การแยกโพลีแซคคาไรด์จากเจลของว่านหางจระเข้โดยการตกตะกอน

นางสาวรัตน์ฉัตร ฉันทวงศ์วุฒิ

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

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ISOLATION OF POLYSACCHARIDE FROM ALOE VERA GEL BY PRECIPITATION

Ms. Ratchat Chantawongvuti

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

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จุฬาลงกรณ์มหาวิทยาลัย

รัตน์ฉัตร ฉันทวงศ์วุฒิ : การแยกโพลีแซคคาไรด์จากเจลของว่านหางจระเข้โดยการตกตะกอน. (ISOLATION OF POLYSACCHARIDE FROM ALOE VERA GEL BY PRECIPITATION) อาจารย์ที่ปรึกษาวิทยานิพนธ์ : อาจารย์ ดร. เหมือนเดือน พิศาลพงศ์, 122 หน้า. ISBN 974-17-4622-9

การศึกษาการแยกโพลีแซคคาไรด์จากเจลของว่านหางจระเข้โดยการตกตะกอน แบ่งออกเป็น 2 ส่วน คือ ส่วนแรกทำการคัดเลือกสารละลายแอลกอฮอล์ที่เหมาะสมในการตกตะกอน พบว่า น้ำหนักแห้งเฉลี่ยของผลิตภัณฑ์เมื่อทำการตกตะกอนเจลว่านหางจระเข้ 100 มิลลิลิตร ด้วยเมธานอล 95% เอธานอล 95% และ ไอโซโพรพานอล 95% 400 มิลลิลิตร มีค่าเท่ากับ 0.0367, 0.0435 และ 0.0517 กรัม ตามลำดับ ปริมาณร้อยละของโพลีแซคคาไรด์เทียบกับน้ำหนักแห้งเฉลี่ยของผลิตภัณฑ์ คือ 17.84, 28.72 และ 20.81 ตามลำดับ

ส่วนที่สองทำการศึกษาปัจจัยที่มีอิทธิพลต่อการแยกโพลีแซคคาไรด์ โดยมีขอบเขตของตัวแปรที่ทำการศึกษา คือ อัตราส่วนของแอลกอฮอล์ที่ใช้ในการตกตะกอนต่อเจลจากว่านหางจระเข้, ค่าความเป็นกรด – ด่างของระบบ, อุณหภูมิ และจำนวนขั้นของการตกตะกอน พบว่า อัตราส่วนของแอลกอฮอล์ที่ใช้ในการตกตะกอนต่อเจลจากว่านหางจระเข้ ที่ให้น้ำหนักแห้งเฉลี่ยของผลิตภัณฑ์มากที่สุด คือ อัตราส่วน 6:1 โดยให้น้ำหนักแห้งเฉลี่ยของผลิตภัณฑ์ที่ได้จากการตกตะกอนเจลว่านหางจระเข้ 100 มิลลิลิตร เท่ากับ 0.1877 กรัม ร้อยละของโพลีแซคคาไรด์เทียบกับน้ำหนักแห้งเฉลี่ยของผลิตภัณฑ์ คือ 26.51 และมีโปรตีนปนเปื้อนร้อยละ 0.28 เทียบกับน้ำหนักแห้งเฉลี่ยของผลิตภัณฑ์ การตกตะกอนโดยไม่ปรับค่าความเป็นกรด – ด่างของระบบ (ค่าความเป็นกรด – ด่างเท่ากับ 5.52) ให้น้ำหนักแห้งเฉลี่ยของผลิตภัณฑ์มากที่สุด อุณหภูมิที่เหมาะสมในการตกตะกอน คือ 32 องศาเซลเซียส (อุณหภูมิห้อง) โดยให้น้ำหนักแห้งของผลิตภัณฑ์ที่ได้จากการตกตะกอนเจลว่านหางจระเข้ 1 ลิตร เท่ากับ 1.8571 กรัม ร้อยละของโพลีแซคคาไรด์เทียบกับน้ำหนักแห้งของผลิตภัณฑ์ คือ 33.74 จากการวิเคราะห์ธาตุองค์ประกอบ มีธาตุคาร์บอนและธาตุไนโตรเจนเป็นองค์ประกอบร้อยละ 32.32 และ 1.26 เทียบกับน้ำหนักแห้งของผลิตภัณฑ์ ตามลำดับ และอัตราส่วนระหว่างธาตุคาร์บอนและธาตุไนโตรเจนมีค่าเท่ากับ 25.74:1 เมื่อทำการตกตะกอนเจลว่านหางจระเข้ 1 ลิตรแบบ 6 ขั้นให้น้ำหนักแห้งของผลิตภัณฑ์เท่ากับ 1.1969 กรัม และได้น้ำหนักแห้งของผลิตภัณฑ์เท่ากับ 1.6664 กรัม เมื่อทำการตกตะกอนแบบขั้นเดียว

ภาควิชา..........วิศวกรรมเคมี........... ลายมือชื่อนิสิต...

สาขาวิชา.........วิศวกรรมเคมี........... ลายมือชื่ออาจารย์ที่ปรึกษา....................................

ปีการศึกษา............2546...................

4370465021 : MAJOR CHEMICAL ENGINEERING
KEY WORD: ALOE VERA / ALOE BARBADENSIS / POLYSACCHARIDE / PRECIPITATION

RATCHAT CHANTAWONGVUTI: ISOLATION OF POLYSACCHARIDE FROM ALOE VERA GEL BY PRECIPITATION. THESIS ADVISOR: MUENDUEN PHISALAPHONG, Ph.D., 122 pp. ISBN 974-17-4622-9

The study of isolation of polysaccharide from aloe vera gel by precipitation can be categorized into 2 sections. For the first section, suitable solvent for the precipitation process was studied. When 100 ml aloe vera gel was precipitated with 400 ml of 95% methanol, 95% ethanol, and 95% iso-propanol, it was found that the average product dry weight of each solvent precipitation were 0.0367, 0.0435, and 0.0517 g, respectively. Polysaccharide content with respect to the average product dry weight of methanol, ethanol, and iso-propanol precipitation were 17.84%, 28.72%, and 20.81%, respectively. Therefore, 95% ethanol was chosen as the solvent for the precipitation. In the second section, various factors affecting polysaccharide separation from the aloe vera gel were investigated. Those factors were solvent to gel ratio, system pH, temperature, and stage of precipitation. It was found that solvent to gel ratio of 6:1 yielded the maximum average product dry weight, 0.1877 g, when 100 ml aloe vera gel was precipitated. Polysaccharide and protein content with respect to the average product dry weight were 26.51% and 0.28%, respectively. Considering pH of the system, the maximum average product dry weight was obtained when pH was not adjusted (pH = 5.52). Focusing on the system temperature, the suitable temperature in the precipitation process was founded to be 32ºC (room temperature). When 1 litre of aloe vera gel was precipitated, the product dry weight was 1.8571 g. Polysaccharide with respect to product dry weight was 33.74%. Elemental analysis showed that the dry product composed of carbon and nitrogen with the contents of 32.32% and 1.26 % by weight, respectively. C:N ratio was 25.74:1. As for the effect of the stage of precipitation, the single stage precipitation yielded 1.6664 g of product dry weight and 1.1969 g was obtained from 6 stages precipitation.

Department…….Chemical Engineering… Student's signature…………………….........
Field of study….Chemical Engineering… Advisor's signature……………………......
Academic year…………2003…………...

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จุฬาลงกรณ์มหาวิทยาลัย

Chapter 1

Introduction

1.1 General Ideas

Aloe vera is one of the oldest healing plants known to mankind. It has a long and illustrious history and has been used by ancient civilizations such as Egyptians, Romans, Greeks, Arabs, and Chinese. The plant is about 96% water. The rest of it contains many natural health promoting substances including vitamins and minerals, enzymes, amino acids, plant sterols, hormones, fatty acids, glycoproteins, anthraquinones, lignin, saponin, and polysaccharides. Taken internally, aloe products have been promoted for insomnia, infection, indigestion, heartburn, constipation, haemorrhoids, ulcers, arthristis, cough, diabetes, cancer, immune – system deficiency, and etc. Used externally, many people attest to the effectiveness of aloe vera in helping to treat burns, sunburn, scalds, psoriasis, eczema, acne, stings, scrapes, abrasions, scalp care, sore muscles, cold sores, bruises, sprains, and many other conditions.

Aloe vera can be separated into two basic products: gel and latex. Aloe vera gel is the mucilaginous gel that obtains from squeezing of a thin clear jelly – like substance of the parenchymal tissue. The gel contains carbohydrate polymers, such as glucomannans or pectic acid, organic and inorganic compound. Aloe latex, commonly referred to as "aloe juice", is a bitter yellow exudate from the pericyclic tubules just beneath the outer skin of the leaves. This latex contains the anthraquinone glycosides, aloin A and B, which are potent laxatives.

The polysaccharides isolated from the aloe vera gel have been claimed as the active ingredients (Eberendu and McAnally, 1996; Grindlay & Reynolds, 1986;

Reynolds & Dweck, 1999; Chevallier, 1996; Ody, 1993; Levetin & McMahon, 1999; McAnally, 1990; Shida et al., 1985; Femenia et al., 1999). These active polysaccharides are referred to as acemannan. Acemannan is an ordered linear polymer of substantially acetylated mannose monomers. The physiological activities of acemannan and its pharmaceutical applications have been investigated. The activity of acemannan as an antiviral agent, an immunomodulator, a means of reducing opportunistic infections, and as a means of stimulating the healing processes has been reported (Eberendu and McAnally, 1996; Grindlay & Reynolds, 1986; Reynolds & Dweck, 1999; Chevallier, 1996; Ody, 1993; McAnally, 1990). Acemannan has been shown in laboratory studies to increase up to 300% in 48 hours the replication of fibroblasts in tissue culture which are known to be responsible for healing burns, ulcers and other wounds of the skin and of the gastrointestinal lining. Acemannan has also been shown to increase DNA synthesis in the nucleus of fibroblasts. The increase in DNA synthesis in turn increases the rate of metabolic activity and cell replication which are fundamental steps in the healing process. In addition, acemannan has been shown in controlled studied to increase the rate of healing in animals, as well, such as an effective treatment for gastric ulcers in animal studies.

This research is to study the effects of separation factors for the isolation of active polysaccharide from aloe vera gel by precipitation. The quality and quantity of the product obtained from the improved process will be analyzed by spectrophotometry.

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1.2 Objective of the study

จุฬาลงกรณ์มหาวิทยาลัย

To determine the separation factors influencing the separation of polysaccharide from aloe vera gel by precipitation.

1.3 Scopes of the study

1. Determine the suitable solvent for the isolation of polysaccharide from aloe vera gel. (Methanol, ethanol, and iso-propanol).
2. Study the effect of separation factors on polysaccharide separation from aloe vera gel as follows:

    2.1 Solvent to gel ratio (3 – 7).

    2.2 Temperature (7°C, 15°C, Room temperature).

    2.3 pH (3 – 9).

    2.4 Number of precipitation stages.

1.4 Research procedure

1. Literature survey
2. Experimentation

    2.1 Perform experiments to determine the suitable solvent in polysaccharide separation.

    2.2 Perform experiments to study the effect of separation factors in the separation process.
3. Result analysis and discussion
4. Conclusion
5. Thesis writing

1.5 Benefits of the study

To develop the process for isolation of polysaccharide from aloe vera gel.

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

Chapter 2

Background and literature reviews

2.1 Background

Aloe vera (*Aloe barbadensis* Miller) is a member of the *Liliacea*. There are about 360 species of aloe plants known (McAnally et al., 1999; McAnally et al., 1992). They seem to thrive in hot, arid areas and are widely scattered from the Mediterranean Sea, Middle East, Africa, China, Japan, Mexico, and the southern U.S.A. The plants belonging to this genus are shrubby and succulent. Aloe vera is a perennial plant with turgid green leaves joined at the stem in a rosette pattern. The leaves of a mature plant have saw-like spikes along their margins, which grow from a short stalk near ground level. Vertical tubular yellow flowers are produced annually in the spring.

Figure 2.1 *Aloe barbadensis* Miller plant

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The mature plants can grow as tall as 2.5 to 4 feet with the average being around 28 to 36 inches in length. Each plant usually has 12 – 16 leaves that may weigh up to 3 pounds when mature. The plants can be harvested every 6 to 8 weeks by removing 3 – 4 lowest leaves per plant.

Slicing the leaf transversely as shown in Figure 2.2 and 2.3 reveals the outer walls of the epidermis (3) covered with thick cuticles, and they are composed of stomata. Beneath the epidermis (3) is the mesophyll which is differentiated into chlorenchymal cells and thinner walled cells known as parenchyma. The parenchymal cells harbour a transparent mucilaginous jelly (1). The vascular bundles (2) with inner bundle sheath cells contain the yellow sap, which has laxative properties, and are sandwiched between the two major cells.

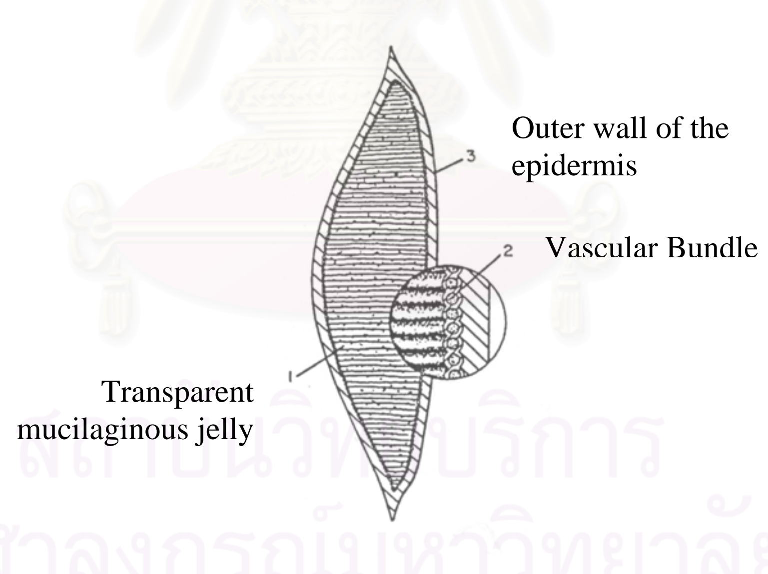

Figure 2.2 Cross sectional surface of aloe vera leaf (McAnally et al., 1999)

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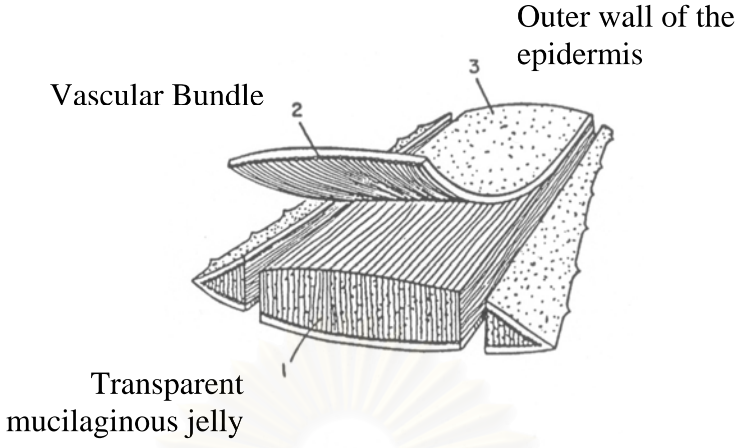

Figure 2.3 Aloe vera leaf (McAnally et al., 1999)

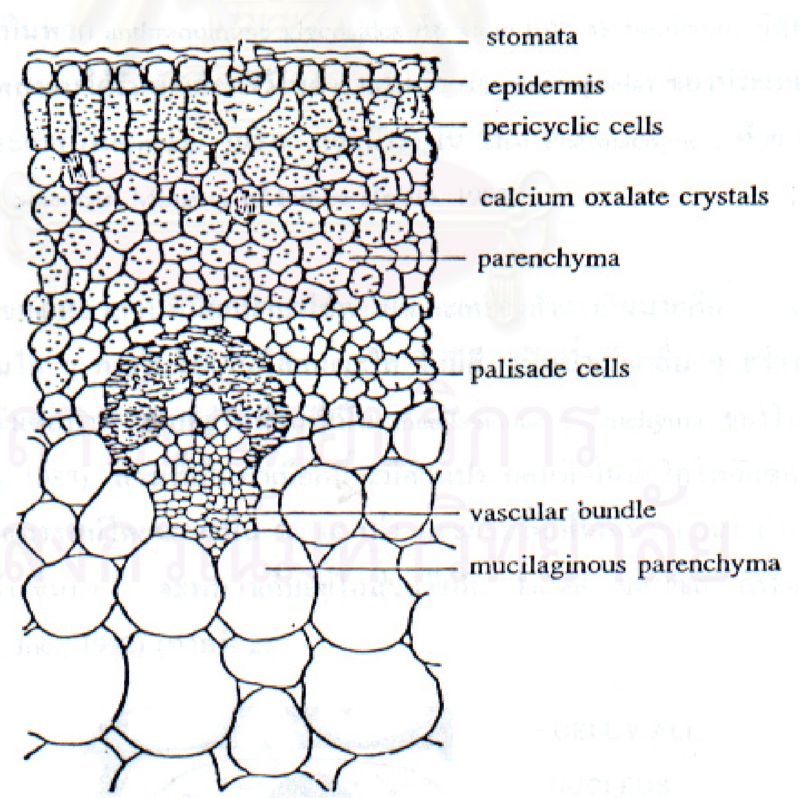

Figure 2.4 Anatomical structure of a transverse section of a mature *Aloe barbadensis* Miller (Prayoonrak, 1995)

Aloe vera contains two major liquid sources, a yellow latex (exudate) and the clear gel (mucilage). The yellow sap contains glycosidic derivatives of anthraquinones such as aloin (barbaloin), aloesin, p-coumaric acid, aloe – emodin (probably produced after oxidation of aloin), etc., which are very bitter in taste (Agarwala, 1997). The dried yellow sap is known as "bitter aloes". It is a strong laxative, useful for short – term constipation (Chevallier, 1996).

The fresh unpreserved gel is about 98.5 to 99.2% water. The total solid that remains after the water has been removed ranges from 0.8 to 1.5% of the total weight (Eberendu and McAnally, 1996; Femenia et al., 1999; McAnally, 1990). The major constituents of that solid are mucilage, fibre, monosaccharides, polysaccharides, proteins, ash, fats, aloin and resin (Eberendu and McAnally, 1996). More than 60% of the remaining solid being made up of polysaccharides (Femenia et al., 1999; McAnally, 1990). Aloe polysaccharides consist of linear chains of $\beta 1 \rightarrow 4$ linked glucose and mannose molecules: owing to the presence of these two simple hexose sugars, they are also called glucomannans, and because there is much more mannose than glucose present, they are also called polymannans. These linear chains range in size from a few molecules to several thousand molecules. By convention the lower limit usually taken as a molecular weight of about 1,000 daltons to qualify as a polysaccharide. Different molecular-sized fractions may posses different physical characteristics and widely differing potential biological activities. Compositions that include enzymes, organic acids, inorganic salts, amino acids and alkaloids have been noted (McAnally, 1990). The aloe gel can be soothing when applied to injured skin by promotes faster healing with less scarring by stimulating cell growth and inhibiting bacterial and fungal infection in injuries ranging from deep dermal burns to radiation burns. Other usages are inhibiting pain, itching, and inflammation, treating skin and mouth ulcers, eczema, psoriasis, ringworm, athlete's foot, and poison ivy rashes. It also used as a powerful purgative for the relief of constipation.

In recent years, aloe vera gel has been used in a cosmetic industry as an emollient and skin conditioner because aloe is a rich source of simple sugars, amino acids (glutamic acid and arginine), lactate salts and organic salts. These components are also known to be the hydrophilic materials that increase the water uptake of

cornified tissue. It can be found in a variety product such as skin creams, shampoos, sunscreen lotions, and bath oils. Recent applications include diapers, baby wipers, tissues, and bandages.

2.2 Literature Reviews

Gowda et al. (1979) studied the structure of polysaccharides from *Aloe vera*. Extraction of the mucilaginous gel from the aloe vera leaves was done by the addition of 50% aqueous ethanol solution. Precipitated with ethanol, a white polysaccharide mixture could be obtained. After dissolution in 0.02 M HCl, dialysis with distilled water, precipitation with ethanol, and dry, the white polysaccharide mixture turned to be a coarse, white, fibrous powder. The yield based on the wet gel weight was 0.7%. The powder composed of glucose and mannose in the 1:6 molar ratio, with traces of galacturonic acid, arabinose, xylose, and galactose. Fractionation of the polysaccharide mixture by graded precipitation with ethanol, from aqueous solution, gave three polysaccharide fractions: A_1, A_2, and B. Further fractionation of A_1 with 0.05M sodium tetraborate gave polysaccharides A_{1a} and A_{1b}. The polysaccharides A_{1b}, A_2, and B were further purified by repeated precipitation from aqueous solution with ethanol, until there was no change in the glucose-to-mannose ratio. Polysaccharide A_{1a} could not be purified, as it was insoluble in water. The yield, specific rotation, *O*-acetyl content, and sugar composition of these polysaccharides are given in Table 2.1. The mucilaginous jelly of Aloe vera contains a mixture of at least four linear glucomannans that differ in their *O*-acetyl content and glucose-to-mannose ratio.

Table 2.1 Composition of the Aloe vera polysaccharides (Gowda et al., 1979)

Polysaccharide fraction	Physical appearance	Molecular weight[a]	$[\alpha]D$[b] (degrees)	Phosphate[c] (%)	*O*-Acetyl[d]	Glc/Man ratio[e]
A_{1a}	Grey powder	-	-	-	1.1	1.5:1 (traces of GalA, Xyl, Ara, and Gal are also present)
A_{1b}	White powder	$> 2 \times 10^5$	-21.5	-	9.25	1:4.5
A_2	White powder	$> 2 \times 10^5$	-32	0.15	10.3	1:13.5
B	Coarse, white, fibrous powder	$> 2 \times 10^5$	-40	0.17	17.2	1:19

[a]By gel filtration. [b]In M sodium hydroxide (c 0.25); values uncorrected for possible deacetylation. [c]Determine by spectrophotometryas the molybdovanadophosphoric acid complex. [d]Determined by saponification. [e]Determined by g.l.c. as the alditol acetates.

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L. A.'t Hart et al. (1989) developed new substances with immunomodulatory activity. This study was aimed at the purification and initial characterization of the active polymer. The production of plant extracts began with lyophilization of the mucilaginous parenchymous tissue that was excised from the fresh leaves and kept under dry condition until used. Then the lyophilized gel was extracted by saline. The supernatant was dialyzed. The non-dialyzable fraction was lyophilized and kept deep frozen till further use. A highly active polysaccharide fraction was isolated by using anion – exchange and gel permeation chromatography. The isolated polysaccharide gave a negative reaction with Bradford's protein reagent and therefore does not contain peptide residues. The positive reaction with Dubois' phenol – sulphuric acid reagent pointed in the direction of polysaccharides. From the gel permeation chromatography, the elution pattern suggested that the carbohydrates have a broad spectrum of chain-lengths. Two peaks could be discerned at M_r 320,000 (B-I) and at M_r 200,000 (B-II) respectively. After dialysis fractions B-I and B-II were methanolysed to determine their monosaccharide composition. The results were shown in Table 2.2.

