, resulted in significant growth of particulate matter over time. Amino acid concentrations of 1–4% in cysteine-added solution with 15 mM/L of calcium gluconate and 5 mM/L of sodium phosphate compatible for up to 30 hours at 27 °C.

Joy et al. (2010) studied the stability of PN solutions containing 1%, 2%, or 3% amino acids with 2.5, 5 mM/L of calcium gluconate and 15, 30 mM/L of sodium phosphate. PN solutions were analyzed for subvisible micro-precipitates using the light obscuration method at 7 time intervals over 48 hours at 30°C. Any precipitated material was characterized by polarized light microscopy and infrared spectroscopy. The PN solutions with the concentration of amino acids 1% and 2% with 5 mM/L of calcium gluconate and 30 mM/L of sodium phosphate showed significant increase in particle counts per milliliter which was similar to the previous study (Parikh et al., 2005). Amino acid concentration ≤ 3% was compatible with 2.5 mM/L of calcium gluconate and 15 mM/L of sodium phosphate.

Organic phosphate might be replaced inorganic phosphate to mix with both form of calcium to ensure the compatibility. Ronchera-oms et al. (1995) studied the stability of PN solutions containing 0.5% amino acids (Vamin® 14 EF) with 4.5 mM/L of calcium chloride and 10 mM/L of glucose-1-phosphate (G1P), 10.47 mM/L of glycerophosphate or 10 mM/L of inorganic phosphate. All PN solutions were stored at 5 ℃ or 22 ℃. Physical stability analysis, including pH measurement, visual inspection and nephelometry were performed at 0, 24, 48 and 72 hours. In PN solutions containing G1P or glycerophosphate were physically stable for 3 days.

Bouchoud et al. (2010) studied the stability of PN solutions containing 0.4% amino acids (Vaminolact®) with sodium phosphate mixed with calcium chloride, sodium phosphate mixed with calcium glubionate, G1P mixed with calcium chloride, and G1P mixed with calcium glubionate. All samples were stored at 4°C for 48 hours and at 32°C for 24 hours. The samples were visually inspected for evidence of precipitation and particle count. Calcium glubionate slightly decreased the risk of precipitation compared to calcium chloride. G1P was associated with a decreased risk of precipitation compared to sodium phosphate. No precipitation occurred when mixing 50 mM/L of G1P with 50 mM/L of calcium chloride or calcium glubionate.

NaGP, another organic phosphate, was also investigated in PN solutions. Oliveira-Ribeiro et al. (2009) studied the stability of PN solutions containing 1% amino acids compounded with 0, 11.63 or 23.25 mM/L of calcium gluconate and 3.67 mM/L

of NaGP. The mixtures were stored at 4°C, 25°C or 37°C and evaluated by visual inspection for 7 days. None of them showed precipitation.

MacKay and Anderson (2015) studied the compatibility of 5, 10, 15, 20, and 25 mM/L of calcium gluconate and corresponding 10, 20, 30, 40, and 50 mM/L of NaGP mixed with 1.5, 4% amino acids (TrophAmine®). PN solutions were stored at room temperature and 37°C for 24 hours. Compatibility was evaluated by visual inspection, microscopy and turbidity. It is recommended that NaGP should be used as a phosphate source instead of sodium phosphate or potassium phosphate in PN solutions to avoid precipitation of calcium phosphate in the solution containing high concentration of mineral contents.

2.5 Testing for calcium phosphate precipitation

Published calcium phosphate compatibility curve are most commonly based on calcium gluconate or a combination of calcium salts. Numerous studies have been conducted to report several factors affecting calcium and phosphate precipitation, resulting in published calcium-phosphate compatibility curve. Methods for determining the presence of calcium phosphate precipitates include automate particle counters, scanning electron microscope (SEM), potentiometric titration, ultraviolet – visible light spectrophotometer (UV-Vis spectrophotometer), pH measurement, visual inspection, turbidity test, microscopic particle count test. Automate particle counters are used to quantify the size and quantity of particulate contamination in fluids (AVL List GmbH Hans-List-Platz, 2010; MP Filtri, 2013). SEM is

not a recommended for routine test of PN compatibility due to high cost and sophisticated sample preparation (Oliver Kim, 2016).

pH values predict forms of phosphoric acid which complex with calcium. The monobasic calcium phosphate ($Ca[H_2PO_4]_2$) is greater soluble than dibasic calcium phosphate ($CaHPO_4$). Phosphoric acid ionizes upon dissolving in water, mainly to give $H_2PO_4^-$ and protons (Lardbucket, 2012).

Visual inspection is one of the primary inspection methods to detect particles in PN solutions. This method is further recommended by United States Pharmacopeia (USP) 797 and Parenteral Quality Control for routine test of PN compatibility. Visual inspection is a quick and easy test to detect particle sizes of about 50 μm. However, other methods are required to detect very small particles (Joy et al., 2010).

Turbidity test is also recommended by USP 797. Turbidity is usually expressed in nephelometric turbidity units (NTU). Turbidity can be measured using either a turbidity tube or a turbidity meter. Turbidity tube is a simple design, low cost method and not easily damaged, however it is less precise and cannot measure very low turbidity (usual minimum is 5 NTU). Turbidity meter can measure scattered light at an angle of 90° (Aqualytic, 2014). It is very accurate and useful for measuring very low turbidity (less than 5 NTU). However, it is expensive, easily damaged and needs

power supply. Slightly large particles and less light scattering solutions could affect the turbidity reading (ReseachGate, 2012).

Microscopic particle count test is another analysis method in USP 797 and Parenteral Quality Control. Microscopic particle count test is a procedure for the determination of particulate matter in PN solutions and setting upper limit acceptable particle size standards at 10 μm and 25 μm. These two sizes are also subsequently determined as standard sizes for particulate matter in small-volume injections.

Each testing method has advantages and disadvantages. This study used several methods which were pH measurement, visual inspection, turbidity test and microscopic particle count test. These methods are less time consuming, easy, available for routine test, very accurate and reliable. However, their costs are also reasonable.

CHAPTER III
MATERIALS AND METHODS

3.1 Research design

This study was an experimental study to investigate the solubility of calcium gluconate and NaGP in infant PN solution. PN solution orders and infant physician's advice were required to identify represented PN formulas of this study. Studied PN formula contained amino acids, dextrose, chloride, magnesium, sodium, calcium and phosphate. Compounding 100-mL PN solutions were triplicately prepared (n = 3/formulation). Each 25-mL admixture was separately tested according to 4 following storage conditions; room temperature for 30 minutes, room temperature for 1 day, 4 ºC for 1 day and 4 ºC for 7 days. Solubility data were finally constructed and applied according to practically prescribed PN orders at Ramathibodi Hospital.

3.2 Materials

10% Aminoven infant® (Fresenius Kabi Austria GmbH, Austria Lot No.16IL2787)

50% dextrose in water (General Hospital Products Public Co., Ltd., Thailand Lot No.1605216)

8.71% Dipotassium phosphate (Thai Otsuka Pharmaceutical Co., Ltd., Thailand Lot No.M6D73)

Sodium glycerophosphate (Fresenius Kabi Austria GmbH, Austria Lot No. 12KFL19)

3% Sodium chloride (General Hospital Products Public Co., Ltd., Thailand Lot No.1606005)

15% Potassium chloride (NIDA Pharma Incorporation, Thailand Lot No.A16188)

24.6% Sodium acetate (Rajavithi Hospital, Thailand Exp.18/11/2017)

29.4% Potassium acetate (Rajavithi Hospital, Thailand Exp.27/10/2017)

Heparin 5,000 units/ml (Leo Pharma @ DKSH .Co. Ltd., Switzerland Lot No.A14037)

Normal saline solution (Thai Otsuka Pharmaceutical Co., Ltd., Thailand Lot No.6L751)

Sterile water for injection (General Hospital Products Public Co., Ltd., Thailand Lot No. 1607004)

Zinc sulfate 1 mg/ml (Rajavithi Hospital, Thailand Exp.1/12/2017)

50% Magnesium sulfate (Atlantic Laboratories, Thailand Lot No.163003)

10% Calcium gluconate (The Government Pharmaceutical Organization, Thailand Lot No.J 590180)

3.3 Instruments

Autoclave (Ikiken Co., Ltd., Japan)

Hot air oven (WTB binder 78532 Tuttlingen, Germany)

Laminar air flow HVB, HH, HV (Boss Scientific Associate L.P., Thailand)

Vortex-Genie (Becthai Bangkok Equipment & Chemical Co., Ltd., Thailand)

Thermometer (Sato, Japan)

Black and white background with LED lamp (Sylvania, China)

pH meter (Mettler Toledo S220, SevenCompact^{TM})

Turbidity and free/Total Chlorine Meter (HI 93414, Hanna Instruments, Italy)

Cellulose Nitrate membrane filter, 0.45 μm, diameter 13 mm (Sartorius Stedim Biotech GmbH, Germany)

Light microscope (Nikon Eclipse E200, Inter Instrument.co., Ltd, Japan)

3.4 Study variables

Variables of this study were as following:

1. Studied variables

   1.1 Concentrations of amino acids in PN solution

   1.2 Storage conditions: room temperature (27-34 °C) for 30 minutes, room temperature for 1 day, 4 °C for 1 day and 4 °C for 7 days

   1.3 Concentrations of sodium in PN solution

   1.4 Concentrations of calcium in PN solution

   1.5 Concentrations of phosphate in PN solution

2. Controlled variables

   The components affecting solubility of calcium and phosphate in PN solutions were added in fixed amounts. According to PN orders for infant patients of Ramathibodi Hospital, the following concentrations were used in the study as controlled variables.

   2.1 Concentrations of dextrose in PN solution

   2.2 Concentrations of chloride in PN solution

   2.3 Concentrations of magnesium in PN solution

3.5 Methods (Figure 3)

3.5.1 Selection of PN solutions

Ramathibodi Hospital infant PN solutions orders during June to August in 2015 were reviewed. All relevant data were recorded including ordered concentrations of amino acid, dextrose, sodium, chloride, magnesium, calcium and phosphate except individual patient's data. Infant physician's advice was required to identify appropriate PN formulas used in this study.

3.5.2 Calculation of working formula

Each formula of PN solution was prepared at 100-mL sample, and composed of amino acids, dextrose, phosphate, sodium, chloride, magnesium and calcium. All compositions were calculated according to available parenteral products in the market. The calculation for working formula was presented in Appendix A.

3.5.3 Compounding PN solutions

PN solutions were aseptically compounded in the laminar airflow hood. Each 100-mL admixture was triplicately prepared in sterile Erlenmeyer flasks. The sequence of compounding was as following: amino acid, dextrose, NaGP or dipotassium phosphate, sodium chloride, sterile water for injection, magnesium sulfate and calcium gluconate. Admixture solutions were thoroughly mixed by vortex mixer at every step of addition. Each 25-mL admixture was separately stored according to 4 following storage conditions; room temperature for 30 minutes, room

temperature for 1 day, 4 ºC for 1 day and 4 ºC for 7 days. Solubility tests were done at each time point.

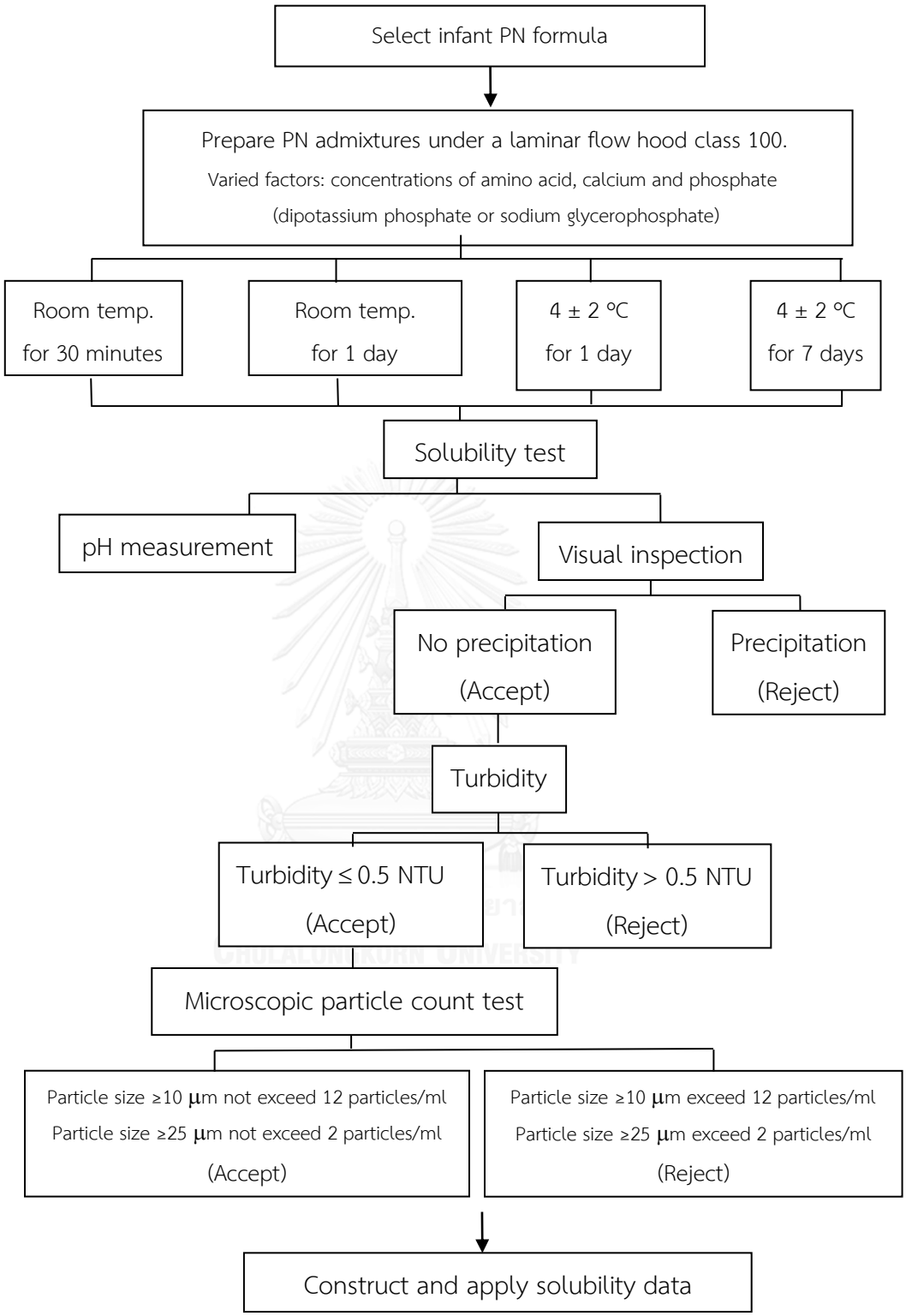


Figure 3 Experimental design of the study

3.5.4 Solubility tests

3.5.4.1 pH measurement

pH value of samples were measured by using a S220 SevenCompact™ pH/Ion pH meter (Mettler Toledo, Switzerland) at room temperature (Bouchoud et al., 2010; ศรีสมร สิทธิกาญจนกุล, 2550). The pH meter was calibrated by using 4.0, 7.0, and 10.0 standard pH buffers prior to measurements. pH value greater than 7.2 was determined as the "arbitrary threshold for precipitation" (Newton and Driscoll, 2008).

3.5.4.2 Visual inspection

All samples were inspected for precipitation, film formation, phase separation and color change at every storage conditions. They were visually inspected against black and white contrast backgrounds with 3-watt LED lamp (Sylvania, China) 220-240 V at 10 inches from the light source. This light intensity specification for visual inspection was quoted under Parenteral Quality Control and USP particulate matter testing (Akers & Larrimore, 2003; The United States Pharmacopeia, 2016). Visual inspection detects limited particle sizes of about 50 μm. Due to the smallest capillary blood vessels have diameter of approximately 4 μm, other methods were required to identify very small particles which human eyes could not detect (Joy et al., 2010).

3.5.4.3 Turbidity test

PN samples which passed the visual inspection were then measured turbidity using Turbidity and free/Total Chlorine Meter (HI 93414, Hanna Instruments, Italy). The standard solutions, HI 93703-0 (represented 0 NTU) and HI 93703-10 (represented 10 NTU), were used as calibrating solutions prior to measurements. Turbidity greater than 0.5 NTU was determined as the "arbitrary threshold for precipitation" (MacKay & Anderson, 2015; Pereira-da-Silva et al., 2003; The United States Pharmacopeia, 2016).

3.5.4.4 Microscopic particle count test

If the PN samples had turbidity less than 0.5 NTU, they were then inspected under light microscope (Nikon Eclipse E200, Inter Instrument.co., Ltd, Japan). Five ml of tested solutions were filtered through 0.45 μm nitrocellulose membrane filters with diameter 13 mm (MacKay & Anderson, 2015). The membranes were microscopically examined according to the United States Pharmacopeia standards for evidence of micro-crystallization under 10X magnification. The particles which were equal to or greater than 10 μm and 25 μm compared to the reference scale were then recorded. The solutions were further analyzed using a microscopic particle count test. The maximal crystal content for each 5-mL test solutions containing less than 60 particles measuring 10 μm in diameter together with less than 10 particles measuring 25 μm in diameter considered as physically compatible (The United States Pharmacopeia, 2016).

3.5.5 Construction and application of solubility data

All results from solubility tests were applied to construct solubility data of calcium gluconate and NaGP. The solubility data were separately drawn by considering different concentrations of amino acids, storage durations and temperatures. They contain 3 concentrations of amino acids corresponding to 4 storage conditions. Y axis was the concentration of calcium gluconate and X axis was the concentration of NaGP. Solubility data of calcium gluconate and dipotassium phosphate were constructed using the same condition as mentioned above to ensure intralaboratory and personnel reliability. Solubility data were made by plotting the maximum concentration of calcium and phosphate which were still compatible in PN solution. The higher concentrations of calcium and phosphate above the line, the greater the probabilities of precipitation would occur.

In practice, infant PN solutions contain other components including potassium chloride, potassium acetate, sodium acetate, zinc sulfate and heparin. In this study, solubility data were applied by Ramathibodi Hospital infant PN solution. PN solutions were underwent solubility tests at difference storage conditions.

3.6 Data collection and analysis

Visual inspection, pH measurement, turbidity, microscopic particle count test were triplicate. The results of visual inspection were recorded as "✓" for clear PN solutions and "✗" for turbid PN solutions. pH value of sample and turbidity were shown as mean and standard deviation (S.D.). The pH value less than 7.2 or turbidity

less than 0.5 NTU were determined as pass. Microscopic particle count test were recorded as maximum particle count per 5 ml. The maximal particle count contain less than 60 particles measuring 10 μm in diameter together with less than 10 particles measuring 25 μm in diameter were determined as pass. All results were represented as pass or fail to the acceptance criteria. Solubility data were finally constructed and applied by Ramathibodi Hospital infant PN solution orders. The test results were coherent that solubility data from this study can be used for prescription and preparation of infant PN solutions.

CHAPTER IV
RESULTS

4.1 Selection PN solutions and calculation working formula

Ramathibodi Hospital infant PN solutions orders during June to August in 2015 were recorded. Total infant PN solutions orders were 610 prescriptions. Concentrations of calcium and NaGP in a range of minimum to maximum and the mostly ordered concentrations of amino acid, dextrose, sodium, chloride, magnesium in PN solution orders were represented in this study. All compositions were calculated according to available parenteral products in the market. PN formulas and working formula used in this study were shown in Table 7. PN formulas of calcium gluconate and dipotassium phosphate used in this study were selected from preliminary study as shown in Appendix B.

Table 7 PN formulas and working formula used in this study

PN solution formula		Working formula	
Components	Concentration	Components	Volume
Amino acids	1.5, 2, or 2.5%	10% amino acid solution	15, 20, or 25 mL
Calcium	0-100 mM/L	10% Ca gluconate	0-44.44 mL
Phosphate	0-150 mM/L	Sodium glycerophosphate	0-15 mL
Sodium	51.3-351.3 mM/L	3% NaCl	10 mL
		Sodium glycerophosphate	0-15 mL
Dextrose	10%	50% dextrose in water	20 mL
Chloride	51.3 mM/L	3% NaCl	10 mL
Magnesium	2.025 mM/L	50% $MgSO_4$	0.1 mL
Sterile water for injection	-	Sterile water for injection	qs to 100 mL

4.2 pH values

The pH values of samples were measured using a S220 SevenCompact™ pH/Ion pH meter (Mettler Toledo, Switzerland) at room temperature. Each PN solution was triplicately measured. The values were expressed in mean ± S.D. (Table 8-10). The PN solutions showed high pH values when the solutions contained low concentrations of calcium and high concentrations of phosphate. Similar results, the PN solutions showed high pH values when PN solutions stored at 4 °C with longer storage duration. The pH values of all PN solutions were less than 7.2. All PN solutions were determined as pass and low risk for precipitation.

Table 8 The pH values of tested PN solutions containing 1.5% amino acid and various concentrations of calcium gluconate and NaGP (mean ± S.D.)

Concentrations of Ca gluconate (mM/L)	Concentrations of NaGP (mM/L)						
	0	25	50	75	100	125	150
Room temperature for 30 minutes							
0	5.820±0.000	6.110±0.000	6.400±0.000	6.550±0.000	6.680±0.000	6.740±0.000	6.827±0.015
25	5.840±0.000	6.090±0.000	6.360±0.000	6.510±0.000	6.653±0.006	6.710±0.000	6.770±0.000
50	5.100±0.100	6.100±0.000	6.310±0.000	6.460±0.000	6.610±0.000	6.687±0.006	6.743±0.006
75	6.060±0.000	6.160±0.000	6.260±0.000	6.430±0.010	6.560±0.000	6.650±0.000	6.720±0.000
100	5.870±0.000	6.040±0.000	6.220±0.000	6.383±0.006	6.520±0.000	NT1	NT1
Room temperature for 1 day							
0	5.830±0.000	6.113±0.006	6.410±0.000	6.553±0.006	6.687±0.006	6.750±0.000	6.840±0.000
25	5.847±0.006	6.100±0.000	6.360±0.000	6.520±0.000	6.660±0.000	6.720±0.000	6.780±0.000
50	5.920±0.000	6.110±0.000	6.320±0.000	6.467±0.006	6.620±0.000	6.690±0.000	6.750±0.000
75	6.070±0.000	6.170±0.000	6.270±0.000	6.440±0.000	6.570±0.000	6.660±0.000	6.733±0.006
100	5.880±0.000	6.047±0.006	6.220±0.000	6.390±0.000	6.527±0.006	NT1	NT1
4 °C for 1 day							
0	5.850±0.000	6.140±0.000	6.440±0.000	6.563±0.012	6.720±0.000	6.770±0.000	6.850±0.020
25	5.870±0.000	6.120±0.000	6.387±0.006	6.543±0.006	6.680±0.000	6.740±0.000	6.800±0.000
50	5.940±0.000	6.127±0.006	6.340±0.000	6.490±0.000	6.640±0.000	6.710±0.000	6.777±0.006
75	6.093±0.006	6.187±0.006	6.277±0.006	6.457±0.006	6.600±0.000	6.680±0.000	6.767±0.015
100	5.900±0.000	6.060±0.010	6.240±0.000	6.413±0.006	6.547±0.006	NT1	NT1
4 °C for 7 days							
0	5.860±0.000	6.140±0.000	6.430±0.000	6.570±0.000	6.720±0.000	6.780±0.000	6.860±0.012
25	5.877±0.006	6.117±0.006	6.390±0.000	6.550±0.000	6.690±0.000	6.750±0.000	6.810±0.000
50	5.940±0.000	6.130±0.000	6.340±0.000	6.500±0.000	6.640±0.000	6.710±0.000	6.780±0.000
75	6.097±0.006	6.190±0.000	6.290±0.000	6.460±0.010	6.600±0.000	6.680±0.000	6.750±0.000
100	5.900±0.000	6.070±0.000	6.250±0.000	6.420±0.000	6.550±0.000	NT1	NT1

Ca = calcium, NaGP = sodium glycerophosphate

NT1 = Not test due to limitation of commercial products concentrations

Table 9 The pH values of tested PN solutions containing 2% amino acid and various concentrations of calcium gluconate and NaGP (mean ± S.D.)

Concentrations of Ca gluconate (mM/L)	Concentrations of NaGP (mM/L)						
	0	25	50	75	100	125	150
Room temperature for 30 minutes							
0	5.513±0.006	6.567±0.006	6.743±0.006	6.847±0.006	6.987±0.006	6.887±0.021	6.940±0.010
25	5.440±0.000	6.343±0.006	6.540±0.000	6.710±0.000	6.813±0.006	6.800±0.000	6.867±0.006
50	5.470±0.010	6.310±0.000	6.413±0.006	6.627±0.006	6.727±0.006	6.750±0.000	6.797±0.006
75	5.523±0.006	6.240±0.000	6.377±0.012	6.600±0.000	6.590±0.010	6.693±0.006	6.777±0.006
100	5.540±0.000	6.230±0.000	6.390±0.010	NT1	NT1	NT1	NT1
Room temperature for 1 Day							
0	5.520±0.000	6.570±0.000	6.747±0.006	6.837±0.006	7.010±0.010	6.90±0.000	6.967±0.023
25	5.420±0.000	6.270±0.000	6.610±0.010	6.710±0.000	6.833±0.006	6.81±0.000	6.870±0.000
50	5.483±0.006	6.297±0.006	6.543±0.006	6.637±0.006	6.750±0.000	6.747±0.006	6.800±0.000
75	5.540±0.000	6.250±0.000	6.423±0.006	6.610±0.000	6.573±0.006	6.687±0.006	6.767±0.015
100	5.570±0.000	6.210±0.000	6.400±0.000	NT1	NT1	NT1	NT1
4 °C for 1 day							
0	5.490±0.000	6.580±0.000	6.773±0.006	6.877±0.012	7.017±0.006	6.940±0.010	6.980±0.010
25	5.483±0.006	6.270±0.000	6.610±0.000	6.763±0.006	6.850±0.000	6.850±0.000	6.913±0.006
50	5.490±0.000	6.327±0.006	6.533±0.006	6.670±0.000	6.753±0.006	6.787±0.006	6.830±0.010
75	5.550±0.000	6.240±0.000	6.520±0.000	6.633±0.006	6.610±0.010	6.727±0.006	6.810±0.000
100	5.573±0.006	6.217±0.006	6.473±0.006	NT1	NT1	NT1	NT1
4 °C for 7 days							
0	5.467±0.006	6.593±0.006	6.737±0.006	6.867±0.006	6.990±0.000	6.933±0.006	6.997±0.006
25	5.433±0.006	6.270±0.000	6.610±0.000	6.727±0.006	6.823±0.006	6.850±0.000	6.903±0.006
50	5.463±0.006	6.283±0.006	6.540±0.000	6.633±0.006	6.750±0.000	6.790±0.000	6.850±0.000
75	5.513±0.006	6.210±0.000	6.457±0.006	6.600±0.000	6.640±0.000	6.730±0.000	6.723±0.006
100	5.533±0.006	6.177±0.006	6.423±0.006	NT1	NT1	NT1	NT1

Ca = calcium, NaGP = sodium glycerophosphate

NT1 = Not test due to limitation of commercial products concentrations

Table 10 The pH values of tested PN solutions containing 2.5% amino acid and various concentrations of calcium gluconate and NaGP (mean ± S.D.)