Table 2.2 Polysaccharide compositions ('t Hart et al., 1989)

Fraction	Mannose (%)	Glucose (%)	Galactose (%)	Arabinose (%)
B-I	92.1	3.2	3.8	0.9
B-II	83.7	3.9	8.8	3.6

Saisuk M. (1991) increased stability of aloe vera gel by spray dried and freeze dried method. It was found that the gel in the dried form, prepared by the aloe vera gel mixed with acacia 1.5% w/v, have nearly the same viscosity and solubility with the dried gel, sale in the market. When it was analyzed by the thin layer chromatography and infrared spectrophotometry, the gel obtained from the spray dried and freeze dried method still had the same characteristic properties of the aloe vera gel. Moreover, the dried gel that was kept in the refrigerator still maintained its quality, longer than the dried gel kept at room temperature during 60 days. The dried gel can be kept longer than 210 days with standard microbial allowance of industrial ministry by dissolved in water containing methyl paraben 0.2% w/v, propyl paraben 0.02% w/v, EDTA 0.05%

w/v and sodium metabisulfite 0.1% w/v. The aloe vera gel containing creams exhibited same quality as the premixed creams except their viscosities were increased.

Angsupanich K. (1993) studied the processing of aloe vera gel powder. The aloe vera gel matrix was pretreated by dipping in 0.5% KMS (Potassium metabisulfite) solution for 2 minutes and blanching in hot water at 80 ^{o}C for 2 minutes. Maltodextrin was added to the aloe vera juice in order to adjust solid content. Then the adjusted aloe vera juice was dried by spray drying and tray drying at various temperatures. The results showed that the powder from spray drying had low dispersibility and the yield lost was greater than from tray drying. Aloe vera juice with 2% solid content, partially evaporated and then dried by tray dryer at 60 ^{o}C for 13 hours, yielded 0.4% of fresh aloe vera leaf. The aloe vera powder colour was ranged from white to yellow. The compositions were 3.04% moisture, 1.27% protein, 0.21% fat, 84.82% carbohydrate, 10.66% ash and trace amount of fibre and 0.22 in water activity (a_w).

Yamaguchi et al. (1993) investigated the main components, including minerals, of the aloe vera gel. The rind of the aloe vera leaf was cut off, and part of the gel was put into bottles and freeze – dried by a Tozai-Tsusho VFD-810 FM-B freeze dryer. 87.5 g of freeze – dried material was obtained from 1.9 kg. of the gel. Part of this freeze-dried gel supplied to Hitachi Analysis Center in Ibaragi Prefecture for detecting the elemental minerals with a Hitachi ICP P-5200 dual-monochlonal emission analyzer. Ten elements were quantitatively detected (in ppm): Al (below 5), B (52), Ba (39), Ca (35400), Fe (4), Mg (7200), Mn (67), Na (1720), P (3210), and Sr (86). Silicon was also quantitatively detected. Freeze – dried gel was extracted with *n*-hexane and then acetone for 20 days each. Both the extracts were then filtered, and the filtrates were each concentrated in a rotary vacuum evaporator to give 8.3 g and 3.39 g of pale yellow oily materials, respectively. The concentrates were independently separated in a silica-gel column (Wako C-300 gel, 230 x 24 mm), eluting with suitable organic solvents. From the fraction eluted with *n*-hexane, β-sitosterol was isolated. The other eluted solutions were combined to afford 5.16 g of oily material from the n-hexane extract, and 150 mg (acetone fraction #1) and 120 mg (acetone fraction #2) of two oily materials from the acetone extract. Each of those

three oily materials was subjected to a GC-MS (JEOL DX-30) analysis. The identified components in the *n*-hexane extract were *n*-hexadecane, decylcyclohexane, *n*-heptadecane, 1-heptadecene, undecylcyclohexane, dodecylbenzene, *n*-octadecane, 1-octadecene, (E)-5-nonadecene, dodecylcyclohexane, tridecylbenzene, *n*-nonadecane, 1-nonadecene, tetradecylbenzene, *n*-icosane, *n*-heneicosane, and *n*-decosane. The components identified in acetone fraction #1 were lauric acid, 2(3H)-benzothiazolone, myristic acid, 4-hydroxy-3,5-di-*t*-butylbenzaldehyde, pentadecanoic acid, palmitoleic acid, palmitic acid, 15-methylhexadecanoic acid, margaric acid, linoleic acid, 7-octadecenoic acid, stearic acid, 1,1-bis(2-hydroxy-3,5-dimethylphenyl)-2-methypropane, arachidic acid, methydehydroabietic acid, monooctyl phthalate, dioctyl phthalate, aloe – emodin, and D : A-friedooleanan-3-one. Acetone fraction #2 consisted of methyl laurate, 3,3'-bis-*p*-menthane, methyl 12-methyltridecanoate, methyl myristate, ethyl myristate, methyl pentadecanoate, methyl 14-methylpentadecanoate, 1,1,2-triphenylcyclopropane, methyl palmitolate, methyl 13-methylpentadecanoate, methyl palmitate, dibutyl phthalate, ethyl palmitate, methyl margarate, 9-propanoylmethoxymethylphenanthrene, methyl 9,12-octadecadienoate, methyl 10,13-octadecadienoate, methyl oleate, methyl 8-oleate, methyl 16-methylheptadecanoate, ethyl linoleate, ethyl oleate, ethyl stearate, 1-octadecene, dehydroabietal, methyl 6,9-octadecanoate, methyl arachate, methyl dehydroabietate, ethyl 4,4-dimethyl-3-(2,4,5-trimethoxyphenyl)-pentanoate, cycloicosane, diheptyl phthalate, methyl heneicosanoate, monooctyl phthalate, diocyl phthalate, methyl behenate, and methyl tricosanate.

Ross et al. (1997) determined the concentration of aloe vera mucilaginous polysaccharide (AVMP) in 18 selected commercial aloe vera products by a qualitative method using a size exclusion chromatographic (SEC) analysis. The Shodex Ohpak KB – 800 series SEC column used for analysis was maintained at 30 ^{o}C with a temperature control module (Water Associates, Milford, MA). The samples were prepared by filtering through an Acrodisc 1.2 μm syringe filter before analysis. The analysis was performed at a flow rate of 1 mL/min, at 30 ^{o}C, with run time, 15 – 30 min, depending on the sample. Of the 18 preparations studied, 9 contained quantitable amounts of AVMP at 0.22 to 1.30 mg/mL, 2 showed trace amounts (<0.2 mg/mL), and 7 preparations showed no detectable levels of AVMP.

Femenia et al. (1999) characterized a complete chemical composition of Aloe vera plant (*Aloe barbadensis* Miller). The aloe vera leaves were dissected in fillet and skin. In addition, a mucilaginous gel extracted form the fillets by the extrusion was also characterized. The thick epidermis accounted for 20% to 30% by weight of the whole leaf and it was formed mainly by lignified cell walls as shown in Figure 2.5.

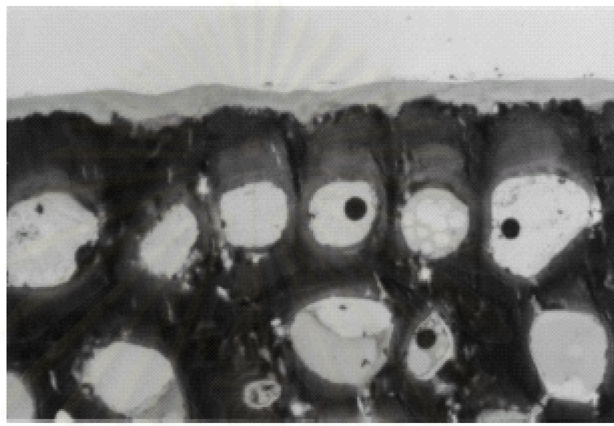

Figure 2.5 Section of skin tissue from Aloe vera leaf (Femenia et al., 1999)

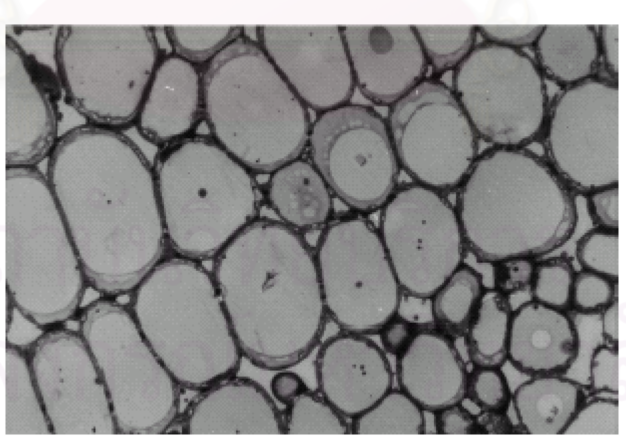

Figure 2.6 Parenchymatous cells from Aloe vera fillet. The small granules attached to the inner surface of the cell walls might contain the bioactive polysaccharide acemannan (Femenia et al., 1999)

This contrasted with the big rounded cells with thin primary walls forming the parenchymatous tissue of the fillet (Figure 2.6), which represented about 65% to 80% by weight of the whole leaf. The yield of the extruded gel in this process was approximately 70% (g gel/100 g fillet). The skin, fillet, and gel were lyophilized and characterized. The results are shown in Table 2.3.

Table 2.3 Chemical characterization of lyophilized Aloe vera plant fractions. (Results are expressed as percentages on dry matter basis; Femenia et al., 1999)

	Skin	Fillet	Gel
Lipids	2.71 ± 0.32	4.21 ± 0.12	5.13 ± 0.23
Proteins	6.33 ± 0.24	7.26 ± 0.33	8.92 ± 0.62
Soluble sugars	11.22 ± 0.73	16.48 ± 0.18	26.81 ± 0.56
Dietary fibres (NSP + lignin)	62.34 ± 1.10	57.14 ± 1.26	35.47 ± 0.62
Ashes	13.46 ± 0.44	15.37 ± 0.32	23.61 ± 0.71
Ca	4.48 ± 0.23	5.34 ± 0.14	3.58 ± 0.42
Mg	0.90 ± 0.12	0.76 ± 0.04	1.22 ± 0.11
Na	1.82 ± 0.09	1.98 ± 0.15	3.66 ± 0.07
K	1.84 ± 0.05	3.06 ± 0.18	4.06 ± 0.21
Proteins	0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0.00
Fe	0.04 ± 0.01	0.04 ± 0.01	0.10 ± 0.02
Cu	0.02 ± 0.01	0.04 ± 0.00	0.06 ± 0.01
Zn	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.00

Note : NSP is non-starch polysaccharides.
Glucose accounted for over 95% of the soluble sugars in all the aloe fractions analyzed.

The lyophilized aloe fractions were extracted with ethanol in order to get the alcohol insoluble residues (AIRs). This extraction allowed to concentrate the major fraction composed of carbohydrate up to 80%. The percentages of carbohydrates, lignins, proteins, ashes, and mineral elements presented in the AIRs were shown in Table 2.4.

Table 2.4 Chemical compositions of the AIRs obtained from the different Aloe vera lyophilized fractions. (Results are expressed as percentage of AIR; Femenia et al., 1999)

	Skin	Fillet	Gel
Carbohydrates	60.34 ± 1.43	79.95 ± 2.12	72.17 ± 1.23
Lignin	19.62 ± 0.97	1.63 ± 0.43	0.34 ± 0.12
Proteins	7.56 ± 0.31	10.12 ± 0.65	15.40 ± 0.87
Ashes	7.12 ± 0.64	7.41 ± 0.54	7.30 ± 0.53
Ca	1.23 ± 0.12	3.12 ± 0.16	3.28 ± 0.22
Mg	0.44 ± 0.02	0.08 ± 0.01	0.26 ± 0.07
Na	0.41 ± 0.07	0.04 ± 0.00	0.03 ± 0.00
K	0.38 ± 0.04	0.11 ± 0.01	0.12 ± 0.01
Proteins	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Fe	0.02 ± 0.00	0.03 ± 0.01	0.03 ± 0.01
Cu	0.002 ± 0.01	0.02 ± 0.01	0.02 ± 0.00
Zn	0.01 ± 0.00	0.01 ± 0.00	0.06 ± 0.01

The composition of the main type of polysaccharides presented in the AIRs was determined and shown in Table 2.5. Mannose and cellulosic glucose were the major polysaccharide components in all AIRs, significant amounts of pectic polysaccharides were also detected.

Table 2.5 Carbohydrate compositions of the polysaccharides present in the AIRs from the different Aloe vera fractions. (Results are expressed as mol%; Femenia et al., 1999)

	Skin	Fillet	Gel
Rhamnose	2.18 ± 0.07	1.69 ± 0.03	0.84 ± 0.01
Fucose	2.54 ± 0.04	1.94 ± 0.03	0.64 ± 0.01
Arabinose	5.88 ± 0.01	1.92 ± 0.06	1.15 ± 0.02
Xylose	11.72 ± 0.05	2.34 ± 0.03	1.38 ± 0.04
Mannose	30.09 ± 1.01	46.07 ± 1.21	52.81 ± 1.33
Galactose	8.43 ± 0.11	4.97 ± 0.06	3.50 ± 0.04
Glucose[a]	25.10 ± 0.71	27.03 ± 0.54	26.68 ± 0.38
Glucose (1 M)[b]	2.89 ± 0.07	5.95 ± 0.02	5.25 ± 0.08
Uronic acids	14.05 ± 0.21	14.04 ± 0.32	13.00 ± 0.25
DE (%)	21 ± 3	76 ± 2	73 ± 3

Note : [a]Glucose determined using the Saeman hydrolysis conditions. (Cellulosic glucose)
[b]Glucose determined using 1 M sulphuric acid. (Non-cellulosic neutral sugars)
DE is degree of esterification of pectic polysaccharides.

Data on the amount of polymers solubilised and the carbohydrate composition obtained after performing the sequential extraction on the aloe skin, fillet, and gel AIRs is shown in Table 2.6.

Table 2.6 Yields, carbohydrate composition and total amount of sugars of the different extracts obtained after sequential extraction of polysaccharides from Aloe vera skin, fillet, and gel portions (Femenia et al., 1999)

Fraction	Yield[a] (%)	Monosaccharides[b] (mol%)								Total sugars (μg/μg)[c]	DE (%)
		Rha	Fuc	Ara	Xyl	Man	Gal	Glc	UA		
H_2O											
Skin	40.6	2.1	2.4	2.0	1.4	5.1	10.0	3.1	74.7	260.1	34
Fillet	41.5	0.2	0.3	1.0	2.1	73.1	1.7	15.7	5.9	578.7	87
Gel	51.2	0.3	0.2	1.1	0.9	76.8	1.4	12.1	7.2	632.4	81
CDTA-1											
Skin	14.4	1.5	0.6	1.4	0.7	23.5	3.1	8.5	60.8	679.7	11
Fillet	14.8	1.5	0.6	2.5	0.7	10.5	4.1	8.5	71.8	679.7	54
Gel	11.3	1.1	0.1	1.0	0.2	13.5	1.2	7.1	75.8	685.2	48
CDTA-2											
Skin	8.3	4.0	1.6	3.8	0.9	35.3	7.1	16.4	31.4	136.7	9
Fillet	9.5	0.8	1.1	2.0	2.5	13.6	5.9	44.2	29.9	295.0	38
Gel	10.1	0.9	0.6	1.1	0.9	7.2	5.8	42.1	41.1	281.4	31
Na_2CO_3-1											
Skin	7.3	1.6	1.1	2.0	0.6	2.2	17.5	3.1	71.9	384.8	-
Fillet	9.5	2.5	2.6	3.3	0.5	4.6	12.1	5.1	69.4	410.4	-
Gel	5.1	2.7	2.0	3.7	0.6	5.2	7.2	2.4	76.2	523.1	-
Na_2CO_3-2											
Skin	3.6	4.8	4.0	6.5	4.8	4.3	35.1	22.9	17.6	234.7	-
Fillet	3.5	4.5	5.5	5.4	6.1	5.6	29.7	26.7	16.5	161.2	-
Gel	2.3	4.3	3.1	6.9	4.3	6.2	30.1	21.2	23.9	154.6	-
0.5 M KOH											
Skin	3.6	1.2	1.6	5.2	40.2	6.2	10.7	29.7	5.3	404.6	-
Fillet	3.2	1.3	2.5	7.1	32.5	12.4	5.8	26.8	11.6	173.0	-
Gel	1.4	1.1	5.2	8.7	18.4	13.4	3.4	15.4	33.0	181.7	-
1 M KOH											
Skin	8.4	1.6	3.2	4.1	32.0	13.1	9.2	32.5	4.3	476.4	-
Fillet	5.1	1.4	2.7	2.3	21.3	21.6	8.3	36.3	6.3	499.1	-
Gel	1.1	1.3	4.6	3.9	19.5	22.6	7.2	24.2	15.6	476.3	-
4 M KOH											
Skin	3.3	3.8	4.5	7.9	22.5	6.5	19.0	26.4	9.4	109.5	-
Fillet	3.0	5.3	3.1	4.7	19.6	10.8	12.0	24.7	19.8	134.4	-
Gel	1.2	2.1	3.8	5.1	23.1	12.4	10.1	21.8	21.6	181.4	-
Final residue											
Skin	10.5	0.6	0.5	2.6	0.6	4.0	12.9	68.0	10.9	845.7	-
Fillet	10.0	0.5	0.4	0.7	0.6	5.1	3.4	76.6	12.7	891.4	-
Gel	9.1	0.3	0.1	0.4	0.5	4.3	4.1	71.3	19.0	875.3	-

Note : [a]Yields are expressed as the percentage of AIR material recovered after each step of the fractionation procedure.

[b]Rha: rhamnose, Fuc: Fucose, Ara: arabinose, Xyl: xylose, Man: mannose, Gal: galactose, Glc: Glucose, and UA: uronic acids.

[c]Total sugars are expressed as μg of total sugar units per mg of material recovered after each extraction.

Yaron (1993) investigated the polysaccharide content and composition and gel consistency as a function of growth conditions in gels obtained from shrubs of *Aloe barbadensis* Miller grown in the Negev region of Israel. Autodegradation of the polysaccharides in the freshly produced gel was also characterized. The fresh gel contained about 1% dry matter. The content of soluble low-molecular-weight sugars was 0.2% to 0.3%, and that of polysaccharides was 0.1% to 0.2%. Leaf composition for the two levels of irrigation is given in Table 2.7. It seemed that irrigation had a greater influence on gel composition than leaf age. The gel obtained from shrubs receiving the low level of irrigation had a higher dry matter content (1.2% vs. 1%, Table 2.7) and higher contents of polysaccharides and soluble sugar at several harvesting dates studied (Table 2.8). The season of harvest had no major effect on the gel polysaccharide or the dry matter content (Table 2.8). The monosugar composition of gels obtained from young and mature leaves grown under the two irrigations levels was shown in Table 2.9. The polysaccharides are composed of two main monosugar, mannose and glucose in a ratio of about 3:1, in all the samples. Only traces of uronic acids were found in the gels. The main difference between the samples from the two irrigation levels laid in the methyl hexoses content, which was lower in the samples from plants receiving low irrigation. Incubation resulted in a drop of the content of polysaccharides. The monosugar profile of the remaining polysaccharide showed a decrease in glucose content and an increase in the mannose/glucose ratio to >10. This change occurred in all the samples irrespective of leaf are or irrigation level.

Table 2.7 Effect of leaf age and level of irrigation on composition of Aloe ver gel (May 1989, Ein Yahav, Negev Desert)[a]. (Yaron, 1993)

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	Low irrigation		High irrigation	
	Young leaves (250 g)	Mature leaves (500 g)	Young leaves (250 g)	Mature leaves (500 g)
Dry matter (%)	1.28 ± 0.10	1.22 ± 0.12	1.08 ± 0.07	0.97 ± 0.08
Polysaccharide (%)	0.21 ± 0.07	0.16 ± 0.01	0.07 ± 0.01	0.10 ± 0.03
Soluble sugars (%)	0.36 ± 0.06	0.30 ± 0.06	0.24 ± 0.07	0.20 ± 0.08

[a] The results are means of samples of six plants ± SE.

Table 2.8 Effect of season at two levels of irrigation on composition of Aloe vera gel (Yaron, 1993)

	Low irrigation			High irrigation		
	23.5.89	23.7.89	30.10.89	23.5.89	23.7.89	30.10.89
Dry matter (%)	1.22 ± 0.12	1.02 ± 0.05	0.96 ± 0.07	0.97 ± 0.08	0.95 ± 0.08	0.79 ± 0.04
Polysaccharide (%)	0.16 ± 0.01	0.14 ± 0.03	0.15 ± 0.10	0.10 ± 0.03	0.12 ± 0.02	0.09 ± 0.01
Soluble sugars (%)	0.30 ± 0.06	0.26 ± 0.10	0.23 ± 0.07	0.20 ± 0.08	0.27 ± 0.10	0.18 ± 0.07

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Table 2.9 Polysaccharide content and monosugar profile in fresh Aloe vera gel and in gel autodegraded by incubation (48 h at 40ºC) (Yaron, 1993)

	High irrigation	Low irrigation	
	Mature leaves[a]	Young leaves[b]	Mature leaves[b]
	Polysaccharide content (%)		
Fresh gel	0.12 ± 0.03	0.33 ± 0.02	0.16 ± 0.04
Incubated gel	0.11 ± 0.03	0.23 ± 0.03	0.16 ± 0.04
	Monosugar profile (percentage of total monosugars)		
	Fresh gel		
Dimethyl hexose	0.2 ± 0.1	10.2 ± 1.4	8.4 ± 0.4
Methyl hexose	-	3.8 ± 1.5	5.3 ± 1.5
Mannose	70.6 ± 5.9	60.3 ± 4.6	61.8 ± 3.4
Glucose	24.5 ± 5.6	22.3 ± 5.2	21.5 ± 0.7
Galactose	4.86 ± 0.3	1.6 ± 0.2	1.5 ± 0.15
Mannose/glucose ratio	2.9	2.7	2.9
	Incubated gel		
Dimethyl hexose	5.3 ± 0.5	1.8 ± 2.0	4.4 ± 0.5
Methyl hexose	1.7 ± 0.1	-	-
Mannose	84.7 ± 2.5	88.2 ± 3.1	86.8 ± 3.3
Glucose	6.3 ± 2.0	5.4 ± 1.0	8.8 ± 1.5
Galactose	1.7 ± 0.5	1.8 ± 0.4	1.8 ± 0.6
Mannose/glucose ratio	13.4	16.3	9.8

[a] The gel apparent viscosity at shear rates of 16 s-1 dropped by incubation from 78 to 30 cps.
[b] The gel apparent viscosity dropped from 235 to 71 cps by incubation of the gel obtained from young leaves and from 86 to 64 cps in the gel from mature leaves.

Chapter 3

Theory

3.1 Precipitation Process

3.1.1 Introduction

Some of the most delicate nondestructive methods available for the separation of similar chemical species depend on a difference in the solubility of the components in the chosen solvent system. Precipitation is probably the most versatile and universally used in separation technique. All the common schemes of qualitative analysis and many of the conventional gravimetric procedures used for the quantitative determination of constituents are based on the selective solubility (or insolubility) of a single chemical substance or a small group of chemically similar substances. Precipitation is the process by which a dissolved substance is converted into an insoluble form which can be separated from the bulk of the solution. Generally, precipitation implies that a chemical change occurred in the transition from the soluble to insoluble form. Actually, the majority of the analytically significant reactions occurs in solution and is influenced by the solubilities of the various constituents. In fact, one of the factors for driving a chemical reaction to completion is the formation of an insoluble product that effectively removes certain reactants from the reaction mixture.