Concentrations of Ca gluconate (mM/L)	Concentrations of NaGP (mM/L)						
	0	25	50	75	100	125	150
Room temperature for 30 minutes							
0	5.563±0.006	6.570±0.000	6.650±0.000	6.867±0.006	6.870±0.010	6.927±0.006	6.937±0.006
25	5.497±0.006	6.263±0.006	6.563±0.006	6.650±0.000	6.760±0.000	6.783±0.006	6.850±0.000
50	5.527±0.006	6.170±0.000	6.500±0.000	6.573±0.006	6.673±0.006	6.720±0.000	6.790±0.000
75	5.563±0.006	6.130±0.000	6.380±0.000	6.523±0.006	6.560±0.000	NT1	NT1
100	5.583±0.006	NT1	NT1	NT1	NT1	NT1	NT1
Room temperature 1 day							
0	5.540±0.000	6.563±0.006	6.660±0.000	6.870±0.000	6.860±0.000	6.900±0.000	6.930±0.000
25	5.490±0.000	6.260±0.000	6.570±0.000	6.657±0.006	6.767±0.006	6.733±0.006	6.850±0.000
50	5.533±0.006	6.180±0.000	6.510±0.000	6.570±0.000	6.680±0.000	6.727±0.006	6.780±0.000
75	5.557±0.006	6.123±0.006	6.393±0.006	6.530±0.000	6.580±0.000	NT1	NT1
100	5.587±0.006	NT1	NT1	NT1	NT1	NT1	NT1
4 °C for 1 day							
0	5.543±0.006	6.573±0.006	6.663±0.006	6.897±0.006	6.887±0.006	6.910±0.000	6.950±0.000
25	5.490±0.000	6.257±0.006	6.577±0.006	6.690±0.000	6.803±0.006	6.770±0.000	6.850±0.000
50	5.530±0.010	6.163±0.006	6.503±0.006	6.597±0.006	6.700±0.000	6.720±0.000	6.770±0.000
75	5.543±0.006	6.140±0.000	6.417±0.006	6.557±0.006	6.593±0.006	NT1	NT1
100	5.573±0.006	NT1	NT1	NT1	NT1	NT1	NT1
4 °C for 7 days							
0	5.570±0.000	6.580±0.000	6.667±0.006	6.877±0.006	6.883±0.006	6.907±0.006	6.943±0.006
25	5.500±0.000	6.260±0.000	6.570±0.000	6.647±0.006	6.800±0.000	6.760±0.010	6.847±0.006
50	5.513±0.006	6.170±0.000	6.510±0.000	6.567±0.006	6.687±0.006	6.720±0.000	6.773±0.006
75	5.520±0.010	6.133±0.006	6.377±0.006	6.527±0.006	6.587±0.006	NT1	NT1
100	5.543±0.006	NT1	NT1	NT1	NT1	NT1	NT1

Ca = calcium, NaGP = sodium glycerophosphate

NT1 = Not test due to limitation of commercial products concentrations

4.3 Visual inspection

Samples were visually inspected against black and white contrast backgrounds at 10 inches from the light source. Visual inspection was recorded as "✓" for clear PN solutions and "✘" for turbid PN solutions. Data were shown in Table 11-13 and all pictures were shown in Appendix C. PN solutions showed precipitation when the solutions contained high concentration of calcium and phosphate. Similar results, PN solutions showed precipitation when the solutions were stored at room temperature with longer storage duration. Samples containing 1.5% amino acid, 75 mM/L of calcium gluconate with 100, 125, 150 mM/L of NaGP and 100 mM/L of calcium gluconate with 100 mM/L of NaGP showed precipitation at room temperature. Similar results, samples containing 2% amino acid, 75 mM/L of calcium gluconate with 100, 125, 150 mM/L of NaGP showed precipitation at room temperature. PN stored at 4 $^{\circ}$C showed no precipitation. Samples containing 2.5% amino acid showed no precipitation at all concentrations of calcium gluconate and NaGP used in this study.

Table 11 Visual inspections of tested PN solutions containing 1.5% amino acid and various concentrations of calcium gluconate and NaGP

Concentrations of Ca gluconate (mM/L)	Concentrations of NaGP (mM/L)						
	0	25	50	75	100	125	150
Room temperature for 30 minutes							
0	✓	✓	✓	✓	✓	✓	✓
25	✓	✓	✓	✓	✓	✓	✓
50	✓	✓	✓	✓	✓	✓	✓
75	✓	✓	✓	✓	✓	✓	✓
100	✓	✓	✓	✓	✓	NT1	NT1
Room temperature for 1 day							
0	✓	✓	✓	✓	✓	✓	✓
25	✓	✓	✓	✓	✓	✓	✓
50	✓	✓	✓	✓	✓	✓	✓
75	✓	✓	✓	✓	✘	✘	✘
100	✓	✓	✓	✓	✘	NT1	NT1
4 °C for 1 day							
0	✓	✓	✓	✓	✓	✓	✓
25	✓	✓	✓	✓	✓	✓	✓
50	✓	✓	✓	✓	✓	✓	✓
75	✓	✓	✓	✓	✓	✓	✓
100	✓	✓	✓	✓	✓	NT1	NT1
4 °C for 7 days							
0	✓	✓	✓	✓	✓	✓	✓
25	✓	✓	✓	✓	✓	✓	✓
100	✓	✓	✓	✓	✓	✓	✓
100	✓	✓	✓	✓	✓	✓	✓
100	✓	✓	✓	✓	✓	NT1	NT1

✓ Clear PN solutions and ✘ Turbid PN solutions

Ca = calcium, NaGP = sodium glycerophosphate

NT1 = Not test due to limitation of commercial products concentrations

Table 12 Visual inspections of tested PN solutions containing 2% amino acid and various concentrations of calcium gluconate and NaGP

Concentrations of Ca gluconate (mM/L)	Concentrations of NaGP (mM/L)						
	0	25	50	75	100	125	150
Room temperature for 30 minutes							
0	✓	✓	✓	✓	✓	✓	✓
25	✓	✓	✓	✓	✓	✓	✓
50	✓	✓	✓	✓	✓	✓	✓
75	✓	✓	✓	✓	✓	✓	✓
100	✓	✓	✓	NT1	NT1	NT1	NT1
Room temperature for 1 day							
0	✓	✓	✓	✓	✓	✓	✓
25	✓	✓	✓	✓	✓	✓	✓
50	✓	✓	✓	✓	✓	✓	✓
75	✓	✓	✓	✓	✗	✗	✗
100	✓	✓	✓	NT1	NT1	NT1	NT1
4 °C for 1 day							
0	✓	✓	✓	✓	✓	✓	✓
25	✓	✓	✓	✓	✓	✓	✓
50	✓	✓	✓	✓	✓	✓	✓
75	✓	✓	✓	✓	✓	✓	✓
100	✓	✓	✓	NT1	NT1	NT1	NT1
4 °C for 7 days							
0	✓	✓	✓	✓	✓	✓	✓
25	✓	✓	✓	✓	✓	✓	✓
50	✓	✓	✓	✓	✓	✓	✓
75	✓	✓	✓	✓	✓	✓	✓
100	✓	✓	✓	NT1	NT1	NT1	NT1

✓ Clear PN solutions and ✗ Turbid PN solutions

Ca = calcium, NaGP = sodium glycerophosphate

NT1 = Not test due to limitation of commercial products concentrations

Table 13 Visual inspections of tested PN solutions containing 2.5% amino acid and various concentrations of calcium gluconate and NaGP

Concentrations of Ca gluconate (mM/L)	Concentrations of NaGP (mM/L)						
	0	25	50	75	100	125	150
Room temperature for 30 minutes							
0	✓	✓	✓	✓	✓	✓	✓
25	✓	✓	✓	✓	✓	✓	✓
50	✓	✓	✓	✓	✓	✓	✓
75	✓	✓	✓	✓	✓	NT1	NT1
100	✓	NT1	NT1	NT1	NT1	NT1	NT1
Room temperature for 1 day							
0	✓	✓	✓	✓	✓	✓	✓
25	✓	✓	✓	✓	✓	✓	✓
50	✓	✓	✓	✓	✓	✓	✓
75	✓	✓	✓	✓	✓	NT1	NT1
100	✓	NT1	NT1	NT1	NT1	NT1	NT1
4 °C for 1 day							
0	✓	✓	✓	✓	✓	✓	✓
25	✓	✓	✓	✓	✓	✓	✓
50	✓	✓	✓	✓	✓	✓	✓
75	✓	✓	✓	✓	✓	NT1	NT1
100	✓	NT1	NT1	NT1	NT1	NT1	NT1
4 °C for 7 days							
0	✓	✓	✓	✓	✓	✓	✓
25	✓	✓	✓	✓	✓	✓	✓
50	✓	✓	✓	✓	✓	✓	✓
75	✓	✓	✓	✓	✓	NT1	NT1
100	✓	NT1	NT1	NT1	NT1	NT1	NT1

✓ Clear PN solutions and ✘ Turbid PN solutions

Ca = calcium, NaGP = sodium glycerophosphate

NT1 = Not test due to limitation of commercial products concentrations

4.4 Turbidity

The PN samples which passed the visual inspection were then measured the turbidity using Turbidity and free/Total Chlorine Meter at room temperature. Turbidity meter can identify very small particles which human eyes cannot detect. PN solutions with no precipitation by visual inspection may be failed by turbidity test. Turbidity of PN solutions less than 0.5 NTU was determined as pass. Each PN solution was triplicately measured. Turbidities of samples were recorded as mean ± S.D. as shown in Table 14-16. PN solutions showed turbidity when the solutions contained high concentrations of calcium and phosphate. Similar results, PN solutions showed turbidity when they were stored at room temperature with longer storage duration.

The samples containing 1.5% amino acid, 75 mM/L of calcium gluconate with 150 mM/L of NaGP at room temperature for 30 minutes showed turbidity greater than 0.5 NTU. The samples containing 1.5% amino acid, 50 mM/L of calcium gluconate with 125, 150 mM/L of NaGP at room temperature for 1 day showed turbidity greater than 0.5 NTU. The samples containing 1.5% amino acid, 50 mM/L of calcium gluconate with 125, 150 mM/L of NaGP and 75 mM/L of calcium gluconate with 100, 125, 150 mM/L of NaGP and 100 mM/L of calcium gluconate with 100 mM/L of NaGP at 4 °C for 1 day also showed turbidity greater than 0.5 NTU. The samples containing 1.5% amino acid, 25 mM/L of calcium gluconate with 125, 150 mM/L of NaGP and 50 mM/L of calcium gluconate with 75 mM/L or more of NaGP

and 75 mM/L of calcium gluconate with 50 mM/L or more of NaGP and 100 mM/L of calcium gluconate with 25 mM/L or more of NaGP at 4 °C for 7 days showed turbidity greater than 0.5 NTU (Table 14).

Turbidity tests of PN solutions containing 2% amino acid showed similar results with 1.5% amino acid at room temperature for 30 minutes and room temperature for 1 day. The samples containing 2% amino acid, 75 mM/L of calcium gluconate with 125, 150 mM/L of NaGP at 4 °C for 1 day showed turbidity greater than 0.5 NTU. The samples containing 2% amino acid, 25 mM/L of calcium gluconate with 150 mM/L of NaGP and 50 mM/L of calcium gluconate with 125, 150 mM/L of NaGP and 75 mM/L of calcium gluconate with 50 mM/L or more of NaGP and 100 mM/L of calcium gluconate with 50 mM/L or more of NaGP at 4 °C for 7 days showed turbidity greater than 0.5 NTU (Table 15).

The samples containing 2.5% amino acid which passed the visual inspection were all passed in turbidity test (Table 16). Maximum concentrations of calcium gluconate and NaGP in PN solutions containing 1.5% and 2% amino acids were shown in Table 17. The samples containing 2.5% amino acid showed no precipitation at all concentrations of calcium gluconate and NaGP used in this study.

Table 14 Turbidity of tested PN solutions containing 1.5% amino acid and various concentrations of calcium gluconate and NaGP (mean ± S.D.)

Concentrations of Ca gluconate (mM/L)	Concentrations of NaGP (mM/L)						
	0	25	50	75	100	125	150
Room temperature for 30 minutes							
0	0.350±0.000	0.300±0.000	0.300±0.000	0.250±0.000	0.283±0.029	0.300±0.000	0.250±0.000
25	0.250±0.000	0.350±0.000	0.300±0.000	0.267±0.029	0.350±0.000	0.300±0.000	0.300±0.000
50	0.217±0.029	0.250±0.000	0.250±0.000	0.350±0.000	0.250±0.000	0.400±0.000	0.400±0.000
75	0.350±0.000	0.350±0.000	0.283±0.029	0.333±0.058	0.350±0.000	0.350±0.000	5.100±0.000*
100	0.317±0.029	0.350±0.000	0.450±0.000	0.367±0.058	0.450±0.000	NT1	NT1
Room temperature for 1 day							
0	0.300±0.000	0.300±0.000	0.333±0.029	0.300±0.000	0.450±0.000	0.450±0.000	0.250±0.000
25	0.267±0.029	0.250±0.000	0.300±0.000	0.300±0.000	0.417±0.058	0.350±0.000	0.250±0.000
50	0.200±0.000	0.250±0.000	0.300±0.000	0.400±0.000	0.250±0.000	2.000±0.000*	4.000±0.000*
75	0.233±0.029	0.250±0.000	0.350±0.000	0.350±0.000	NT2	NT2	NT2
100	0.300±0.000	0.300±0.000	0.300±0.000	0.300±0.000	NT2	NT1	NT1
4 °C for 1 day							
0	0.317±0.029	0.300±0.000	0.317±0.029	0.417±0.029	0.400±0.000	0.400±0.000	0.333±0.029
25	0.250±0.000	0.267±0.029	0.300±0.000	0.400±0.000	0.433±0.029	0.383±0.029	0.350±0.000
50	0.200±0.000	0.250±0.000	0.300±0.000	0.400±0.000	0.300±0.000	0.850±0.180*	1.083±0.126*
75	0.250±0.000	0.283±0.029	0.350±0.000	0.383±0.029	1.017±0.375*	4.717±0.225*	5.133±0.058*
100	0.250±0.000	0.300±0.000	0.350±0.000	0.400±0.000	1.283±0.465*	NT1	NT1
4 °C for 7 days							
0	0.350±0.000	0.283±0.029	0.250±0.000	0.400±0.000	0.400±0.000	0.400±0.000	0.400±0.000
25	0.300±0.000	0.300±0.000	0.417±0.029	0.450±0.000	0.400±0.000	0.750±0.000*	0.900±0.000*
50	0.250±0.000	0.250±0.000	0.333±0.029	0.700±0.050*	1.500±0.000*	1.500±0.000*	1.100±0.000*
75	0.250±0.000	0.333±0.058	1.200±0.000*	1.700±0.000*	1.800±0.000*	3.500±0.000*	3.800±0.000*
100	0.400±0.000	0.650±0.050*	1.500±0.000*	2.400±0.000*	2.600±0.00*	NT1	NT1

Ca = calcium, NaGP = sodium glycerophosphate

*PN solutions showed turbidity greater than 0.5 NTU

NT1 = Not test due to limitation of commercial products concentrations

NT2 = Not test due to fail by visual inspection

Table 15 Turbidity of tested PN solutions containing 2% amino acid and various concentrations of calcium gluconate and NaGP (mean ± S.D.)

Concentrations of Ca gluconate (mM/L)	Concentrations of NaGP (mM/L)						
	0	25	50	75	100	125	150
Room temperature for 30 minutes							
0	0.150±0.000	0.200±0.000	0.217±0.029	0.283±0.076	0.217±0.029	0.183±0.029	0.233±0.058
25	0.150±0.000	0.233±0.058	0.250±0.050	0.217±0.029	0.283±0.029	0.257±0.012	0.367±0.029
50	0.200±0.000	0.233±0.029	0.267±0.029	0.200±0.000	0.233±0.029	0.383±0.076	0.333±0.058
75	0.300±0.100	0.250±0.000	0.200±0.000	0.333±0.058	0.267±0.115	0.383±0.076	4.000±0.000*
100	0.233±0.029	0.300±0.000	0.200±0.000	NT1	NT1	NT1	NT1
Room temperature for 1 day							
0	0.200±0.000	0.183±0.029	0.200±0.000	0.300±0.000	0.217±0.029	0.200±0.000	0.300±0.000
25	0.200±0.000	0.167±0.029	0.250±0.000	0.200±0.000	0.283±0.029	0.267±0.029	0.367±0.029
50	0.300±0.000	0.217±0.029	0.200±0.000	0.317±0.029	0.250±0.000	1.600±0.000*	3.233±0.058*
75	0.300±0.000	0.217±0.029	0.350±0.000	0.300±0.000	NT2	NT2	NT2
100	0.233±0.029	0.350±0.050	0.283±0.029	NT1	NT1	NT1	NT1
4 °C for 1 day							
0	0.167±0.029	0.267±0.029	0.233±0.029	0.283±0.029	0.200±0.000	0.333±0.029	0.383±0.029
25	0.217±0.029	0.317±0.029	0.300±0.000	0.217±0.029	0.317±0.029	0.350±0.000	0.383±0.029
50	0.267±0.029	0.317±0.029	0.317±0.029	0.300±0.000	0.300±0.000	0.450±0.000	0.367±0.029
75	0.267±0.058	0.233±0.029	0.333±0.076	0.300±0.000	0.400±0.000	4.200±0.100*	4.567±0.741*
100	0.233±0.029	0.350±0.000	0.400±0.000	NT1	NT1	NT1	NT1
4 °C for 7 days							
0	0.267±0.029	0.250±0.000	0.333±0.029	0.333±0.029	0.367±0.029	0.217±0.029	0.383±0.076
25	0.200±0.000	0.350±0.000	0.400±0.000	0.367±0.029	0.300±0.000	0.350±0.000	0.783±0.058*
50	0.183±0.029	0.433±0.029	0.383±0.029	0.233±0.029	0.300±0.100	0.800±0.050*	0.917±0.029*
75	0.417±0.029	0.217±0.029	0.800±0.100*	0.867±0.058*	0.950±0.050*	3.267±0.029*	3.500±0.000*
100	0.400±0.000	0.300±0.000	0.950±0.050*	NT1	NT1	NT1	NT1

Ca = calcium, NaGP = sodium glycerophosphate

*PN solutions showed turbidity greater than 0.5 NTU

NT1 = Not test due to limitation of commercial products concentrations

NT2 = Not test due to fail by visual inspection

Table 16 Turbidity of tested PN solutions containing 2.5% amino acid and various concentrations of calcium gluconate and NaGP (mean ± S.D.)

Concentrations of Ca gluconate (mM/L)	Concentrations of NaGP (mM/L)						
	0	25	50	75	100	125	150
Room temperature for 30 minutes							
0	0.283±0.029	0.367±0.029	0.183±0.029	0.317±0.029	0.267±0.029	0.217±0.029	0.250±0.000
25	0.233±0.029	0.200±0.000	0.300±0.050	0.300±0.000	0.300±0.000	0.300±0.000	0.217±0.029
50	0.233±0.029	0.317±0.029	0.250±0.000	0.317±0.029	0.317±0.029	0.300±0.050	0.317±0.029
75	0.250±0.000	0.200±0.000	0.350±0.000	0.317±0.058	0.400±0.000	NT1	NT1
100	0.300±0.000	NT1	NT1	NT1	NT1	NT1	NT1
Room temperature for 1 day							
0	0.250±0.000	0.333±0.029	0.200±0.000	0.300±0.000	0.250±0.000	0.250±0.000	0.333±0.029
25	0.200±0.000	0.183±0.029	0.283±0.029	0.300±0.000	0.300±0.000	0.317±0.029	0.283±0.029
50	0.250±0.000	0.300±0.000	0.250±0.000	0.300±0.000	0.300±0.000	0.300±0.000	0.300±0.000
75	0.283±0.029	0.167±0.029	0.300±0.000	0.283±0.029	0.500±0.000	NT1	NT1
100	0.300±0.000	NT1	NT1	NT1	NT1	NT1	NT1
4 °C for 1 day							
0	0.233±0.029	0.317±0.029	0.183±0.029	0.333±0.029	0.283±0.029	0.250±0.000	0.300±0.000
25	0.200±0.000	0.200±0.000	0.300±0.000	0.317±0.029	0.317±0.029	0.350±0.000	0.300±0.000
50	0.267±0.029	0.317±0.029	0.217±0.029	0.333±0.029	0.333±0.029	0.350±0.000	0.333±0.029
75	0.267±0.029	0.200±0.000	0.317±0.029	0.300±0.000	0.450±0.000	NT1	NT1
100	0.317±0.029	NT1	NT1	NT1	NT1	NT1	NT1
4 °C for 7 days							
0	0.250±0.000	0.300±0.000	0.200±0.000	0.267±0.058	0.267±0.029	0.250±0.000	0.150±0.000
25	0.300±0.000	0.200±0.000	0.300±0.000	0.283±0.029	0.300±0.000	0.150±0.000	0.200±0.000
50	0.267±0.029	0.300±0.000	0.250±0.000	0.300±0.000	0.300±0.000	0.150±0.000	0.183±0.029
75	0.283±0.029	0.200±0.000	0.267±0.029	0.250±0.000	0.350±0.000	NT1	NT1
100	0.333±0.029	NT1	NT1	NT1	NT1	NT1	NT1

Ca = calcium, NaGP = sodium glycerophosphate

NT1 = Not test due to limitation of commercial products concentrations

Table 17 Maximum concentrations of calcium gluconate and NaGP in PN solutions containing 1.5% and 2% amino acids

Conditions	Concentrations			
	1.5% amino acid		2% amino acid	
	Ca	NaGP	Ca	NaGP
Room temperature for 30 minutes	25	150	25	150
	50	150	50	150
	75	125	75	125
	100	100	100	50
Room temperature for 1 day	25	150	25	150
	50	100	50	100
	75	75	75	75
	100	75	100	50
4 °C for 1 day	25	150	25	150
	50	100	50	150
	75	75	75	100
	100	75	100	50
4 °C for 7 days	25	100	25	125
	50	50	50	100
	75	25	75	25
	100	0	100	25

Ca = concentration of calcium gluconate (mM/L)

NaGP = concentration of sodium glycerophosphate (mM/L)

4.5 Microscopic particle count

The PN samples that had turbidity less than 0.5 NTU were then inspected under light microscope. Microscopic particle counts were recorded as maximum particle count per 5 ml of sample solutions. Maximum particle counts for triplicates per conditions were shown in Appendix D. The maximum particle counts of all PN solutions were less than 60 particles measuring 10 μm in diameter together with less than 10 particles measuring 25 μm in diameter. All PN solutions which passed turbidity test were passed in microscopic particle count test.

4.6 Construction of solubility data

The solubility data of calcium gluconate and NaGP including 1.5% and 2% amino acid stored at room temperature for 30 minutes, room temperature for 1 day, 4 °C for 1 day, and 4 °C for 7 days were shown in figure 4 and figure 5. The samples containing 2.5% amino acid showed no precipitation at all concentrations of calcium gluconate and NaGP in this study. Solubility data of calcium gluconate and NaGP with 2.5% amino acid at every storage conditions were shown in figure 6.

As parallel, PN solutions containing calcium gluconate and dipotassium phosphate were underwent solubility tests. The solubility data of calcium gluconate and dipotassium phosphate were constructed. All results of solubility tests and solubility data of calcium gluconate and dipotassium phosphate were shown in Appendix E.

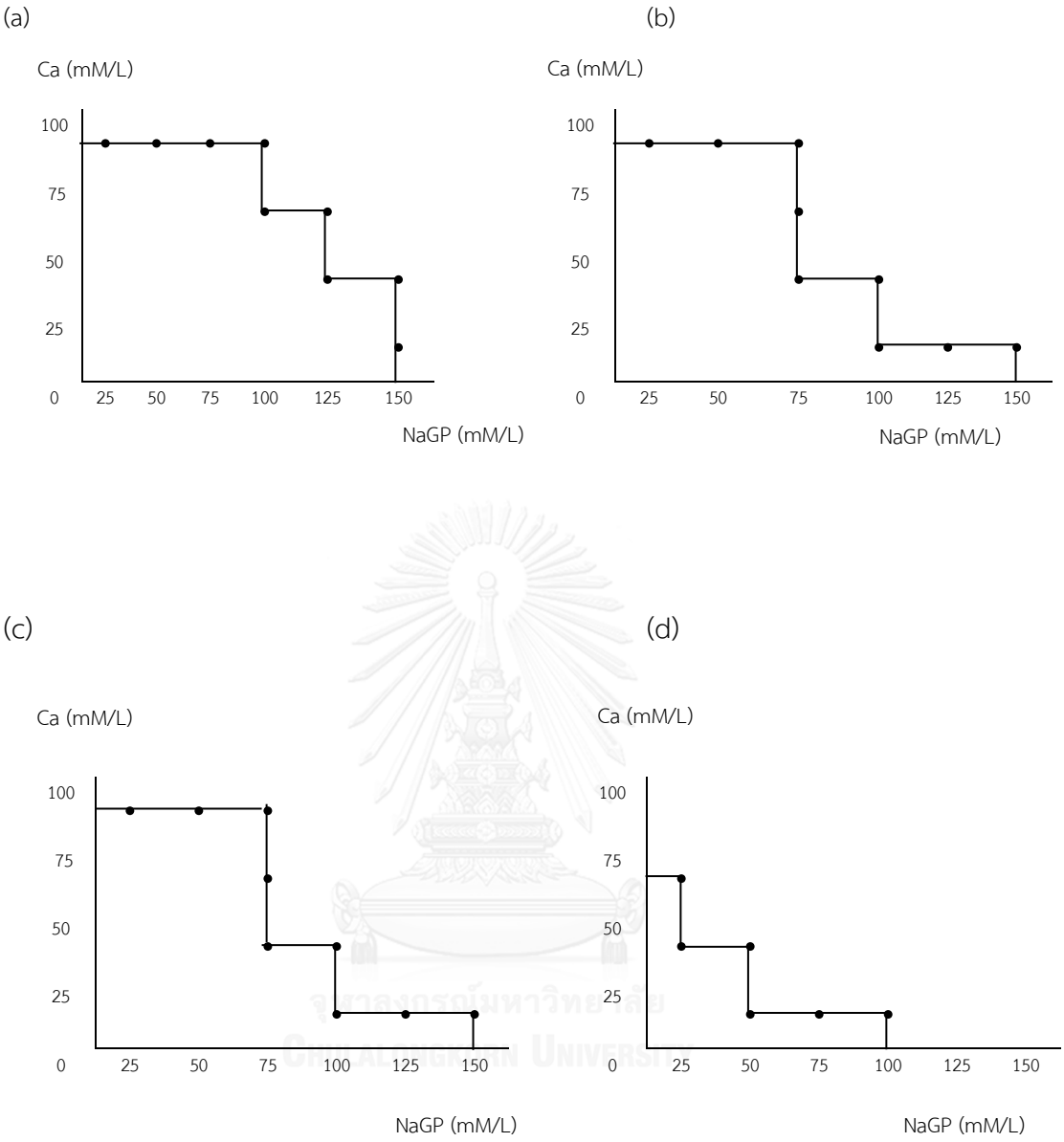


Figure 4 Solubility data of 1.5% amino acid stored at (a) room temperature for 30 minutes (b) room temperature for 1 day (c) 4 °C for 1 day and (d) 4 °C for 7 days

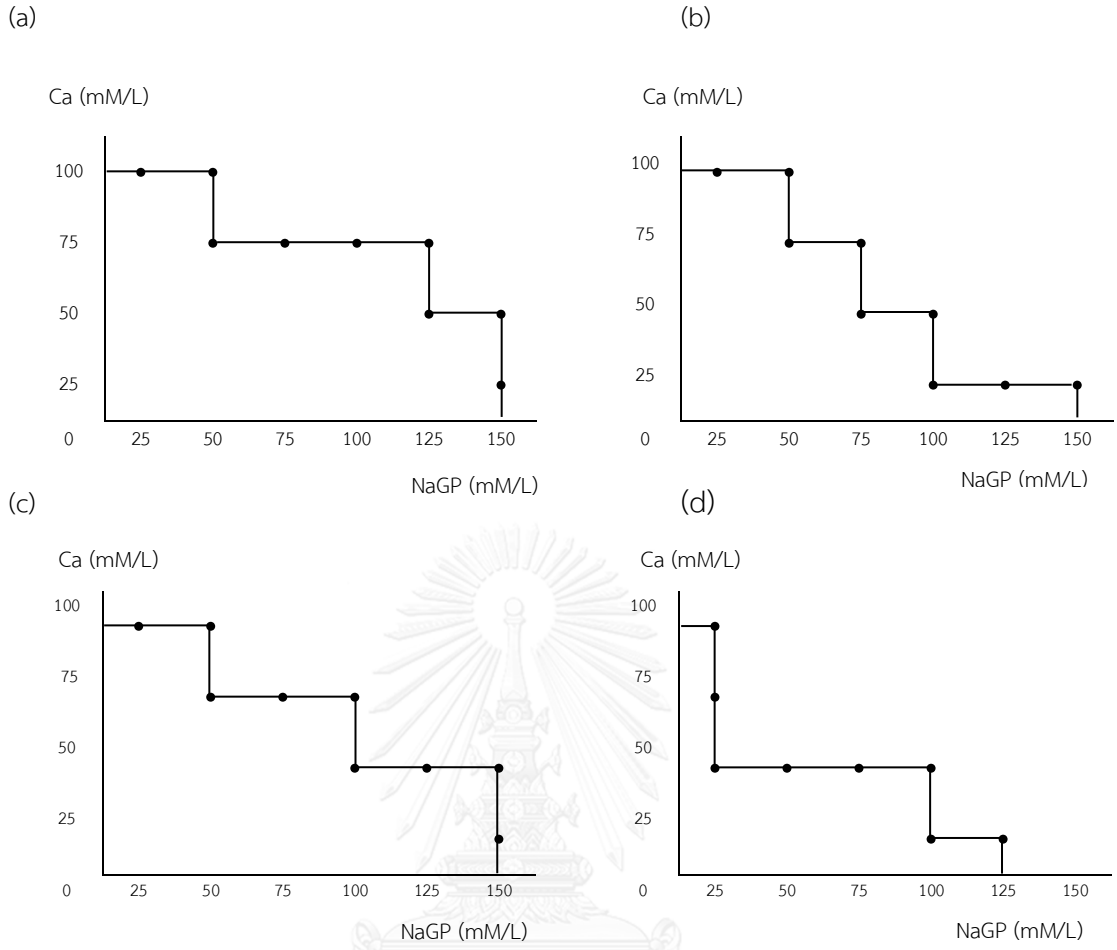


Figure 5 Solubility data of 2% amino acid stored at (a) room temperature for 30 minutes (b) room temperature for 1 day (c) 4 °C for 1 day and (d) 4 °C for 7 days

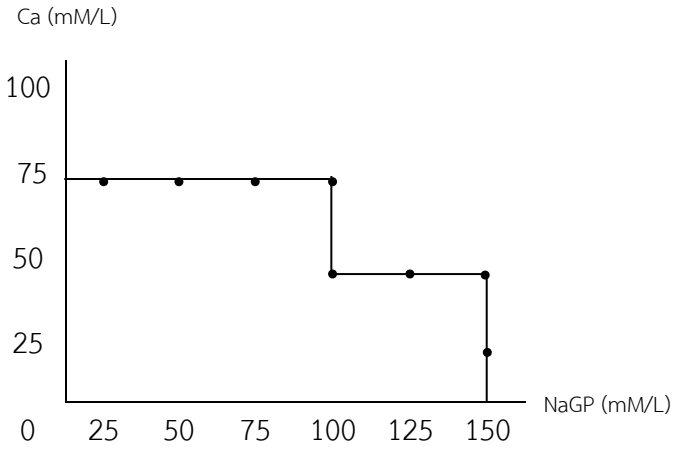
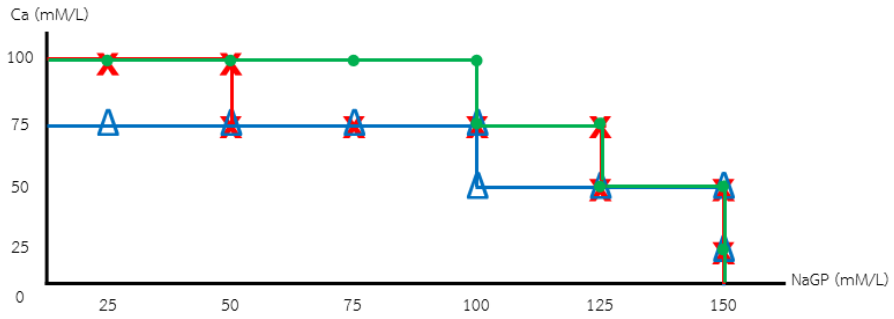
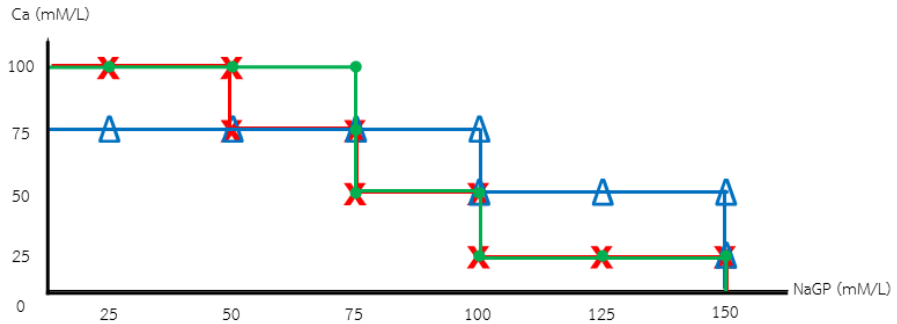


Figure 6 Solubility data of 2.5% amino acid stored at room temperature for 30 minutes, room temperature for 1 day, 4 °C for 1 day and 4 °C for 7 days

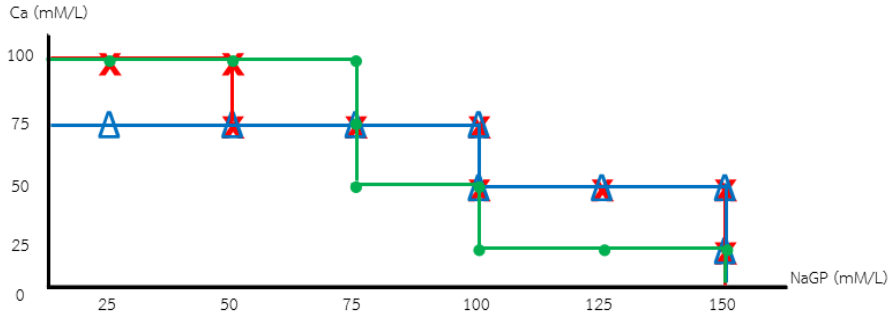
(a)



(b)



(c)



(d)

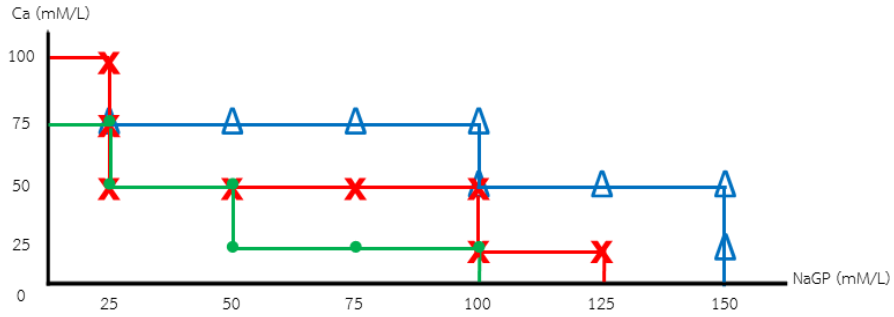


Figure 7 Solubility data of 1.5% amino acid 2% amino acid and 2.5% amino acid stored at (a) room temperature for 30 minutes (b) room temperature for 1 day (c) 4 °C for 1 day and (d) 4 °C for 7 days

4.7 Application of solubility data

In this study, solubility data were applied according to practically prescribed infant PN orders at Ramathibodi Hospital contain amino acid (Aminoven infant®). Ramathibodi Hospital infant PN solution orders during July to December in 2016 were reviewed. Total infant PN solutions orders were 31 prescriptions as shown in Table

18. All PN solutions were underwent solubility tests at various storage conditions according to the study protocol. All PN solutions were determined as pass in pH measurement (Table 19), visual inspection, turbidity test (Table 20) and microscopic particle count test (Table 21). In this study, PN solutions contained other components including potassium chloride, potassium acetate, sodium acetate, zinc sulfate and heparin were determined as pass and no precipitation. The results were consistent with solubility data from this study at 1.09-30.82 mM/L of calcium and 1.19-40.00 mM/L of phosphate (figures 4-7). The solubility data from this study can be used for prescription and preparation of infant PN solutions.

Table 18 PN solution formulas used for solubility data application

Components	Formulation number							
	1	2	3	4	5	6	7	8
Amino acid (%)	2.50	1.50	2.04	2.05	1.50	1.46	2.00	2.04
Dextrose (%)	8.40	12.50	15.00	9.59	6.00	6.77	7.71	13.54
Phosphate (mM/L)	2.36	31.25	7.61	13.70	3.13	7.29	7.29	16.67
Sodium (mM/L)	38.92	222.81	30.83	167.95	47.40	92.70	92.70	108.33
Potassium (mM/L)	16.67	9.72	87.50	21.74	340.02	18.75	18.75	25.00
Chloride (mM/L)	34.20	170.03	103.11	162.29	349.29	40.61	40.61	-
Acetate (mM/L)	33.33	-	-	-	63.75	112.50	112.50	200.00
Heparin (units)	360	40	230	72	800	720	720	720
Zinc (mg/L)	4.17	10.00	3.26	34.25	1.25	3.13	3.13	4.17
Magnesium (mM/L)	1.69	-	1.76	13.87	2.53	2.53	2.53	2.11
Calcium (mM/L)	1.09	28.13	6.85	30.82	4.50	1.88	1.88	3.28
Sterile water	qs to total volume							

Table 18 (continued)

Components	Formulation number							
	9	**10**	**11**	**12**	**13**	**14**	**15**	**16**
Amino acid (%)	2.50	1.45	2.46	2.53	2.51	2.03	2.00	2.02
Dextrose (%)	13.54	7.75	11.20	15.67	10.00	9.71	25.00	8.96
Phosphate (mM/L)	20.83	12.50	11.11	26.67	8.11	10.14	37.50	3.13
Sodium (mM/L)	67.32	25.00	36.47	109.76	31.47	41.11	113.48	18.75
Potassium (mM/L)	43.75	33.33	20.00	44.44	40.00	10.81	5.80	6.25
Chloride (mM/L)	25.65	33.33	34.25	100.87	55.25	31.63	44.27	-
Acetate (mM/L)	87.50	-	-	-	-	-	-	37.50
Heparin (units)	480	200	135	150	190	345	40	240
Zinc (mg/L)	4.17	6.25	3.70	10.00	2.70	2.90	6.25	3.13
Magnesium (mM/L)	4.22	2.03	1.50	4.05	1.64	2.93	2.53	1.69
Calcium (mM/L)	3.75	11.25	6.25	24.75	3.34	9.13	16.88	1.88
Sterile water	qs to total volume							

Table 18 (continued)

Components	Formulation number							
	17	**18**	**19**	**20**	**21**	**22**	**23**	**24**
Amino acid (%)	2.00	2.54	2.53	2.53	2.03	2.04	1.51	1.46
Dextrose (%)	24.64	12.69	13.50	11.27	10.63	17.81	5.00	12.50
Phosphate (mM/L)	28.57	7.69	8.33	11.39	10.94	10.42	5.33	4.63
Sodium (mM/L)	86.46	22.29	33.77	29.93	29.89	28.31	72.23	49.63
Potassium (mM/L)	33.33	42.86	30.77	16.67	14.06	25.00	62.50	5.33
Chloride (mM/L)	62.65	49.76	47.87	23.81	8.02	7.48	124.06	45.71
Acetate (mM/L)	-	-	-	-	28.13	50.00	-	-
Heparin (units)	70	130	147	195	160	120	375	432
Zinc (mg/L)	10.71	3.85	3.33	2.53	3.13	2.08	4.00	4.63
Magnesium (mM/L)	7.23	0.87	1.35	1.54	0.63	0.84	2.70	1.41
Calcium (mM/L)	25.71	3.46	7.50	6.27	3.16	6.09	4.50	4.43
Sterile water	qs to total volume							

Table 18 (continued)

Components	Formulation number						
	25	**26**	**27**	**28**	**29**	**30**	**31**
Amino acid (%)	2.50	2.03	2.53	2.47	2.04	2.46	2.05
Dextrose (%)	26.88	9.83	7.06	24.17	9.06	11.11	9.82
Phosphate (mM/L)	12.50	8.33	5.88	40.00	16.67	2.78	1.19
Sodium (mM/L)	66.68	39.75	26.85	100.52	95.83	77.70	142.50
Potassium (mM/L)	6.94	30.00	13.33	14.12	28.13	37.50	46.43
Chloride (mM/L)	48.63	23.09	28.42	34.64	-	24.23	57.98
Acetate (mM/L)	-	60.00	-	-	181.25	170.83	257.14
Heparin (units)	40	150	85	15	480	720	840
Zinc (mg/L)	6.25	1.67	2.94	3.33	4.17	4.17	4.76
Magnesium (mM/L)	-	2.03	1.19	6.75	2.11	2.81	2.41
Calcium (mM/L)	8.44	6.75	6.62	15.00	3.28	1.56	2.68
Sterile water	qs to total volume						

Table 19 The pH values of applied PN solutions (mean ± S.D.)

Formulation no.	Room temperature for 30 minutes	Room temperature for 1 day	4 °C for 1 day	4 °C for 7 days
1	4.753±0.006	4.870±0.000	4.927±0.015	4.937±0.006
2	6.040±0.000	6.313±0.006	6.320±0.010	6.367±0.015
3	5.920±0.000	6.053±0.006	6.070±0.026	6.070±0.017
4	5.887±0.006	6.063±0.006	6.123±0.021	6.080±0.010
5	4.677±0.006	4.853±0.006	4.913±0.012	4.847±0.015
6	4.540±0.000	4.680±0.000	4.703±0.021	4.687±0.025
7	4.580±0.000	4.713±0.012	4.740±0.010	4.773±0.015
8	4.553±0.006	4.717±0.015	4.763±0.025	4.800±0.010
9	4.650±0.000	4.783±0.006	4.790±0.010	4.807±0.015
10	6.137±0.006	6.337±0.015	6.337±0.021	6.367±0.021
11	5.920±0.000	6.093±0.006	6.143±0.025	6.173±0.006
12	6.127±0.006	6.263±0.015	6.303±0.006	6.313±0.025
13	6.020±0.000	6.173±0.006	6.197±0.006	6.210±0.010
14	5.960±0.000	6.070±0.020	6.073±0.021	6.093±0.015
15	6.123±0.006	6.283±0.006	6.310±0.010	6.323±0.015
16	4.683±.0006	4.853±0.006	4.883±0.015	4.863±0.015
17	5.830±0.000	5.943±0.006	5.957±0.015	5.963±0.015
18	5.700±0.000	5.810±0.000	5.813±0.015	5.833±0.006
19	5.867±0.006	5.933±0.029	5.980±0.010	5.977±0.015
20	5.950±0.000	5.963±0.015	5.963±0.015	5.997±0.012
21	5.000±0.000	5.163±0.015	5.190±0.000	5.170±0.010
22	4.817±0.006	4.903±0.015	4.957±0.006	4.960±0.010
23	6.090±0.000	6.177±0.006	6.237±0.006	6.247±0.006
24	6.080±0.000	6.243±0.012	6.317±0.015	6.273±0.021
25	5.557±0.006	5.713±0.015	5.713±0.023	5.743±0.015
26	4.830±0.000	4.953±0.006	4.960±0.000	4.973±0.015
27	6.010±0.000	6.030±0.010	6.077±0.025	6.103±0.021
28	6.180±0.000	6.307±0.006	6.313±0.015	6.303±0.006
29	4.560±0.000	4.683±0.006	4.710±0.000	4.717±0.006
30	4.540±0.000	4.597±0.012	4.667±0.015	4.653±0.025
31	4.520±0.000	4.667±0.006	4.710±0.017	4.690±0.020

Table 20 Turbidity of applied PN solutions (mean ± S.D.)

Formulation no.	Room temperature for 30 minutes	Room temperature for 1 day	4 °C for 1 day	4 °C for 7 days
1	0.433±0.029	0.450±0.000	0.450±0.000	0.467±0.029
2	0.350±0.000	0.383±0.029	0.400±0.000	0.417±0.029
3	0.400±0.000	0.400±0.000	0.383±0.029	0.417±0.029
4	0.400±0.000	0.400±0.000	0.417±0.029	0.450±0.000
5	0.450±0.000	0.450±0.000	0.417±0.029	0.450±0.000
6	0.450±0.000	0.450±0.000	0.417±0.029	0.433±0.029
7	0.450±0.050	0.450±0.000	0.433±0.029	0.450±0.000
8	0.450±0.000	0.450±0.000	0.450±0.000	0.467±0.029
9	0.400±0.000	0.417±0.029	0.400±0.000	0.433±0.029
10	0.367±0.029	0.400±0.000	0.383±0.029	0.400±0.000
11	0.450±0.000	0.450±0.000	0.433±0.029	0.450±0.000
12	0.417±0.029	0.450±0.000	0.400±0.000	0.417±0.029
13	0.400±0.000	0.417±0.029	0.417±0.029	0.450±0.000
14	0.383±0.029	0.400±0.000	0.383±0.029	0.417±0.029
15	0.417±0.029	0.450±0.000	0.417±0.029	0.433±0.029
16	0.400±0.050	0.433±0.029	0.400±0.000	0.417±0.029
17	0.417±0.058	0.383±0.058	0.383±0.029	0.400±0.000
18	0.400±0.000	0.433±0.029	0.400±0.000	0.433±0.029
19	0.433±0.029	0.450±0.000	0.400±0.000	0.433±0.029
20	0.400±0.000	0.433±0.076	0.417±0.029	0.450±0.000
21	0.417±0.029	0.433±0.029	0.400±0.000	0.433±0.029
22	0.417±0.029	0.450±0.000	0.400±0.000	0.417±0.029
23	0.400±0.000	0.417±0.029	0.400±0.000	0.400±0.000
24	0.400±0.000	0.400±0.000	0.417±0.029	0.450±0.000
25	0.367±0.029	0.400±0.000	0.417±0.029	0.433±0.029
26	0.417±0.029	0.450±0.000	0.400±0.000	0.450±0.000
27	0.417±0.029	0.450±0.000	0.400±0.000	0.433±0.029
28	0.367±0.029	0.400±0.000	0.400±0.000	0.417±0.029
29	0.350±0.000	0.383±0.029	0.400±0.000	0.433±0.029
30	0.400±0.000	0.417±0.029	0.433±0.029	0.450±0.000
31	0.450±0.000	0.433±0.029	0.417±0.076	0.433±0.029

Table 21 Maximum particle count of applied PN solutions*

Formulation no.	Conditions	PC <10 μm	PC ≥10 μm	PC ≥25 μm
1	Day 0[a]	0	3	4
	Day 1[a]	1	4	0
	Day 1[b]	1	2	0
	Day 7[b]	3	3	1
2	Day 0[a]	0	0	1
	Day 1[a]	0	3	0
	Day 1[b]	0	1	0
	Day 7[b]	0	0	2
3	Day 0[a]	0	2	1
	Day 1[a]	1	1	2
	Day 1[b]	0	0	1
	Day 7[b]	0	1	1
4	Day 0[a]	0	4	0
	Day 1[a]	0	1	1
	Day 1[b]	0	0	1
	Day 7[b]	0	1	2
5	Day 0[a]	0	1	0
	Day 1[a]	1	2	0
	Day 1[b]	0	0	1
	Day 7[b]	0	1	2
6	Day 0[a]	0	1	0
	Day 1[a]	0	2	0
	Day 1[b]	0	2	0
	Day 7[b]	1	5	0

a = room temperature, b = 4 °C

*Maximum particle count: ≥10 μm less than 60 particles/5mL, ≥25 μm less than 10 particles/5mL

Table 21 (continued)*

Formulation no.	Conditions	PC <10 μm	PC ≥10 μm	PC ≥25 μm
7	Day 0[a]	0	0	1
	Day 1[a]	0	0	1
	Day 1[b]	0	1	1
	Day 7[b]	0	0	1
8	Day 0[a]	0	0	1
	Day 1[a]	0	0	1
	Day 1[b]	0	1	0
	Day 7[b]	0	0	1
9	Day 0[a]	0	1	0
	Day 1[a]	7	4	0
	Day 1[b]	0	1	0
	Day 7[b]	0	0	2
10	Day 0[a]	1	1	0
	Day 1[a]	0	2	0
	Day 1[b]	2	1	0
	Day 7[b]	0	0	2
11	Day 0[a]	0	1	2
	Day 1[a]	1	3	1
	Day 1[b]	0	1	0
	Day 7[b]	1	0	2
12	Day 0[a]	1	4	0
	Day 1[a]	1	3	1
	Day 1[b]	0	3	0
	Day 7[b]	0	2	1
13	Day 0[a]	0	2	1
	Day 1[a]	0	3	0
	Day 1[b]	0	1	0
	Day 7[b]	0	0	2

a = room temperature, b = 4 °C

*Maximum particle count: ≥10 μm less than 60 particles/5mL, ≥25 μm less than 10 particles/5mL

Table 21 (continued)*

Formulation no.	Conditions	PC <10 μm	PC ≥10 μm	PC ≥25 μm
14	Day 0[a]	1	2	0
	Day 1[a]	0	0	1
	Day 1[b]	0	2	0
	Day 7[b]	0	0	1
15	Day 0[a]	1	0	1
	Day 1[a]	0	0	1
	Day 1[b]	0	2	0
	Day 7[b]	2	0	1
16	Day 0[a]	0	2	0
	Day 1[a]	0	0	1
	Day 1[b]	1	1	0
	Day 7[b]	0	0	2
17	Day 0[a]	0	1	1
	Day 1[a]	0	3	0
	Day 1[b]	0	1	1
	Day 7[b]	0	2	1
18	Day 0[a]	0	2	1
	Day 1[a]	0	1	2
	Day 1[b]	0	2	0
	Day 7[b]	0	2	1
19	Day 0[a]	0	2	1
	Day 1[a]	1	2	1
	Day 1[b]	0	1	0
	Day 7[b]	0	2	1
20	Day 0[a]	0	3	0
	Day 1[a]	0	2	1
	Day 1[b]	0	1	0
	Day 7[b]	0	1	2

a = room temperature, b = 4 °C

*Maximum particle count: ≥10 μm less than 60 particles/5mL, ≥25 μm less than 10 particles/5mL

Table 21 (continued)*

Formulation no.	Conditions	PC <10 μm	PC ≥10 μm	PC ≥25 μm
21	Day 0[a]	0	1	1
	Day 1[a]	0	1	1
	Day 1[b]	0	1	0
	Day 7[b]	0	1	0
22	Day 0[a]	0	2	0
	Day 1[a]	1	1	1
	Day 1[b]	0	2	0
	Day 7[b]	1	1	1
23	Day 0[a]	0	2	0
	Day 1[a]	0	0	1
	Day 1[b]	1	1	1
	Day 7[b]	0	0	2
24	Day 0[a]	0	1	0
	Day 1[a]	0	3	0
	Day 1[b]	0	4	0
	Day 7[b]	0	0	1
25	Day 0[a]	0	1	0
	Day 1[a]	0	1	1
	Day 1[b]	0	1	0
	Day 7[b]	0	9	1
26	Day 0[a]	1	2	0
	Day 1[a]	1	3	0
	Day 1[b]	0	2	0
	Day 7[b]	1	0	2
27	Day 0[a]	1	2	0
	Day 1[a]	0	3	0
	Day 1[b]	0	1	0
	Day 7[b]	0	1	3

a = room temperature, b = 4 °C

*Maximum particle count: ≥10 μm less than 60 particles/5mL, ≥25 μm less than 10 particles/5mL

Table 21 (continued)*

Formulation no.	Conditions	PC <10 μm	PC ≥10 μm	PC ≥25 μm
28	Day 0[a]	0	1	0
	Day 1[a]	0	2	1
	Day 1[b]	0	0	1
	Day 7[b]	0	2	0
29	Day 0[a]	1	2	0
	Day 1[a]	0	5	0
	Day 1[b]	0	1	0
	Day 7[b]	0	0	2
30	Day 0[a]	0	1	2
	Day 1[a]	0	3	0
	Day 1[b]	0	2	0
	Day 7[b]	0	1	2
31	Day 0[a]	0	0	1
	Day 1[a]	2	3	0
	Day 1[b]	0	0	1
	Day 7[b]	0	0	2

a = room temperature, b = 4 °C

*Maximum particle count: ≥10 μm less than 60 particles/5mL, ≥25 μm less than 10 particles/5mL

CHAPTER V
DISCUSSION

The calcium phosphate incompatibility is a problem in infant PN solution. Low PN volume, low concentrations of amino acids and high amounts of calcium and phosphate result in calcium phosphate salts precipitation in PN solution (Prinzivalli & Ceccarelli, 1999).

Previous study reported solubility curves of calcium gluconate and dipotassium phosphate in various concentrations of amino acid to facilitate PN compounding (Mo-suwan et al., 1997). In this study, PN solutions containing calcium gluconate and dipotassium phosphate were tested for intralaboratory and personnel reliability. Solubility data from this study were consistent with previous study which referred to the method and environments were suitable for analysis.

NaGP is a new parenteral phosphate product available in the market. It contains organic phosphate claimed to increase solubility compared to inorganic phosphate even in a solution containing high concentration of calcium or high pH value. (Ronchera-Oms et al., 1995). Several clinical studies showed the advantages of NaGP in PN compounding. Rodell and Linden (1989) compared the compatibility of hydrogen phosphate or glycerophosphate and calcium in amino acid solutions. They found that NaGP provided much better compatibility, allowing the addition of up to 100 mM/L phosphorus and 40 mM/L calcium without any precipitation. Ronchera-

oms et al. (1995) reported the stability of PN solutions containing glucose-1-phosphate or glycerophosphate which showed physically and chemically stable for 3 days under refrigeration or controlled room temperature without light protection.

MacKay and Anderson (2015) studied the compatibility of 5, 10, 15, 20, and 25 mM/L of calcium gluconate with 10, 20, 30, 40, and 50 mM/L of NaGP. It was recommended that NaGP should be used as a phosphate source instead of sodium phosphate or potassium phosphate in PN solutions to avoid precipitation of calcium phosphate in the solution containing high concentration of mineral contents.

This study found that even though NaGP was used instead of dipotassium phosphate, the precipitation still occurred in the PN solutions with high concentrations of calcium gluconate and NaGP. However, the concentrations of calcium gluconate and NaGP that showed precipitation were extremely higher than the requirement for preterm infants. The maximum concentrations of calcium gluconate and NaGP in PN solutions without precipitation were greater than the ESPGHAN recommendation (Koletzko et al., 2005).

This study was the first study which reported the compatibility of PN solution containing high concentrations of calcium gluconate and NaGP. The results were shown as solubility data of calcium gluconate and NaGP in various concentrations of amino acid and conditions.

The final pH of the solution was a factor determining calcium phosphate compatibility in PN solutions. The pH was mainly influenced by the concentration of the amino acid and the concentration of glucose in the solution. The higher the concentrations of amino acids and glucose, the greater the amount of calcium and phosphate were allowed to mix in the solution without causing precipitation. The final pH of the solution interfered the dissociation of ions in solution therefore it predicted the forms of phosphoric acid which are able to form a calcium-phosphate complex. pH values of PN solutions in this study at every condition were less than 7.2. PN solutions were determined as pass and low risk for precipitation due to highly soluble calcium phosphate salts formation. While pH value of the PN solution was determined as pass, some PN solutions showed turbidity greater than 0.5 NTU. (Allwood & Kearney, 1998; Niemiec & Vanderveen, 1984).

Visual inspection was a simple but extremely important method to evaluate the physicochemical compatibility. Color alterations, presence of precipitate and instability phases were evaluated and confirmed sensitivity methods in this step (Akers & Larrimore, 2003; Joy et al., 2010; The United States Pharmacopeia, 2016). Turbid PN solutions were observed in solutions stored at room temperature, but no alteration was observed at 4 °C. Turbid alteration was related not only to the temperature, but also the duration, which was the reason why we found a great difference in turbid between PN solutions kept at room temperature, and at 4 °C. This study used microscope to observe particle sizes and particle counts. All PN

solutions which passed turbidity test were passed in microscopic particle count test (MacKay & Anderson, 2015).

After solubility data were constructed, all of them were applied by Ramathibodi Hospital infant PN solution. The PN formula from the clinical practice contained amino acid, dextrose, sodium chloride, NaGP, potassium chloride, potassium acetate, sodium acetate, zinc sulfate, heparin, magnesium sulfate and calcium gluconate. The results were consistent with solubility data from this study. The solubility data from this study can be used for prescription and preparation of infant PN solutions.

NaGP contains a phosphate group, which is covalently bonded to an organic structure (glycerol) therefore precipitation of inorganic calcium phosphate should not occur. The use of organic phosphate seems to solve calcium phosphate incompatibility, thus enabling the PN formulation, compounding and storage. The absences of precipitation were reducing of admixture wastage, avoided of catheter occlusion and prevented of clinical complications. However, NaGP was more expensive than inorganic phosphate. Therefore this product should only be prescribed to the patients who required high amounts of calcium and phosphate in the same PN bag or clinical situations where calcium phosphate incompatibility problem was foreseen or suspected (i.e. preterm VLBW infants, adult patients with

high calcium or phosphate requirements). The cost effectiveness of NaGP use in PN solution should be further evaluated.

A standard dosage of calcium at 1.3–3.0 mM/kg/day and phosphorus at 1.0–2.3 mM/kg/day were commonly recommended in newborn infants. The concentrations of calcium and phosphorus in these prescriptions rarely exceeded 15 mM/L of calcium and 30 mM/L of phosphorus. Exceeding these limits using inorganic calcium and phosphorus can result in particulate formation; however it depended on other factors in PN formulation. The result of this study indicated stability of 100 mM/L of calcium and 150 mM/L of phosphorus (over the requirement for preterm infants) in various concentrations of amino acids and storage conditions. The data indicated that using organic phosphorus reduced the need of saturation data and the probability of calcium and phosphorus precipitates.