The criterion that satisfied the separation is that the solution must become supersaturated with respect to the solute. The new phase is the result of either direct chemical action which forms a new chemical species, that is insoluble in the solution, or changes in the system which decrease the solubility of the solute in the parent

solution. The factors which determine the solubility of solid solutes to the greatest extent are temperature, pressure, particle size, degree of supersaturation, solvent – solute interactions, variations in pH, and salt concentration.

3.1.2 Typical precipitation processes

3.1.2.1 Precipitation with a nonsolvent

In this case, a solvent is simply added to a feed solution. This solvent is miscible with the feed but causes solute to precipitate. The cause of precipitation can be seen by considering the solute's chemical potential. At equilibrium, the chemical potential of the solid precipitate is the same as that in solution (Belter et al., 1988):

$$\mu_i(ppt) = \mu_i(solution)$$
$$\mu_i(ppt) = \mu_i^0(solution) + RT \ln y_i \qquad (3.1)$$

where μ_i is the chemical potential,
μ_i is the chemical potential at the standard state,
y_i is the concentration of solute *i* in the solution,
R is the gas constant
T is the temperature,
and *ppt* is the subscription for precipitate.

When the nonsolvent is added, the standard state chemical potential is raised, as suggested by Figure 3.1. The result is that the solution becomes supersaturated, and precipitation occurs until a new saturated concentration is reached.

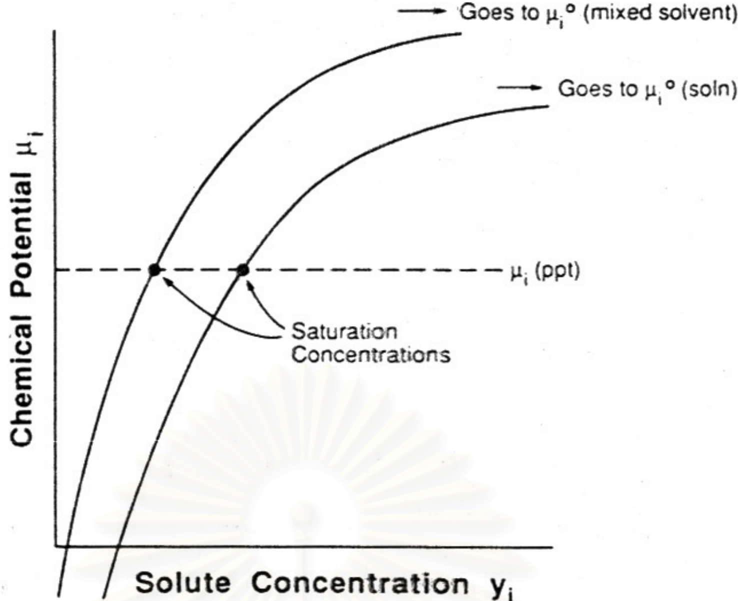

Figure 3.1 Precipitation in a mixed solvent. Adding a second solvent will often increase the solute's chemical potential and hence reduce its solubility. Such nonsolvents often have an effect which is highly dependent on pH (Belter et al., 1988).

The nonsolvent is frequently a water miscible organic solvent like acetone or ethanol. Water soluble polymers, especially polyethylene glycol, are popular, especially if they produce precipitation without excessive viscosity.

3.1.2.2 Precipitation with salts

The alternative to precipitate with nonsolvents is the precipitation with salts, called "salting out". Precipitation of simple electrolytes is described below.

Potassium nitrate is an example of a simple electrolyte which can be precipitated by salting out. Its solubility in water is given by the equation

$$K = \left[K^{+}\right]\left[NO_3^{-}\right] \tag{3.2}$$

where K is an equilibrium solubility.

The numerical value of K depends on the chemical potentials of potassium ion and nitrate ion in the system.

$$\mu_i(solids) = \mu_i(solution)$$
$$\mu_i(solids) = \mu_i^0(solution) + RT\ln[i] + Z_i F\phi \qquad (3.3)$$

where [*i*] is the concentration of the species *i*,
Zi is its ionic charge,
F is a Faraday's constant,
and ϕ is the electrostatic potential.

Rearrange the equation in the form of Equation (3.2)

$$\ln K = \frac{\mu_{K^+}(solid) - \mu_{K^+}^0(solution) + \mu_{NO_3^-}(solid) - \mu_{NO_3^-}^0(solution)}{RT} \qquad (3.4)$$

This equation (Belter et al., 1988) shows that the solubility product is an exponential function of the energy in the solid minus that in solution. More exactly, the logarithmic form of the solubility product is proportional to the difference in the standard state chemical potentials between the solid and the solution. If sodium nitrate, which is more soluble than potassium nitrate, is added to the potassium nitrate solution which is slightly below saturation, a potassium nitrate will precipitate. When sodium nitrate is added, there are more nitrate ions in the solution. The potassium ion concentration must drop, by precipitate, in order to maintain the value of solubility product, K, in the Equation (3.2).

Salts choosing

1. Effective order of anions:

   Citrate > PO_4^{3-} > SO_4^{2-} > CH_3COO^- > Cl^- > NO_3^-
2. Effective order of cations :

   NH_4^+ > K^+ > Na^+
3. Cheap salt.
4. Choose a salt that the density of the precipitate is different from the density of the solution.
5. Add a solid salt rather than a salt solution to minimize dilution.

3.1.2.3 Precipitation with temperature

Precipitation by increasing the temperature is focusing on protein purification, where temperature increases can selectively denature and precipitate the protein. Temperature decreases can crystallize solutes of moderate molecular weight, like antibiotics (Belter et al., 1988).

The analysis of temperature induced precipitation is based on the assumption that denaturation follows the first order chemical kinetics with an Arrhenius temperature dependence:

$$\frac{d[P]}{dt} = -\kappa[P] \tag{3.5}$$

where [P] is the dissolved protein concentration

κ is the rate constant given by

$$\kappa = \kappa_0 e^{-E/RT} \tag{3.6}$$

where κ_o is a characteristic constant

E is the activation energy of the denaturation.

Because E appears exponentially in Equation (3.6), it can have a large effect when temperature is slightly changed. The activation energy can be manipulated by changes in pH or solvent.

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3.1.3 Important factors on solute solubility

3.1.3.1 The effect of temperature on solute solubility

The basic equation that can calculate the solubility of insoluble strong electrolytes (common precipitates) at different temperatures is the Van't Hoff equation (Berg, 1963).

$$\log\frac{K_1}{K_2} = -\frac{\Delta H}{R}\left(\frac{1}{T_1} - \frac{1}{T_2}\right) \tag{3.7}$$

where ΔH is the heat of reaction or dissolution,
and K is the equilibrium constant (K_{sp}).

It is apparent that the influence of temperature on the solubility of solids in liquids is a function of the differential heat of solution. The dissolution of most salts is endothermic, which accounts for their greater solubility at higher temperatures. However, there are some salts that occur into solution by an exothermic process and are less soluble at higher temperature.

3.1.3.2 The effect of pressure on solute solubility

The ideal equation relating pressure and solubility (Berg, 1963) is

$$\frac{d\ln x}{dP} = \frac{V^S - V^L}{RT} \tag{3.8}$$

where x is the solubility or mole fraction
V^S is the molal volume of the solid
V^L is the molal volume of the solid in the pure liquid state.

Integration of Equation (3.8) between limits of p_1 and p_2 gives

$$\ln\frac{x_1}{x_2} = \frac{V^S - V^L}{RT}\left(p_2 - p_1\right) \tag{3.9}$$

From this equation, the solubility decreases by increasing of the pressure. However, the effect of pressure on the solubility of solids is usually small and can be neglected for most purposes unless the pressure is quite large.

3.1.3.3 The effect of particle size on solute solubility

Many colloidal and other very finely divided precipitates undergo a spontaneous change on digestion in contact with the solution. The fine particles become coarser and fewer in number with the smallest disappearing. This phenomenon is known as *Ostwald ripening*. The process is not obviously an equilibrium because the change of solute in the state of aggregation to another is spontaneous.

The Ostwald – Freundlich equation (Berg, 1963) that expresses the relationship between particle size and solubility is

$$RT\ln\frac{S}{S^0} = \frac{2\gamma M}{rd} \tag{3.10}$$

where S is the solubility of microparticles,
S^0 is the solubility of macroparticles,
γ is the surface tension of the solid,
M is the molecular weight of the solute,
r is the particle radius,
and d is the density.

Following this equation, only the extremely small particle size can affect the solubility. The Ostwald – Freundlich equation can be used for qualitatively predicting solubilities on the basis of particle size only if the particles are very small less than 1 μ in diameter.

3.1.3.4 The effect of supersaturation on solute solubility

When a reagent is added to an analyte solution forming a sparingly soluble compound, the solubility product for that compound is immediately exceeded and the solution is supersaturated. A supersaturated solution is an unstable solution that contains more solutes than a saturated solution. With time, supersaturation is relieved by precipitation of the excess solute.

Von Weimarn proposed the theory that the speed of precipitation depends on the absolute concentration of solute and its equilibrium solubility. Von Weimarn's equation (Berg, 1963) is

$$D = K\frac{Q-S}{S} \tag{3.11}$$

where D is the dispersion coefficient (precipitation speed),
Q is the total concentration of substance in solution,
S is the equilibrium solubility of coarse crystals,
Q – S is the supersaturation at the moment of nucleation,
(Q-S)/S is the degree of supersaturation,
and K is a constant.

Von Weimarn's equation indicates that a precipitation can be slowed down by increasing the solubility of the precipitate or by decreasing the reactants concentration. The precipitation rate is dependent on two factors; the rate of nucleation and the rate of particle growth. These two factors are largely independent of each other, but both are dependent on the solute solubility.

Mechanism of precipitate formation is divided into 2 steps. The first one, nucleation, is a process that a minimum number of atoms, ions or molecules aggregate together to give a stable solid (Skoog et al., 1996). There are 2 types of nucleation (Fifield & Kealey, 1994):

1. Homogeneous nucleation is the aggregation of molecules or ions.
2. Heterogeneous nucleation is developed by particulate impurities initially aggregate in the solution.

The homogeneous nucleation process depends exponentially on the precipitation rate, the heterogeneous nucleation process is largely independent of it.

After nucleation the precipitation continues by particle growth, with further ions or molecules adding to the aggregates. The rate of particle growth will be dependent on the precipitation rate and on the surface area of the particles.

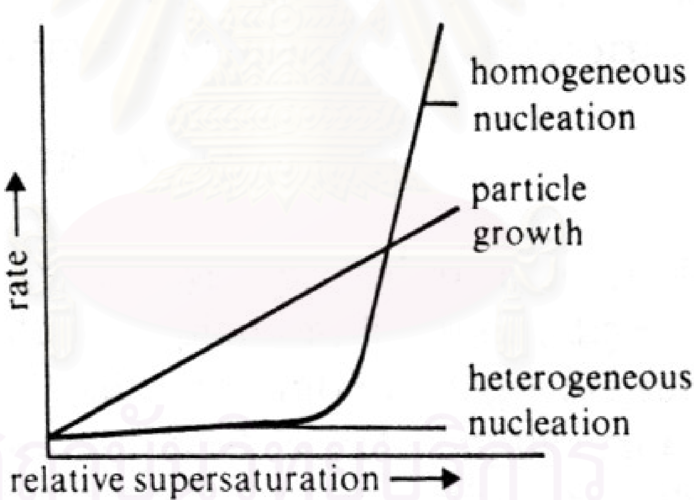

Figure 3.2 Nucleation and particle growth rates related to relative supersaturation. (Fifield & Kealey, 1994)

3.1.3.5 The effect of solvent on solute solubility

The influence of solvent on solute solubility can be realized by considering the dissolution process as consisting of two steps: the complete separation of crystal components and the solvation of the independent particles. Since the solvation energy is a function of both the solvent and the solute, the effect of the solvent on the solubility of a solute is determined by the magnitude of the solvation energy relative to the lattice energy. It is generally conceded that polar substances are more soluble in polar solvents than in nonpolar solvents. The converse is also true. Polar compounds are usually characterized by high lattice energies, high melting points, high solvation energies, and appreciable solubility in water or other highly polar solvent. Relative to the polar substances, the nonpolar substances are characterized by generally lower lattice energies, lower melting points. The lower solvation energies, results in much lower solubilities in water and other polar solvent. As would be expected, polar compounds are quite insoluble in nonpolar solvents because the lattice energies are high and the solvation energies low. The molecular interaction decreases as the solvent polarity decreases.

3.1.3.6 The effect of salt concentration on solute solubility

For the solubility equilibrium;

$$AB \leftrightarrow A + B \tag{3.12}$$

$$K_{sp} = [A][B] = \gamma_A C_A \gamma_B C_B \tag{3.13}$$

where γ is the activity coefficient,

and C is the concentration of the species.

The activity coefficient may be calculated from the Debye – Hückel equation (Fifield & Kealey, 1994) at 298 K,

$$-\log_{10}\gamma_A = 0.51Z_A^2\mu^{1/2} \tag{3.14}$$

where Z is the charge on A,

and μ is the ionic strength of its solution (mol kg^{-1}).

Thus, γ_A, K_{sp} and in turn the solubility of AB will increase with the ionic strength of the solution environment.

The addition of A or B to the AB system will shift the equilibrium to the left and will reduce the solubility of AB.

3.1.3.7 The effect of pH on solute solubility

The anions of all weak acids are associated with protons in aqueous solutions to form the weak acid and an equilibrium mixture. Sparingly soluble salts of weak acids such as calcium oxalate, barium carbonate, and silver chromate are soluble in dilute solutions of strong acids. In each case, the anion is removed from the salt to form the weak electrolyte in the following manner:

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$$CaC_2O_4\downarrow \rightleftharpoons Ca^{++} + C_2O_4^{=}$$
$$C_2O_4^{=} + H^+ \rightleftharpoons HC_2O_4^-$$

$$Ag_2CrO_4\downarrow \rightleftharpoons 2Ag^+ + CrO_4^{=}$$
$$CrO_4^{=} + H^+ \rightleftharpoons HCrO_4^-$$

Figure 3.3 Examples of weak acid equilibrium mixture (Berg, 1963).

The criterion for the dissolution of the sparingly soluble salt is that the equilibrium concentration of the anion of the weak acid is less than the equilibrium concentration of the anion of the salt. Under such conditions the formation of the weak acid is favored and the salt must dissolve to supply the necessary anionic component.

3.2 Carbohydrates

3.2.1 Introduction

Carbohydrates or sugars occupy a central position in plant metabolism so that methods for their detection and estimation are very important to the plant scientist. Not only are sugars the first complex organic compounds formed in the plant as a result of photosynthesis, but also they provide a major source of respiratory energy. They provide a means of storing energy (as starch) and transport of energy (as sucrose) and also the building blocks of the cell wall (cellulose). In addition, many other classes of plant constituent, e.g. the nucleic acids and the plant glycosides, contain sugars as essential features of their structures. Finally, sugars play a number of ecological roles, in plant-animal interactions (flower nectars are mainly sugar), in protection from wounding and infection and in the detoxification of foreign substances.

Sugars are conveniently classified into three groups, on the basis of molecular size: the simple monosaccharides (e.g. glucose, fructose) and their derivatives; the oligosaccharides, formed by condensation of two or more monosaccharide units (e.g. sucrose); and the polysaccharides which consist of long chains of monosaccharide units, joined head to tail, either as straight chains or with branching.

In their chemistry, low-molecular-weight sugars have many properties in common. They are optically active, aliphatic polyhydroxy compounds, which are usually very water-soluble. They are often difficult to crystallize, even when pure, and are frequently isolated as derivatives (e.g. as osazones by reaction with

phenylhydrazine). The sugars are relatively labile, and they easily undergo isomerization (enzymic or otherwise), and/or ring opening during extraction and the concentration of such extracts. Therefore, care must be taken to avoid extremes of heat or pH when isolating them.

Sugars are colourless substances and when present in micro amounts have to be detected by reaction with a suitable chromogenic reagent. Reducing sugars such as glucose, classically detected by a yellow-red precipitate with Fehling's solution, are easily detected on chromatograms by using one of a range of phenolic or amine reagents (e.g. resorcinol-H_2SO_4 or aniline hydrogen phthalate). Non-reducing sugars are less responsive to these reagents and are usually detected by their rapid oxidation with periodate or lead tetracetate. A general reagent for all sugars is alkaline $AgNO_3$, but this is not entirely specific for sugars since it also reacts with certain other plant substances, such as phenols.

Chemically, macromolecules consist of long chains of small structural units or 'building blocks', linked covalently in a number of different ways. Chemical characterization in the first instance therefore depends on identifying these smaller units. Polysaccharides are similarly derived from the union of simple sugar units, such as glucose, joined through ether (-O-) links. There are compounds peculiar to plants which are random polymers, formed by oxidative, non-enzymic polymerization of simple phenolic units.

3.2.2 Monosaccharides

The major free sugars in plants are the monosaccharides, glucose and fructose (and the disaccharide sucrose), together with traces of xylose, rhamnose and galactose. Other sugars present in trace amounts are the sugar phosphates, which are involved in metabolism; these are very easily hydrolyzed during manipulation to the parent sugars and special procedures are required to detect them. The bulk of carbohydrate occurs in plants in bound form, as oligo- or polysaccharide, or attached to a range of different aglycones, as plant glycoside. Since the free sugar pool is relatively uniform in higher plants, analysis of monosaccharides is therefore most

frequently concerned with the identification of sugars in hydrolysates of plant glycosides, oligosaccharides or polysaccharides.

Five sugars are commonly found as components of glycosides and polysaccharides and most plant analyses are concerned with their separation and identification. Two are hexoses, glucose and galactose, two are pentoses, xylose and arabinose, and one is a methylpentose, rhamnose. Of fairly common occurrence are the uronic acids, glucuronic and galacturonic acids and a third hexose, mannose, is not uncommon in polysaccharides. The pentose sugars ribose and deoxyribose, components of RNA and DNA respectively should be mentioned here, but these sugars are rarely encountered in plants in any other association. The only common keto sugar is fructose, not often present in plant glycoside, but a frequent component of oligosaccharides (e.g. sucrose) and of the polysaccharides known as fructans. A range of rarer sugars occur in plant glycosides, one example being the five-carbon branched sugar apiose, present as the flavone glycoside apiin in parsley seed.

The structures of most of the monosaccharides mentioned above are given in Figure 3.4. It is to be remembered that each sugar can exist as more than optically active isomer; however, in plants, only one form is normally encountered. Thus, glucose is usually the β-D-isomer, rhamnose the α-L form and so on. Chromatography does not normally distinguish between optical enantiomers. Again, each sugar can theoretically exist in both a pyrano-(six-membered) and furano-(five-membered ring) form, although one or other is usually favoured. Thus, glucose normally takes up the pyrano-configuration, whereas fructose is usually the furano-form (see Figure 3.4).

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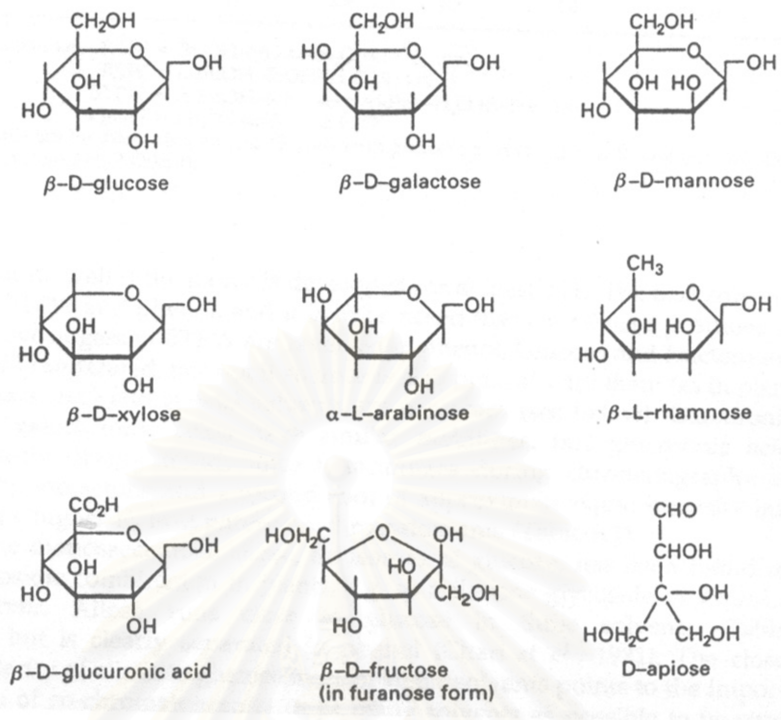

Figure 3.4 Formulae of common monosaccharides

3.2.3 Oligosaccharides

Most of the common plant oligosaccharides contain from two (e.g. sucrose, maltose) to five (e.g. verbascose) monosaccharide units. Even when there are only two units, these can be joined together by ether links in a number of different ways (i.e. through different hydroxyls and by α- or β-links) so that one of the main problems in oligosaccharide identification is distinguishing different isomer. In the case of disaccharides containing glucose, eight isomeric structures are possible and all are known.

The number of oligosaccharides which accumulate as such in plants are relatively few. Sucrose (2−α-glucosylfructose) is the only one which is of universal occurrence. Fairly common are α,α-trehalose (α-glucosyl-α-glucose), raffinose (6^G-α-galactosylsucrose), stachyose (6^G-α-digalactosylsucrose) and verbascose (6^G-α-trigalactosylsucrose). Raffinose and stachyose are present, for example, in many

legume seeds. One of the more uncommon oligosaccharides is umbelliferose (2^G-α-galactosylsucrose), which is mainly restricted in its distribution to members of the Umbelliferae.

A large number of the known plant oligosaccharides are not present in the free state, but occur combined to other organic molecules as plant glycosides. Among flavonoid pigments, for example, the following oligosaccharides are frequently found: rutinose (6-α-rhamnosylglucose), neohesperidose (2-α-rhamnosylglucose), sophorose (2-β-glucosylglucose), sambubiose (2-β-xylosylglucose) and robinobiose (2-α-rhamnosylgalactose). Other groups of plant glycoside containing many different oligosaccharides are the saponins and steroidal alkaloids. Structures of a few oligosaccharides are illustrated in Figure 3.5. Oligosaccharides resemble monosaccharides in their chemical properties.

Figure 3.5 Structures of oligosaccharides

3.2.4 Polysaccharides

The chemistry of polysaccharides is simple, since these polymers contain only a few simple sugars in their structure. Indeed, the best known polysaccharides – cellulose and starch – are polymers of a single sugar, glucose. The structural complexity of polysaccharides is due to the fact that two sugar units can be linked together, through an ether linkage, in a number of different ways. The reducing end of one sugar (C1) can condense with any hydroxyl of a second sugar (at C2, C3, C4 or C6) so that during polymerization some sugars may be substituted in two positions, giving rise to branched chain structures. Furthermore, the ether linkage can have either an α- or a β-configuration, due to the stereochemistry of simple sugars, and both types of linkage can co-exist in the same molecule.

Although a few polysaccharides (e.g. cellulose) are, in fact, simple straight-chain polymers, the majority have partly branched structures. It is very difficult to determine complete sequences in such branched polysaccharides and, at present, it is only possible to define their structures in terms of a 'repeating unit' of oligosaccharide, a large number of which are linked together to produce the complete macromolecule. Some of the structural variation that can occur among polysaccharides is shown in Figure 3.6. The structures of the individual monosaccharides from which plant polysaccharides are composed have been given in an earlier section (see Figure 3.4).