Although PN solutions were determined as pass in pH measurement, visual inspection, turbidity test and microscopic particle count test, some PN solutions were found the particles when inspected under light microscope. Therefore, it was recommended to use filters when administered PN solutions in order to confirm patient safety.

Aminoven infant® is the most common amino acid prescribed for all infants including preterm infants in Thailand. In practice, commercial amino acid solutions, such as Amiparen®, Aminoplasmal®, Aminoleban®, and Kidmin®, have similar pH values (approximate 5.5-7.5). Solubility data for these amino acid solutions have

been established. However, solubility test of calcium gluconate and NaGP in different amino acid solutions should be constructed to ensure the results.

Limitation of this study was that we used a single dextrose concentration of 10% for all solutions. There were no significant effect of dextrose concentration on calcium and phosphate compatibility in previous study when compare 5% dextrose to 10% dextrose (Parikh et al., 2005; Singh et al., 2009). Experimental laboratory were modified from USP 797. USP 797 requires sterile product analysis to be performed in a class 100 in 10000 environment, while laboratory room at the Department of Food and Pharmaceutical Sciences is a class 100 in unlimited number of particles environment. There was a possibility that environment in this study contained other particles and slightly effect on the microscopic particle count in PN solutions.

CHAPTER VI
CONCLUSION

The PN solutions containing low concentration of amino acid, high concentrations of calcium and phosphate and long storage duration enhanced risks of precipitation in PN solutions. This study reported maximum concentration of calcium gluconate and NaGP in PN solutions. The PN solutions containing 1.5% and 2% amino acid showed precipitation at high concentration of calcium and phosphate. The concentrations of calcium gluconate and NaGP that showed precipitation were extremely higher than the requirement for preterm infants. The PN solutions containing 2.5% amino acid passed all solubility tests at studied concentrations of calcium gluconate and NaGP. However, some PN solutions were found the particles when inspected under light microscope, it was recommended to use filters when administered PN solutions to ensure patient safety. NaGP may be a good choice of phosphate source for patients to meet the requirements of phosphate instead of dipotassium phosphate. Solubility data from this study can be used for prescription and preparation of infant PN solutions.

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APPENDICES

APPENDIX A

CALCULATION OF WORKING FORMULA

Table A-1 Concentrations of chemicals for PN preparation

Commercial product	Concentration per ml
10% Amino acid	Amino acid 0.1 g
50% Dextrose	Dextrose 0.5 g
Sodium glycerophosphate	Sodium 2 mM
	Phosphate 1 mM
8.71% Dipotassium phosphate	Potassium 1 mM
	Phosphate 0.5 mM
3% Sodium chloride	Sodium 0.513 mM
	Chloride 0.513 mM
15% Potassium chloride	Potassium 2 mM
	Chloride 2 mM
24.6% Sodium acetate	Sodium 3 mM
	Acetate 6 mM
29.4% Potassium acetate	Potassium 3 mM
	Acetate 6 mM
Heparin	Heparin 5,000 units
Zinc sulfate	Zinc 1 mg
50% Magnesium sulfate	Magnesium 2.025 mM
10% Calcium gluconate	Calcium 0.225 mM

Calculation of working formula, each formula of PN solution was prepared at 100 ml sample, and composed of 1.5% amino acid, 10% dextrose, sodium glycerophosphate 150 mM/L or dipotassium phosphate 20 mM/L, sodium 351.3 mM/L, chloride 51.3 mM/L, potassium chloride 45 mM/L, sodium acetate 50 mM/L,

potassium acetate 25mM/L, heparin 1000 units/L, zinc 5 mg/L, magnesium 2.025 mM/L, calcium 100 mM/L

(1) The volume of amino acid solution 10% to make up 1.5% amino acids in PN solution

100 mL was calculated as following

1.5% amino acids; 100 mL solution contain 1.5 g of amino acids

Amino acid solution 10%; 10 g of amino acids are in 100 mL solution

1.5 g of amino acids are obtained from 100×1.5/10 = 15 mL

∴ Take 15 mL of amino acid solution 10% to make up 3% amino acids in PN solution 100 mL

(2) The volume of dextrose 50% in water to make up 10% dextrose in PN solution 100

mL was calculated as following

10% dextrose; 100 mL solution contain 10 g of dextrose

Dextrose 50% in water; 50 g of dextrose are in 100 mL solution

10 g of dextrose are obtained from 100×10/50 = 20 mL

∴ Take 20 mL of dextrose 50% in water to make up 10% dextrose in PN solution 100 mL

(3) The volume of sodium glycerophosphate (Glycophos®) to make up phosphate 0-150

mM/L with each increment of 25 mM/L or 8.71% dipotassium phosphate to make up phosphate 0-20 mM/L with each increment of 5 mM/L in PN solution 100 mL was calculated as following

Phosphate 150 mM/L; 1000 mL solution contain 150 mM of phosphate

100 mL solution contain 150×100/1000 = 15 mM of phosphate

Sodium glycerophosphate; 1 mM of phosphate are in 1 mL solution

15 mM of phosphate are obtained from 1×15/1 = 15 mL

∴ Take 15 mL of sodium glycerophosphate (Glycophos®) to make up phosphate 150 mM/L in PN solution 100 mL

Phosphate 20 mM/L; 1000 mL solution contain 20 mM of phosphate

100 mL solution contain 20×100/1000 = 2 mM of phosphate

8.71% dipotassium phosphate; 0.5 mM of phosphate are in 1 mL solution

2 mM of phosphate are obtained from 1×2/0.5 = 4 mL

∴ Take 4 mL of 8.71% dipotassium phosphate to make up phosphate 20 mM/L in PN solution 100 mL

(4) The volume of NaCl solution 3% to make up chloride 51.3 mM/L in PN solution 100

mL was calculated as following

Chloride 51.3 mM/L; 1000 mL solution contain 51.3 mM of chloride

100 mL solution contain 51.3×100/1000 = 5.13 mM of chloride

NaCl solution 3%; 0.513 mM of chloride are in 1 mL solution

5.13 mM of chloride are obtained from 1×5.13/0.513 = 10 mL

∴ Take 10 mL of NaCl solution 3% to make up chloride 51.3 mM/L in PN solution 100 mL

(5) The volume of potassium chloride solution to make up magnesium 45 mM/L in PN solution 100 mL was calculated as following

Potassium 45 mM/L; 1000 mL solution contain 45 mM of potassium

100 mL solution contain 45×100/1000 = 4.5 mM of magnesium

Potassium chloride solution; 2 mM of potassium are in 1 mL solution

4.5 mM of potassium are obtained from 1×4.5/2 = 2.25 mL

∴ Take 2.25 mL of Potassium chloride solution to make up magnesium 45 mM/L in PN solution 100 mL

(6) The volume of 24.6% sodium acetate solution to make up sodium 50 mM/L in PN solution 100 mL was calculated as following

Sodium 50 mM/L; 1000 mL solution contain 50 mM of sodium

100 mL solution contain 50×100/1000 = 5 mM of sodium

24.6% sodium acetate solution; 3 mM of sodium are in 1 mL solution

5 mM of sodium are obtained from 1×5/3 = 1.67 mL

∴ Take 1.67 mL of 24.6% sodium acetate solution to make up sodium 50 mM/L in PN solution 100 mL

(7) The volume of 29.4% potassium acetate solution to make up potassium 25 mM/L

in PN solution 100 mL was calculated as following

Potassium 25 mM/L; 1000 mL solution contain 25 mM of potassium

100 mL solution contain 25×100/1000 = 2.5 mM of potassium

29.4% potassium acetate solution; 3 mM of sodium are in 1 mL solution

2.5 mM of potassium are obtained from 1×2.5/3 = 0.83 mL

∴ Take 0.83 mL of 29.4% potassium acetate solution to make up potassium 25 mM/L in PN solution 100 mL

(8) The volume of heparin solution to make up heparin 100 units

in PN solution 100 mL was calculated as following

Heparin 100 units; 100 mL solution contain 100 units of heparin

Heparin solution; 5,000 units of heparin are in 1 mL solution

100 units of heparin are obtained from 1×100/5,000 = 0.02 mL

∴ Take 0.02 mL of heparin solution to make up heparin 100 units in PN solution 100 mL

(9) The volume of zinc sulfate solution to make up zinc 5 mg/L

in PN solution 100 mL was calculated as following

Zinc 5 mg/L; 1000 mL solution contain 5 mg of zinc

100 mL solution contain 5×100/1000 = 0.5 mg of zinc

Zinc sulfate solution; 1 mg of zinc are in 1 mL solution

0.5mg of zinc are obtained from 1×0.5/1 = 0.5 mL

∴ Take 0.5 mL of zinc sulfate solution to make up zinc 5 mg/L in PN solution 100 mL

(10) The volume of $MgSO_4$ solution 50% to make up magnesium 2.025 mM/L in PN solution 100 mL was calculated as following

Magnesium 2.025 mM/L; 1000 mL solution contain 2.025 mM of magnesium

100 mL solution contain 2.025×100/1000 = 0.2025 mM of magnesium

$MgSO_4$ solution 50%; 2.025 mM of magnesium are in 1 mL solution

0.2025 mM of magnesium are obtained from 1×0.2025/2.025 = 0.1 mL

∴ Take 0.1 mL of $MgSO_4$ solution 50% to make up magnesium 2.025 mM/L in PN solution 100 mL

(11) The volume of 10% Ca gluconate solution to make up calcium 0-100 mM/L with

each increment of 25 mM/L in PN solution 100 mL was calculated as following

Calcium 100 mM/L; 1000 mL solution contain 100 mM of calcium

100 mL solution contain 100×100/1000 = 10 mM of calcium

10% Ca gluconate solution; 0.225 mM of calcium are in 1 mL solution

10 mM of phosphate are obtained from 1×10/0.225 = 44.44 mL

∴ Take 44.44 mL of 10% Ca gluconate solution to make up calcium 100 mM/L in PN solution 100 mL

Other formulas calculated as the above example.

APPENDIX B

PRELIMINARY STUDY OF PN FORMULAS CONTAINING CALCIUM GLUCONATE AND DIPOTASSIUM PHOSPHATE

Inorganic phosphate; dipotassium phosphate is currently used as a parenteral phosphate product for PN solutions. There is limited of solubility in a solution containing high concentration of calcium or high pH. Previous study reported solubility data of calcium gluconate and dipotassium phosphate in various concentrations of amino acid. In this study, PN solutions containing calcium gluconate and dipotassium phosphate were solubility tested for intralaboratory and personnel reliability.

Methods

Preparation of the PN solutions studies

Ten PN solutions formulas for neonatal use were aseptically prepared under a laminar flow hood. Standard formula consisted of dextrose at the concentration of 10% (Dextrose 50% in water, Thai Otsuka Pharmaceutical Co.,Ltd., Thailand) and amino acid at the concentration of 2% (Aminoven infant® 10%, Fresenius Kabi, Austria) according to Ramathibodi Hospital infant PN formulation. In this study, calcium gluconate (The Government Pharmaceutical Organization, Thailand) at the concentrations of 0 and 30 mM/L and dipotassium phosphate (Thai Otsuka Pharmaceutical Co.,Ltd., Thailand) or sodium glycerophosphate (Glycophos®, Fresenius Kabi, Austria) at the concentrations of 0 and 50 mM/L, used calcium

gluconate concentrations at 20 mM/L and dipotassium phosphate or sodium glycerophosphate concentrations at 20 mM/L equally of each, were orderly mixed into the standard formula.

Physicochemical assessments

Each PN solutions separately tested according to 4 following conditions; room temperature for 30 minutes, room temperature for 1 day, 4°C for 1 day and 4°C for 7 days. Visual inspection against a black and white contrast background was examined to detect any color change, precipitation, film formation and phase separation with 3-watt LED lamp 220-240 V at 10 inches from the light source (Akers & Larrimore, 2003; MacKay & Anderson, 2015; Ronchera-Oms et al., 1995). Sample pH solution was measured using pH meter (Ultra Basic, Denver Instrument, USA). Samples were examined for light scattering by UV spectrophotometer (ThermoSpectronic, Becthai Bangkok Equipment & Chemical Co.,Ltd., Thailand) at 600 nm against sterile water for injection blank. An absorbance of greater than 0.06 was determined as the "arbitrary threshold for precipitation" (Hanning et al., 1989). For particle size evaluation, one milliliter of each sample was analyzed using a laser instrument (Zetasizer Nano ZS, Malvern Instruments Ltd, DKSH, Thailand) to determine the hydrodynamic diameter of the particles in a given solution (the Z average particle size). An average particle size of greater than 4 μm was determined as the "arbitrary threshold for precipitation" (Huston et al., 2014).

Results

Visual inspection was evaluated any color change, precipitation, film formation and phase separation for 10 PN solutions formulas over 7 days storage duration, as shown in table A-2 and figure A-1. There was no visual precipitation in all solutions containing NaGP. On the other hand, samples containing 50 mM/L of inorganic phosphate and 30 mM/L of calcium gluconate or inorganic phosphate and calcium gluconate 20 mM/L equally of each, precipitations were observed in PN solutions. Table A-3 showed means and S.D. of pH values for PN solutions during 7 days of study. In all cases, pH of the PN admixtures slightly increased regarding to longer storage duration.

Table A-2 Visual inspection of PN solutions

Types of phosphate	Concentrations (mM/L)	Room temperature for 30 minutes	Room temperature for 1 day	4 °C for 1 day	4 °C for 7 days
Inorganic	Blank	✓	✓	✓	✓
	Ca 0 P 0	✓	✓	✓	✓
	Ca 0 P 50	✓	✓	✓	✓
	Ca 20 P 20	✗	✗	✗	✗
	Ca 30 P 0	✓	✓	✓	✓
	Ca 30 P 50	✗	✗	✗	✗
Organic	Ca 0 NaGP 0	✓	✓	✓	✓
	Ca 0 NaGP 50	✓	✓	✓	✓
	Ca 20 NaGP 20	✓	✓	✓	✓
	Ca 30 NaGP 0	✓	✓	✓	✓
	Ca 30 NaGP 50	✓	✓	✓	✓

✓ No precipitation ✗ Precipitation, Ca = calcium gluconate, P = dipotassium phosphate, NaGP = sodium glycerophosphate

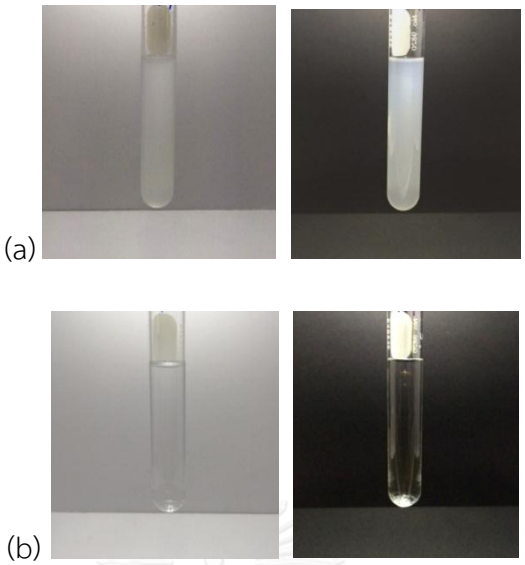


Figure A-1 Visual inspection after mixing 30 mM/L of calcium gluconate with (a) 50 mM/L of dipotassium phosphate or (b) 50 mM/L of sodium glycerophosphate

Table A-3 The pH values of PN solutions

Types of phosphate	Concentrations (mM/L)	Room temperature for 30 minutes	Room temperature for 1 day	4 °C for 1 day	4 °C for 7 days
Inorganic	Blank	7.77±0.01	7.76±0.01	7.77±0.01	7.77±0.01
	Ca 0 P 0	6.19±0.03	6.22±0.03	6.24±0.05	6.26±0.04
	Ca 0 P 50	5.69±0.02	5.79±0.14	5.80±0.12	5.82±0.12
	Ca 20 P 20	6.13±0.02	6.09±0.07	6.12±0.09	6.14±0.09
	Ca 30 P 0	5.25±0.01	5.47±0.39	5.47±0.32	5.48±0.34
	Ca 30 P 50	5.80±0.02	5.98±0.15	6.04±0.09	5.11±0.05
Organic	Ca 0 NaGP 0	6.71±0.01	6.72±0.02	6.74±0.02	6.74±0.04
	Ca 0 NaGP 50	6.62±0.01	6.65±0.05	6.67±0.04	6.68±0.02
	Ca 20 NaGP 20	6.80±0.02	6.83±0.02	6.85±0.03	6.87±0.03
	Ca 30 NaGP 0	6.33±0.02	6.35±0.02	6.36±0.02	6.38±0.01
	Ca 30 NaGP 50	6.89±0.04	6.91±0.04	6.91±0.02	6.95±0.03

Mean±S.D. Ca = calcium gluconate, P = dipotassium phosphate, NaGP = sodium glycerophosphate

According to the absorbance of PN solutions from spectrophotometer (table A-4) and average particle size from Zetasizer Nano ZS (table A-5), the results found that two sample formulas, containing calcium gluconate 30 mM/L and inorganic phosphate 50 mM/L and calcium gluconate 20 mM/L and inorganic phosphate 20 mM/L showed the precipitation during 7 days of analysis at any temperature.

Table A-4 Absorbance of PN solutions from spectrophotometer

Types of phosphate	Concentrations (mM/L)	Room temperature for 30 minutes	Room temperature for 1 day	4 °C for 1 day	4 °C for 7 days
	Blank	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000
	Ca 0 P 0	0.002±0.002	0.041±0.001	0.040±0.001	0.043±0.002
	Ca 0 P 50	0.001±0.001	0.042±0.001	0.039±0.001	0.042±0.001
Inorganic	Ca 20 P 20	0.910±0.110	0.779±0.002	0.837±0.002	0.839±0.001
	Ca 30 P 0	0.001±0.001	0.042±0.002	0.042±0.001	0.042±0.001
	Ca 30 P 50	1.014±0.205	1.031±0.002	1.094±0.002	1.097±0.002
	Ca 0 NaGP 0	0.047±0.001	0.042±0.001	0.041±0.001	0.043±0.002
	Ca 0 NaGP 50	0.042±0.002	0.040±0.001	0.041±0.002	0.042±0.001
Organic	Ca 20 NaGP 20	0.044±0.001	0.041±0.001	0.039±0.001	0.040±0.001
	Ca 30 NaGP 0	0.041±0.001	0.040±0.001	0.038±0.001	0.041±0.001
	Ca 30 NaGP 50	0.042±0.001	0.039±0.001	0.039±0.001	0.039±0.001

Mean±S.D. Ca = calcium gluconate, P = dipotassium phosphate, NaGP = sodium glycerophosphate

Table A-5 Average particle size of PN solutions (μm)

Types of phosphate	Concentrations (mM/L)	Room temperature for 30 minutes	Room temperature for 1 day	4 °C for 1 day	4 °C for 7 days
	Blank	0.291±23.129	0.543±255.860	0.149±120.555	0.500±151.552
	Ca 0 P 0	0.348±216.031	0.126±17.753	0.134±70.579	0.067±21.112
	Ca 0 P 50	0.268±146.604	0.112±16.157	0.094±22.873	0.091±24.350
Inorganic	Ca 20 P 20	4.885±662.409	3.866±2261.200	4.049±668.518	3.552±1786.624
	Ca 30 P 0	0.372±361.215	0.290±201.388	0.326±95.634	0.342±101.137
	Ca 30 P 50	5.526±670.499	3.903±611.842	5.733±1618.815	5.319±1553.279
	Ca 0 NaGP 0	0.139±3.877	0.146±83.320	0.046±16.553	0.121±14.435
	Ca 0 NaGP 50	0.196±19.717	0.154±81.837	0.152±62.693	0.207±139.322
Organic	Ca 20 NaGP 20	0.391±95.527	0.292±68.379	0.181±17.226	0.397±119.155
	Ca 30 NaGP 0	0.476±364.063	0.253±126.058	0.282±215.478	0.576±396.278
	Ca 30 NaGP 50	0.142±49.910	0.131±79.826	0.047±31.956	0.106±26.705

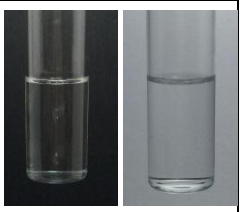
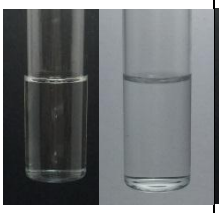
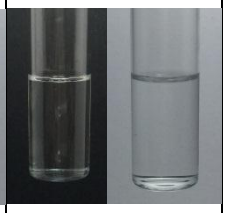
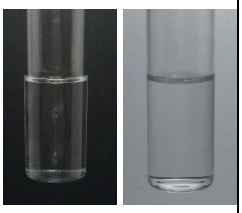
Mean±S.D. Ca = calcium gluconate, P = dipotassium phosphate, NaGP = sodium glycerophosphate

This preliminary result showed the compatibility of calcium and NaGP at the concentrations normally used in practice. All NaGP-containing formulas exhibited no precipitation along 7 days storage duration at both 4 °C and room temperature while formulas containing dipotassium phosphate concentrations at 20 mM/L and calcium gluconate concentrations at 20 mM/L equally of each obviously showed precipitation. Inorganic phosphate; dipotassium phosphate, is easily dissociated and resulted in calcium monohydrogen phosphate precipitation.

APPENDIX C

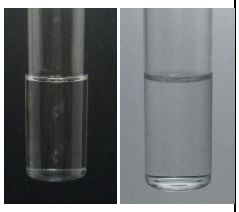
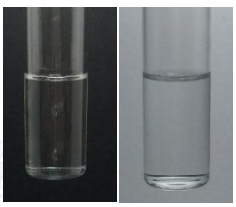
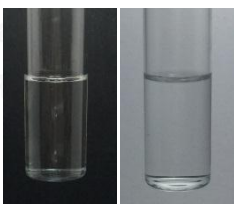
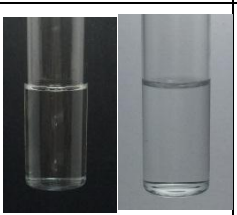
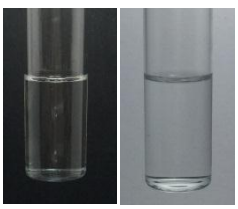
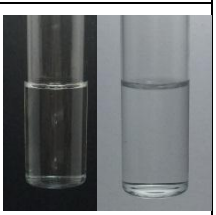
VISUAL INSPECTION

Table A-6 Visual inspections of tested PN solutions containing 1.5% amino acid and various concentrations of calcium gluconate and NaGP

Conditions / Formula		Room temperature for 30 minutes	Room temperature for 1 day	4 °C for 1 day	4 °C for 7 days
NaGP 0	Ca 0				
	Ca 25				
	Ca 50				
	Ca 75				
	Ca 100				

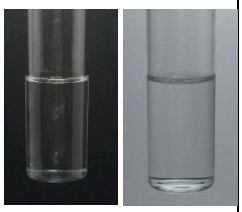
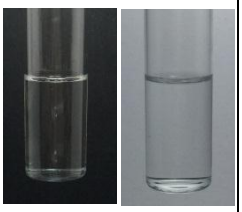
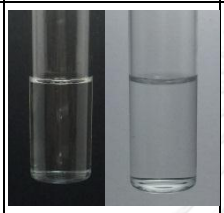
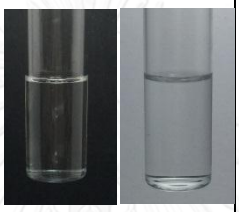
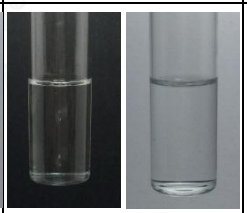
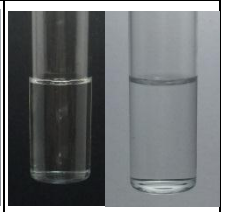
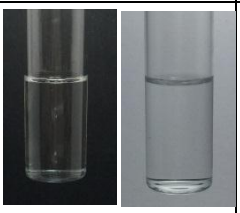
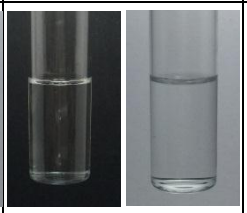
Ca = concentration of calcium gluconate, NaGP = concentration of sodium glycerophosphate

Table A-6 (continued)

Conditions / Formula		Room temperature for 30 minutes	Room temperature for 1 day	4 °C for 1 day	4 °C for 7 days
NaGP 25	Ca 0				
	Ca 25				
	Ca 50				
	Ca 75				
	Ca 100				

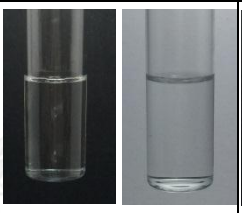
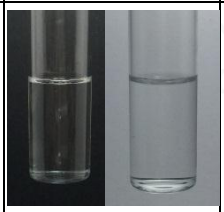
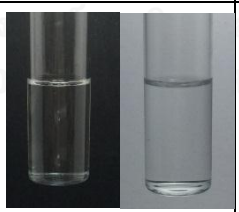
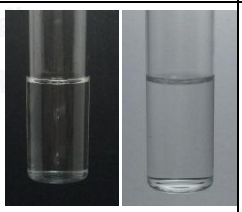
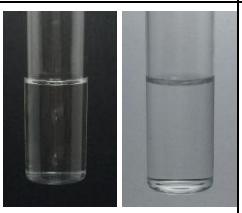
Ca = concentration of calcium gluconate, NaGP = concentration of sodium glycerophosphate

Table A-6 (continued)

Formula \ Conditions		Room temperature for 30 minutes	Room temperature for 1 day	4 °C for 1 day	4 °C for 7 days
NaGP 50	Ca 0				
	Ca 25				
	Ca 50				
	Ca 75				
	Ca 100				

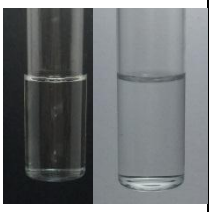
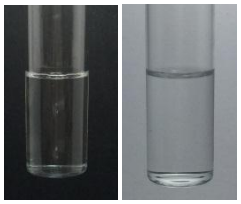
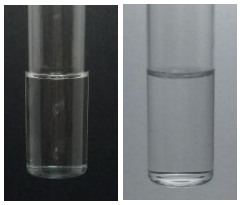
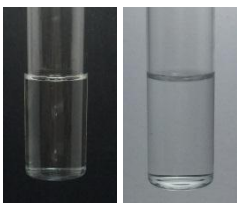
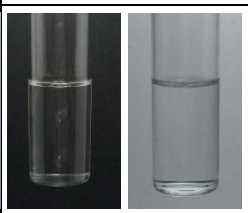
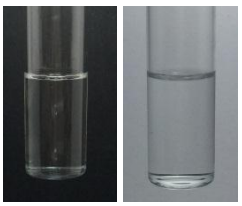
Ca = concentration of calcium gluconate, NaGP = concentration of sodium glycerophosphate

Table A-6 (continued)

Formula \ Conditions		Room temperature for 30 minutes	Room temperature for 1 day	4 °C for 1 day	4 °C for 7 days
NaGP 75	Ca 0				
	Ca 25				
	Ca 50				
	Ca 75				
	Ca 100				

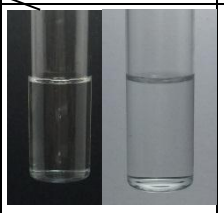
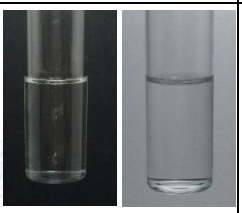
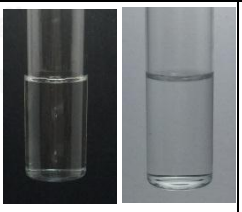
Ca = concentration of calcium gluconate, NaGP = concentration of sodium glycerophosphate

Table A-6 (continued)

Formula \ Conditions		Room temperature for 30 minutes	Room temperature for 1 day	4 °C for 1 day	4 °C for 7 days
NaGP 100	Ca 0				
	Ca 25				
	Ca 50				
	Ca 75				
	Ca 100				

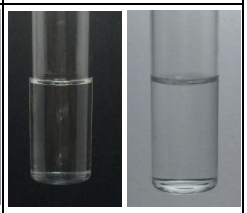
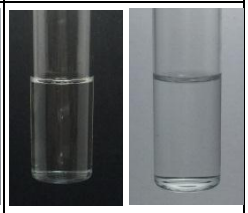
Ca = concentration of calcium gluconate, NaGP = concentration of sodium glycerophosphate

Table A-6 (continued)

Formula \ Conditions		Room temperature for 30 minutes	Room temperature for 1 day	4 °C for 1 day	4 °C for 7 days
NaGP 125	Ca 0				
	Ca 25				
	Ca 50				
	Ca 75				

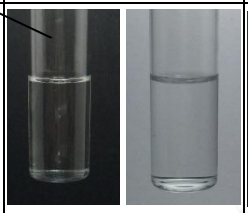
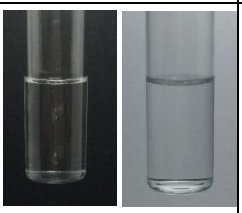
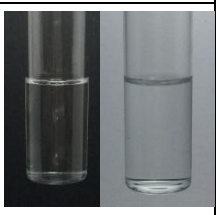
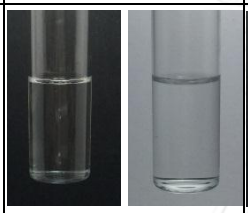
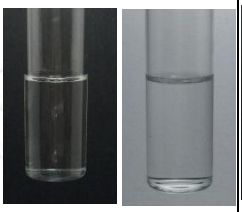
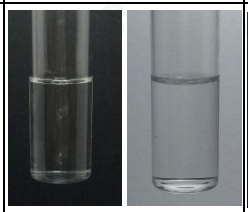
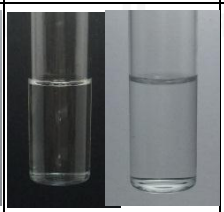
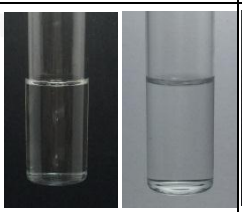
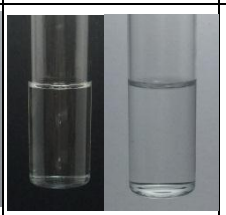
Ca = concentration of calcium gluconate, NaGP = concentration of sodium glycerophosphate

Table A-6 (continued)

Formula \ Conditions		Room temperature for 30 minutes	Room temperature for 1 day	4 °C for 1 day	4 °C for 7 days
NaGP 150	Ca 0				
	Ca 25				
	Ca 50				
	Ca 75				

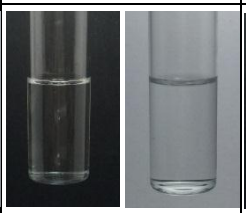
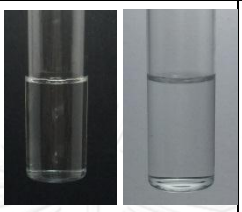
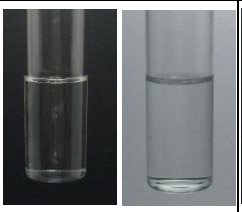
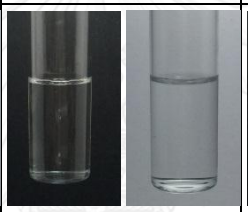
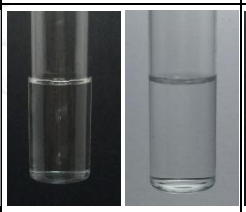
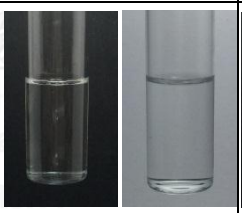
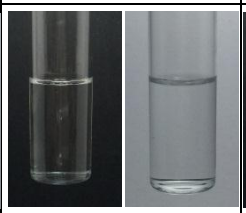
Ca = concentration of calcium gluconate, NaGP = concentration of sodium glycerophosphate

Table A-7 Visual inspections of tested PN solutions containing 2% amino acid and various concentrations of calcium gluconate and NaGP

Formula \ Conditions		Room temperature for 30 minutes	Room temperature for 1 day	4 °C for 1 day	4 °C for 7 days
NaGP 0	Ca 0				
	Ca 25				
	Ca 50				
	Ca 75				
	Ca 100				

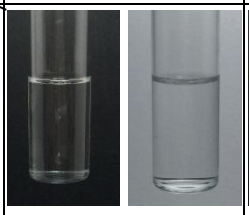
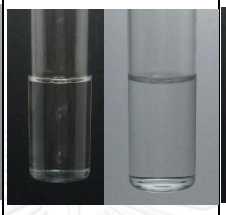
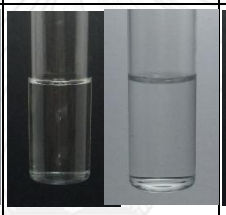
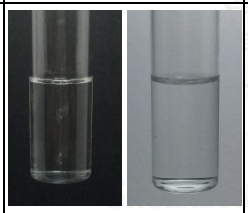
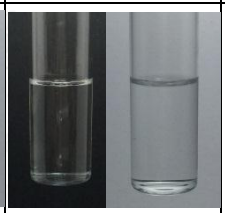
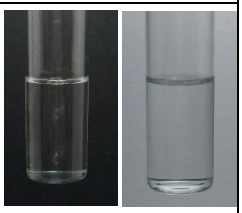
Ca = concentration of calcium gluconate, NaGP = concentration of sodium glycerophosphate

Table A-7 (continued)

Formula \ Conditions		Room temperature for 30 minutes	Room temperature for 1 day	4 °C for 1 day	4 °C for 7 days
NaGP 25	Ca 0				
	Ca 25				
	Ca 50				
	Ca 75				
	Ca 100				

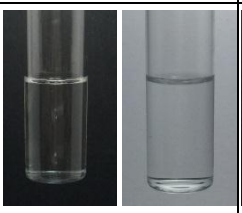
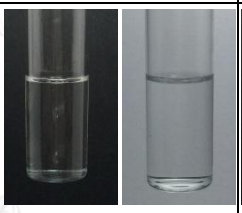
Ca = concentration of calcium gluconate, NaGP = concentration of sodium glycerophosphate

Table A-7 (continued)

Formula \ Conditions		Room temperature for 30 minutes	Room temperature for 1 day	4 °C for 1 day	4 °C for 7 days
NaGP 50	Ca 0				
	Ca 25				
	Ca 50				
	Ca 75				
	Ca 100				

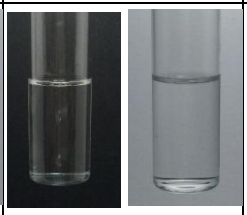
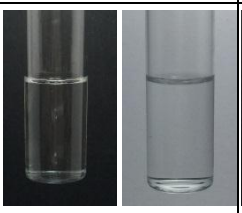
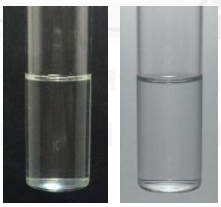
Ca = concentration of calcium gluconate, NaGP = concentration of sodium glycerophosphate

Table A-7 (continued)

Formula \ Conditions		Room temperature for 30 minutes	Room temperature for 1 day	4 °C for 1 day	4 °C for 7 days
NaGP 75	Ca 0				
	Ca 25				
	Ca 50				
	Ca 75				

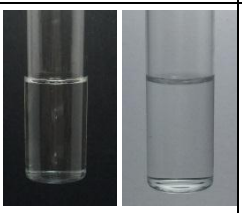
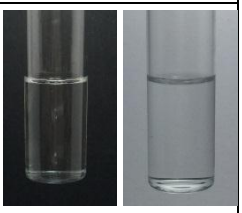
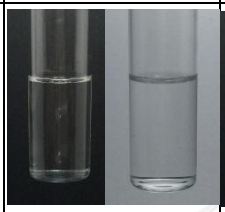
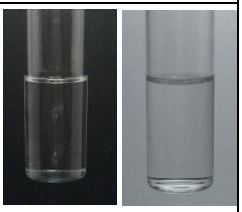
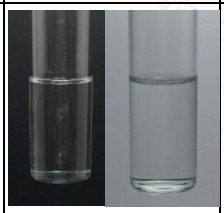
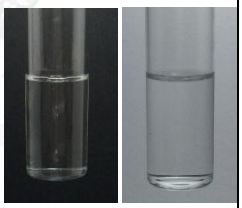
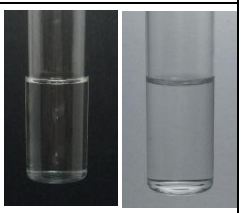
Ca = concentration of calcium gluconate, NaGP = concentration of sodium glycerophosphate

Table A-7 (continued)

Formula \ Conditions		Room temperature for 30 minutes	Room temperature for 1 day	4 °C for 1 day	4 °C for 7 days
NaGP 100	Ca 0				
	Ca 25				
	Ca 50				
	Ca 75				

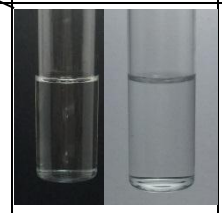
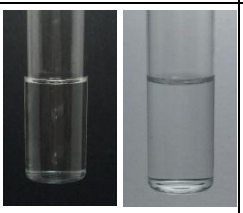
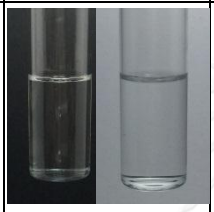
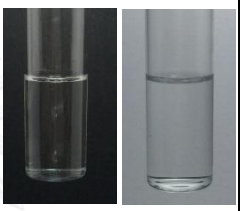
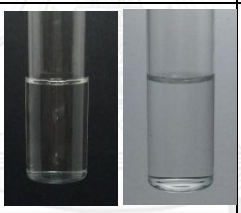
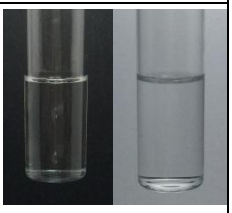
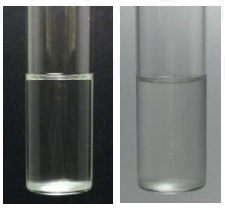
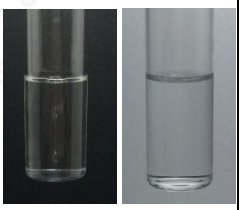
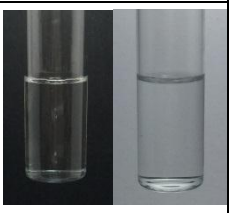
Ca = concentration of calcium gluconate, NaGP = concentration of sodium glycerophosphate

Table A-7 (continued)

Formula \ Conditions		Room temperature for 30 minutes	Room temperature for 1 day	4 °C for 1 day	4 °C for 7 days
NaGP 125	Ca 0				
	Ca 25				
	Ca 50				
	Ca 75				

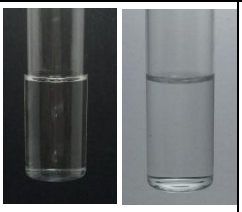
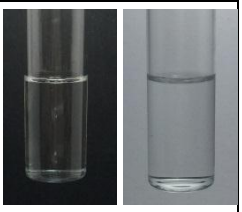
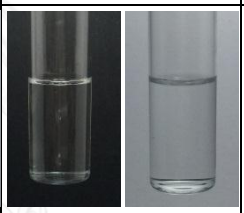
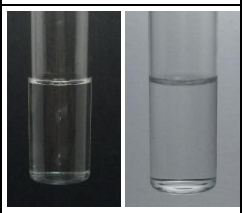
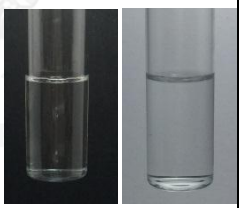
Ca = concentration of calcium gluconate, NaGP = concentration of sodium glycerophosphate

Table A-7 (continued)

Formula \ Conditions		Room temperature for 30 minutes	Room temperature for 1 day	4 °C for 1 day	4 °C for 7 days
NaGP 150	Ca 0				
	Ca 25				
	Ca 50				
	Ca 75				

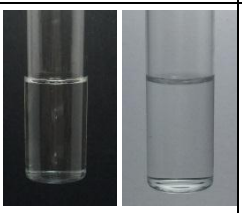
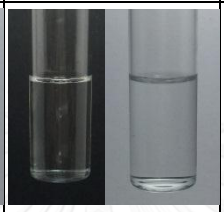
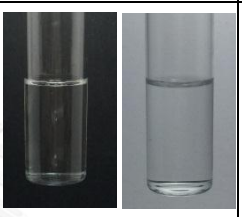
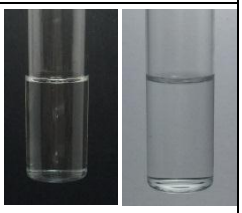
Ca = concentration of calcium gluconate, NaGP = concentration of sodium glycerophosphate

Table A-8 Visual inspections of tested PN solutions containing 2.5% amino acid and various concentrations of calcium gluconate and NaGP

Formula \ Conditions		Room temperature for 30 minutes	Room temperature for 1 day	4 °C for 1 day	4 °C for 7 days
NaGP 0	Ca 0				
	Ca 25				
	Ca 50				
	Ca 75				
	Ca 100				

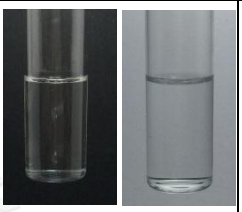
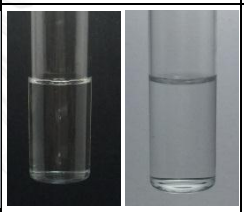
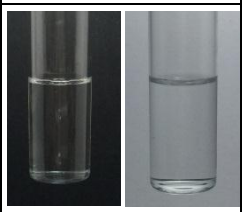
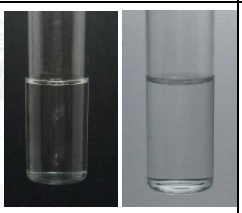
Ca = concentration of calcium gluconate, NaGP = concentration of sodium glycerophosphate

Table A-8 (continued)

Formula \ Conditions		Room temperature for 30 minutes	Room temperature for 1 day	4 °C for 1 day	4 °C for 7 days
NaGP 25	Ca 0				
	Ca 25				
	Ca 50				
	Ca 75				

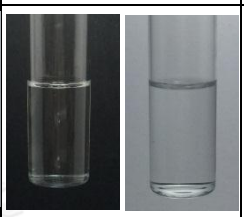
Ca = concentration of calcium gluconate, NaGP = concentration of sodium glycerophosphate

Table A-8 (continued)

Formula \ Conditions		Room temperature for 30 minutes	Room temperature for 1 day	4 °C for 1 day	4 °C for 7 days
NaGP 50	Ca 0				
	Ca 25				
	Ca 50				
	Ca 75				

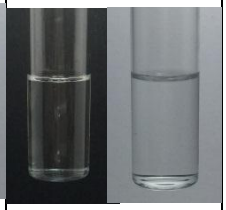
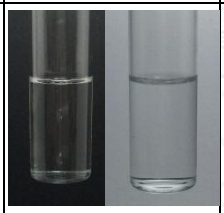
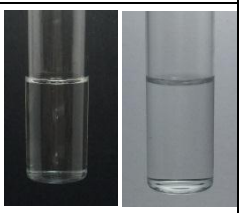
Ca = concentration of calcium gluconate, NaGP = concentration of sodium glycerophosphate

Table A-8 (continued)

Formula \ Conditions		Room temperature for 30 minutes	Room temperature for 1 day	4 °C for 1 day	4 °C for 7 days
NaGP 75	Ca 0				
	Ca 25				
	Ca 50				
	Ca 75				

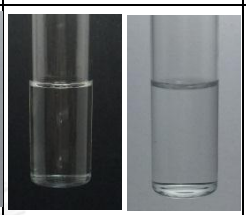
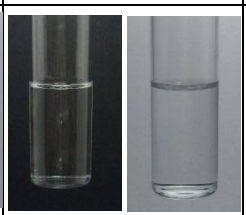
Ca = concentration of calcium gluconate, NaGP = concentration of sodium glycerophosphate

Table A-8 (continued)

Formula \ Conditions		Room temperature for 30 minutes	Room temperature for 1 day	4 °C for 1 day	4 °C for 7 days
NaGP 100	Ca 0				
	Ca 25				
	Ca 50				
	Ca 75				

Ca = concentration of calcium gluconate, NaGP = concentration of sodium glycerophosphate

Table A-8 (continued)

Formula \ Conditions		Room temperature for 30 minutes	Room temperature for 1 day	4 °C for 1 day	4 °C for 7 days
NaGP 125	Ca 0				
	Ca 25				
	Ca 50				
NaGP 150	Ca 0				
	Ca 25				
	Ca 50				

Ca = concentration of calcium gluconate, NaGP = concentration of sodium glycerophosphate

APPENDIX D

MICROSCOPIC PARTICLE COUNT TEST

Table A-9 Maximum particle count of tested PN solutions containing 1.5% amino acid and various concentrations of calcium gluconate and NaGP*

Formulations		Conditions	PC <10 μm	PC ≥10 μm	PC ≥25 μm
NaGP 0	Ca 0	Day 0[a]	0	0	0
		Day 1[a]	0	0	0
		Day 1[b]	0	0	0
		Day 7[b]	0	0	0
NaGP 0	Ca 25	Day 0[a]	0	0	0
		Day 1[a]	0	0	0
		Day 1[b]	0	0	0
		Day 7[b]	0	0	0
NaGP 0	Ca 50	Day 0[a]	0	0	0
		Day 1[a]	0	0	0
		Day 1[b]	0	0	0
		Day 7[b]	0	0	0
NaGP 0	Ca 75	Day 0[a]	0	0	0
		Day 1[a]	0	0	0
		Day 1[b]	0	0	0
		Day 7[b]	0	1	0
NaGP 0	Ca 100	Day 0[a]	0	0	0
		Day 1[a]	0	0	0
		Day 1[b]	0	0	0
		Day 7[b]	0	1	0

a = room temperature, b = 4 °C, Ca = concentration of calcium gluconate (mM/L), NaGP = concentration of sodium glycerophosphate (mM/L)

*Maximum particle count: ≥10 μm less than 60 particles/5mL, ≥25 μm less than 10 particles/5mL

Table A-9 (continued)*

Formulations		Conditions	PC <10 μm	PC ≥10 μm	PC ≥25 μm
NaGP 25	Ca 0	Day 0[a]	0	0	0
		Day 1[a]	0	0	0
		Day 1[b]	0	0	0
		Day 7[b]	0	0	0
NaGP 25	Ca 25	Day 0[a]	0	0	0
		Day 1[a]	0	0	0
		Day 1[b]	0	0	0
		Day 7[b]	0	0	0
NaGP 25	Ca 50	Day 0[a]	0	0	0
		Day 1[a]	0	0	0
		Day 1[b]	0	0	0
		Day 7[b]	0	1	0
NaGP 25	Ca 75	Day 0[a]	1	0	0
		Day 1[a]	0	0	1
		Day 1[b]	0	1	0
		Day 7[b]	0	1	0
NaGP 25	Ca 100	Day 0[a]	0	1	0
		Day 1[a]	0	1	0
		Day 1[b]	0	1	0
		Day 7[b]	NT3	NT3	NT3

a = room temperature, b = 4 °C, Ca = concentration of calcium gluconate (mM/L), NaGP = concentration of sodium glycerophosphate (mM/L)

NT3 = Not test due to fail by turbidity measurement

*Maximum particle count: ≥10 μm less than 60 particles/5mL, ≥25 μm less than 10 particles/5mL

Table A-9 (continued)*

Formulations		Conditions	PC <10 μm	PC ≥10 μm	PC ≥25 μm
NaGP 50	Ca 0	Day 0[a]	0	0	0
		Day 1[a]	0	0	0
		Day 1[b]	0	0	0
		Day 7[b]	0	0	0
NaGP 50	Ca 25	Day 0[a]	0	0	0
		Day 1[a]	0	0	0
		Day 1[b]	0	0	0
		Day 7[b]	0	0	0
NaGP 50	Ca 50	Day 0[a]	0	0	0
		Day 1[a]	0	0	0
		Day 1[b]	0	0	0
		Day 7[b]	0	1	0
NaGP 50	Ca 75	Day 0[a]	0	1	0
		Day 1[a]	0	1	0
		Day 1[b]	0	1	0
		Day 7[b]	NT3	NT3	NT3
NaGP 50	Ca 100	Day 0[a]	2	2	0
		Day 1[a]	0	1	1
		Day 1[b]	0	1	0
		Day 7[b]	NT3	NT3	NT3

a = room temperature, b = 4 °C, Ca = concentration of calcium gluconate (mM/L), NaGP = concentration of sodium glycerophosphate (mM/L)

NT3 = Not test due to fail by turbidity measurement

*Maximum particle count: ≥10 μm less than 60 particles/5mL, ≥25 μm less than 10 particles/5mL

Table A-9 (continued)*

Formulations		Conditions	PC <10 μm	PC ≥10 μm	PC ≥25 μm
NaGP 75	Ca 0	Day 0[a]	0	0	0
		Day 1[a]	0	0	0
		Day 1[b]	0	0	0
		Day 7[b]	0	1	0
NaGP 75	Ca 25	Day 0[a]	0	0	0
		Day 1[a]	0	0	0
		Day 1[b]	0	0	0
		Day 7[b]	1	1	3
NaGP 75	Ca 50	Day 0[a]	0	0	0
		Day 1[a]	0	1	0
		Day 1[b]	0	1	0
		Day 7[b]	NT3	NT3	NT3
NaGP 75	Ca 75	Day 0[a]	1	0	0
		Day 1[a]	1	2	0
		Day 1[b]	0	0	1
		Day 7[b]	NT3	NT3	NT3
NaGP 75	Ca 100	Day 0[a]	0	1	0
		Day 1[a]	0	0	3
		Day 1[b]	1	1	2
		Day 7[b]	NT3	NT3	NT3

a = room temperature, b = 4 °C, Ca = concentration of calcium gluconate (mM/L), NaGP = concentration of sodium glycerophosphate (mM/L)

NT3 = Not test due to fail by turbidity measurement

*Maximum particle count: ≥10 μm less than 60 particles/5mL, ≥25 μm less than 10 particles/5mL

Table A-9 (continued)*

Formulations		Conditions	PC <10 μm	PC ≥10 μm	PC ≥25 μm
NaGP 100	Ca 0	Day 0[a]	0	0	0
		Day 1[a]	0	0	0
		Day 1[b]	0	0	0
		Day 7[b]	0	1	0
NaGP 100	Ca 25	Day 0[a]	0	0	0
		Day 1[a]	0	2	0
		Day 1[b]	0	0	0
		Day 7[b]	0	1	3
NaGP 100	Ca 50	Day 0[a]	0	0	1
		Day 1[a]	0	2	1
		Day 1[b]	0	0	1
		Day 7[b]	NT3	NT3	NT3
NaGP 100	Ca 75	Day 0[a]	0	1	0
		Day 1[a]	NT3	NT3	NT3
		Day 1[b]	NT3	NT3	NT3
		Day 7[b]	NT3	NT3	NT3
NaGP 100	Ca 100	Day 0[a]	0	1	0
		Day 1[a]	NT3	NT3	NT3
		Day 1[b]	NT3	NT3	NT3
		Day 7[b]	NT3	NT3	NT3

a = room temperature, b = 4 °C, Ca = concentration of calcium gluconate (mM/L), NaGP = concentration of sodium glycerophosphate (mM/L)

NT3 = Not test due to fail by turbidity measurement

*Maximum particle count: ≥10 μm less than 60 particles/5mL, ≥25 μm less than 10 particles/5mL

Table A-9 (continued)*

Formulations		Conditions	PC <10 μm	PC ≥10 μm	PC ≥25 μm
NaGP 125	Ca 0	Day 0[a]	0	0	0
		Day 1[a]	0	0	0
		Day 1[b]	0	0	0
		Day 7[b]	0	0	1
NaGP 125	Ca 25	Day 0[a]	0	0	0
		Day 1[a]	0	1	0
		Day 1[b]	0	0	0
		Day 7[b]	NT3	NT3	NT3
NaGP 125	Ca 50	Day 0[a]	1	0	0
		Day 1[a]	NT3	NT3	NT3
		Day 1[b]	NT3	NT3	NT3
		Day 7[b]	NT3	NT3	NT3
NaGP 125	Ca 75	Day 0[a]	0	0	1
		Day 1[a]	NT3	NT3	NT3
		Day 1[b]	NT3	NT3	NT3
		Day 7[b]	NT3	NT3	NT3

a = room temperature, b = 4 °C, Ca = concentration of calcium gluconate (mM/L), NaGP = concentration of sodium glycerophosphate (mM/L)

NT3 = Not test due to fail by turbidity measurement

*Maximum particle count: ≥10 μm less than 60 particles/5mL, ≥25 μm less than 10 particles/5mL

Table A-9 (continued)*

Formulations		Conditions	PC <10 μm	PC ≥10 μm	PC ≥25 μm
NaGP 150	Ca 0	Day 0[a]	0	0	0
		Day 1[a]	0	0	0
		Day 1[b]	0	0	0
		Day 7[b]	0	0	1
NaGP 150	Ca 25	Day 0[a]	0	0	0
		Day 1[a]	0	1	0
		Day 1[b]	0	0	0
		Day 7[b]	NT3	NT3	NT3
NaGP 150	Ca 50	Day 0[a]	0	1	1
		Day 1[a]	NT3	NT3	NT3
		Day 1[b]	NT3	NT3	NT3
		Day 7[b]	NT3	NT3	NT3
NaGP 150	Ca 75	Day 0[a]	NT3	NT3	NT3
		Day 1[a]	NT3	NT3	NT3
		Day 1[b]	NT3	NT3	NT3
		Day 7[b]	NT3	NT3	NT3

a = room temperature, b = 4 °C, Ca = concentration of calcium gluconate (mM/L), NaGP = concentration of sodium glycerophosphate (mM/L)

NT3 = Not test due to fail by turbidity measurement

*Maximum particle count: ≥10 μm less than 60 particles/5mL, ≥25 μm less than 10 particles/5mL

Table A-10 Maximum particle count of tested PN solutions containing 2% amino acid and various concentrations of calcium gluconate and NaGP*

Formulations		Conditions	PC <10 μm	PC ≥10 μm	PC ≥25 μm
NaGP 0	Ca 0	Day 0[a]	0	4	2
		Day 1[a]	5	4	3
		Day 1[b]	4	6	2
		Day 7[b]	1	2	0
NaGP 0	Ca 25	Day 0[a]	1	3	2
		Day 1[a]	2	2	3
		Day 1[b]	1	4	3
		Day 7[b]	2	2	0
NaGP 0	Ca 50	Day 0[a]	1	4	3
		Day 1[a]	1	1	3
		Day 1[b]	3	5	2
		Day 7[b]	4	2	1
NaGP 0	Ca 75	Day 0[a]	4	2	3
		Day 1[a]	0	2	2
		Day 1[b]	0	2	3
		Day 7[b]	0	1	2
NaGP 0	Ca 100	Day 0[a]	6	5	3
		Day 1[a]	3	2	2
		Day 1[b]	4	3	5
		Day 7[b]	1	2	1

a = room temperature, b = 4 °C, Ca = concentration of calcium gluconate (mM/L), NaGP = concentration of sodium glycerophosphate (mM/L)

*Maximum particle count: ≥10 μm less than 60 particles/5mL, ≥25 μm less than 10 particles/5mL

Table A-10 (continued)*

Formulations		Conditions	PC <10 μm	PC ≥10 μm	PC ≥25 μm
NaGP 25	Ca 0	Day 0[a]	1	2	3
		Day 1[a]	1	5	7
		Day 1[b]	3	1	3
		Day 7[b]	0	2	5
NaGP 25	Ca 25	Day 0[a]	3	2	3
		Day 1[a]	2	2	4
		Day 1[b]	0	3	2
		Day 7[b]	1	2	3
NaGP 25	Ca 50	Day 0[a]	0	3	3
		Day 1[a]	0	2	3
		Day 1[b]	0	1	2
		Day 7[b]	0	2	3
NaGP 25	Ca 75	Day 0[a]	0	0	2
		Day 1[a]	1	2	2
		Day 1[b]	0	1	1
		Day 7[b]	0	2	4
NaGP 25	Ca 100	Day 0[a]	0	4	4
		Day 1[a]	1	2	3
		Day 1[b]	3	3	1
		Day 7[b]	1	2	3

a = room temperature, b = 4 °C, Ca = concentration of calcium gluconate (mM/L), NaGP = concentration of sodium glycerophosphate (mM/L)

*Maximum particle count: ≥10 μm less than 60 particles/5mL, ≥25 μm less than 10 particles/5mL