The most familiar plant polysaccharides are cellulose and starch, but many other kinds are known, which have other sugars besides, or in addition to, glucose (Table 3.1). Cellulose represents a very large percentage of the combined carbon implants and is the most abundant organic compound of all. It is the fibrous material of the cell wall and is responsible, with lignin, for the structural rigidity of plants. Cellulose occurs in almost pure form (98%) in cotton fibres; the wood of trees is a less abundant source (40 – 50%) but the most important commercially for cellulose production. Chemically, cellulose is a β-glucan and consists of long chains of $\beta 1 \rightarrow 4$

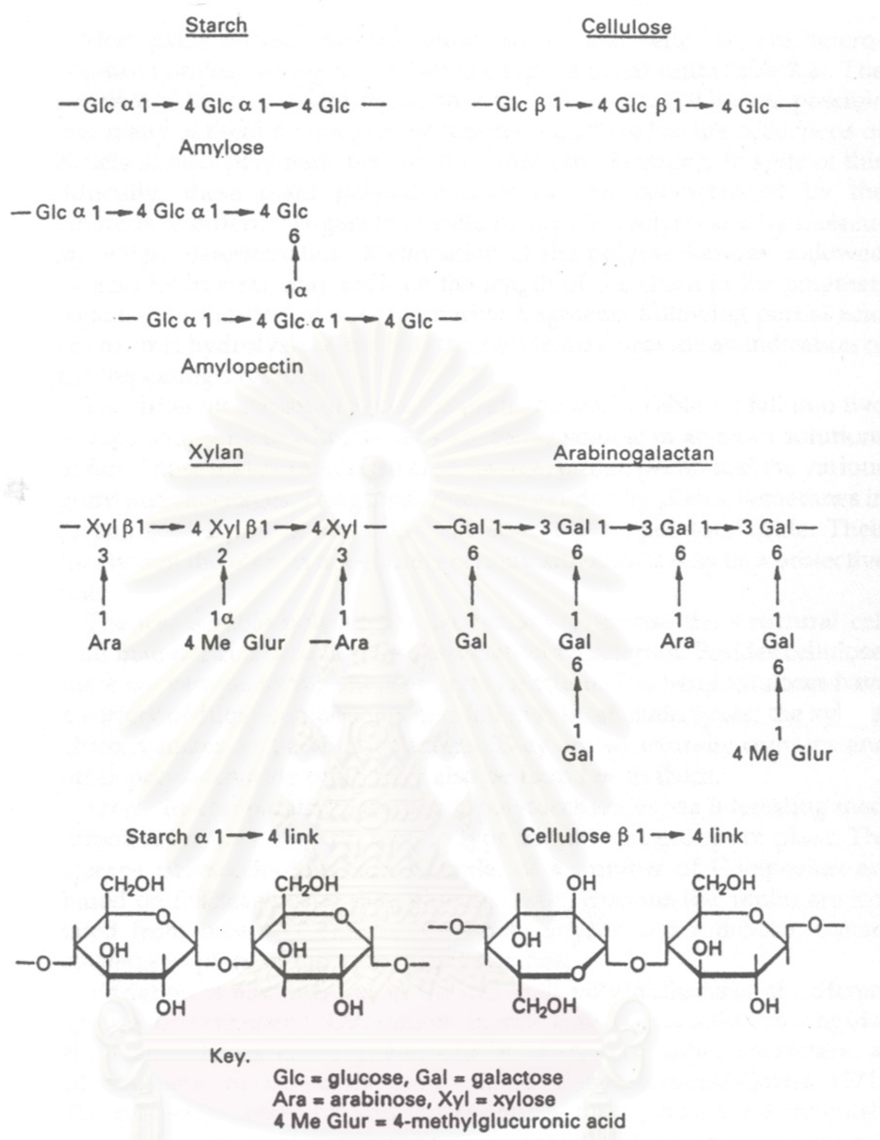

Figure 3.6 Structure 'repeating units' of some common polysaccharides

linked glucose units (Figure 3.6), the molecular weight varying from 100,000 to 200,000. Cellulose occurs in the plant cell as a crystalline lattice, in which long straight chains of polymer lie side by side linked by hydrogen bonding. Starch differs from cellulose in having the linkage between the glucose units $\alpha1\rightarrow4$ and not $\beta1\rightarrow4$ and also in having some branching in the chain. Starch, in fact, comprises two components, amylase and amylopectin, which can be separated from each other. Amylase (*ca.* 20% of the total starch) contains about 300 glucose units linked in a simple chain $\alpha1\rightarrow4$, which exists *in vivo* in the form of an α-helix. Amylopectin (*ca.* 80%) contains $\alpha1\rightarrow4$ chains, with regular branching of the main chain by secondary

$\alpha 1 \rightarrow 6$ linkages (see Figure 3.6). Its structure is thus of a randomly multibranched type. The two forms of starch are easily distinguished by their response to iodine, amylase giving a blue and amylopectin a reddish purple colour. Starch, itself, is the essential storage form of energy in the plant and starch granules are frequently located within the chloroplast close to the site of photosynthesis.

Most plant polysaccharides, unlike starch and cellulose, are heteropolysaccharides, having more than one type of sugar unit (Table 3.1). The problem of structural determination is an acute one and it is even possible that many of them do not possess unique sequences but are collections of closely similar polymers, produced by random branching. In spite of this difficulty, these plant polysaccharides can be characterized by the amounts of different sugars they yield on acid hydrolysis and by molecular weight determination. Methylation of the polysaccharides, followed by acid hydrolysis, may indicate the length of the chain in the polymer. Again, identification of oligosaccharide fragments following partial acid or enzymic hydrolysis of the polysaccharide may provide an indication of the 'repeating unit' present.

The different classes of polysaccharide shown in Table 3.1 fall into two groups according to whether they are easily soluble in aqueous solutions or not. Those that are soluble include starch, inulin, pectin and the various gums and mucilages. The gums which are exuded by plants, sometimes in response to injury or infection, are almost pure polysaccharide. Their function in the plant is not entirely certain, although it may be a protective one.

The less soluble polysaccharides usually comprise the structural cell wall material and occur in close association with lignin. Besides cellulose, there are various hemicelluloses in this fraction. The hemicelluloses have a variety of sugar components and fall into three main types: the xylans, glucomannans and arabinogalactans. They are structurally complex and other polysaccharide types may also be found with them.

Table 3.1 The main classes of polysaccharides in higher plants, algae and fungi

Class name	Sugar unit(s)	Linkage	Distribution
HIGHER PLANTS			
Cellulose	glucose	β1→4	universal as cell wall material
Starch-amylose	glucose	α1→4	universal as storage material
Starch-amylopectin	glucose	α1→4, α1→6	universal as storage material
Fructan	fructose (some glucose)	β2→1	in artichoke chicory, etc.
Xylan	xylose (some arabinose and uronic acid)	β1→4	widespread, e.g. in grasses
Glucomannan	glucose, mannose	β1→4	widespread, but especially in coniferous wood
Arabinogalactan	arabinose, galactose	1→3, 1→6	widespread, but especially in coniferous wood
Pectin	galacturonic acid (some others)	α1→4	widespread
Galactomannan	mannose, galactose	β1→4, α1→6	seed mucilages
Gum	arabinose, rhamnose, galactose, glucuronic acid	highly branched	in *Acacia* and *Prunus* species
ALGAE (Seaweeds)			
Laminaran	glucose	β1→3	
Polysaccharide sulphate	fucose (and others)	-	Phaeophyceae (brown algae)
Alginic acid	mannuronic and guluronic acids	-	
Amylopectin	glucose	α1→4, 1→6	Rhodophyceae (red algae)
Galactan	galactose	1→3, 1→4	Rhodophyceae (red algae)
Starch	glucose	α1→4, α1→6	
Polysaccharide sulphate	rhamnose, xylose, glucuronic acid	-	Chlorophyceae (green algae)
FUNGI*			
Chitin	*N*-acetylglucosamine	β1→4	widespread
Chitosan	glucosamine	β1→4	Zygomycetes
β-Glucan	glucose	β1→3, β1→6	widespread
α-Glucan	glucose	α1→3, 1→4	widespread
Mannan	mannose	α1→3, 1→6, 1→3	mainly Ascomycetes

* By contrast, bacterial cell wall is particularly complex. The polysaccharide material may contain, in addition to the usual sugars, such components as glycerol, ribitol, phosphate, amino acid and N-acetylglucosamine.

From the comparative viewpoint, polysaccharides are interesting macromolecules since they do vary in type in different groups of plant. The storage polysaccharides, for example, of a number of Composites are based on fructose rather than glucose. Thus, fructans like inulin are isolated from tubers of chicory, *Cichorium intybus* and artichoke, *Cynarascolymus* in place of the more usual starches.

Variation is also marked in the cell wall polysaccharides of different groups of organism. Differences in cell wall composition distinguish the major classes of fungi and can be used, with other characters, as phytogenetic markers in the group (Table 3.1). The cell wall polysaccharides of ferns and gymnosperms are distinguishable (from the angiosperms) by their frequent high mannose content. Within the angiosperms, the differences in cell wall materials are less pronounced, but they are sufficient to be detected by simple methods. For example, the xylan fraction in the hemicelluloses of monocotyledons is usually composed of arabinoxylans, whereas in dicotyledons it consists of chains of xyloglucans interspersed with 4-*O*-methylglucuronic acid residues.

Finally, the marked variation in polysaccharide composition between different algae (seaweed) must be mentioned. Three of the main classes – brown, red, and green – are clearly distinct in the types of polysaccharide they have (Table 3.1). Polysaccharides have been obtained from other classes (e.g. the blue – greens) but insufficient species have so far been examined to draw any taxonomic conclusion.

Chapter 4

Materials & Methods

4.1 Experimental Apparatus

1. Magnetic Stirrer/hot plate, RCT Basic, Ika Labortechnik, Germany.
2. Centrifuge, Kubota 5100, Kubota Corporation, Japan.
3. Spectrophotometer, Spectronic 20 Genesys, Spectronic Instruments, USA.
4. 4-digits balance, AB204, Mettler Toledo, Switzerland.
5. 1-digits balance, PT1200, Sartorius.
6. Household Blender, Cucina, Philips.
7. Centrifuge, Kubota 7820, Kubota Corporation, Japan.
8. Vortex mixer, Genie 2, Scientific Industries, INC., USA.
9. Hot air oven, ULM500, Memmert, Germany.
10. pH meter, MP220, Mettler Toledo, Switzerland.

4.2 Experimental Chemicals

1. Ethanol 95%, GPO, Thailand.
2. Glucose, Carlo Erba Reagenti, Italy.
3. Mannose, Fluka Chemical, Switzerland.
4. Suphuric Acid, BDH,
5. Sodium hydroxide, Carlo Erba Reagenti, Italy.
6. 3,5 dinitrosaliylic acid (DNS), Fluka Chemical, Switzerland.
7. Sodium potassium tartrate tetrahydrate, Carlo Erba Reagenti, Italy.
8. Copper sulphate, Carlo Erba Reagenti, Italy.

9. Sodium carbonate, Ajax Chemical, Australia.
10. Folin-Ciocalteau Reagent, Carlo Erba Reagenti, Italy.
11. Bovine serum albumin: BSA, Fluka Chemical, Switzerland.

4.3 Experimental Methods

4.3.1 Preparation aloe vera gel for precipitation process

For this investigation, whole aloe vera (Aloe barbadensis) leaves are the starting material.

1. Whole leaves were washed and the spikes were removed before slicing the leaf to separate the epidermis or green skin from the fillet, gel matrix.

2. The gel matrixes were washed extensively to remove the exudates from their surface.

3. The washed gel matrixes were homogenized in the household blender and the homogenate was filtered to yield a mucilaginous gel leaving a remaining fibrous fraction which was discards.

4.3.2 Precipitation process

1. The aloe vera gel was precipitated by adding solvent to the prepared gel and slowly stirred until the precipitate occurred as following conditions in Table 4.1.

2. The precipitate was removed by centrifugation and dried at 40ºC in hot air oven.

3. The dried material was ground with pestle and mortar to obtain a powder.

4. The powder – like product was analyzed with DNS method to obtain the carbohydrate fraction and with Lowry’s method to obtain the protein fraction.

4.3.3 Experimental conditions

Table 4.1 Experimental conditions

Parameters	Conditions
Solvent	Methanol, Ethanol and Iso-propanol
Solvent:Gel Ratio	3, 4, 5, 6 and 7
pH	3, 4, 5, 6, 7, 8 and 9*
Temperature, ºC	7, 15 and 32 (room temperature)

* pH of the system was adjusted by 0.1, 1 M hydrochloric acid and 0.1, 1 M sodium hydroxide.

Chapter 5

Results and Discussion

This chapter presents the experimental results and the discussion dealing with the precipitation of polysaccharide at various conditions. The fresh leaves of *Aloe barbadensis* Miller used in this investigation were collected from Luksong, Banpaew, Nakornpathom Province. Whole leaves were washed and the spikes, placed along their margins, were removed. The green covering of the leaves was carefully removed and the colourless, thick jelly was washed extensively to remove exudates from its surface. Further, the fillets were homogenized and filtered to yield the aloe vera gel ready for the precipitation process. After separation of precipitate from solvent, the precipitate was dried at 40ºC in a hot air oven and ground with pestle and mortar. The polysaccharide powder was hydrolyzed and quantified as monosaccharide against the standard curve of mannose (all data are presented in this chapter) and glucose (all data are shown in Appendix D). Soluble sugar in precipitate was also quantified without hydrolysis to check for free sugars presence in product. After that, total polysaccharide quantity was obtained from the monosaccharide quantity of hydrolyzed polysaccharide minus with soluble sugar quantity in precipitate. In addition, product impurity as protein in samples was investigated by Lowry's Method using Bovine Serum Albumin (BSA) as a standard material.

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5.1 Solvent Selection

Polysaccharide could be normally precipitated by alcohol. In this experiment, aloe vera gel was precipitated with 3 different solvents, namely methanol, ethanol, and iso-propanol. 400 ml of each solvent was added to 100 ml of aloe vera gel. The experiments were done in triplicate. The comparison of the average product dry weight and the polysaccharide quantity of various solvent treatments was presented in

Figure 5.1. As can be seen in Figure 5.1, precipitation with iso-propanol gave the highest average product dry weight. However, the maximum polysaccharide content and %polysaccharide with respect to average product dry weight were obtained when aloe vera gel was precipitated with ethanol as shown in Figure 5.1. In this case, the highest average product dry weight was 0.0517 g and the maximum polysaccharide content (as mannose) was 1.2407E-02 g. Therefore, ethanol was chosen as the solvent for the following study. Ethanol was also used as the precipitation solvent of aloe vera gel according to the prior works of Mandal and Das, 1980, Gowda et al., 1979, and Femenia et al., 1999.

Table 5.1 Solvent selection data

Solvent	Average Product Dry Weight, g	Polysaccharide Content, g (as Mannose)	% Polysaccharide per dry weight
Methanol	0.0367 ± 4.7149E-03	7.1344E-03	17.8361
Ethanol	0.0435 ± 1.5177E-03	1.2407E-02	28.7189
Iso-propanol	0.0517 ± 1.3051E-03	1.0675E-02	20.8098

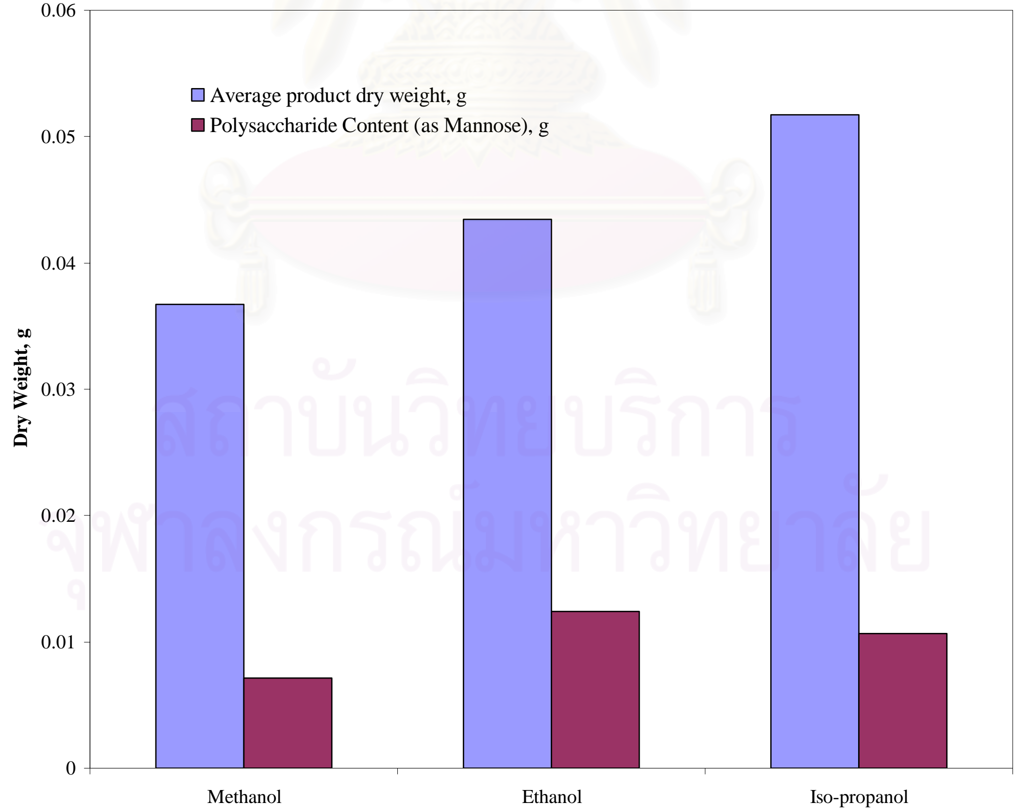

Figure 5.1 The effect of solvent on average product dry weight and polysaccharide content

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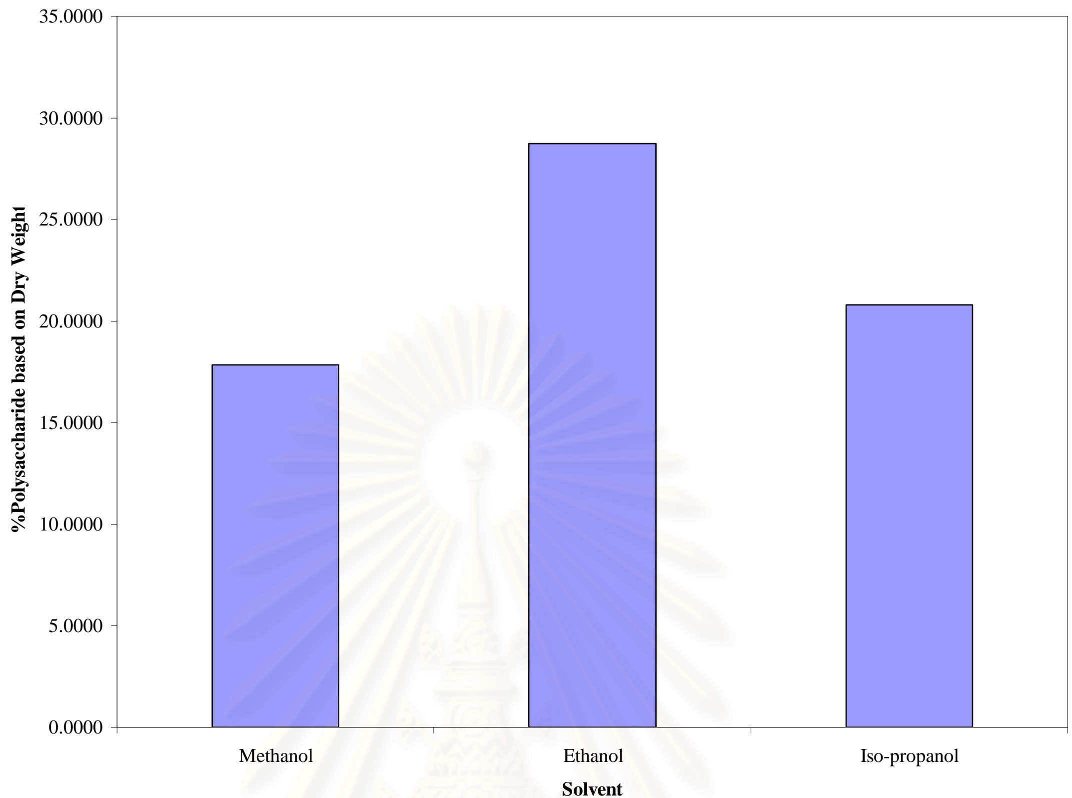

Figure 5.2 %Polysaccharide based on the average product dry weight of different solvents

5.2 Separation Factors

5.2.1 Solvent to gel ratio

The polysaccharide composition of the aloe vera gel was influenced by growing conditions, e.g. irrigation, leaf age, season (Yaron, 1993), and irradiance (Paez et al., 2000), therefore the solvent volume used in the precipitation process was determined in order to obtain the suitable ratio for this plant material. In this experiment, 100 ml of aloe vera gel was precipitated with ethanol in the solvent to gel ratio ranging from 3 to 7. The experiments were done in triplicate. Table 5.2 showed the average product dry weight and polysaccharide content of each solvent to gel ratio. In order to compare the results, Figure 5.3 was presented. As can be seen in Figure 5.3, when the solvent volume increased the average product dry weight also

increased until the solvent to gel ratio at 6, it tended to decrease at solvent to gel ratio 7. Focusing on the polysaccharide content, solvent to gel ratio ranging from 3 to 6 gave a slightly difference of the polysaccharide content in the product dry weight but the polysaccharide content of solvent to gel ratio 7 was lower. The maximum product dry weight and polysaccharide content were obtained from solvent to gel ratio 6. On the contrary, the protein content was determined as a product impurity. As can be seen in Figure 5.5, the tendency of protein content was higher when the ratio was increased, however, separation with solvent to gel ratio ranged from 4 to 6 showed insignificant difference in percentages of protein content. In term of %protein in dry product, the lowest %protein yielded from solvent to gel ratio 6. In this case, the highest average product dry weight was 0.1877 g and polysaccharide content (as mannose) was 4.9761E-02 g. Considering maximum yield of product dry weight, polysaccharide content and low impurity, the suitable solvent to gel ratio for the precipitation was 6.