Table A-10 (continued)*

Formulations		Conditions	PC <10 μm	PC ≥10 μm	PC ≥25 μm
NaGP 50	Ca 0	Day 0[a]	0	1	3
		Day 1[a]	1	2	4
		Day 1[b]	2	1	3
		Day 7[b]	0	1	1
NaGP 50	Ca 25	Day 0[a]	4	3	2
		Day 1[a]	0	2	1
		Day 1[b]	0	3	1
		Day 7[b]	0	3	1
NaGP 50	Ca 50	Day 0[a]	0	2	4
		Day 1[a]	0	1	2
		Day 1[b]	0	3	1
		Day 7[b]	2	2	1
NaGP 50	Ca 75	Day 0[a]	0	3	2
		Day 1[a]	2	6	5
		Day 1[b]	1	1	1
		Day 7[b]	NT3	NT3	NT3
NaGP 50	Ca 100	Day 0[a]	2	3	1
		Day 1[a]	1	3	3
		Day 1[b]	1	1	0
		Day 7[b]	NT3	NT3	NT3

a = room temperature, b = 4 °C, Ca = concentration of calcium gluconate (mM/L), NaGP = concentration of sodium glycerophosphate (mM/L)

NT3 = Not test due to fail by turbidity measurement

*Maximum particle count: ≥10 μm less than 60 particles/5mL, ≥25 μm less than 10 particles/5mL

Table A-10 (continued)*

Formulations		Conditions	PC <10 μm	PC ≥10 μm	PC ≥25 μm
NaGP 75	Ca 0	Day 0[a]	0	2	3
		Day 1[a]	1	2	3
		Day 1[b]	1	3	0
		Day 7[b]	2	4	5
NaGP 75	Ca 25	Day 0[a]	0	0	1
		Day 1[a]	1	4	2
		Day 1[b]	2	1	0
		Day 7[b]	2	6	4
NaGP 75	Ca 50	Day 0[a]	2	1	2
		Day 1[a]	3	4	2
		Day 1[b]	0	1	1
		Day 7[b]	2	5	3
NaGP 75	Ca 75	Day 0[a]	0	2	2
		Day 1[a]	1	2	2
		Day 1[b]	4	0	0
		Day 7[b]	NT3	NT3	NT3

a = room temperature, b = 4 °C, Ca = concentration of calcium gluconate (mM/L), NaGP = concentration of sodium glycerophosphate (mM/L)

NT3 = Not test due to fail by turbidity measurement

*Maximum particle count: ≥10 μm less than 60 particles/5mL, ≥25 μm less than 10 particles/5mL

Table A-10 (continued)*

Formulations		Conditions	PC <10 μm	PC ≥10 μm	PC ≥25 μm
NaGP 100	Ca 0	Day 0[a]	3	2	1
		Day 1[a]	2	3	2
		Day 1[b]	2	2	1
		Day 7[b]	0	2	5
NaGP 100	Ca 25	Day 0[a]	1	0	1
		Day 1[a]	2	2	0
		Day 1[b]	0	0	0
		Day 7[b]	1	2	3
NaGP 100	Ca 50	Day 0[a]	0	2	3
		Day 1[a]	3	5	2
		Day 1[b]	1	3	0
		Day 7[b]	0	4	4
NaGP 100	Ca 75	Day 0[a]	0	0	2
		Day 1[a]	NT3	NT3	NT3
		Day 1[b]	1	1	2
		Day 7[b]	NT3	NT3	NT3

a = room temperature, b = 4 °C, Ca = concentration of calcium gluconate (mM/L), NaGP = concentration of sodium glycerophosphate (mM/L)

NT3 = Not test due to fail by turbidity measurement

*Maximum particle count: ≥10 μm less than 60 particles/5mL, ≥25 μm less than 10 particles/5mL

Table A-10 (continued)*

Formulations		Conditions	PC <10 μm	PC ≥10 μm	PC ≥25 μm
NaGP 125	Ca 0	Day 0[a]	0	0	0
		Day 1[a]	0	1	1
		Day 1[b]	0	1	1
		Day 7[b]	1	3	1
NaGP 125	Ca 25	Day 0[a]	0	0	1
		Day 1[a]	0	0	0
		Day 1[b]	0	0	0
		Day 7[b]	1	4	3
NaGP 125	Ca 50	Day 0[a]	1	2	2
		Day 1[a]	NT3	NT3	NT3
		Day 1[b]	0	1	2
		Day 7[b]	NT3	NT3	NT3
NaGP 125	Ca 75	Day 0[a]	0	0	1
		Day 1[a]	NT3	NT3	NT3
		Day 1[b]	NT3	NT3	NT3
		Day 7[b]	NT3	NT3	NT3

a = room temperature, b = 4 °C, Ca = concentration of calcium gluconate (mM/L), NaGP = concentration of sodium glycerophosphate (mM/L)

NT3 = Not test due to fail by turbidity measurement

*Maximum particle count: ≥10 μm less than 60 particles/5mL, ≥25 μm less than 10 particles/5mL

Table A-10 (continued)*

Formulations		Conditions	PC <10 μm	PC ≥10 μm	PC ≥25 μm
NaGP 150	Ca 0	Day 0[a]	0	1	1
		Day 1[a]	0	0	1
		Day 1[b]	0	1	2
		Day 7[b]	5	3	3
NaGP 150	Ca 25	Day 0[a]	0	2	1
		Day 1[a]	0	1	1
		Day 1[b]	0	0	0
		Day 7[b]	NT3	NT3	NT3
NaGP 150	Ca 50	Day 0[a]	0	0	2
		Day 1[a]	NT3	NT3	NT3
		Day 1[b]	2	0	2
		Day 7[b]	NT3	NT3	NT3
NaGP 150	Ca 75	Day 0[a]	NT3	NT3	NT3
		Day 1[a]	NT3	NT3	NT3
		Day 1[b]	NT3	NT3	NT3
		Day 7[b]	NT3	NT3	NT3

a = room temperature, b = 4 °C, Ca = concentration of calcium gluconate (mM/L), NaGP = concentration of sodium glycerophosphate (mM/L)

NT3 = Not test due to fail by turbidity measurement

*Maximum particle count: ≥10 μm less than 60 particles/5mL, ≥25 μm less than 10 particles/5mL

Table A-11 Maximum particle count of tested PN solutions containing 2.5% amino acid and various concentrations of calcium gluconate and NaGP*

Formulations		Conditions	PC <10 μm	PC ≥10 μm	PC ≥25 μm
NaGP 0	Ca 0	Day 0[a]	2	4	1
		Day 1[a]	1	2	1
		Day 1[b]	4	5	2
		Day 7[b]	3	1	0
NaGP 0	Ca 25	Day 0[a]	1	4	4
		Day 1[a]	1	4	3
		Day 1[b]	2	2	3
		Day 7[b]	1	2	1
NaGP 0	Ca 50	Day 0[a]	0	2	2
		Day 1[a]	0	2	2
		Day 1[b]	1	2	0
		Day 7[b]	0	3	1
NaGP 0	Ca 75	Day 0[a]	0	1	2
		Day 1[a]	1	2	2
		Day 1[b]	2	3	0
		Day 7[b]	2	1	1
NaGP 0	Ca 100	Day 0[a]	0	1	2
		Day 1[a]	1	4	2
		Day 1[b]	2	3	0
		Day 7[b]	1	2	2

a = room temperature, b = 4 °C, Ca = concentration of calcium gluconate (mM/L), NaGP = concentration of sodium glycerophosphate (mM/L)

*Maximum particle count: ≥10 μm less than 60 particles/5mL, ≥25 μm less than 10 particles/5mL

Table A-11 (continued)*

Formulations		Conditions	PC <10 μm	PC ≥10 μm	PC ≥25 μm
NaGP 25	Ca 0	Day 0[a]	1	1	0
		Day 1[a]	0	2	1
		Day 1[b]	1	1	0
		Day 7[b]	1	1	0
NaGP 25	Ca 25	Day 0[a]	1	1	0
		Day 1[a]	1	2	2
		Day 1[b]	0	1	1
		Day 7[b]	1	1	0
NaGP 25	Ca 50	Day 0[a]	1	1	0
		Day 1[a]	0	1	1
		Day 1[b]	0	1	0
		Day 7[b]	0	1	0
NaGP 25	Ca 75	Day 0[a]	0	1	1
		Day 1[a]	1	1	1
		Day 1[b]	2	2	0
		Day 7[b]	1	1	0

a = room temperature, b = 4 °C, Ca = concentration of calcium gluconate (mM/L), NaGP = concentration of sodium glycerophosphate (mM/L)

*Maximum particle count: ≥10 μm less than 60 particles/5mL, ≥25 μm less than 10 particles/5mL

Table A-11 (continued)*

Formulations		Conditions	PC <10 μm	PC ≥10 μm	PC ≥25 μm
NaGP 50	Ca 0	Day 0[a]	0	1	1
		Day 1[a]	1	1	0
		Day 1[b]	0	0	0
		Day 7[b]	1	0	0
NaGP 50	Ca 25	Day 0[a]	1	1	1
		Day 1[a]	0	2	0
		Day 1[b]	0	1	0
		Day 7[b]	1	0	0
NaGP 50	Ca 50	Day 0[a]	1	0	1
		Day 1[a]	0	1	3
		Day 1[b]	0	1	0
		Day 7[b]	0	1	0
NaGP 50	Ca 75	Day 0[a]	1	3	3
		Day 1[a]	1	1	2
		Day 1[b]	1	2	1
		Day 7[b]	1	1	1

a = room temperature, b = 4 °C, Ca = concentration of calcium gluconate (mM/L), NaGP = concentration of sodium glycerophosphate (mM/L)

*Maximum particle count: ≥10 μm less than 60 particles/5mL, ≥25 μm less than 10 particles/5mL

Table A-11 (continued)*

Formulations		Conditions	PC <10 μm	PC ≥10 μm	PC ≥25 μm
NaGP 75	Ca 0	Day 0[a]	1	4	0
		Day 1[a]	1	2	0
		Day 1[b]	1	2	0
		Day 7[b]	0	1	0
NaGP 75	Ca 25	Day 0[a]	0	1	2
		Day 1[a]	0	2	1
		Day 1[b]	1	1	0
		Day 7[b]	1	2	0
NaGP 75	Ca 50	Day 0[a]	0	3	1
		Day 1[a]	1	2	2
		Day 1[b]	1	2	1
		Day 7[b]	1	1	0
NaGP 75	Ca 75	Day 0[a]	1	3	0
		Day 1[a]	0	2	1
		Day 1[b]	1	1	0
		Day 7[b]	1	1	1

a = room temperature, b = 4 °C, Ca = concentration of calcium gluconate (mM/L), NaGP = concentration of sodium glycerophosphate (mM/L)

*Maximum particle count: ≥10 μm less than 60 particles/5mL, ≥25 μm less than 10 particles/5mL

Table A-11 (continued)*

Formulations		Conditions	PC <10 μm	PC ≥10 μm	PC ≥25 μm
NaGP 100	Ca 0	Day 0[a]	1	3	1
		Day 1[a]	0	0	1
		Day 1[b]	0	0	0
		Day 7[b]	1	2	1
NaGP 100	Ca 25	Day 0[a]	1	2	1
		Day 1[a]	1	2	2
		Day 1[b]	0	2	0
		Day 7[b]	1	2	0
NaGP 100	Ca 50	Day 0[a]	2	2	1
		Day 1[a]	1	2	1
		Day 1[b]	1	1	0
		Day 7[b]	0	2	0
NaGP 100	Ca 75	Day 0[a]	1	1	1
		Day 1[a]	1	1	2
		Day 1[b]	2	2	0
		Day 7[b]	0	2	1

a = room temperature, b = 4 °C, Ca = concentration of calcium gluconate (mM/L), NaGP = concentration of sodium glycerophosphate (mM/L)

*Maximum particle count: ≥10 μm less than 60 particles/5mL, ≥25 μm less than 10 particles/5mL

Table A-11 (continued)*

Formulations		Conditions	PC <10 μm	PC ≥10 μm	PC ≥25 μm
NaGP 125	Ca 0	Day 0[a]	1	1	1
		Day 1[a]	1	3	0
		Day 1[b]	0	2	0
		Day 7[b]	2	2	1
NaGP 125	Ca 25	Day 0[a]	1	2	1
		Day 1[a]	1	3	1
		Day 1[b]	2	2	0
		Day 7[b]	2	2	0
NaGP 125	Ca 50	Day 0[a]	3	1	1
		Day 1[a]	1	1	2
		Day 1[b]	0	2	0
		Day 7[b]	1	1	0
NaGP 150	Ca 0	Day 0[a]	1	2	0
		Day 1[a]	0	3	0
		Day 1[b]	1	1	0
		Day 7[b]	1	2	1
NaGP 150	Ca 25	Day 0[a]	1	4	1
		Day 1[a]	0	1	1
		Day 1[b]	1	1	0
		Day 7[b]	1	2	1
NaGP 150	Ca 50	Day 0[a]	0	5	0
		Day 1[a]	1	2	2
		Day 1[b]	1	1	0
		Day 7[b]	1	3	1

a = room temperature, b = 4 °C, Ca = concentration of calcium gluconate (mM/L), NaGP = concentration of sodium glycerophosphate (mM/L)

*Maximum particle count: ≥10 μm less than 60 particles/5mL, ≥25 μm less than 10 particles/5mL

APPENDIX E

SOLUBILITY OF CALCIUM GLUCONATE AND DIPOTASSIUM PHOSPHATE

Table A-12 The pH values of tested PN solutions containing 1.5% amino acid and various concentrations of calcium gluconate and dipotassium phosphate (mean ± S.D.)

Concentrations of Ca gluconate (mM/L)	Concentrations of P (mM/L)				
	0	5	10	15	20
Room temperature for 30 minutes					
0	5.340±0.000	5.940±0.000	6.440±0.000	6.730±0.000	6.820±0.000
5	5.280±0.000	5.920±0.000	6.310±0.000	6.530±0.000	6.483±0.006
10	5.250±0.000	5.820±0.000	6.300±0.000	6.220±0.000	6.120±0.000
15	5.250±0.000	5.790±0.000	6.260±0.000	6.030±0.000	5.980±0.000
20	5.260±0.000	5.780±0.000	6.240±0.000	5.870±0.000	5.760±0.000
Room temperature for 1 day					
0	5.357±0.006	5.950±0.000	6.450±0.000	6.740±0.000	6.830±0.000
5	5.290±0.000	5.930±0.000	6.320±0.000	6.540±0.000	6.490±0.000
10	5.267±0.006	5.840±0.000	6.290±0.000	6.230±0.000	6.123±0.006
15	5.260±0.000	5.790±0.000	6.270±0.000	6.040±0.000	5.980±0.000
20	5.270±0.000	5.787±0.006	6.250±0.000	5.870±0.000	5.770±0.000
4 °C for 1 day					
0	5.360±0.000	5.950±0.000	6.460±0.000	6.740±0.000	6.850±0.000
5	5.290±0.000	5.940±0.000	6.320±0.000	6.550±0.000	6.510±0.000
10	5.270±0.000	5.850±0.000	6.310±0.000	6.240±0.000	6.150±0.000
15	5.270±0.000	5.810±0.000	6.280±0.000	6.040±0.000	6.010±0.000
20	5.280±0.000	5.800±0.000	6.260±0.000	5.880±0.000	5.787±0.006
4 °C for 7 days					
0	5.380±0.000	5.970±0.000	6.470±0.000	6.760±0.000	6.857±0.006
5	5.310±0.000	5.950±0.000	6.330±0.000	6.560±0.000	6.520±0.000
10	5.280±0.000	5.840±0.000	6.330±0.000	6.243±0.006	6.150±0.000
15	5.280±0.000	5.817±0.006	6.293±0.006	6.060±0.000	6.017±0.006
20	5.287±0.006	5.810±0.000	6.267±0.006	5.907±0.006	5.790±0.000

Ca = calcium, P = dipotassium phosphate

Table A-13 The pH values of tested PN solutions containing 2% amino acid and various concentrations of calcium gluconate and dipotassium phosphate (mean ± S.D.)

Concentrations of Ca gluconate (mM/L)	Concentrations of P (mM/L)				
	0	5	10	15	20
Room temperature for 30 minutes					
0	6.190±0.000	6.347±0.006	6.443±0.006	6.600±0.000	6.707±0.006
5	6.023±0.006	6.200±0.000	6.350±0.000	6.410±0.000	6.690±0.000
10	5.947±0.006	6.070±0.000	6.330±0.000	6.390±0.000	6.407±0.006
15	5.900±0.000	6.040±0.000	6.297±0.006	6.090±0.000	6.200±0.000
20	5.870±0.000	6.000±0.000	6.290±0.000	6.020±0.000	6.050±0.000
Room temperature 1 day					
0	6.200±0.000	6.340±0.000	6.447±0.006	6.600±0.000	6.707±0.006
5	6.030±0.000	6.203±0.006	6.353±0.006	6.407±0.006	6.693±0.006
10	5.953±0.012	6.080±0.000	6.333±0.006	6.397±0.006	6.400±0.000
15	5.903±0.006	6.043±0.006	6.300±0.000	6.097±0.006	6.203±0.006
20	5.877±0.006	6.003±0.006	6.290±0.000	6.023±0.006	6.057±0.006
4 °C for 1 day					
0	6.207±0.006	6.353±0.006	6.480±0.000	6.630±0.000	6.733±0.006
5	6.027±0.006	6.210±0.000	6.377±0.006	6.443±0.006	6.697±0.006
10	5.950±0.000	6.087±0.006	6.350±0.000	6.427±0.006	6.413±0.006
15	5.910±0.000	6.050±0.000	6.333±0.006	6.120±0.000	6.207±0.006
20	5.880±0.000	6.040±0.000	6.320±0.000	6.050±0.000	6.060±0.000
4 °C for 7 days					
0	6.230±0.000	6.390±0.000	6.480±0.000	6.640±0.000	6.740±0.000
5	6.057±0.006	6.240±0.000	6.380±0.000	6.450±0.000	6.723±0.006
10	5.980±0.000	6.107±0.006	6.357±0.006	6.420±0.000	6.440±0.000
15	5.937±0.006	6.077±0.006	6.330±0.000	6.120±0.000	6.233±0.006
20	5.903±0.006	6.043±0.006	6.323±0.006	6.060±0.000	6.083±0.006

Ca = calcium, P = dipotassium phosphate

Table A-14 The pH values of tested PN solutions containing 2.5% amino acid and various concentrations of calcium gluconate and dipotassium phosphate (mean ± S.D.)

Concentrations of	Concentrations of P (mM/L)				
Ca gluconate (mM/L)	0	5	10	15	20
Room temperature for 30 minutes					
0	6.083±0.006	6.247±0.006	6.420±0.000	6.550±0.000	6.660±0.000
5	5.920±0.000	6.107±0.006	6.343±0.006	6.403±0.006	6.443±0.006
10	5.900±0.000	5.990±0.000	6.210±0.000	6.340±0.000	6.360±0.000
15	5.777±0.006	5.940±0.000	6.257±0.006	6.260±0.000	6.077±0.006
20	5.740±0.000	5.850±0.000	6.270±0.000	6.230±0.000	6.010±0.000
Room temperature for 1 day					
0	6.090±0.000	6.250±0.000	6.423±0.006	6.560±0.000	6.667±0.006
5	5.950±0.000	6.110±0.000	6.340±0.000	6.417±0.006	6.450±0.000
10	5.903±0.006	6.000±0.000	6.220±0.000	6.343±0.006	6.363±0.006
15	5.790±0.000	5.950±0.000	6.270±0.000	6.270±0.000	6.080±0.000
20	5.750±0.000	5.857±0.006	6.283±0.006	6.240±0.000	6.020±0.000
4 °C for 1 day					
0	6.130±0.000	6.270±0.000	6.450±0.000	6.580±0.000	6.697±0.006
5	5.960±0.010	6.130±0.000	6.367±0.006	6.440±0.000	6.477±0.006
10	5.930±0.000	6.023±0.006	6.230±0.000	6.370±0.000	6.387±0.006
15	5.810±0.000	5.970±0.000	6.290±0.000	6.290±0.000	6.103±0.006
20	5.773±0.006	5.877±0.006	6.307±0.006	6.260±0.000	6.043±0.006
4 °C for 7 days					
0	6.130±0.000	6.270±0.000	6.457±0.006	6.583±0.006	6.710±0.000
5	5.970±0.000	6.140±0.000	6.370±0.000	6.447±0.006	6.480±0.000
10	5.940±0.000	6.030±0.000	6.230±0.000	6.380±0.000	6.390±0.000
15	5.817±0.006	5.970±0.000	6.300±0.000	6.297±0.006	6.110±0.000
20	5.783±0.006	5.880±0.000	6.313±0.006	6.260±0.000	6.050±0.000

Ca = calcium, P = dipotassium phosphate

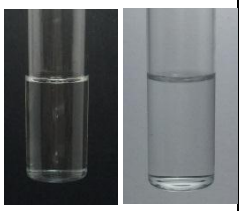
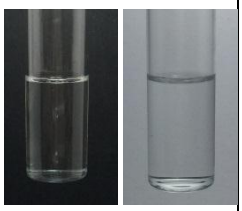
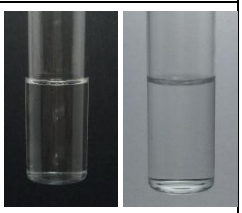
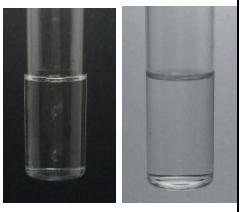
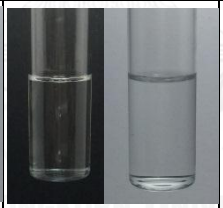
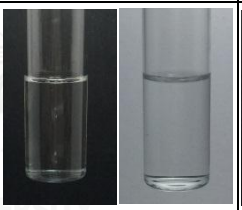
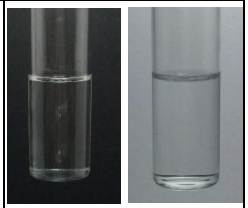
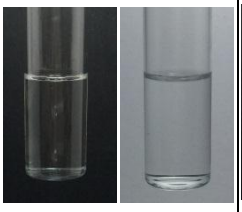
Table A-15 Visual inspections of tested PN solutions containing 1.5% amino acid and various concentrations of calcium gluconate and dipotassium phosphate

Concentrations of	Concentrations of P (mM/L)				
Ca gluconate (mM/L)	0	5	10	15	20
Room temperature for 30 minutes					
0	✓	✓	✓	✓	✓
5	✓	✓	✓	✗	✗
10	✓	✓	✓	✗	✗
15	✓	✓	✓	✗	✗
20	✓	✓	✓	✗	✗
Room temperature for 1 day					
0	✓	✓	✓	✓	✓
5	✓	✓	✓	✗	✗
10	✓	✓	✗	✗	✗
15	✓	✓	✗	✗	✗
20	✓	✓	✗	✗	✗
4 °C for 1 day					
0	✓	✓	✓	✓	✓
5	✓	✓	✓	✗	✗
10	✓	✓	✓	✗	✗
15	✓	✓	✓	✗	✗
20	✓	✓	✓	✗	✗
4 °C for 7 days					
0	✓	✓	✓	✓	✓
5	✓	✓	✓	✗	✗
10	✓	✓	✗	✗	✗
15	✓	✓	✗	✗	✗
20	✓	✓	✗	✗	✗

✓ Clear PN solutions and ✗ Turbid PN solutions

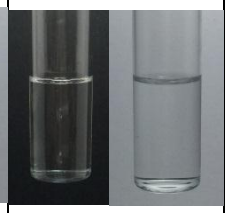
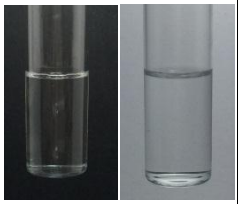
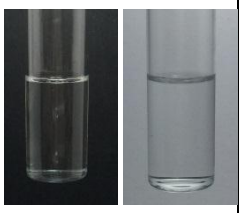
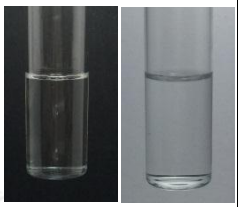
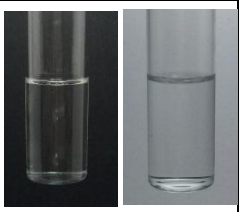
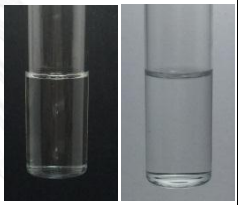
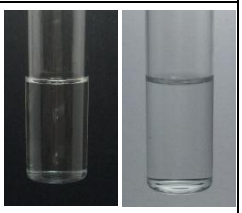
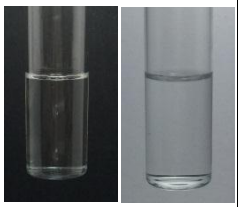
Ca = calcium, P = dipotassium phosphate

Table A-15 (continued)

Formula \ Conditions		Room temperature for 30 minutes	Room temperature for 1 day	4 °C for 1 day	4 °C for 7 days
P 0	Ca 0				
	Ca 5				
	Ca 10				
	Ca 15				
	Ca 20				

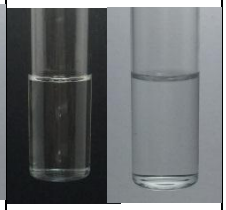
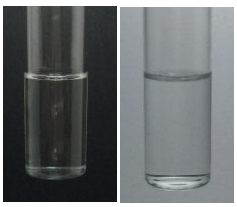
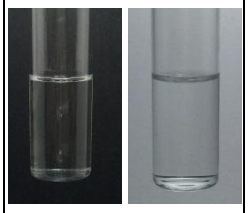
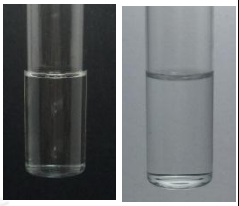
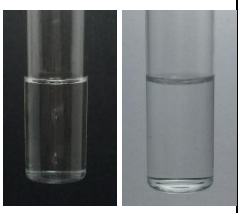
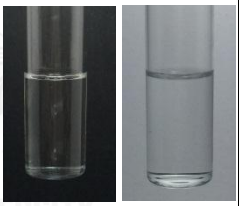
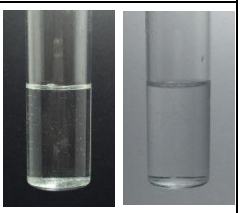
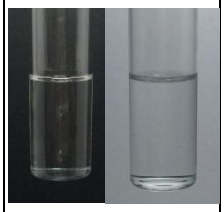
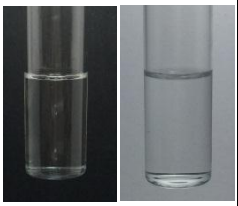
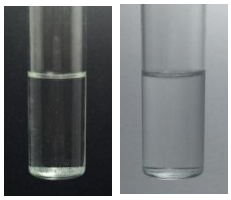
Ca = concentration of calcium gluconate, P = concentration of dipotassium phosphate

Table A-15 (continued)

Formula \ Conditions		Room temperature for 30 minutes	Room temperature for 1 day	4 °C for 1 day	4 °C for 7 days
P 5	Ca 0				
	Ca 5				
	Ca 10				
	Ca 15				
	Ca 20				

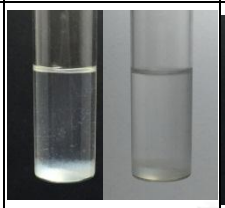
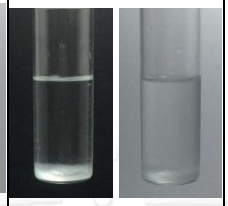
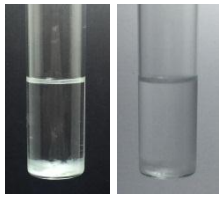
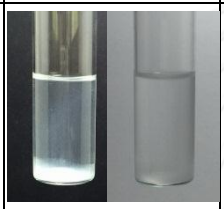
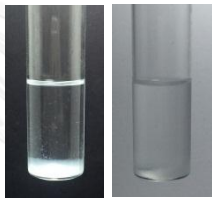
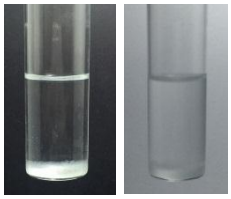
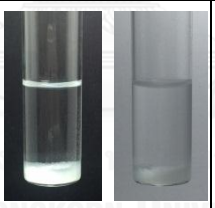
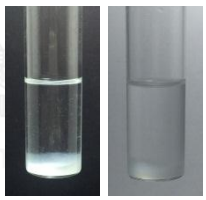
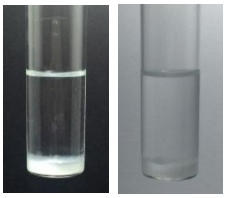
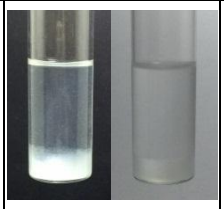
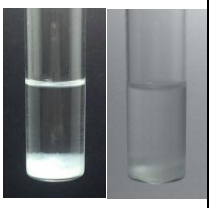
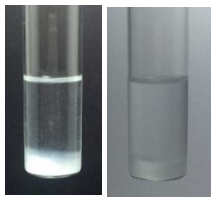
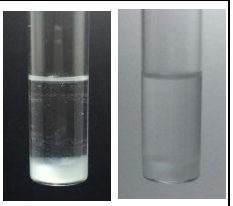
Ca = concentration of calcium gluconate, P = concentration of dipotassium phosphate

Table A-15 (continued)

Formula \ Conditions		Room temperature for 30 minutes	Room temperature for 1 day	4 °C for 1 day	4 °C for 7 days
P 10	Ca 0				
	Ca 5				
	Ca 10				
	Ca 15				
	Ca 20				

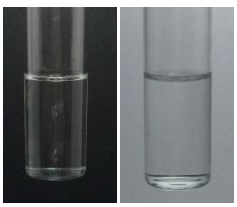
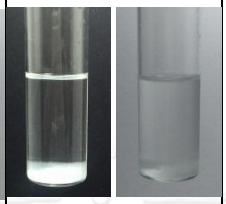
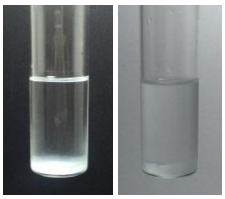
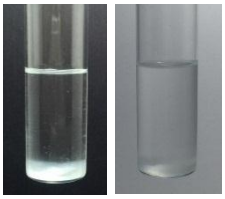
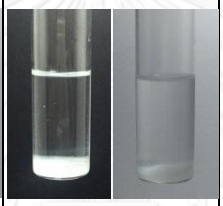
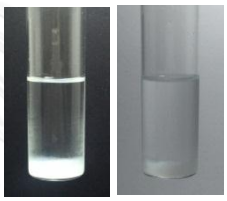
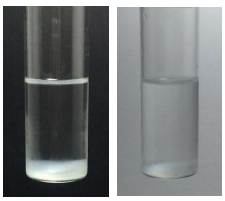
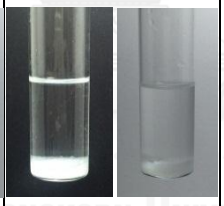
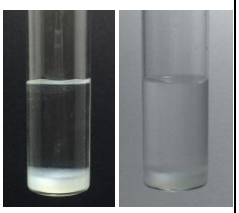
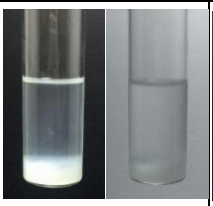
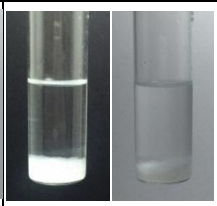
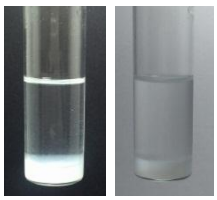
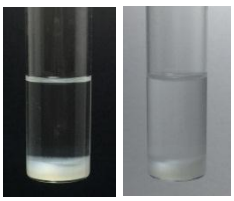
Ca = concentration of calcium gluconate, P = concentration of dipotassium phosphate

Table A-15 (continued)

Formula \ Conditions		Room temperature for 30 minutes	Room temperature for 1 day	4 °C for 1 day	4 °C for 7 days
P 15	Ca 0				
	Ca 5				
	Ca 10				
	Ca 15				
	Ca 20				

Ca = concentration of calcium gluconate, P = concentration of dipotassium phosphate

Table A-15 (continued)

Formula \ Conditions		Room temperature for 30 minutes	Room temperature for 1 day	4 °C for 1 day	4 °C for 7 days
P 20	Ca 0				
	Ca 5				
	Ca 10				
	Ca 15				
	Ca 20				

Ca = concentration of calcium gluconate, P = concentration of dipotassium phosphate

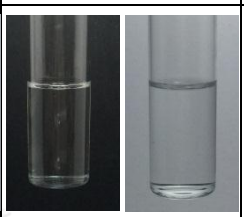
Table A-16 Visual inspections of tested PN solutions containing 2% amino acid and various concentrations of calcium gluconate and dipotassium phosphate

Concentrations of	Concentrations of P (mM/L)				
Ca gluconate (mM/L)	0	5	10	15	20
Room temperature for 30 minutes					
0	✓	✓	✓	✓	✓
5	✓	✓	✓	✗	✗
10	✓	✓	✓	✗	✗
15	✓	✓	✓	✗	✗
20	✓	✓	✓	✗	✗
Room temperature for 1 day					
0	✓	✓	✓	✓	✓
5	✓	✓	✓	✗	✗
10	✓	✓	✗	✗	✗
15	✓	✓	✗	✗	✗
20	✓	✓	✗	✗	✗
4 °C for 1 day					
0	✓	✓	✓	✓	✓
5	✓	✓	✓	✗	✗
10	✓	✓	✓	✗	✗
15	✓	✓	✓	✗	✗
20	✓	✓	✓	✗	✗
4 °C for 7 days					
0	✓	✓	✓	✓	✓
5	✓	✓	✓	✗	✗
10	✓	✓	✗	✗	✗
15	✓	✓	✗	✗	✗
20	✓	✓	✗	✗	✗

✓ Clear PN solutions and ✗ Turbid PN solutions

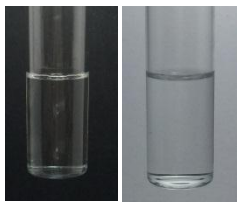
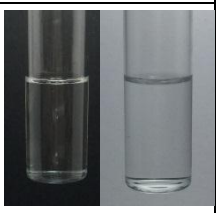
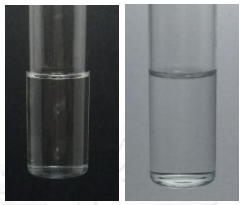
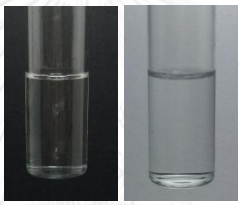
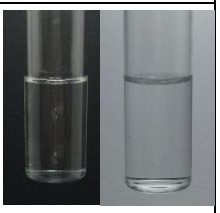
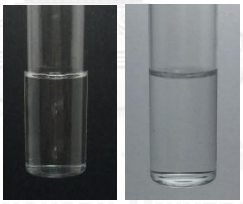
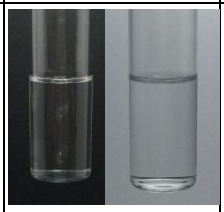
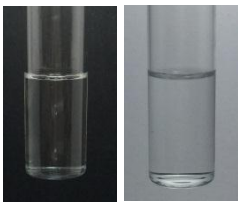
Ca = calcium, P = dipotassium phosphate

Table A-16 (continued)

Formula \ Conditions		Room temperature for 30 minutes	Room temperature for 1 day	4 °C for 1 day	4 °C for 7 days
P 0	Ca 0				
	Ca 5				
	Ca 10				
	Ca 15				
	Ca 20				

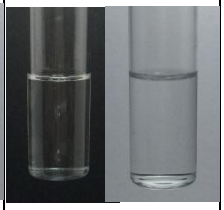
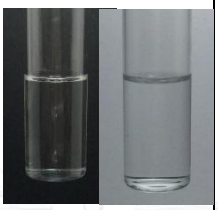
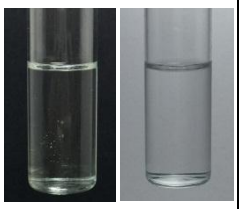
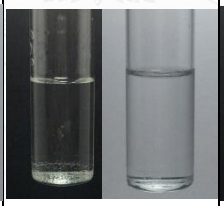
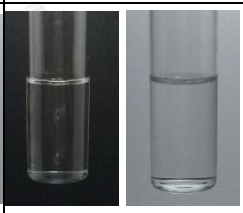
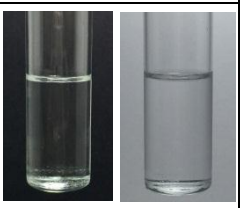
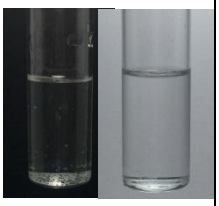
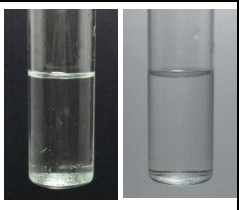
Ca = concentration of calcium gluconate, P = concentration of dipotassium phosphate

Table A-16 (continued)

Formula \ Conditions		Room temperature for 30 minutes	Room temperature for 1 day	4 °C for 1 day	4 °C for 7 days
P 5	Ca 0				
	Ca 5				
	Ca 10				
	Ca 15				
	Ca 20				

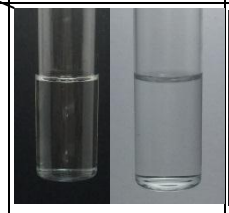
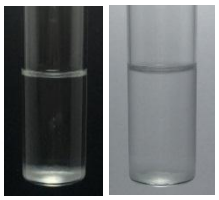
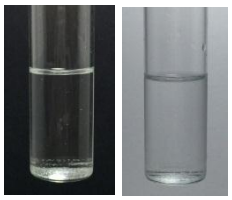
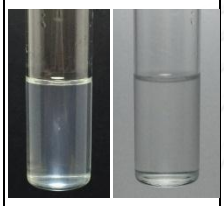
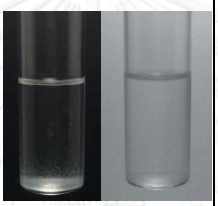
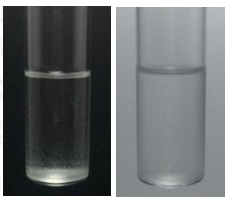
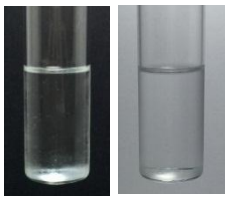
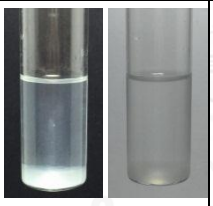
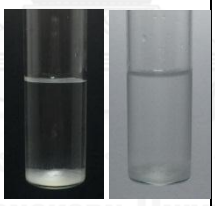
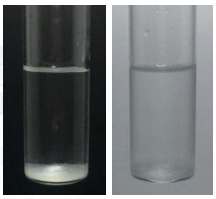
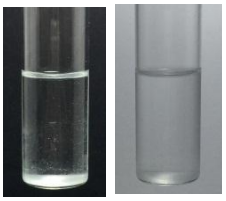
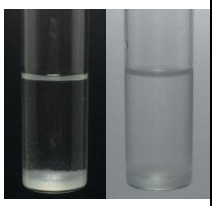
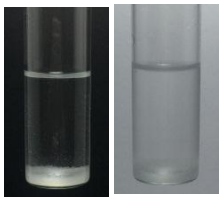
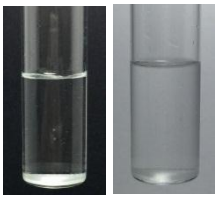
Ca = concentration of calcium gluconate, P = concentration of dipotassium phosphate

Table A-16 (continued)

Formula \ Conditions		Room temperature for 30 minutes	Room temperature for 1 day	4 °C for 1 day	4 °C for 7 days
P 10	Ca 0				
	Ca 5				
	Ca 10				
	Ca 15				
	Ca 20				

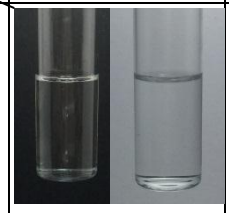
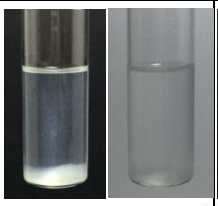
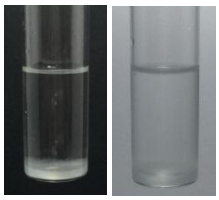
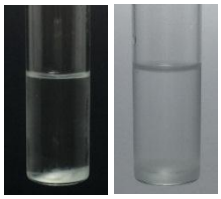
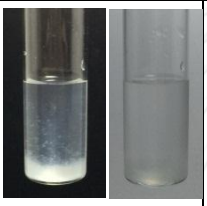
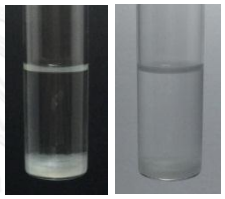
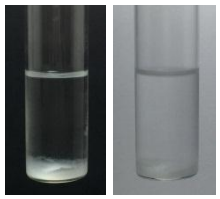
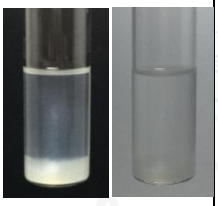
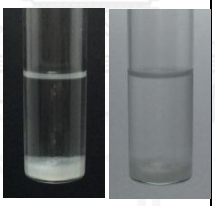
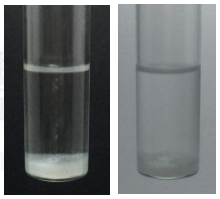
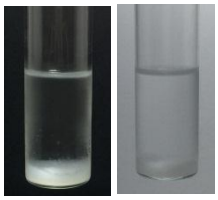
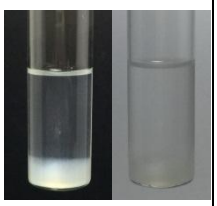
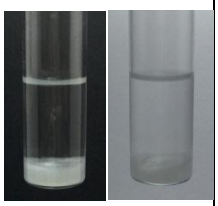
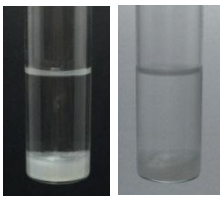
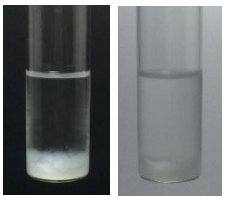
Ca = concentration of calcium gluconate, P = concentration of dipotassium phosphate

Table A-16 (continued)

Formula \ Conditions		Room temperature for 30 minutes	Room temperature for 1 day	4 °C for 1 day	4 °C for 7 days
P 15	Ca 0				
	Ca 5				
	Ca 10				
	Ca 15				
	Ca 20				

Ca = concentration of calcium gluconate, P = concentration of dipotassium phosphate

Table A-16 (continued)

Formula \ Conditions		Room temperature for 30 minutes	Room temperature for 1 day	4 °C for 1 day	4 °C for 7 days
P 20	Ca 0				
	Ca 5				
	Ca 10				
	Ca 15				
	Ca 20				

Ca = concentration of calcium gluconate, P = concentration of dipotassium phosphate

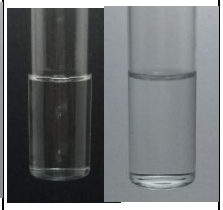
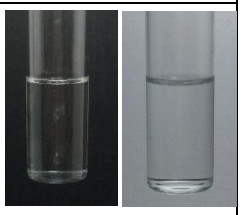
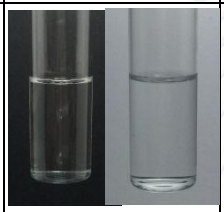
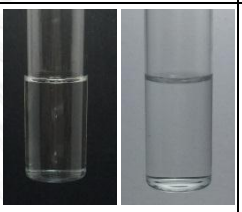
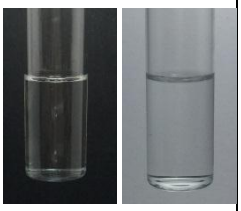
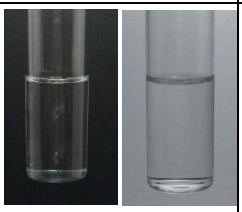
Table A-17 Visual inspections of tested PN solutions containing 2.5% amino acid and various concentrations of calcium gluconate and dipotassium phosphate

Concentrations of	Concentrations of P (mM/L)				
Ca gluconate (mM/L)	0	5	10	15	20
Room temperature for 30 minutes					
0	✓	✓	✓	✓	✓
5	✓	✓	✓	✓	✗
10	✓	✓	✓	✗	✗
15	✓	✓	✓	✗	✗
20	✓	✓	✓	✗	✗
Room temperature for 1 day					
0	✓	✓	✓	✓	✓
5	✓	✓	✓	✗	✗
10	✓	✓	✗	✗	✗
15	✓	✓	✗	✗	✗
20	✓	✓	✗	✗	✗
4 °C for 1 day					
0	✓	✓	✓	✓	✓
5	✓	✓	✓	✓	✗
10	✓	✓	✓	✓	✗
15	✓	✓	✓	✗	✗
20	✓	✓	✓	✗	✗
4 °C for 7 days					
0	✓	✓	✓	✓	✓
5	✓	✓	✓	✗	✗
10	✓	✓	✓	✗	✗
15	✓	✓	✓	✗	✗
20	✓	✓	✗	✗	✗

✓ Clear PN solutions and ✗ Turbid PN solutions

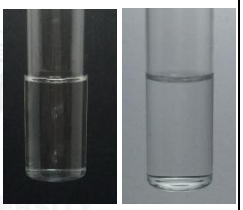
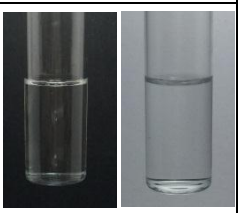
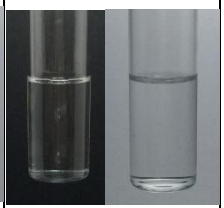
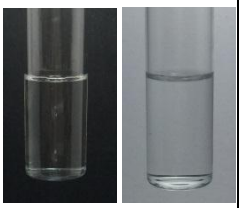
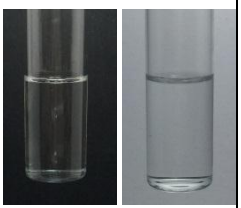
Ca = calcium, P = dipotassium phosphate

Table A-17 (continued)

Formula \ Conditions		Room temperature for 30 minutes	Room temperature for 1 day	4 °C for 1 day	4 °C for 7 days
P 0	Ca 0				
	Ca 5				
	Ca 10				
	Ca 15				
	Ca 20				

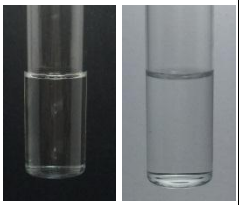
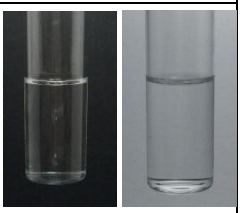
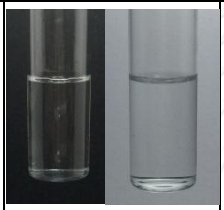
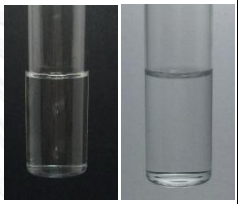
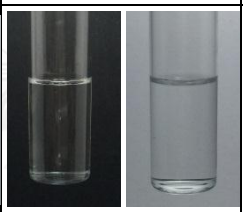
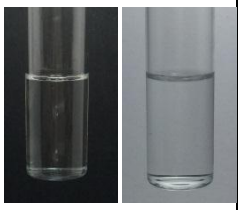
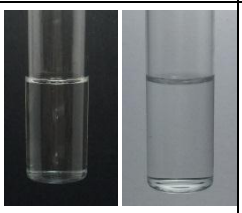
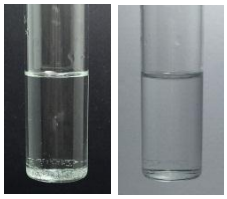
Ca = concentration of calcium gluconate, P = concentration of dipotassium phosphate

Table A-17 (continued)

Formula \ Conditions		Room temperature for 30 minutes	Room temperature for 1 day	4 °C for 1 day	4 °C for 7 days
P 5	Ca 0				
	Ca 5				
	Ca 10				
	Ca 15				
	Ca 20				

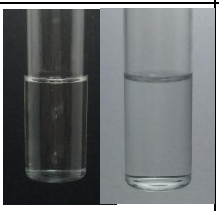
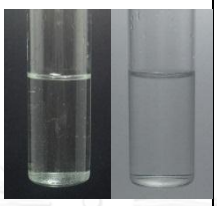
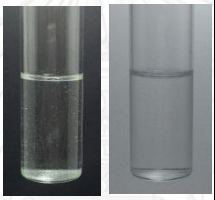
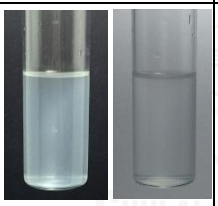
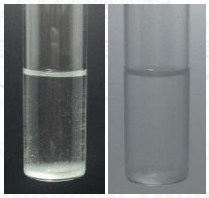
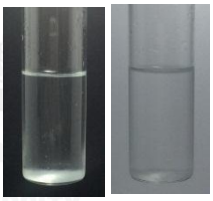
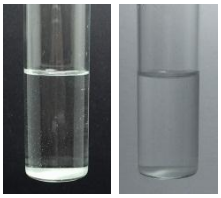
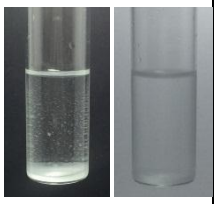
Ca = concentration of calcium gluconate, P = concentration of dipotassium phosphate

Table A-17 (continued)

Formula \ Conditions		Room temperature for 30 minutes	Room temperature for 1 day	4 °C for 1 day	4 °C for 7 days
P 10	Ca 0				
	Ca 5				
	Ca 10				
	Ca 15				
	Ca 20				

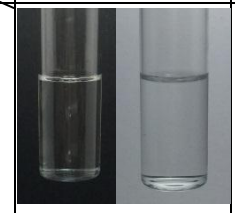
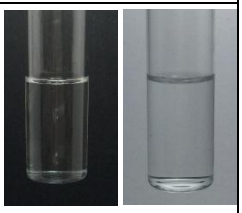
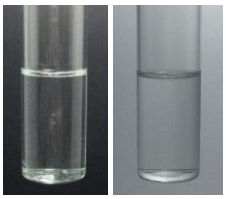
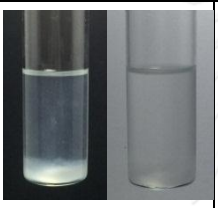
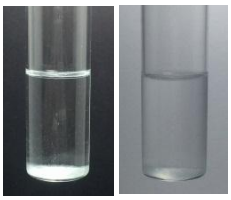
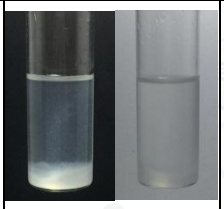
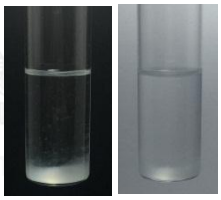
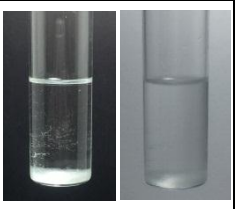
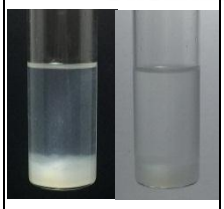
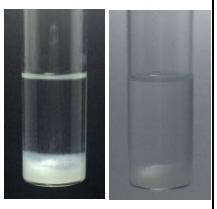
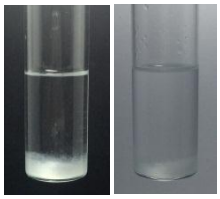
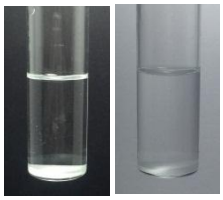
Ca = concentration of calcium gluconate, P = concentration of dipotassium phosphate

Table A-17 (continued)

Formula \ Conditions		Room temperature for 30 minutes	Room temperature for 1 day	4 °C for 1 day	4 °C for 7 days
P 15	Ca 0				
	Ca 5				
	Ca 10				
	Ca 15				
	Ca 20				

Ca = concentration of calcium gluconate, P = concentration of dipotassium phosphate

Table A-17 (continued)

Formula \ Conditions		Room temperature for 30 minutes	Room temperature for 1 day	4 °C for 1 day	4 °C for 7 days
P 20	Ca 0				
	Ca 5				
	Ca 10				
	Ca 15				
	Ca 20				

Ca = concentration of calcium gluconate, P = concentration of dipotassium phosphate

Table A-18 Turbidity of tested PN solutions containing 1.5% amino acid and various concentrations of calcium gluconate and dipotassium phosphate (mean ± S.D.)

Concentrations of Ca gluconate (mM/L)	Concentrations of P (mM/L)				
	0	5	10	15	20
Room temperature for 30 minutes					
0	0.200±0.000	0.250±0.000	0.300±0.000	0.267±0.029	0.300±0.000
5	0.250±0.000	0.267±0.029	0.300±0.000	NT2	NT2
10	0.250±0.000	0.250±0.000	7.500±0.000	NT2	NT2
15	0.250±0.000	0.250±0.000	10.00±0.000	NT2	NT2
20	0.300±0.000	0.300±0.000	11.00±0.000	NT2	NT2
Room temperature for 1 day					
0	0.217±0.029	0.250±0.000	0.300±0.000	0.300±0.000	0.317±0.029
5	0.267±0.029	0.300±0.000	0.350±0.000	NT2	NT2
10	0.267±0.029	0.300±0.000	NT2	NT2	NT2
15	0.250±0.000	0.300±0.000	NT2	NT2	NT2
20	0.300±0.000	0.350±0.000	NT2	NT2	NT2
4 °C for 1 day					
0	0.200±0.000	0.250±0.000	0.300±0.000	0.300±0.000	0.300±0.000
5	0.250±0.000	0.300±0.000	0.350±0.000	NT2	NT2
10	0.250±0.000	0.300±0.000	7.700±0.000	NT2	NT2
15	0.250±0.000	0.300±0.000	11.00±0.000	NT2	NT2
20	0.300±0.000	0.350±0.000	10.00±0.000	NT2	NT2
4 °C for 7 days					
0	0.300±0.000	0.267±0.029	0.450±0.000	0.333±0.029	0.283±0.029
5	0.250±0.000	0.367±0.029	1.200±0.000	NT2	NT2
10	0.300±0.000	0.300±0.000	NT2	NT2	NT2
15	0.300±0.000	0.400±0.000	NT2	NT2	NT2
20	0.383±0.058	0.700±0.000	NT2	NT2	NT2

Ca = calcium, P = dipotassium phosphate, NT2 = Not test due to fail by visual inspection

Table A-19 Turbidity of tested PN solutions containing 2% amino acid and various concentrations of calcium gluconate and dipotassium phosphate (mean ± S.D.)

Concentrations of Ca gluconate (mM/L)	Concentrations of P (mM/L)				
	0	5	10	15	20
Room temperature for 30 minutes					
0	0.200±0.000	0.200±0.000	0.250±0.000	0.250±0.000	0.250±0.000
5	0.183±0.029	0.217±0.058	0.350±0.000	NT2	NT2
10	0.200±0.000	0.250±0.000	1.933±0.058	NT2	NT2
15	0.200±0.000	0.350±0.000	3.533±0.058	NT2	NT2
20	0.200±0.000	0.350±0.000	7.400±0.000	NT2	NT2
Room temperature for 1 day					
0	0.200±0.000	0.200±0.000	0.300±0.000	0.300±0.000	0.283±0.029
5	0.200±0.000	0.250±0.000	0.400±0.000	NT2	NT2
10	0.217±0.029	0.250±0.000	NT2	NT2	NT2
15	0.200±0.000	0.333±0.029	NT2	NT2	NT2
20	0.200±0.000	0.400±0.000	NT2	NT2	NT2
4 °C for 1 day					
0	0.200±0.000	0.233±0.029	0.217±0.029	0.250±0.000	0.250±0.000
5	0.217±0.029	0.233±0.029	0.350±0.000	NT2	NT2
10	0.200±0.000	0.267±0.029	2.500±0.000	NT2	NT2
15	0.200±0.000	0.317±0.029	4.733±0.058	NT2	NT2
20	0.200±0.000	0.217±0.029	9.033±0.058	NT2	NT2
4 °C for 7 days					
0	0.250±0.000	0.250±0.000	0.200±0.000	0.200±0.000	0.250±0.000
5	0.250±0.000	0.267±0.029	0.383±0.029	NT2	NT2
10	0.250±0.000	0.300±0.000	NT2	NT2	NT2
15	0.250±0.000	0.267±0.029	NT2	NT2	NT2
20	0.250±0.000	0.317±0.076	NT2	NT2	NT2

Ca = calcium, P = dipotassium phosphate, NT2 = Not test due to fail by visual inspection

Table A-20 Turbidity of tested PN solutions containing 2.5% amino acid and various concentrations of calcium gluconate and dipotassium phosphate (mean ± S.D.)