Table 5.2 Solvent to gel ratio data

Solvent to gel ratio	Average Product Dry Weight, g	Polysaccharide Content, g (as Mannose)	% Polysaccharide per dry weight
3	0.1524 ± 3.0610E-03	4.5398E-02	29.79
4	0.1690 ± 4.9642E-03	4.6367E-02	27.44
5	0.1755 ± 1.5044E-03	4.7642E-02	27.15
6	0.1877 ± 1.8175E-03	4.9761E-02	26.51
7	0.1844 ± 9.0266E-03	4.2474E-02	23.03

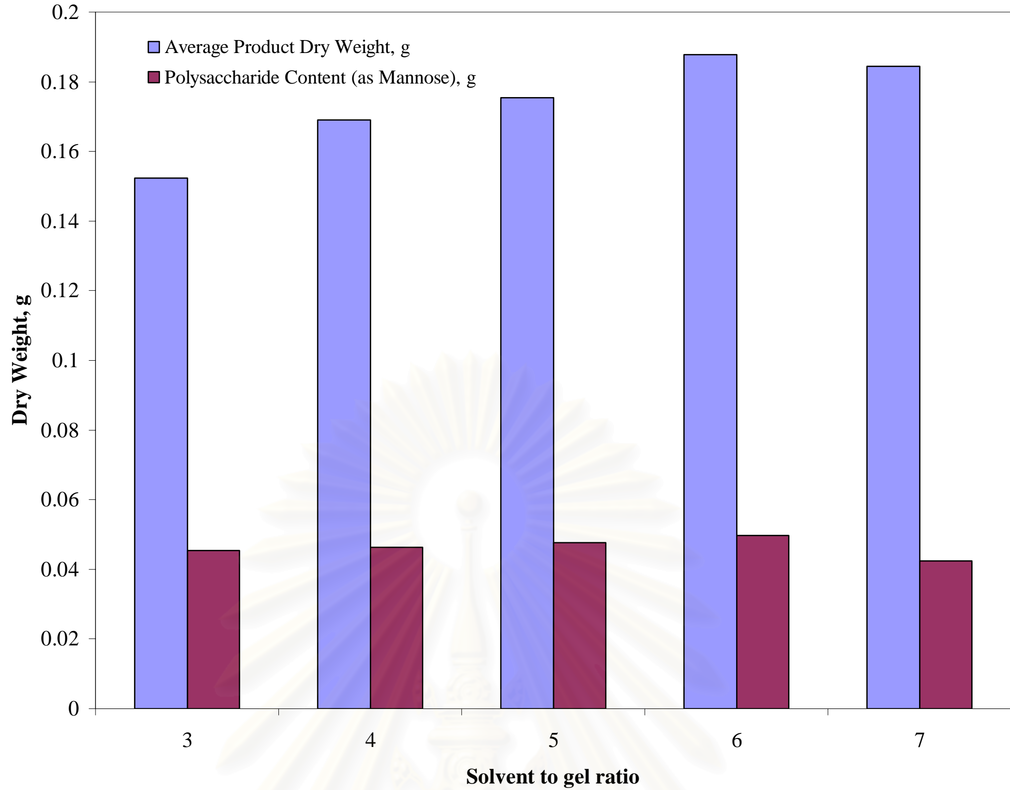

Figure 5.3 The effect of solvent to gel ratio on average product dry weight and polysaccharide content

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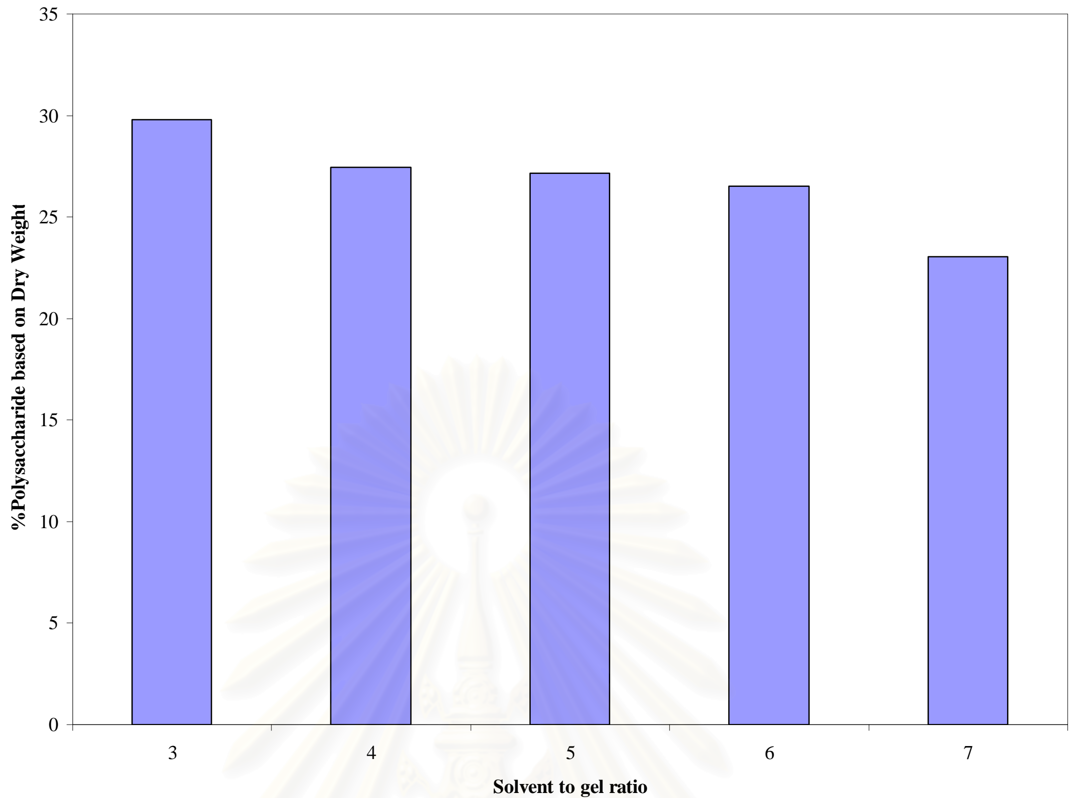

Figure 5.4 %Polysaccharide based on the average product dry weight of solvent to gel ratio ranging from 3 to 7

Table 5.3 Protein content at several solvent to gel ratio

Solvent to gel ratio	Average Product Dry Weight, g	Protein Content, g (as BSA)	% Protein per dry weight
3	0.1524 ± 3.0610E-03	4.0669E-04	0.27
4	0.1690 ± 4.9642E-03	5.1822E-04	0.31
5	0.1755 ± 1.5044E-03	5.1723E-04	0.29
6	0.1877 ± 1.8175E-03	5.3017E-04	0.28
7	0.1844 ± 9.0266E-03	7.4467E-04	0.40

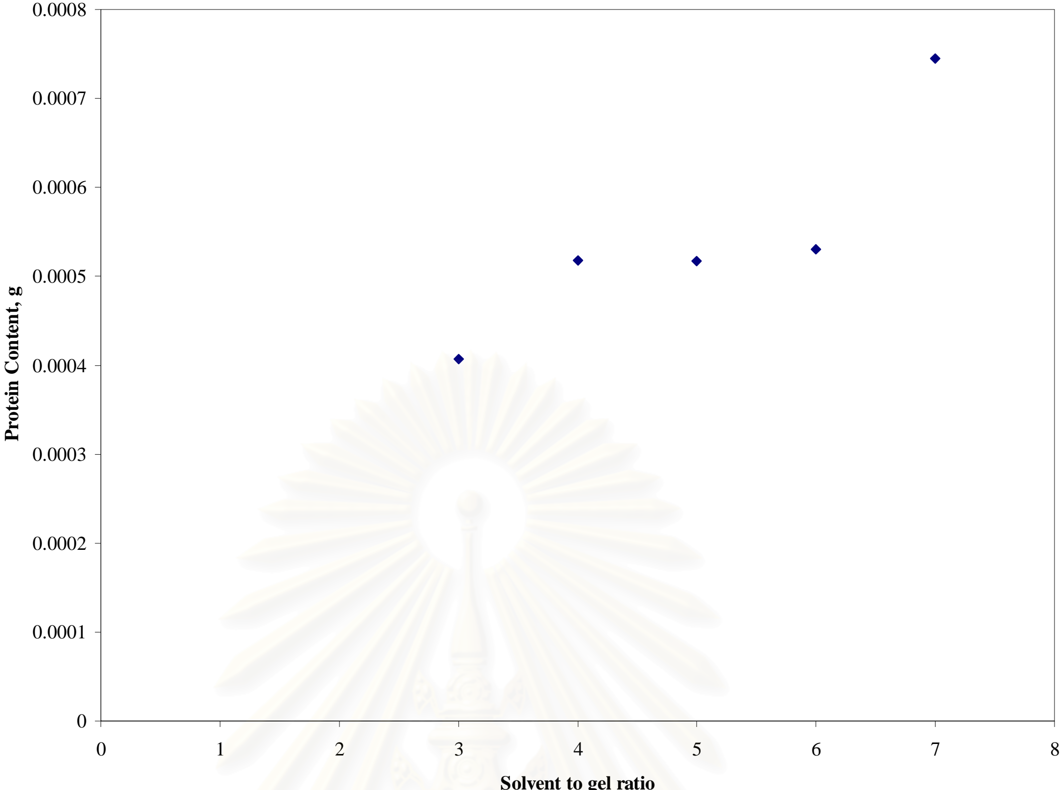

Figure 5.5 The effect of solvent to gel ratio on protein content

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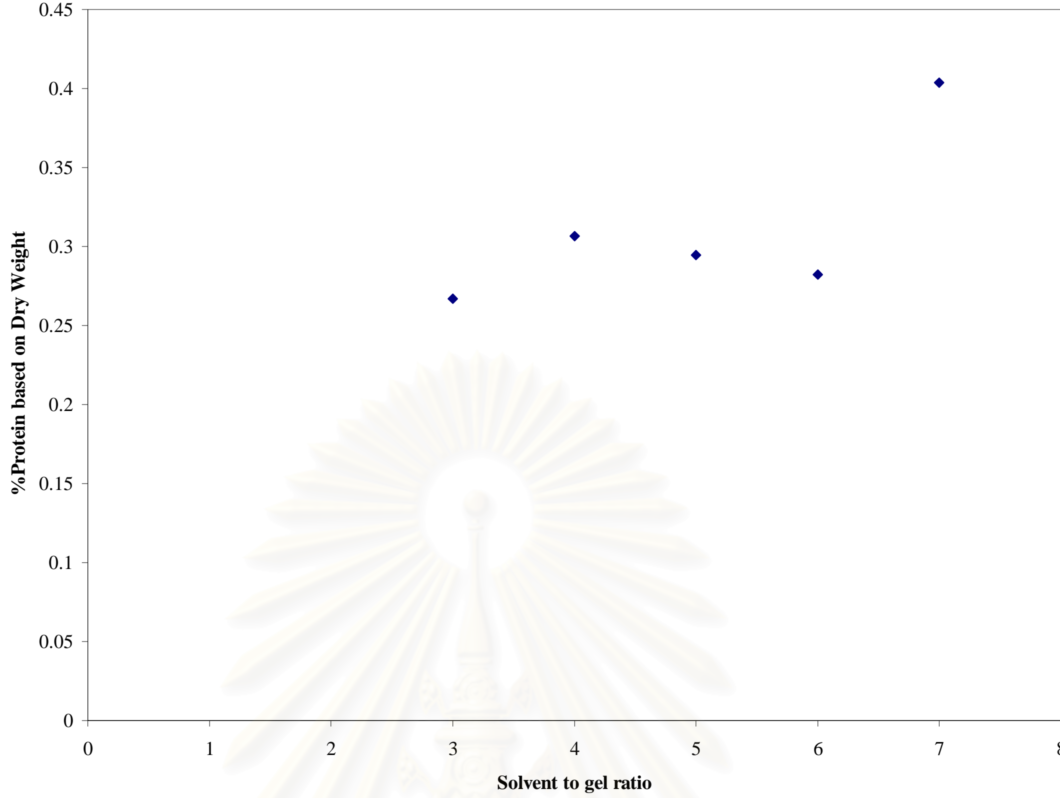

Figure 5.6 %Protein based on the average product dry weight of solvent to gel ratio ranging from 3 to 7

5.2.2 pH

The experiments in this section focused on the effect of pH on solute solubility and also protein solubility. When pH was equal to pI, protein solubility was lowest due to zero net charge of protein molecule and lowest intermolecular force. In this study, aloe vera gel was precipitated with ethanol at solvent to gel ratio 6, following the data obtained from section 5.1 and 5.2.1. In each treatment, 600 ml of ethanol was added into the aloe vera gel, 100 ml, in order to obtain the precipitate. Then pH of the systems was adjusted to be varied from 3 to 9. The experiments were done in triplicate for each pH treatment with non-adjusted pH precipitations as the control. The data obtained from the experimental study was summarized in Table 5.4. When the system pH was adjusted to 3 – 4, the total product dry weight and polysaccharide content from the precipitation was lowest, although, %polysaccharide per dry weight was highest at pH 3. The maximum precipitation of dry weight was obtained when the system pH was adjusted to 7 – 8, nevertheless no significant difference of

polysaccharide content from the precipitation was observed when the system pH was changed from 5 – 9. Therefore, although the pH of system affected the overall solid precipitation, changing pH has not affected polysaccharide precipitation, but may cause hydrolysis especially at very low pH. On the contrary, the protein tendency was not the same as the average product dry weight. At pH 3, the precipitation of protein was maximum and then protein precipitation decreased when system pH was higher as shown in Figure 5.9. From system pH ranging from 4 to 9 and the control experiment, the very small difference in protein content was observed. Considering the results from Table 5.4, precipitation at pH 7 gave the highest average product dry weight but the maximum polysaccharide content was reported at the control experiment, non-adjusted pH precipitation. Therefore, non-adjusted pH precipitation was chosen for the following experimentation.

Table 5.4 pH data

pH	Average Product Dry Weight, g	Polysaccharide Content, g (as Mannose)	% Polysaccharide per dry weight
3	0.0932 ± 9.7893E-03	3.8418E-02	41.22
4	0.1373 ± 2.1385E-03	3.3047E-02	24.06
5	0.1896 ± 1.4731E-03	4.5933E-02	24.23
6	0.2080 ± 2.4583E-03	4.6149E-02	22.18
7	0.2139 ± 1.0214E-03	4.4793E-02	20.94
8	0.2105 ± 9.6421E-03	4.5196E-02	21.47
9	0.1987 ± 3.9950E-03	4.8870E-02	24.60
non-adjusted pH (5.52)	0.1877 ± 1.8175E-03	4.9761E-02	26.51

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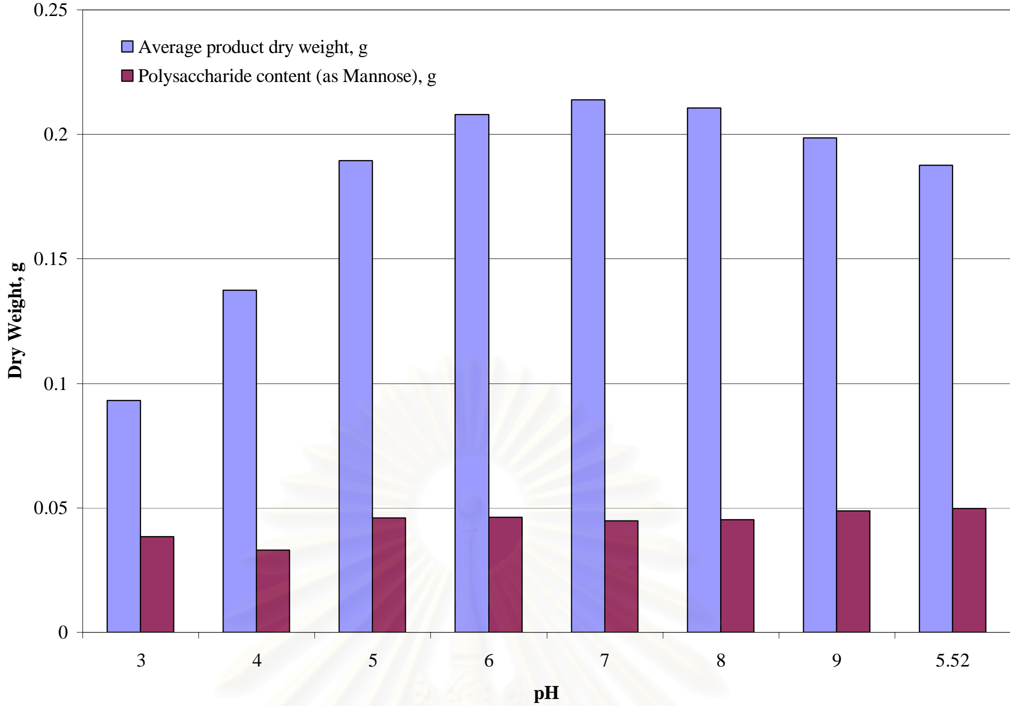

Figure 5.7 The effect of pH on average product dry weight and polysaccharide content

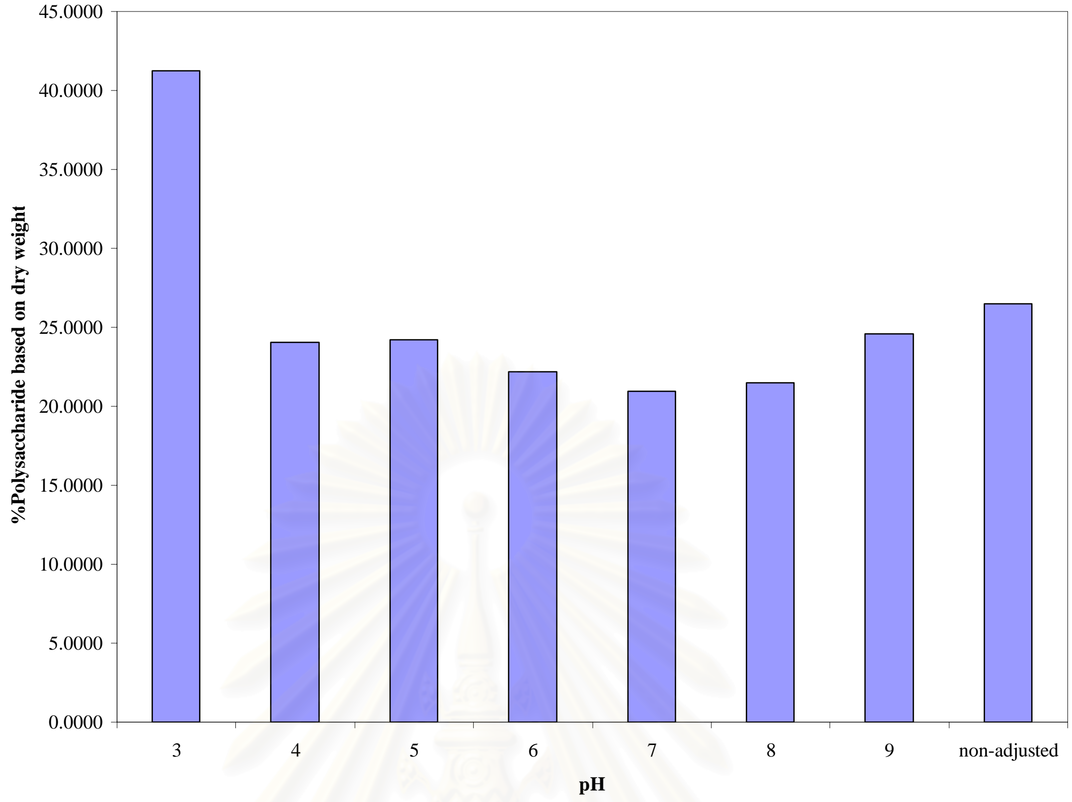

Figure 5.8 %Polysaccharide based on the average product dry weight of pH ranging from 3 to 9 and non-adjusted pH precipitation

Table 5.5 Protein content at several pH

pH	Average Product Dry Weight, g	Protein Content, g (as BSA)	% Protein per dry weight
3	0.0932 ± 9.7893E-03	7.2626E-04	0.78
4	0.1373 ± 2.1385E-03	4.1715E-04	0.30
5	0.1896 ± 1.4731E-03	4.7652E-04	0.25
6	0.2080 ± 2.4583E-03	4.7168E-04	0.23
7	0.2139 ± 1.0214E-03	4.3521E-04	0.20
8	0.2105 ± 9.6421E-03	4.6688E-04	0.22
9	0.1987 ± 3.9950E-03	4.5803E-04	0.23
non-adjusted pH (5.52)	0.1877 ± 1.8175E-03	5.3017E-04	0.28

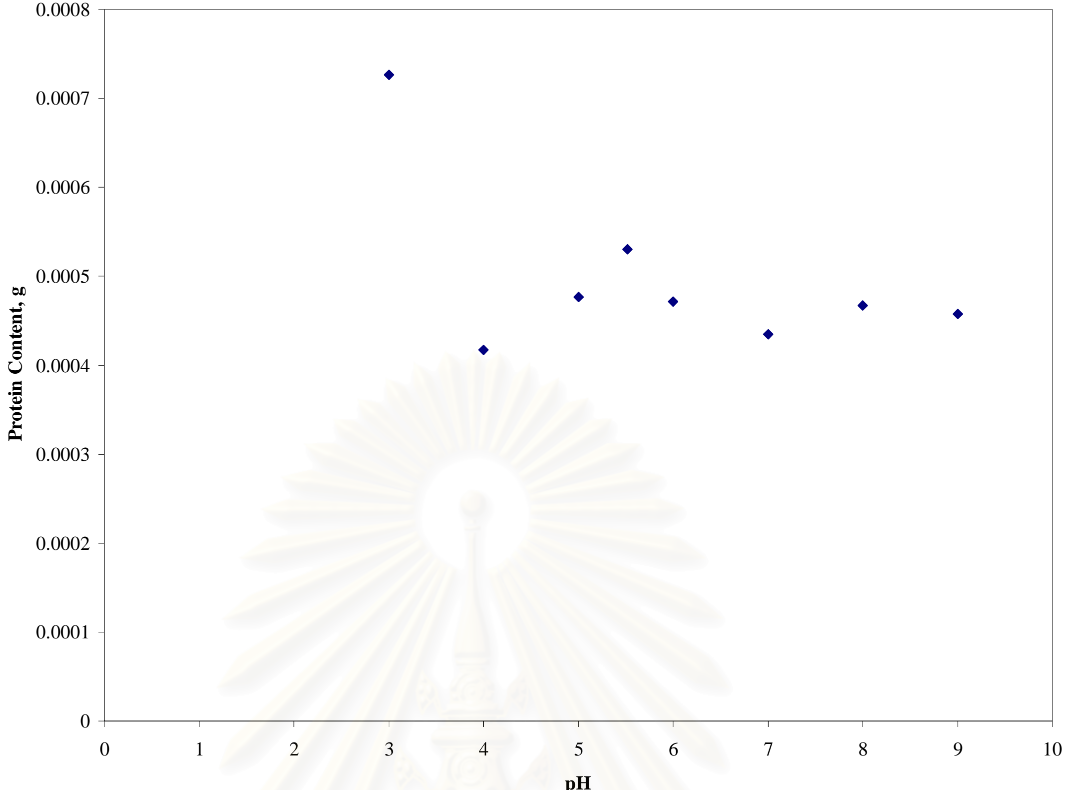

Figure 5.9 The effect of pH on protein content

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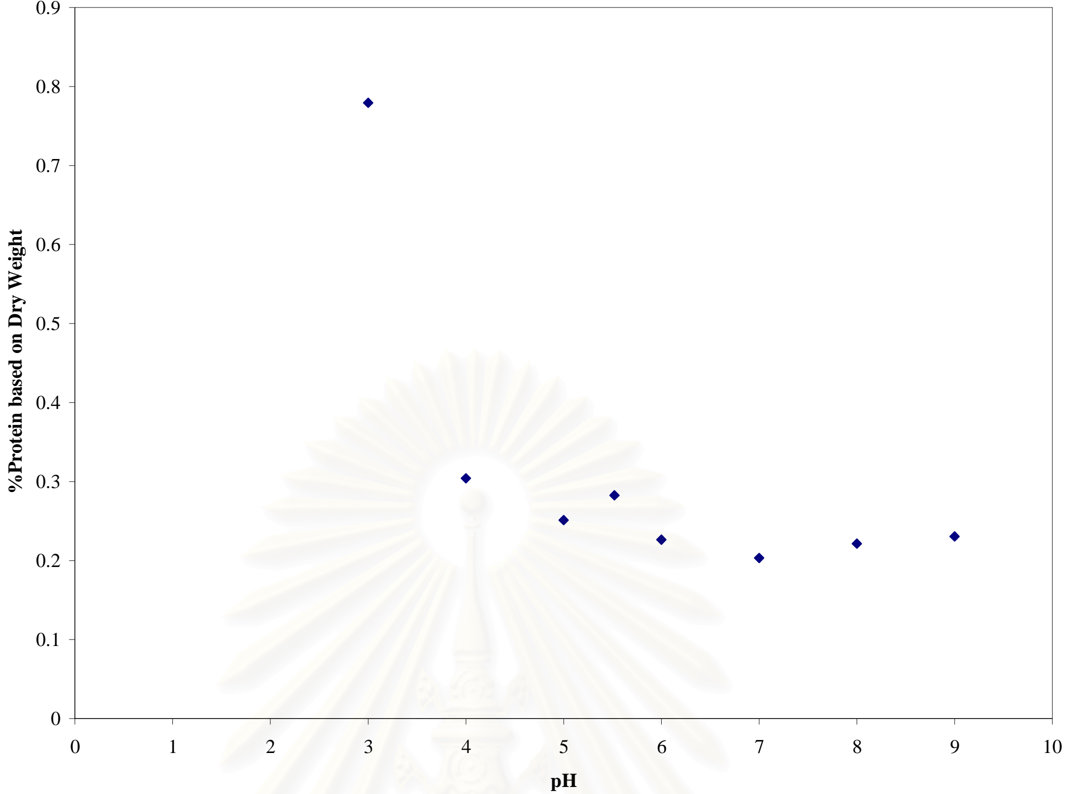

Figure 5.10 %Protein based on the average product dry weight of pH ranging from 3 to 9 and non-adjusted pH precipitation

5.2.3 Temperature

The effect of temperature on precipitation process was studied by precipitating aloe vera gel at 3 different temperatures. Firstly, the ethanol was kept in the cooling bath to lower the temperature to 15°C and in the refrigerator in order to lower temperature to 7°C. After that 1 litre of aloe vera gel was added into the prepared ethanol, 6 litres, and the precipitation temperature was controlled at 7, 15, and 32°C (room temperature). It was found that there were two different precipitates which can be seen obviously. The first one was a fluffy fibrous white material floating at the surface of the solvent and the other were small white particles at the bottom of the precipitating container. From this observation, the samples were taken separately on the different precipitates and named “Float precipitate” and “Bottom precipitate”. Finally, the products were elemental analyzed for carbon, hydrogen, and nitrogen content by CHNS /O Analyzer, Perkin Elmer PE 2400 Series II, as shown in

Appendix E. From Table 5.6, the maximum total product dry weight, 1.8571 grams, occurred at 32°C and the maximum float and bottom precipitates also obtained at this condition. At each temperature, the floating material dry weight was higher than the bottom one. Generally, operating temperature has influence on the solubility of solids in liquids depended on the heat of dissolution. However, from the experimental studies, the polysaccharide precipitation was only slightly increased at 32ºC. At operating temperature ranging from 7 – 32ºC, no significant effect of temperature on precipitation was observed; only at 15ºC that a lower ratio of C:N was noticed.

Considering on the elemental analysis, the carbon component represented the polysaccharide content. The protein was also investigated and presented in term of nitrogen component. The results were reported in Table 5.10 – 5.12. For carbon component, the maximum C component in total precipitate was 0.6003 g, Table 5.12. In the float part (Table 5.10) and bottom precipitate (Table 5.11) were 0.3334 and 0.2705 g, respectively. Focusing on the temperature effect, the temperature conditions of the maximum C component were summarized in Table 5.14. At each temperature, C component in float was higher than that in bottom precipitate. For nitrogen component, the minimum N component in total precipitate (Table 5.12) was 2.2401E-02 g. In the float (Table 5.10) and bottom (Table 5.11) were 1.3992E-02 and 8.4085E-03 g, respectively. Focusing on the temperature effect, the temperature conditions of the minimum N component were tabulated in Table 5.15. At each temperature, N component in float was higher than bottom precipitate.

Table 5.6 Product dry weight at various temperatures

Temperature, °C	Product Dry Weight, g		
	Float Precipitate	Bottom Precipitate	Total Precipitate
7	0.9086	0.8317	1.7403
15	0.9279	0.6722	1.6001
32	1.0211	0.8360	1.8571

Table 5.7 Polysaccharide content in float precipitate

Temperature, ºC	Product Dry Weight, g	Polysaccharide Content, g (as Mannose)	% Polysaccharide per dry weight
7	0.9086	NA	NA
15	0.9279	3.3967E-01	36.61
32	1.0211	3.8583E-01	37.79

Table 5.8 Polysaccharide content in bottom precipitate

Temperature, ºC	Product Dry Weight, g	Polysaccharide Content, g (as Mannose)	% Polysaccharide per dry weight
7	0.8317	NA	NA
15	0.6722	1.6035E-01	23.86
32	0.8360	2.4075E-01	28.80

Table 5.9 Polysaccharide content in total precipitate

Temperature, ºC	Product Dry Weight, g	Polysaccharide Content, g (as Mannose)	% Polysaccharide per dry weight
7	1.7403	NA	NA
15	1.6001	5.0003E-01	31.25
32	1.8571	6.2657E-01	33.74

Total 5.10 Elemental component in float precipitate

Temperature, ºC	Product Dry Weight, g	Component in Dry weight, g		
		Carbon	Hydrogen	Nitrogen
7	0.9086	0.3277	5.4162E-02	1.3992E-02
15	0.9279	0.3334	6.0740E-02	1.8679E-02
32	1.0211	0.3297	4.6858E-02	1.4775E-02

Table 5.11 Elemental component in bottom precipitate

Temperature, ºC	Product Dry Weight, g	Component in Dry weight, g		
		Carbon	Hydrogen	Nitrogen
7	0.8317	0.2559	4.5885E-02	8.4085E-03
15	0.6722	0.1983	2.7096E-02	8.6176E-03
32	0.8360	0.2705	3.1592E-02	8.5439E-03

Table 5.12 Elemental component in total precipitate

Temperature, ºC	Product Dry Weight, g	Component in Dry weight, g		
		Carbon	Hydrogen	Nitrogen
7	1.7403	0.5836	1.0005E-01	2.2401E-02
15	1.6001	0.5318	8.7837E-02	2.7296E-02
32	1.8571	0.6003	7.8451E-02	2.3319E-02

Table 5.13 C:N ratio of precipitates of several temperatures

Temperature, ºC	C : N Ratio		
	Float Precipitate	Bottom Precipitate	Total Precipitate
7	23.42 : 1	30.43 : 1	26.05 : 1
15	17.85 : 1	23.02 : 1	19.48 : 1
32	22.32 : 1	31.67 : 1	25.74 : 1

Table 5.14 Temperature of maximum carbon component in precipitates

Maximum Carbon Component in precipitate	Temperature, °C
Total precipitate	32
Float precipitate	15
Bottom precipitate	32

Table 5.15 Temperature of minimum nitrogen component in precipitates

Minimum N Component in precipitate	Temperature, °C
Total precipitate	7
Float precipitate	7
Bottom precipitate	7

5.2.4 Stage of Precipitation

This section investigated the effect of precipitation stage on the separation. Ethanol 6000 ml was divided into 6 equal parts and then added into 1000 ml aloe vera gel, part by part (6 stage precipitation). The results were compared with the test with the single stage precipitation with 6000 ml ethanol.

Table 5.16 Product dry weight at each stage

Stage	Product Dry Weight, g		
	Float Precipitate	Bottom Precipitate	Total Precipitate
1	0.0000	0.0000	0.0000
2	0.0000	0.1683	0.1683
3	0.0967	0.4919	0.5886
4	0.0000	0.4400	0.4400
5	0.0000	0.0000	0.0000
6	0.0000	0.0000	0.0000
Total	0.0967	1.1002	1.1969
Single Stage	1.0999	0.5665	1.6664

As can be seen in Table 5.16, float precipitate and total product dry weight of 6 stage precipitation was lower than the single stage precipitation which could be the cause from the material loss during the operation. The step precipitation gave more bottom precipitate dry weight but less float precipitation dry weight. From the

experimental study, no more precipitate occurred after the 4th stage of the precipitation (with 4000 ml total volume of 95% ethanol).

5.2.5 Difference between homogenized aloe vera gel and dripped aloe vera gel

In this experiment, aloe vera leaves were prepared as the same procedure described in 4.3.1.1 and 4.3.1.2 in order to obtain gel matrix. Then the matrix was sliced into 0.5 cm in diameter of cylindrical pieces by machine and put in a plastic basket to allow the gel to drip. For comparison, the preparing of aloe vera gel followed the method in Chapter 4 was made. Then the homogenized and dripped aloe vera gel viscosity were measured by U-tube viscometer. The data was shown in Table 5.17. In the next step, the prepared aloe vera gel samples were precipitated with ethanol at solvent to gel ratio 6, room temperature and non-adjusted pH. The precipitation obtained from the float and bottom parts were used for the analysis. After drying and grinding process, the products were analyzed with Kjeldahl machine in order to determine the nitrogen component in the precipitate, as shown in Appendix E. Table 5.18 presented the product dry weight of each precipitate. From the data shown, the difference between float and bottom dry weight of the dripped gel is higher. The nitrogen component in precipitate was summarized in Table 5.19. The total nitrogen component of the homogenized gel was higher than the dripped gel. Considering nitrogen ratio in the precipitates, the N content difference between float and bottom material of the homogenized gel was lower than the dripped gel. This may be due to the effect of homogenization on gel matrix. When the gel matrix was homogenized, the long chain polysaccharide in the gel might be cut by blading and this shorten the chain structure and homogenized the gel matrix. On the other hand, the dripped gel was obtained from the non – homogeneous process. The polysaccharide chain in the gel was almost maintained as the same structure as the natural gel matrix. When the long chain polysaccharide was precipitated, more floating part was obtained at the surface of the container because of its low density when compared to the density of solvent as observed from precipitation of dripped gel. For homogenized gel, the higher release of N compounds (could be proteins) into

the bottom precipitate was observed. This could be explained from the change of the structure of gel matrix after homogenized step.

Table 5.17 Homogenized and dripped gel viscosity

	Viscosity, cp
Homogenized Aloe vare gel	4.0425
Dripped Aloe vare gel	7.7177

Table 5.18 Product dry weight for homogenized gel and dripped gel

Aloe vera gel	Product Dry Weight, g		
	Float Precipitate	Bottom Precipitate	Total Precipitate
Homogenized	4.1568	4.4056	8.5624
Dripped	6.6382	1.3300	7.9682

Table 5.19 Nitrogen content of precipitates from homogenized and dripped gel

Aloe vera gel	Nitrogen Content, g		
	Float Precipitate	Bottom Precipitate	Total Precipitate
Homogenized	0.0759	0.0537	0.1296
Dripped	0.1042	0.0060	0.1102

Chapter 6

Conclusions and Recommendations

6.1 Conclusions

The investigation in this work leads to the following conclusions:

1. The suitable solvent for precipitation of polysaccharide from aloe vera gel was 95% ethanol. The average product dry weight 0.0435 g was obtained from the precipitation of 100 ml of aloe vera gel in 400 ml of solvent. The product was composed of polysaccharide 28.72% by weight.
2. Important separation factors were investigated. The solvent (95% ethanol) to gel ratio 6:1 yielded the maximum product dry weight of 0.1877 g when 100 ml aloe vera gel was precipitated. Polysaccharide and protein content with respect to the average product dry weight were 26.51% and 0.28%, respectively.
3. Considering pH of the system, the maximum product dry weight was obtained when pH was not adjusted (pH = 5.52).
4. Focusing on the system temperature, the suitable temperature in precipitation process was 32ºC (room temperature). When 1 litre of aloe vera gel was precipitated by 6 litres of 95% ethanol, the product dry weight was 1.8571 g. %Polysaccharide with respect to product dry weight was 33.74. From elemental analysis, the dry product was composed of carbon and nitrogen with the contents of 32.32 and 1.26% by weight, respectively. C:N ratio was 25.74:1.
5. As for the effect of the stage of precipitation, for precipitation of 1000 ml of aloe vera gel in 6000 ml of 95% ethanol, the single stage precipitation yielded 1.6664 g of product dry weight and 1.1969 g was obtained from 6 stages precipitation.

6. Homogenization might shorten the structure chain of polysaccharide. More release of N compound in bottom precipitate was observed when homogenization was used to prepare gel matrix compared to dripping process.

6.2 Recommendations

1. The factor that effect on impurity should be further observed.
2. The dry product should be further analyzed in order to obtain information of all components.
3. All experiments should be done from the same aloe vera gel to eliminate the plant material diversity.
4. The aloe vera leaf age for harvest should be highly considered to allow the least variety of plant material.

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จุฬาลงกรณ์มหาวิทยาลัย

APPENDICES

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

APPENDIX A

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

Appendix A

A.1 DNS Method

Chemicals

1. Glucose
2. Mannose
3. Sulphuric acid
4. Sodium hydroxide
5. DNS
6. Sodium potassium tartrate tetrahydrate

DNS Reagent Preparation

Dissolve 10 grams DNS (3,5 dinitrosalicylic acid) in 200 ml 2M sodium hydroxide with warming and vigorous stirring. Dissolve 300 grams sodium potassium tartrate tetrahydrate in 500 ml distilled water. Mix these two solutions and make up to 1 litre with distilled water

A.1.1 Soluble Sugar Analysis

Method

1. Weigh accurately about 0.1 – 0.2 grams of the powdered samples.
2. Add 20 ml of RO water, mix and leave the solutions overnight.
3. Centrifuge at 3000 rpm for 10 minutes in order to get supernatant.
4. Pipette 1.0 ml of the supernatant into a test tube and add 1.0 ml DNS reagent
5. Heat all tubes in boiling water for 5 minutes to allow the reaction occur. Cool in an ice bath.
6. Add 4 ml of RO water and mix well.
7. Read an absorbance at 540 nm.

A.1.2 Polysaccharide Analysis

Method

1. Weigh accurately about 0.1 – 0.2 g of the powdered sample into a test tube.
2. Add 10 ml of 1.5 M sulphuric acid and heat in boiling water for 40 minutes (Hydrolyzed time study was done and all data was shown in the following part). Cool in an ice bath.
3. Add carefully 12 ml 10% sodium hydroxide and mix.
4. Centrifuge at 3000 rpm for 10 minutes in order to get a supernatant.
5. Pipette 1.0 ml supernatant and add RO water to make 25% supernatant solution.
6. Pipette 1.0 ml 25% supernatant solution into a test tube and add 1.0 ml DNS reagent.
7. Heat all tubes in boiling water for 5 minutes to allow the reaction occurred. Cool in an ice bath.
8. Add 10 ml RO water and mix well.
9. Read an absorbance at 540 nm.

A.1.3 Hydrolyzed time for DNS Method

Table A1 Glucose standard curve data

Item	Glucose Concentration, mg/ml	Absorbance @ 540 nm
1	0.00	0.000
2	0.25	0.038
3	0.50	0.101
4	1.00	0.218
5	1.25	0.294
6	1.50	0.366
7	2.00	0.483

Table A2 Mannose standard curve data

Item	Mannose Concentration, mg/ml	Absorbance @ 540 nm
1	0	0
2	0.1	0.007
3	0.2	0.031
4	0.3	0.054
5	0.4	0.078
6	0.5	0.112
7	0.6	0.14
8	0.7	0.167
9	0.8	0.197
10	0.9	0.218
11	1	0.251

Table A3 Polysaccharide content in dry product

Item	Hydrolyzed time, min	%Polysaccharide	
		As glucose	As mannose
1	20	17.66	19.20
2	40	21.70	23.78
3	60	19.82	22.30

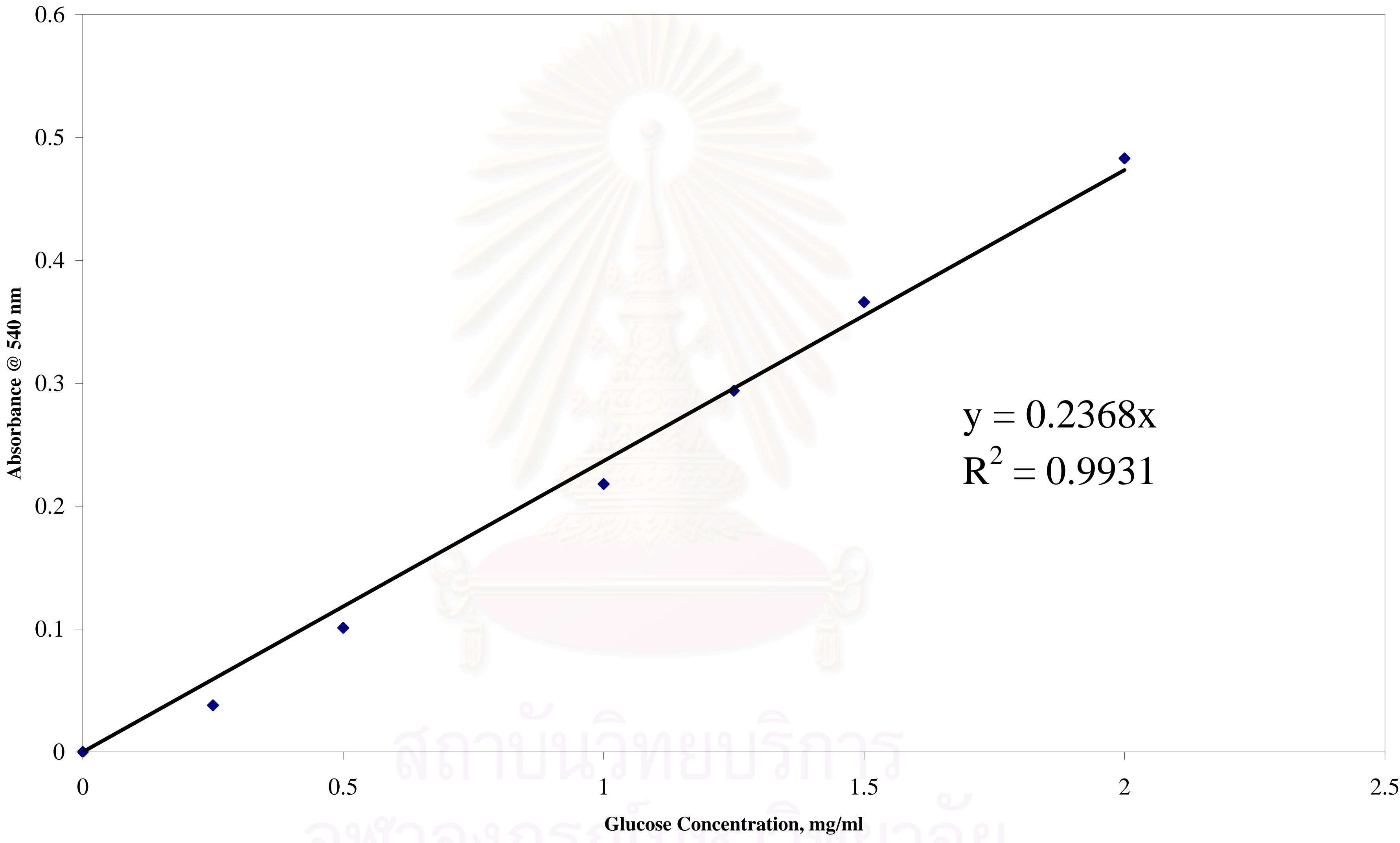

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Figure A1 Glucose standard curve for hydrolized polysaccharide analysis

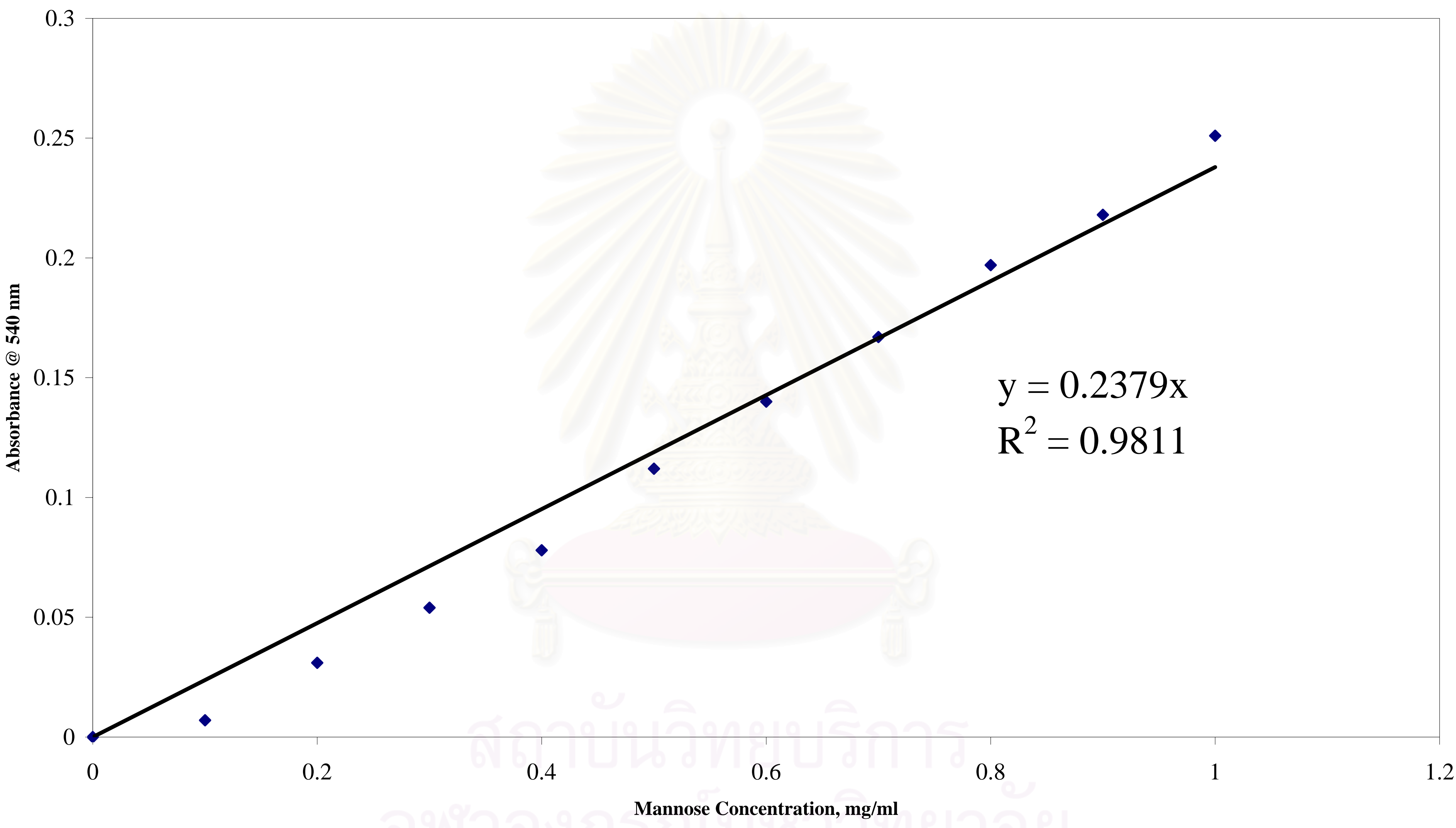

Figure A2 Mannose standard curve for hydrolyzed polysaccharide analysis

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A.2 Lowry's method

Chemicals

1. Bovine serum albumin
2. Copper sulphate
3. Sodium potassium tartrate
4. Sodium hydroxide
5. Sodium carbonate
6. Folin-Ciocalteau reagent

Solutions preparation

1. Solution A is 1% w/v copper sulphate solution.
2. Solution B is 2% w/v sodium potassium tartrate solution.
3. Solution C is 0.2 M sodium hydroxide.
4. Solution D is 4% w/v sodium carbonate.
5. Solution E is a fresh reagent make from 49 ml solution C, 49 ml solution D, 1 ml solution A, and 1 ml solution B.
6. Solution F is 50% v/v folin-ciocalteau reagent solution. (Dilute 1:1 with RO water)

Method

1. Pipette 0.5 ml sample (protein < 0.5 mg) into a test tube and add 2.5 ml solution E. Mix and wait for 10 minutes.
2. Then add 0.25 ml solution F and leave for 30 minutes.
3. Read an absorbance at 750 nm.
4. Standard curve. BSA 0.05 – 0.3 mg/ml.