Concentrations of Ca gluconate (mM/L)	Concentrations of P (mM/L)				
	0	5	10	15	20
Room temperature for 30 minutes					
0	0.200±0.000	0.200±0.000	0.200±0.000	0.250±0.000	0.300±0.000
5	0.200±0.000	0.217±0.029	0.250±0.000	1.833±0.058	NT2
10	0.217±0.029	0.250±0.000	0.417±0.029	NT2	NT2
15	0.233±0.029	0.267±0.029	0.400±0.000	NT2	NT2
20	0.250±0.000	0.267±0.058	1.200±0.000	NT2	NT2
Room temperature for 1 day					
0	0.250±0.000	0.200±0.000	0.233±0.029	0.300±0.000	0.350±0.000
5	0.250±0.000	0.217±0.029	0.283±0.029	NT2	NT2
10	0.267±0.029	0.283±0.029	NT2	NT2	NT2
15	0.300±0.000	0.300±0.000	NT2	NT2	NT2
20	0.300±0.000	0.300±0.000	NT2	NT2	NT2
4 °C for 1 day					
0	0.217±0.029	0.250±0.000	0.250±0.000	0.300±0.000	0.317±0.029
5	0.233±0.029	0.250±0.000	0.300±0.000	1.500±0.000	NT2
10	0.300±0.000	0.300±0.000	1.000±0.000	1.600±0.000	NT2
15	0.300±0.000	0.333±0.029	1.700±0.000	NT2	NT2
20	0.333±0.029	0.300±0.000	1.800±0.000	NT2	NT2
4 °C for 7 days					
0	0.250±0.000	0.250±0.000	0.250±0.000	0.300±0.000	0.400±0.000
5	0.250±0.000	0.250±0.000	0.283±0.029	NT2	NT2
10	0.300±0.000	0.300±0.000	0.717±0.029	NT2	NT2
15	0.300±0.000	0.350±0.000	2.200±0.000	NT2	NT2
20	0.350±0.000	0.400±0.000	NT2	NT2	NT2

Ca = calcium, P = dipotassium phosphate, NT2 = Not test due to fail by visual inspection

Table A-21 Maximum particle count of tested PN solutions containing 1.5% amino acid and various concentrations of calcium gluconate and dipotassium phosphate*

Formulations		Conditions	PC <10 μm	PC ≥10 μm	PC ≥25 μm
P 0	Ca 0	Day 0[a]	0	0	0
		Day 1[a]	0	0	0
		Day 1[b]	0	0	0
		Day 7[b]	0	0	0
P 0	Ca 5	Day 0[a]	0	0	0
		Day 1[a]	0	0	0
		Day 1[b]	0	0	0
		Day 7[b]	0	0	0
P 0	Ca 10	Day 0[a]	0	0	0
		Day 1[a]	0	0	0
		Day 1[b]	0	0	0
		Day 7[b]	0	0	0
P 0	Ca 15	Day 0[a]	0	0	0
		Day 1[a]	0	0	0
		Day 1[b]	0	0	0
		Day 7[b]	0	0	0
P 0	Ca 20	Day 0[a]	0	1	0
		Day 1[a]	0	0	1
		Day 1[b]	0	1	0
		Day 7[b]	0	0	1

a = room temperature, b = 4 °C, Ca = concentration of calcium gluconate (mM/L), P = concentration of dipotassium phosphate (mM/L)

*Maximum particle count: ≥10 μm less than 60 particles/5mL, ≥25 μm less than 10 particles/5mL

Table A-21 (continued)*

Formulations		Conditions	PC <10 μm	PC ≥10 μm	PC ≥25 μm
P 5	Ca 0	Day 0[a]	0	0	0
		Day 1[a]	0	0	0
		Day 1[b]	0	0	0
		Day 7[b]	0	0	0
P 5	Ca 5	Day 0[a]	0	0	0
		Day 1[a]	0	0	0
		Day 1[b]	0	0	0
		Day 7[b]	0	1	0
P 5	Ca 10	Day 0[a]	0	0	0
		Day 1[a]	0	0	0
		Day 1[b]	0	0	0
		Day 7[b]	0	2	0
P 5	Ca 15	Day 0[a]	1	0	0
		Day 1[a]	0	0	1
		Day 1[b]	0	1	0
		Day 7[b]	0	0	2
P 5	Ca 20	Day 0[a]	0	1	0
		Day 1[a]	0	2	1
		Day 1[b]	0	0	1
		Day 7[b]	NT3	NT3	NT3

a = room temperature, b = 4 °C, Ca = concentration of calcium gluconate (mM/L), P = concentration of dipotassium phosphate (mM/L), NT3 = Not test due to fail by turbidity measurement

*Maximum particle count: ≥10 μm less than 60 particles/5mL, ≥25 μm less than 10 particles/5mL

Table A-21 (continued)*

Formulations		Conditions	PC <10 μm	PC ≥10 μm	PC ≥25 μm
P 10	Ca 0	Day 0[a]	0	0	1
		Day 1[a]	0	1	0
		Day 1[b]	0	0	0
		Day 7[b]	0	1	0
P 10	Ca 5	Day 0[a]	1	3	0
		Day 1[a]	0	1	2
		Day 1[b]	0	1	0
		Day 7[b]	NT3	NT3	NT3
P 10	Ca 10	Day 0[a]	NT3	NT3	NT3
		Day 1[a]	NT3	NT3	NT3
		Day 1[b]	NT3	NT3	NT3
		Day 7[b]	NT3	NT3	NT3
P 10	Ca 15	Day 0[a]	NT3	NT3	NT3
		Day 1[a]	NT3	NT3	NT3
		Day 1[b]	NT3	NT3	NT3
		Day 7[b]	NT3	NT3	NT3
P 10	Ca 20	Day 0[a]	NT3	NT3	NT3
		Day 1[a]	NT3	NT3	NT3
		Day 1[b]	NT3	NT3	NT3
		Day 7[b]	NT3	NT3	NT3

a = room temperature, b = 4 °C, Ca = concentration of calcium gluconate (mM/L), P = concentration of dipotassium phosphate (mM/L), NT3 = Not test due to fail by turbidity measurement

*Maximum particle count: ≥10 μm less than 60 particles/5mL, ≥25 μm less than 10 particles/5mL

Table A-21 (continued)*

Formulations		Conditions	PC <10 μm	PC ≥10 μm	PC ≥25 μm
P 15	Ca 0	Day 0[a]	0	0	1
		Day 1[a]	1	1	0
		Day 1[b]	0	0	0
		Day 7[b]	0	1	0
P 15	Ca 5	Day 0[a]	NT3	NT3	NT3
		Day 1[a]	NT3	NT3	NT3
		Day 1[b]	NT3	NT3	NT3
		Day 7[b]	NT3	NT3	NT3
P 15	Ca 10	Day 0[a]	NT3	NT3	NT3
		Day 1[a]	NT3	NT3	NT3
		Day 1[b]	NT3	NT3	NT3
		Day 7[b]	NT3	NT3	NT3
P 15	Ca 15	Day 0[a]	NT3	NT3	NT3
		Day 1[a]	NT3	NT3	NT3
		Day 1[b]	NT3	NT3	NT3
		Day 7[b]	NT3	NT3	NT3
P 15	Ca 20	Day 0[a]	NT3	NT3	NT3
		Day 1[a]	NT3	NT3	NT3
		Day 1[b]	NT3	NT3	NT3
		Day 7[b]	NT3	NT3	NT3

a = room temperature, b = 4 °C, Ca = concentration of calcium gluconate (mM/L), P = concentration of dipotassium phosphate (mM/L), NT3 = Not test due to fail by turbidity measurement

*Maximum particle count: ≥10 μm less than 60 particles/5mL, ≥25 μm less than 10 particles/5mL

Table A-21 (continued)*

Formulations		Conditions	PC <10 μm	PC ≥10 μm	PC ≥25 μm
P 20	Ca 0	Day 0[a]	0	0	1
		Day 1[a]	0	0	1
		Day 1[b]	0	0	1
		Day 7[b]	0	0	1
P 20	Ca 5	Day 0[a]	NT3	NT3	NT3
		Day 1[a]	NT3	NT3	NT3
		Day 1[b]	NT3	NT3	NT3
		Day 7[b]	NT3	NT3	NT3
P 20	Ca 10	Day 0[a]	NT3	NT3	NT3
		Day 1[a]	NT3	NT3	NT3
		Day 1[b]	NT3	NT3	NT3
		Day 7[b]	NT3	NT3	NT3
P 20	Ca 15	Day 0[a]	NT3	NT3	NT3
		Day 1[a]	NT3	NT3	NT3
		Day 1[b]	NT3	NT3	NT3
		Day 7[b]	NT3	NT3	NT3
P 20	Ca 20	Day 0[a]	NT3	NT3	NT3
		Day 1[a]	NT3	NT3	NT3
		Day 1[b]	NT3	NT3	NT3
		Day 7[b]	NT3	NT3	NT3

a = room temperature, b = 4 °C, Ca = concentration of calcium gluconate (mM/L), P = concentration of dipotassium phosphate (mM/L), NT3 = Not test due to fail by turbidity measurement

*Maximum particle count: ≥10 μm less than 60 particles/5mL, ≥25 μm less than 10 particles/5mL

Table A-22 Maximum particle count of tested PN solutions containing 2% amino acid and various concentrations of calcium gluconate and dipotassium phosphate*

Formulations		Conditions	PC <10 μm	PC ≥10 μm	PC ≥25 μm
P 0	Ca 0	Day 0[a]	0	1	0
		Day 1[a]	1	1	0
		Day 1[b]	0	1	0
		Day 7[b]	0	0	0
P 0	Ca 5	Day 0[a]	0	1	0
		Day 1[a]	2	1	0
		Day 1[b]	0	1	0
		Day 7[b]	0	0	0
P 0	Ca 10	Day 0[a]	2	1	0
		Day 1[a]	1	0	1
		Day 1[b]	0	1	0
		Day 7[b]	0	0	0
P 0	Ca 15	Day 0[a]	1	1	0
		Day 1[a]	0	2	1
		Day 1[b]	0	0	0
		Day 7[b]	0	0	0
P 0	Ca 20	Day 0[a]	0	2	0
		Day 1[a]	2	2	0
		Day 1[b]	0	0	0
		Day 7[b]	0	1	0

a = room temperature, b = 4 °C, Ca = concentration of calcium gluconate (mM/L), P = concentration of dipotassium phosphate (mM/L)

*Maximum particle count: ≥10 μm less than 60 particles/5mL, ≥25 μm less than 10 particles/5mL

Table A-22 (continued)*

Formulations		Conditions	PC <10 μm	PC ≥10 μm	PC ≥25 μm
P 5	Ca 0	Day 0[a]	0	0	0
		Day 1[a]	2	1	1
		Day 1[b]	0	0	0
		Day 7[b]	0	0	0
P 5	Ca 5	Day 0[a]	0	2	0
		Day 1[a]	0	2	1
		Day 1[b]	0	0	0
		Day 7[b]	0	0	0
P 5	Ca 10	Day 0[a]	0	1	0
		Day 1[a]	1	1	0
		Day 1[b]	0	0	0
		Day 7[b]	0	1	1
P 5	Ca 15	Day 0[a]	1	1	0
		Day 1[a]	0	0	1
		Day 1[b]	0	0	0
		Day 7[b]	0	1	0
P 5	Ca 20	Day 0[a]	1	0	0
		Day 1[a]	0	1	0
		Day 1[b]	0	0	0
		Day 7[b]	0	0	0

a = room temperature, b = 4 °C, Ca = concentration of calcium gluconate (mM/L), P = concentration of dipotassium phosphate (mM/L)

*Maximum particle count: ≥10 μm less than 60 particles/5mL, ≥25 μm less than 10 particles/5mL

Table A-22 (continued)*

Formulations		Conditions	PC <10 μm	PC ≥10 μm	PC ≥25 μm
P 10	Ca 0	Day 0[a]	0	1	0
		Day 1[a]	1	0	0
		Day 1[b]	0	0	0
		Day 7[b]	0	0	0
P 10	Ca 5	Day 0[a]	1	1	0
		Day 1[a]	1	1	0
		Day 1[b]	0	0	0
		Day 7[b]	2	1	1
P 10	Ca 10	Day 0[a]	NT3	NT3	NT3
		Day 1[a]	NT3	NT3	NT3
		Day 1[b]	NT3	NT3	NT3
		Day 7[b]	NT3	NT3	NT3
P 10	Ca 15	Day 0[a]	NT3	NT3	NT3
		Day 1[a]	NT3	NT3	NT3
		Day 1[b]	NT3	NT3	NT3
		Day 7[b]	NT3	NT3	NT3
P 10	Ca 20	Day 0[a]	NT3	NT3	NT3
		Day 1[a]	NT3	NT3	NT3
		Day 1[b]	NT3	NT3	NT3
		Day 7[b]	NT3	NT3	NT3

a = room temperature, b = 4 °C, Ca = concentration of calcium gluconate (mM/L), P = concentration of dipotassium phosphate (mM/L), NT3 = Not test due to fail by turbidity measurement

*Maximum particle count: ≥10 μm less than 60 particles/5mL, ≥25 μm less than 10 particles/5mL

Table A-22 (continued)*

Formulations		Conditions	PC <10 μm	PC ≥10 μm	PC ≥25 μm
P 15	Ca 0	Day 0[a]	0	1	0
		Day 1[a]	0	1	0
		Day 1[b]	0	0	0
		Day 7[b]	0	0	0
P 15	Ca 5	Day 0[a]	NT3	NT3	NT3
		Day 1[a]	NT3	NT3	NT3
		Day 1[b]	NT3	NT3	NT3
		Day 7[b]	NT3	NT3	NT3
P 15	Ca 10	Day 0[a]	NT3	NT3	NT3
		Day 1[a]	NT3	NT3	NT3
		Day 1[b]	NT3	NT3	NT3
		Day 7[b]	NT3	NT3	NT3
P 15	Ca 15	Day 0[a]	NT3	NT3	NT3
		Day 1[a]	NT3	NT3	NT3
		Day 1[b]	NT3	NT3	NT3
		Day 7[b]	NT3	NT3	NT3
P 15	Ca 20	Day 0[a]	NT3	NT3	NT3
		Day 1[a]	NT3	NT3	NT3
		Day 1[b]	NT3	NT3	NT3
		Day 7[b]	NT3	NT3	NT3

a = room temperature, b = 4 °C, Ca = concentration of calcium gluconate (mM/L), P = concentration of dipotassium phosphate (mM/L), NT3 = Not test due to fail by turbidity measurement

*Maximum particle count: ≥10 μm less than 60 particles/5mL, ≥25 μm less than 10 particles/5mL

Table A-22 (continued)*

Formulations		Conditions	PC <10 μm	PC ≥10 μm	PC ≥25 μm
P 20	Ca 0	Day 0[a]	0	1	0
		Day 1[a]	0	1	0
		Day 1[b]	0	0	0
		Day 7[b]	1	0	0
P 20	Ca 5	Day 0[a]	NT3	NT3	NT3
		Day 1[a]	NT3	NT3	NT3
		Day 1[b]	NT3	NT3	NT3
		Day 7[b]	NT3	NT3	NT3
P 20	Ca 10	Day 0[a]	NT3	NT3	NT3
		Day 1[a]	NT3	NT3	NT3
		Day 1[b]	NT3	NT3	NT3
		Day 7[b]	NT3	NT3	NT3
P 20	Ca 15	Day 0[a]	NT3	NT3	NT3
		Day 1[a]	NT3	NT3	NT3
		Day 1[b]	NT3	NT3	NT3
		Day 7[b]	NT3	NT3	NT3
P 20	Ca 20	Day 0[a]	NT3	NT3	NT3
		Day 1[a]	NT3	NT3	NT3
		Day 1[b]	NT3	NT3	NT3
		Day 7[b]	NT3	NT3	NT3

a = room temperature, b = 4 °C, Ca = concentration of calcium gluconate (mM/L), P = concentration of dipotassium phosphate (mM/L), NT3 = Not test due to fail by turbidity measurement

*Maximum particle count: ≥10 μm less than 60 particles/5mL, ≥25 μm less than 10 particles/5mL

Table A-23 Maximum particle count of tested PN solutions containing 2.5% amino acid and various concentrations of calcium gluconate and dipotassium phosphate*

Formulations		Conditions	PC <10 μm	PC ≥10 μm	PC ≥25 μm
P 0	Ca 0	Day 0[a]	0	0	0
		Day 1[a]	0	0	0
		Day 1[b]	0	0	0
		Day 7[b]	0	0	0
P 0	Ca 5	Day 0[a]	0	1	0
		Day 1[a]	0	0	0
		Day 1[b]	0	0	0
		Day 7[b]	0	0	0
P 0	Ca 10	Day 0[a]	0	1	0
		Day 1[a]	0	0	0
		Day 1[b]	0	0	0
		Day 7[b]	0	1	0
P 0	Ca 15	Day 0[a]	0	1	0
		Day 1[a]	0	1	0
		Day 1[b]	0	0	0
		Day 7[b]	0	1	0
P 0	Ca 20	Day 0[a]	0	1	1
		Day 1[a]	0	1	0
		Day 1[b]	0	0	0
		Day 7[b]	0	1	1

a = room temperature, b = 4 °C, Ca = concentration of calcium gluconate (mM/L), P = concentration of dipotassium phosphate (mM/L)

*Maximum particle count: ≥10 μm less than 60 particles/5mL, ≥25 μm less than 10 particles/5mL

Table A-23 (continued)*

Formulations		Conditions	PC <10 μm	PC ≥10 μm	PC ≥25 μm
P 5	Ca 0	Day 0[a]	0	0	0
		Day 1[a]	0	0	0
		Day 1[b]	0	0	0
		Day 7[b]	0	0	0
P 5	Ca 5	Day 0[a]	0	1	0
		Day 1[a]	0	0	0
		Day 1[b]	0	0	0
		Day 7[b]	0	1	0
P 5	Ca 10	Day 0[a]	0	2	0
		Day 1[a]	0	1	1
		Day 1[b]	0	0	0
		Day 7[b]	0	2	0
P 5	Ca 15	Day 0[a]	0	0	2
		Day 1[a]	0	0	1
		Day 1[b]	0	0	0
		Day 7[b]	0	2	1
P 5	Ca 20	Day 0[a]	1	0	0
		Day 1[a]	0	1	1
		Day 1[b]	0	0	0
		Day 7[b]	0	1	2

a = room temperature, b = 4 °C, Ca = concentration of calcium gluconate (mM/L), P = concentration of dipotassium phosphate (mM/L)

*Maximum particle count: ≥10 μm less than 60 particles/5mL, ≥25 μm less than 10 particles/5mL

Table A-23 (continued)*

Formulations		Conditions	PC <10 μm	PC ≥10 μm	PC ≥25 μm
P 10	Ca 0	Day 0[a]	0	0	0
		Day 1[a]	0	0	0
		Day 1[b]	0	0	0
		Day 7[b]	0	0	0
P 10	Ca 5	Day 0[a]	0	0	0
		Day 1[a]	0	1	0
		Day 1[b]	0	0	0
		Day 7[b]	0	0	1
P 10	Ca 10	Day 0[a]	1	1	0
		Day 1[a]	NT3	NT3	NT3
		Day 1[b]	NT3	NT3	NT3
		Day 7[b]	NT3	NT3	NT3
P 10	Ca 15	Day 0[a]	0	0	1
		Day 1[a]	NT3	NT3	NT3
		Day 1[b]	NT3	NT3	NT3
		Day 7[b]	NT3	NT3	NT3
P 10	Ca 20	Day 0[a]	NT3	NT3	NT3
		Day 1[a]	NT3	NT3	NT3
		Day 1[b]	NT3	NT3	NT3
		Day 7[b]	NT3	NT3	NT3

a = room temperature, b = 4 °C, Ca = concentration of calcium gluconate (mM/L), P = concentration of dipotassium phosphate (mM/L), NT3 = Not test due to fail by turbidity measurement

*Maximum particle count: ≥10 μm less than 60 particles/5mL, ≥25 μm less than 10 particles/5mL

Table A-23 (continued)*

Formulations		Conditions	PC <10 μm	PC ≥10 μm	PC ≥25 μm
P 15	Ca 0	Day 0[a]	0	0	0
		Day 1[a]	0	0	0
		Day 1[b]	0	0	0
		Day 7[b]	0	0	0
P 15	Ca 5	Day 0[a]	NT3	NT3	NT3
		Day 1[a]	NT3	NT3	NT3
		Day 1[b]	NT3	NT3	NT3
		Day 7[b]	NT3	NT3	NT3
P 15	Ca 10	Day 0[a]	NT3	NT3	NT3
		Day 1[a]	NT3	NT3	NT3
		Day 1[b]	NT3	NT3	NT3
		Day 7[b]	NT3	NT3	NT3
P 15	Ca 15	Day 0[a]	NT3	NT3	NT3
		Day 1[a]	NT3	NT3	NT3
		Day 1[b]	NT3	NT3	NT3
		Day 7[b]	NT3	NT3	NT3
P 15	Ca 20	Day 0[a]	NT3	NT3	NT3
		Day 1[a]	NT3	NT3	NT3
		Day 1[b]	NT3	NT3	NT3
		Day 7[b]	NT3	NT3	NT3

a = room temperature, b = 4 °C, Ca = concentration of calcium gluconate (mM/L), P = concentration of dipotassium phosphate (mM/L), NT3 = Not test due to fail by turbidity measurement

*Maximum particle count: ≥10 μm less than 60 particles/5mL, ≥25 μm less than 10 particles/5mL

Table A-23 (continued)*

Formulations		Conditions	PC <10 μm	PC ≥10 μm	PC ≥25 μm
P 20	Ca 0	Day 0[a]	0	0	0
		Day 1[a]	0	1	0
		Day 1[b]	0	0	0
		Day 7[b]	0	0	0
P 20	Ca 5	Day 0[a]	NT3	NT3	NT3
		Day 1[a]	NT3	NT3	NT3
		Day 1[b]	NT3	NT3	NT3
		Day 7[b]	NT3	NT3	NT3
P 20	Ca 10	Day 0[a]	NT3	NT3	NT3
		Day 1[a]	NT3	NT3	NT3
		Day 1[b]	NT3	NT3	NT3
		Day 7[b]	NT3	NT3	NT3
P 20	Ca 15	Day 0[a]	NT3	NT3	NT3
		Day 1[a]	NT3	NT3	NT3
		Day 1[b]	NT3	NT3	NT3
		Day 7[b]	NT3	NT3	NT3
P 20	Ca 20	Day 0[a]	NT3	NT3	NT3
		Day 1[a]	NT3	NT3	NT3
		Day 1[b]	NT3	NT3	NT3
		Day 7[b]	NT3	NT3	NT3

a = room temperature, b = 4 °C, Ca = concentration of calcium gluconate (mM/L), P = concentration of dipotassium phosphate (mM/L), NT3 = Not test due to fail by turbidity measurement

*Maximum particle count: ≥10 μm less than 60 particles/5mL, ≥25 μm less than 10 particles/5mL

Construction solubility data

Solubility data of calcium gluconate and dipotassium phosphate include 1.5%, 2% and 2.5% amino acid stored at room temperature for 30 minutes, room temperature for 1 day, 4 °C for 1 day, 4 °C for 7 days, as shown in Table A-2, A-3 and A-4

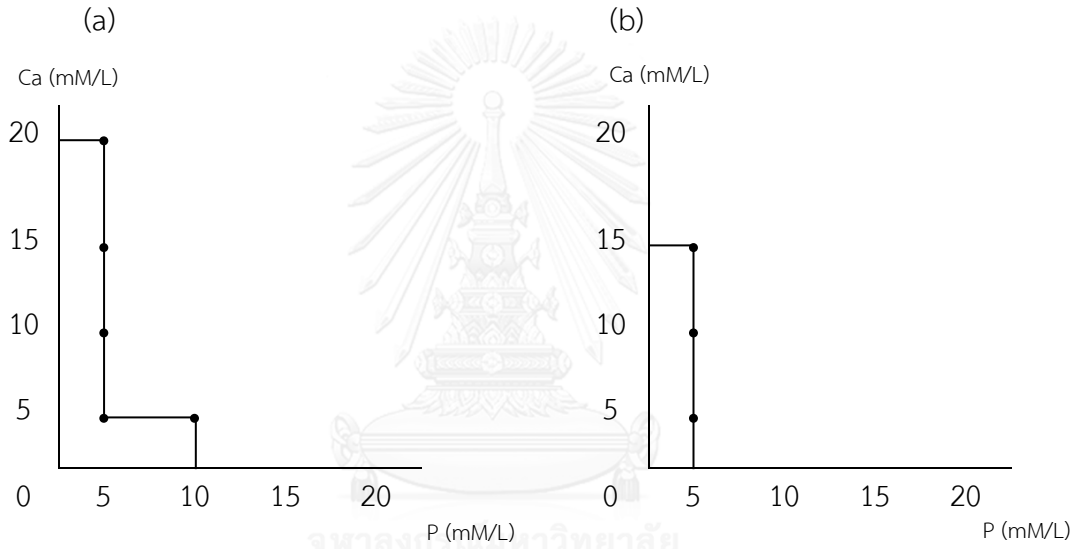


Figure A-2 Solubility data of 1.5% amino acid stored at (a) room temperature for 30 minutes, room temperature for 1 day, 4 °C for 1 day and (b) 4 °C for 7 days

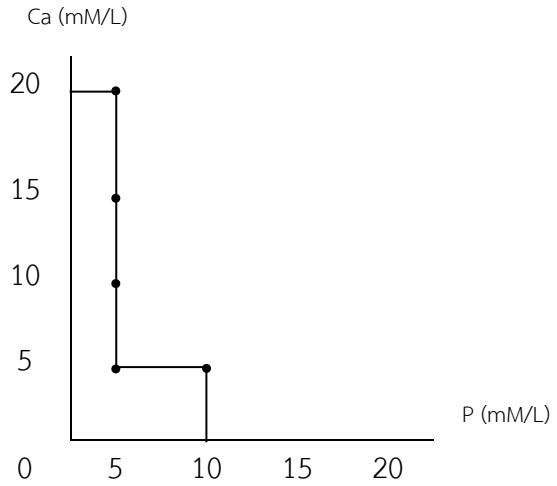


Figure A-3 Solubility data of 2% amino acid stored at room temperature for 30 minutes, room temperature for 1 day and 4 °C for 1 day and 4 °C for 7 days

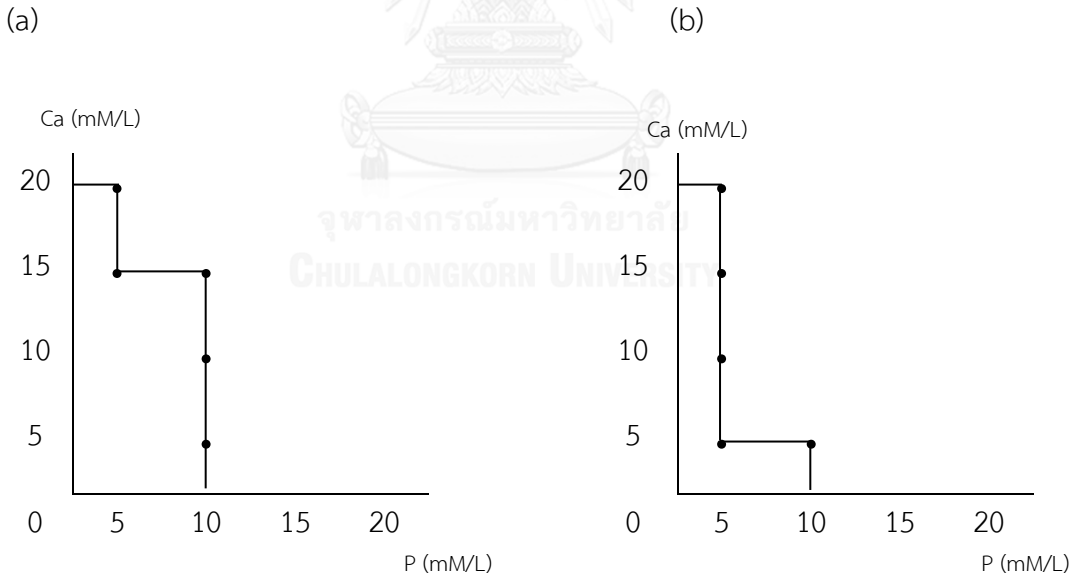


Figure A-4 Solubility data of 2.5% amino acid stored at (a) room temperature for 30 minutes and (b) room temperature for 1 day, 4 °C for 1 day and 4 °C for 7 days

VITA

Miss Nattaporn Thowladda was born in January 1, 1989 in Bangkok, Thailand. She received a Bachelor of Pharmacy from the Faculty of Pharmaceutical Sciences, Silpakorn University, Thailand in 2011. After graduation, she has been working as a pharmacist at Drug compounding unit, Pharmacy Department, Ramathibodi Hospital, Thailand. Her responsibilities include drug information services, extemporaneous preparation, total parenteral nutrition preparation, intravenous admixture preparation and chemotherapy preparation.