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APPENDIX B

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Appendix B

Table B1 Raw material for solvent selection

Aloe vera leaves weight	10,000	g
Aloe vera gel matrix weight	6,100	g
Aloe vera gel volume	4,700	ml
Aloe vera gel density	0.9820	g/ml

Table B2 Raw material for solvent to gel ratio and pH experiments

Aloe vera leaves weight	20,000	g
Aloe vera gel matrix weight	12,000	g
Aloe vera gel volume	8,100	ml
Aloe vera gel density	0.9812	g/ml

Table B3 Raw material for temperature experiment

Aloe vera leaves weight	6,800	g
Aloe vera gel matrix weight	4,120	g
Aloe vera gel volume	3,035	ml
Aloe vera gel density	0.9838	g/ml

Table B4 Raw material for stage of precipitation experiment

Aloe vera leaves weight	10,000	g
Aloe vera gel matrix weight	5,900	g
Aloe vera gel volume	4,200	ml
Aloe vera gel density	0.9906	g/ml

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Table B5 Raw material for difference between homogenized and dripped aloe vera gel

Item	Value	Unit
Homogenized aloe vera gel		
Aloe vera leaves weight	6,800	g
Aloe vera gel matrix weight	4,120	g
Aloe vera gel volume	3,035	ml
Aloe vera gel density	0.9838	g/ml
Dripped aloe vera gel		
Aloe vera leaves weight	7,000	g
Aloe vera gel matrix weight	4,240	g
Aloe vera gel volume	1,500	ml
Aloe vera gel density	0.9906	g/ml

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APPENDIX C

สถาบันวิทยบริการ
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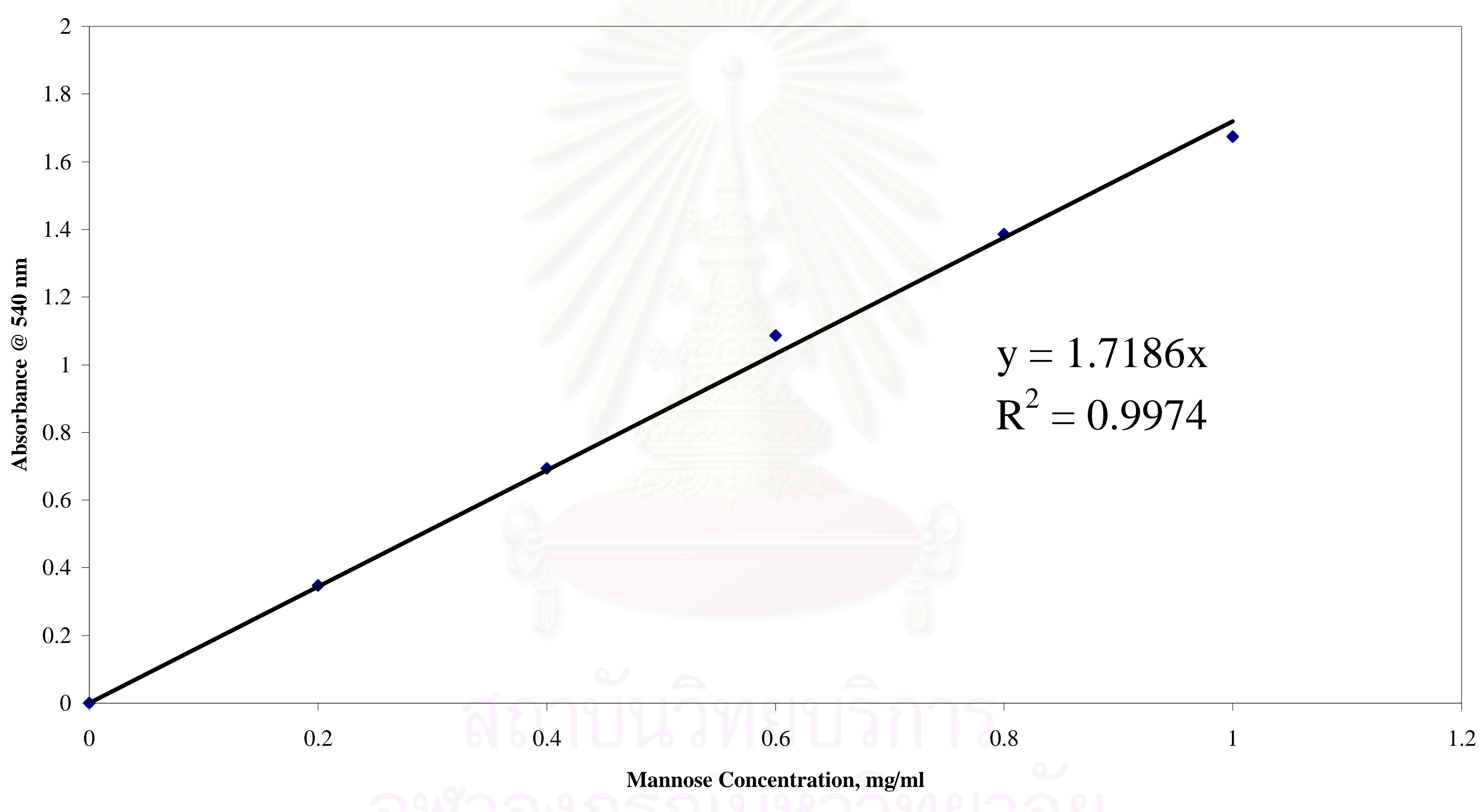

Figure C1 Mannose standard curve for solvent selection analysis

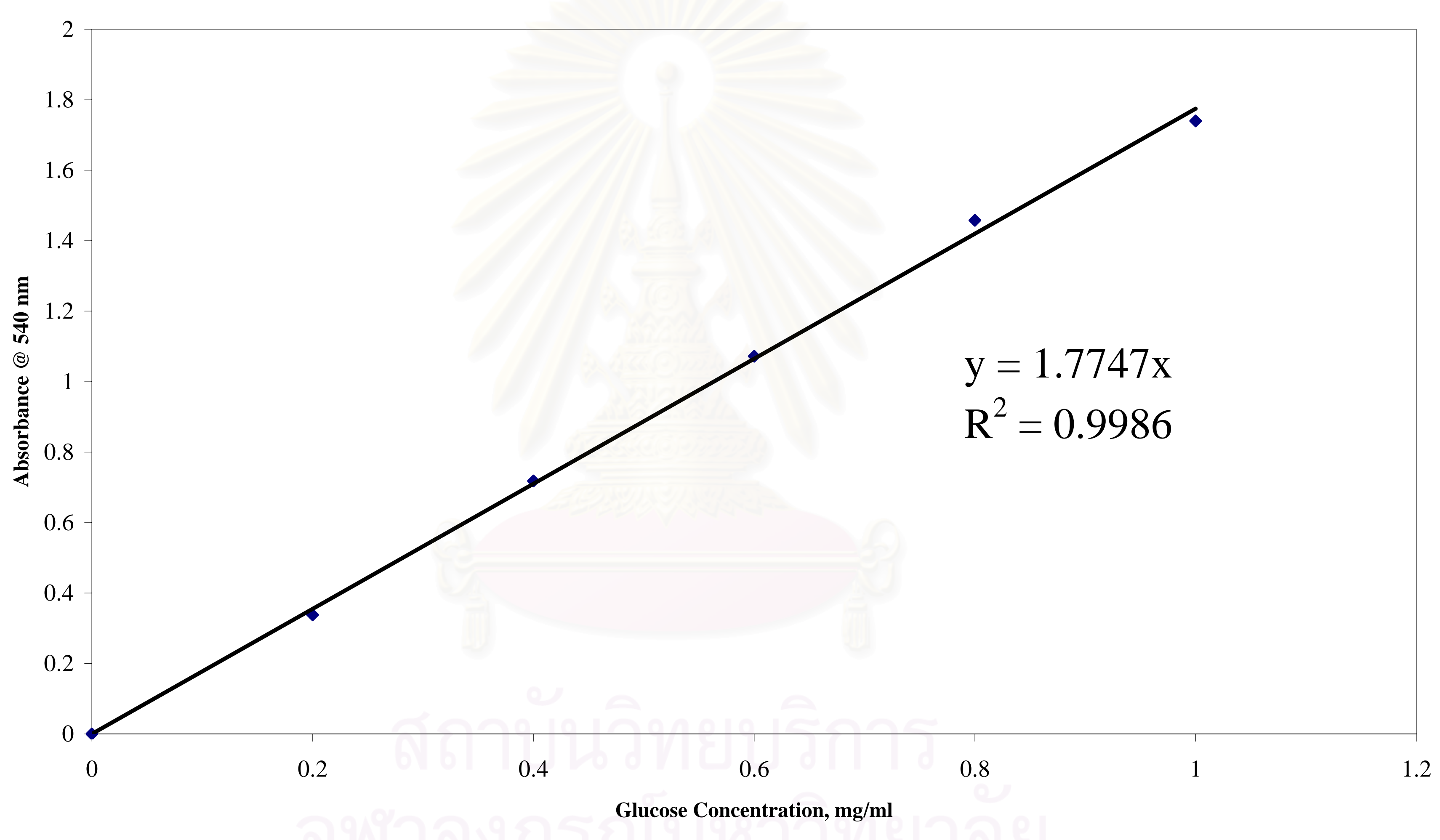

Figure C2 Glucose standard curve for solvent selection analysis

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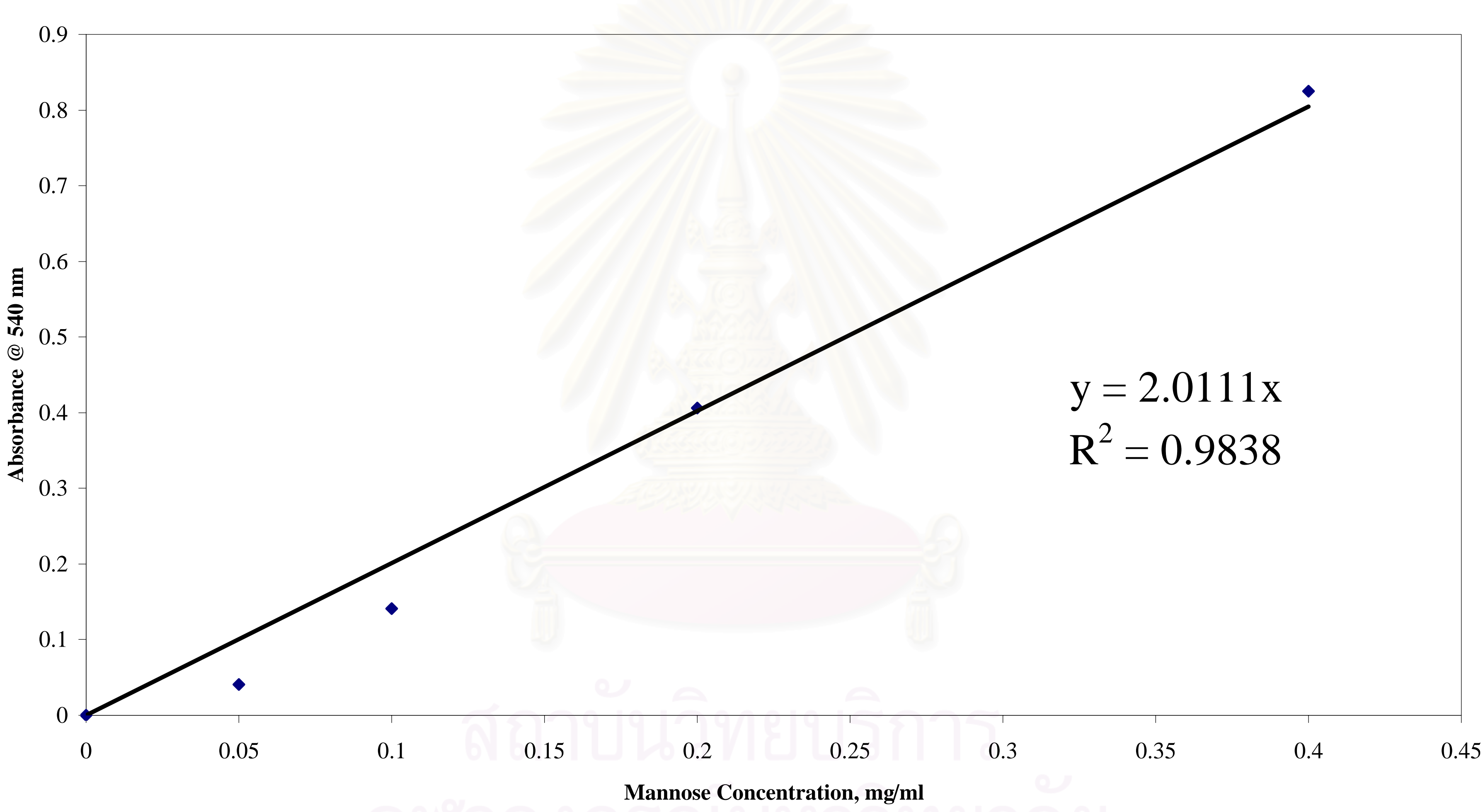

Figure C3 Mannose standard curve for soluble sugar analysis in solvent to gel ratio and pH experiment

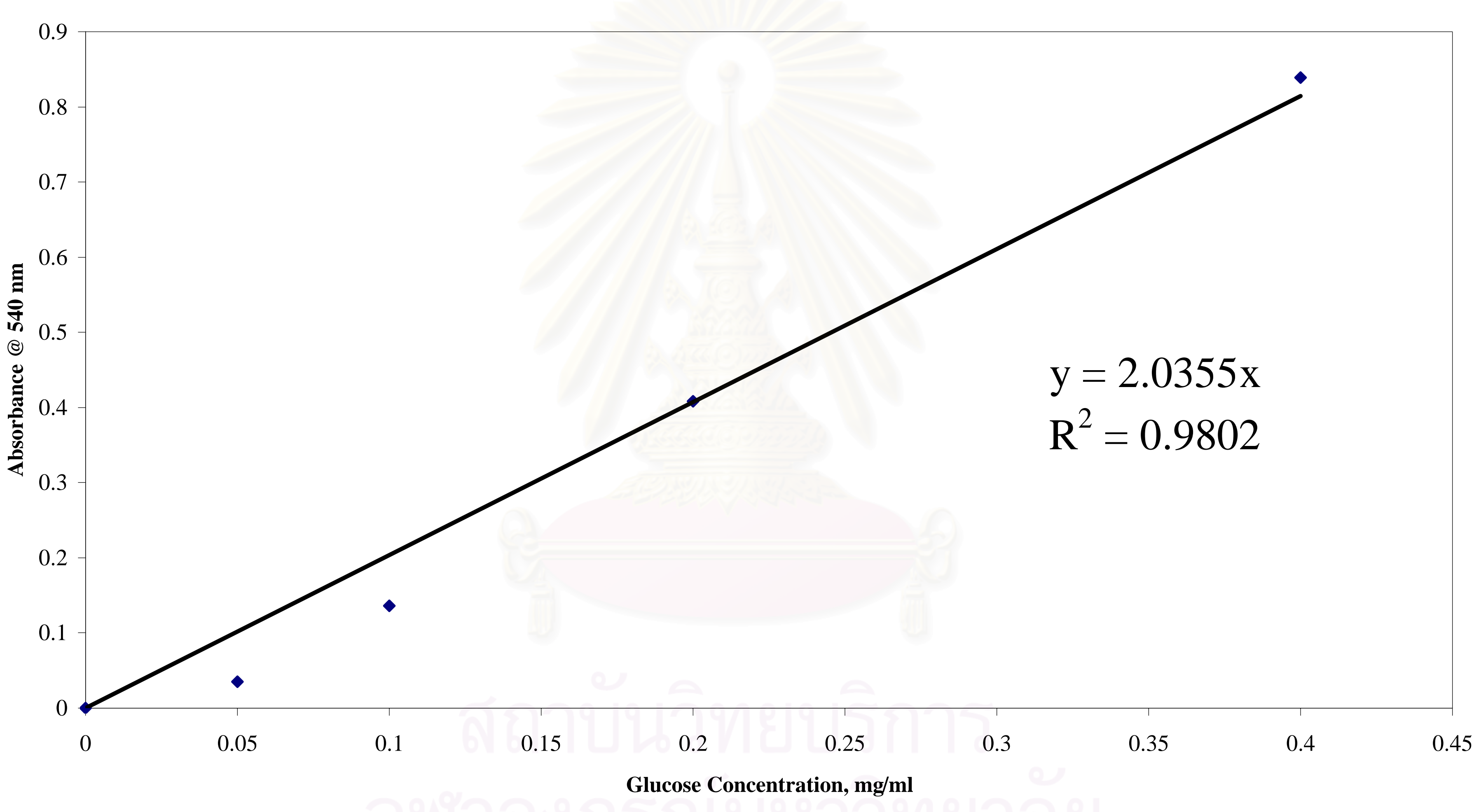

Figure C4 Glucose standard curve for soluble sugar analysis in solvent to gel ratio and pH experiment

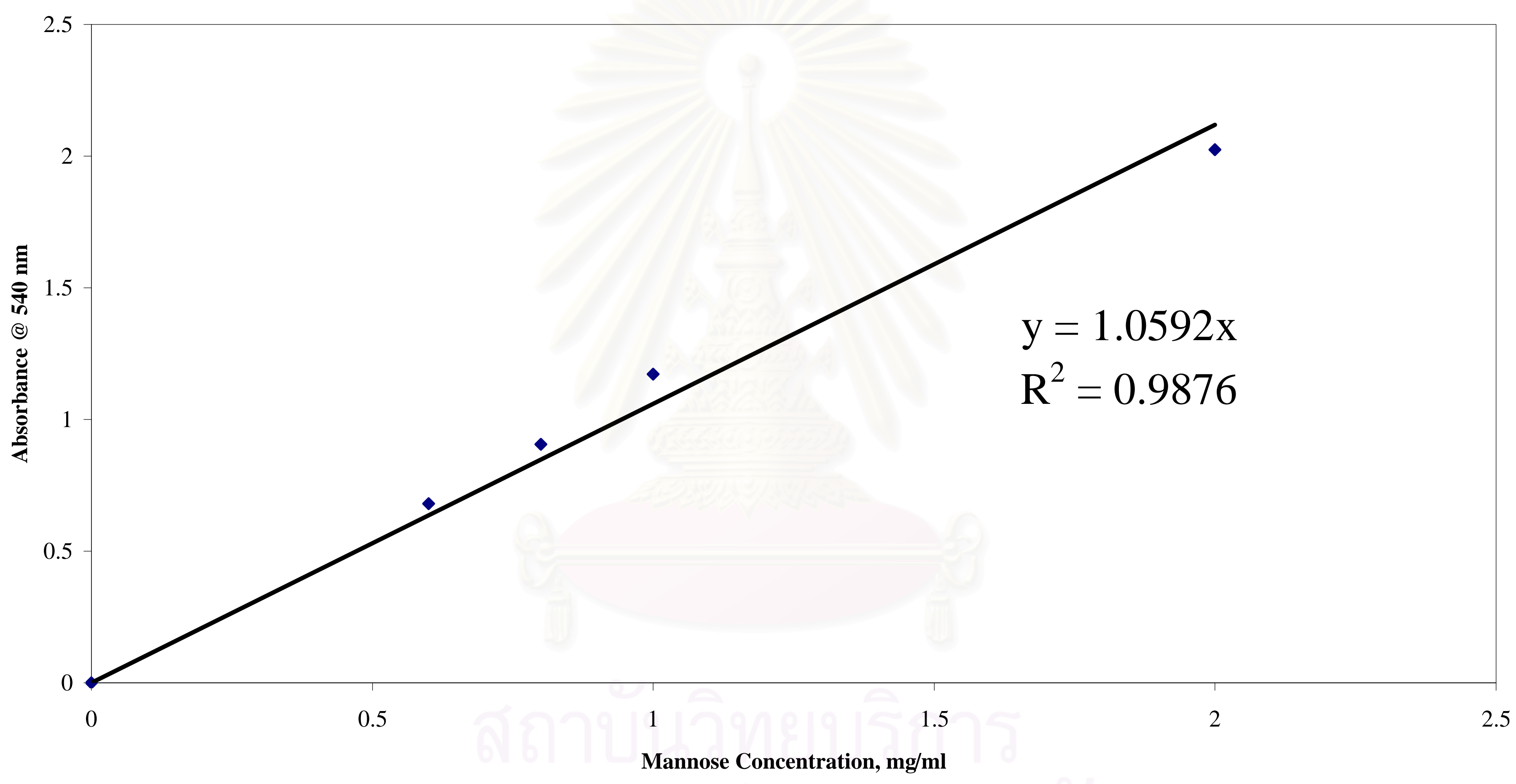

Figure C5 Mannose standard curve for hydrolized polysaccharide analysis in solvent to gel ratio and pH experiment

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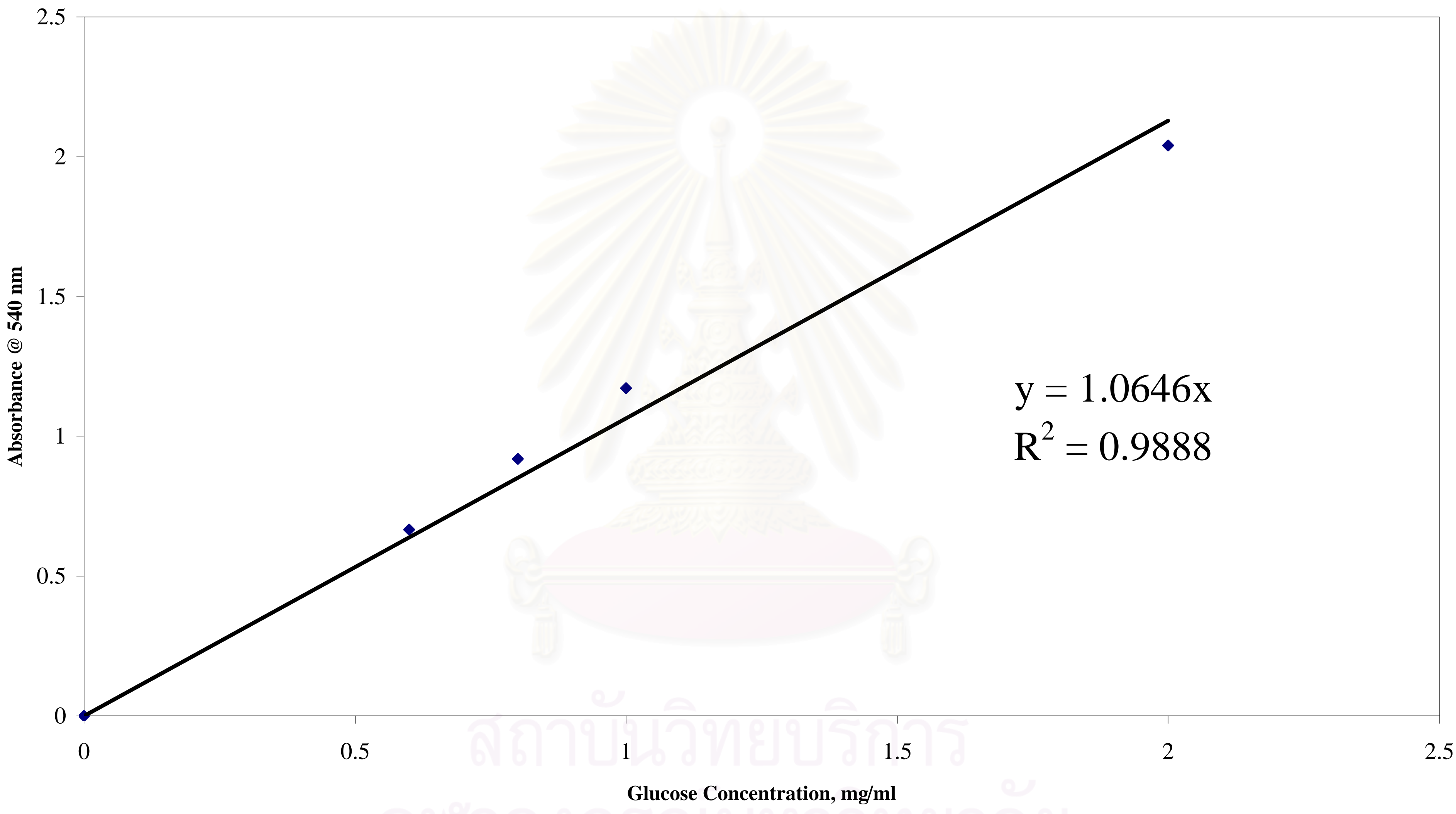

Figure C6 Glucose standard curve for hydrolized polysaccharide analysis in solvent to gel ratio and pH experiment

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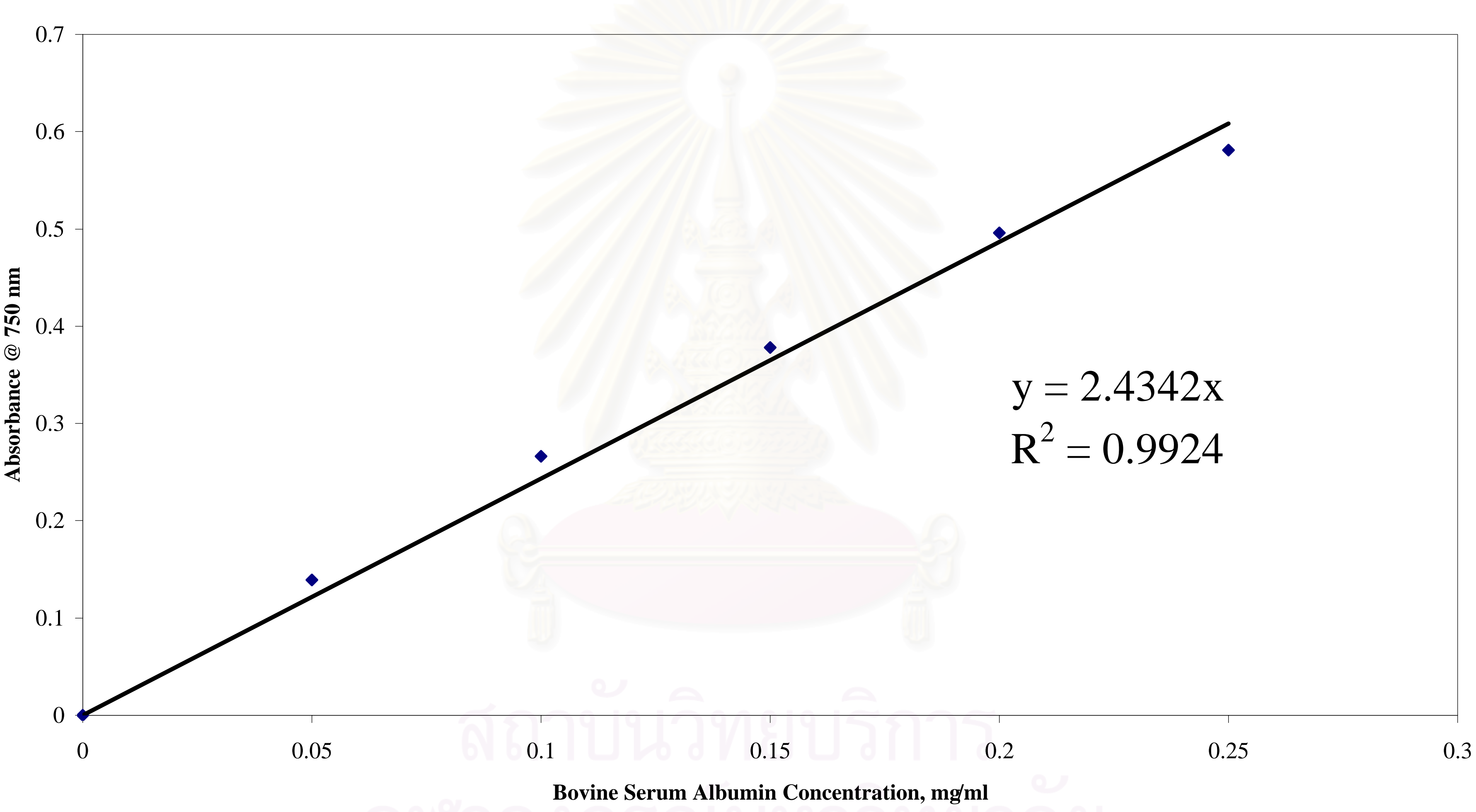

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Figure C7 BSA standard curve for solvent to gel ratio and pH experiment

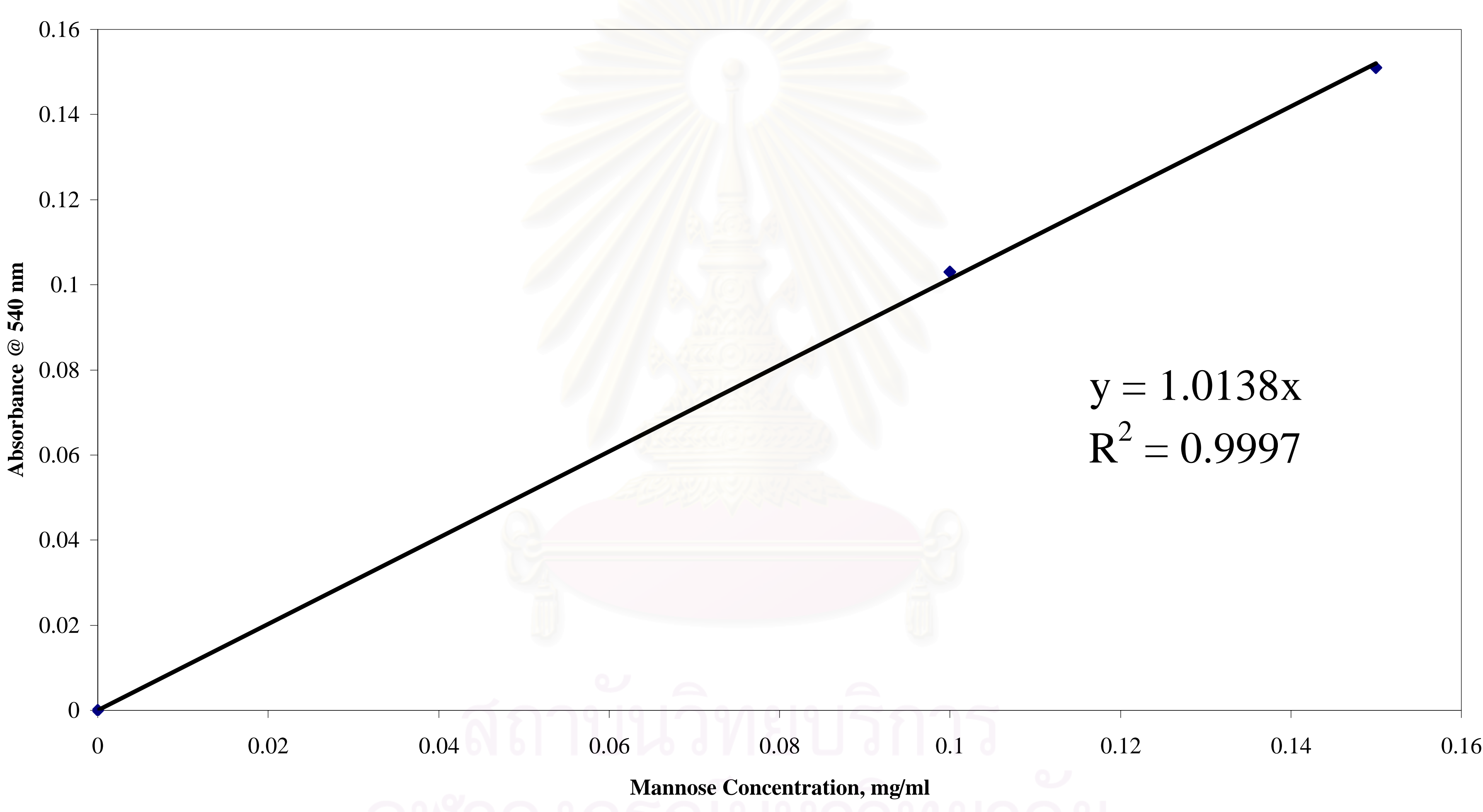

Figure C8 Mannose standard curve for soluble sugar analysis in temperature experiment

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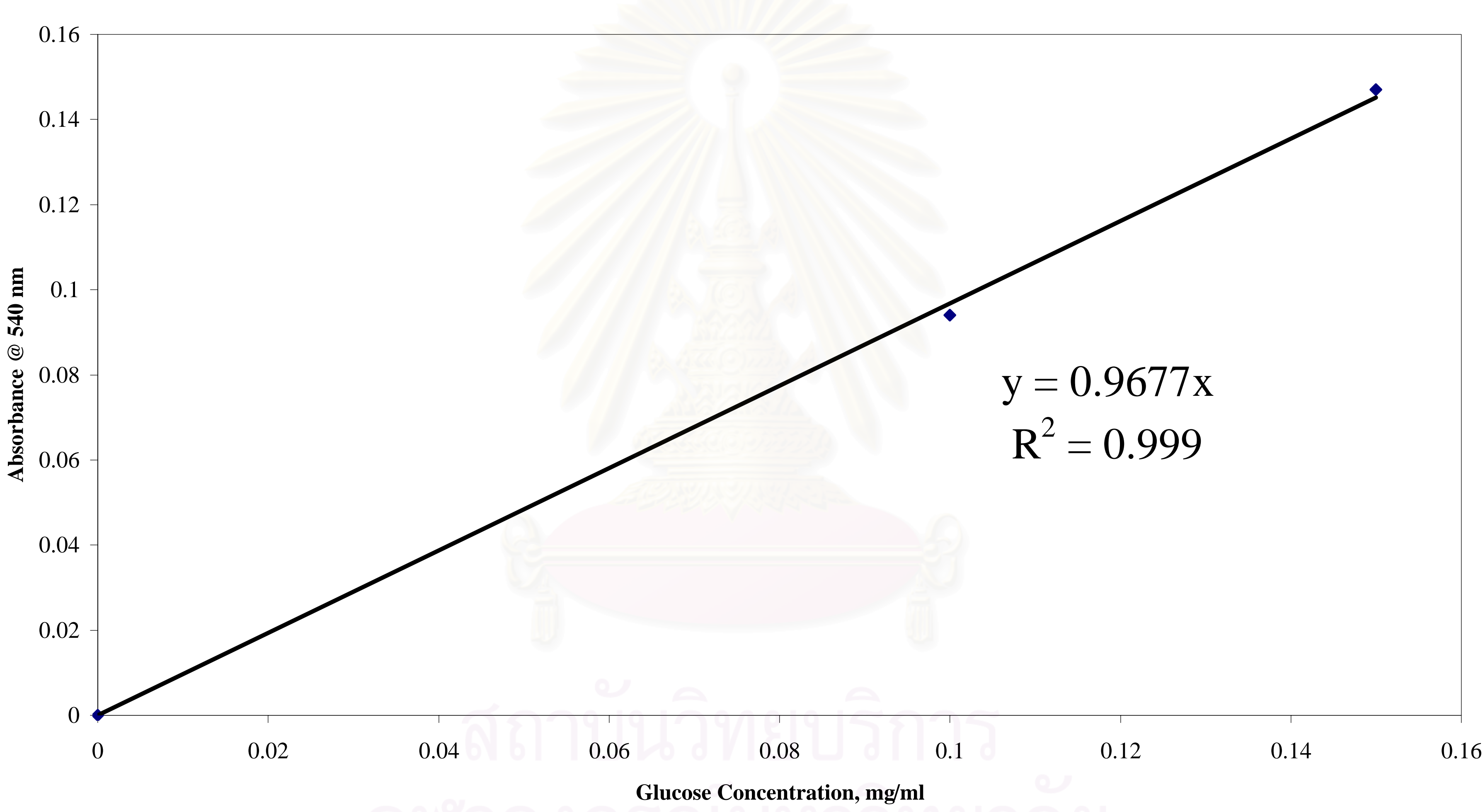

Figure C9 Glucose standard curve for soluble sugar analysis in temperature experiment

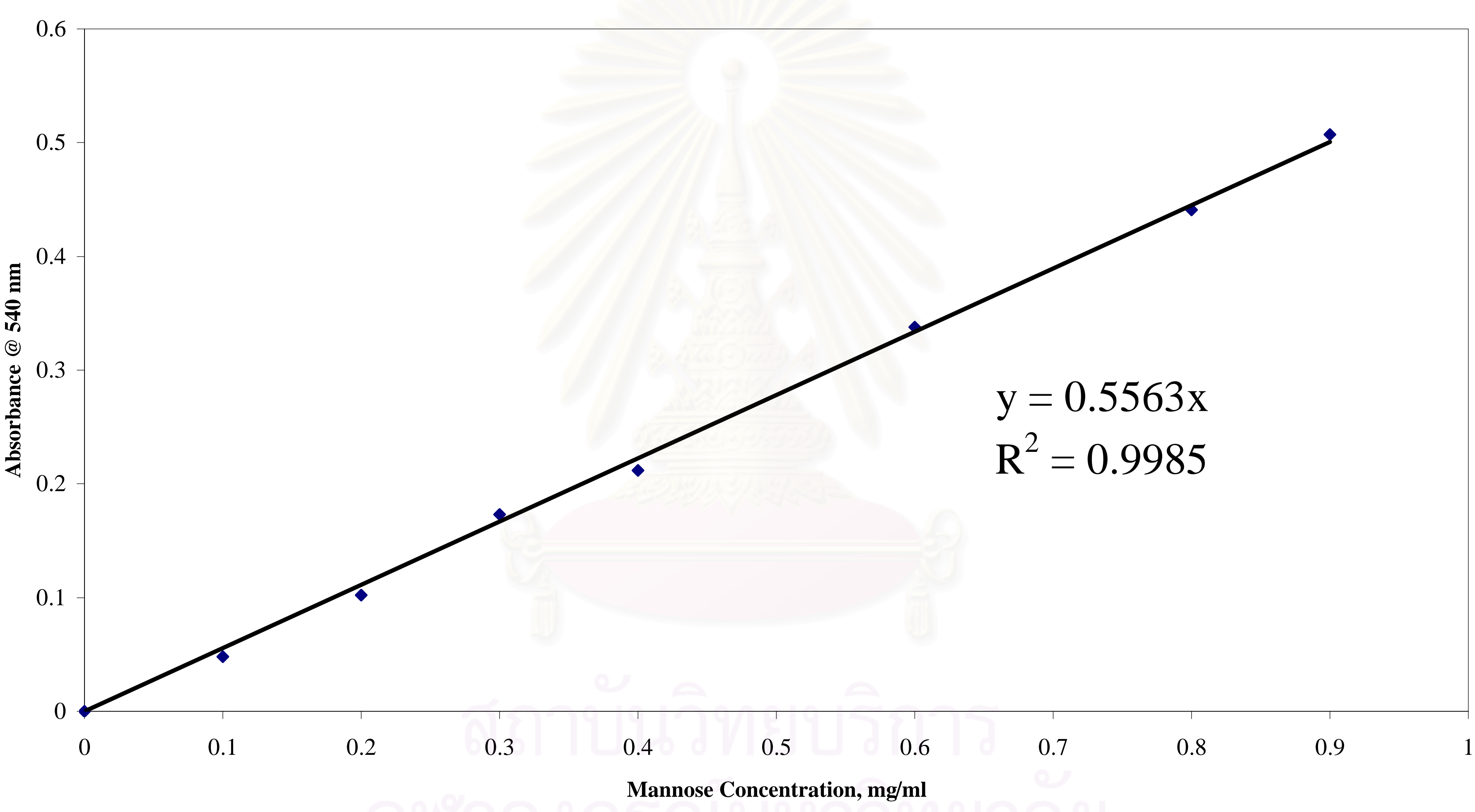

Figure C10 Mannose standard curve for hydrolized polysaccharide analysis in temperature experiment

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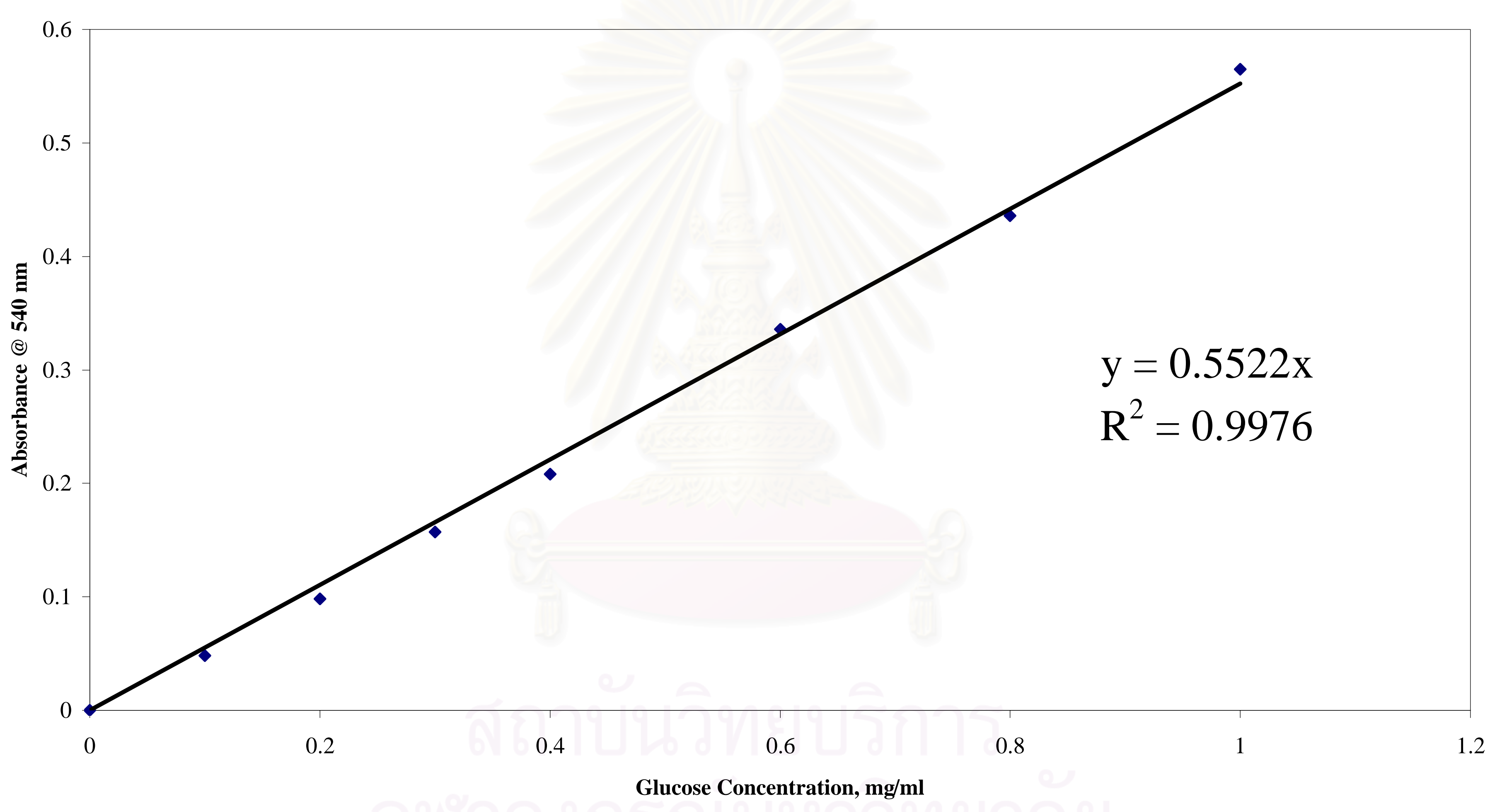

Figure C11 Glucose standard curve for hydrolized polysaccharide analysis in temperature experiment

APPENDIX D

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

Appendix D

Table D1 Solvent selection data

Solvent	Average Product Dry Weight, g	Polysaccharide Content, g (as Glucose)	% Polysaccharide per dry weight
Methanol	0.0367 ± 4.7149E-03	6.1306E-03	15.3265
Ethanol	0.0435 ± 1.5177e-03	1.0661E-02	24.6781
Iso-propanol	0.0517 ± 1.3051E-03	9.1734E-03	17.8818

Table D2 Solvent to gel ratio data

Solvent to gel ratio	Average Product Dry Weight, g	Polysaccharide Content, g (as Glucose)	% Polysaccharide per dry weight
3	0.1524 ± 3.0610E-03	4.5178E-02	29.6444
4	0.1690 ± 4.9642E-03	4.6137E-02	27.3057
5	0.1755 ± 1.5044E-03	4.7407E-02	27.0178
6	0.1877 ± 1.8175E-03	4.9515E-02	26.3750
7	0.1844 ± 9.0266E-03	4.2267E-02	22.9212

Table D3 pH data

pH	Average Product Dry Weight, g	Polysaccharide Content, g (as Glucose)	% Polysaccharide per dry weight
3	0.0932 ± 9.7893E-03	3.8227E-02	41.0163
4	0.1373 ± 2.1385E-03	3.2884E-02	23.9447
5	0.1896 ± 1.4731E-03	4.5709E-02	24.1082
6	0.2080 ± 2.4583E-03	4.5921E-02	22.0739
7	0.2139 ± 1.0214E-03	4.4573E-02	20.8350
8	0.2105 ± 9.6421E-03	4.4972E-02	21.3644
9	0.1987 ± 3.9950E-03	4.8632E-02	24.4750
non-adjusted pH (5.52)	0.1877 ± 1.8175E-03	4.9515E-02	26.3750

Table D4 Polysaccharide content in float precipitate

Temperature, °C	Product Dry Weight, g	Polysaccharide Content, g (as Glucose)	% Polysaccharide per dry weight
7	0.9086	NA	NA
15	0.9279	3.4179E-01	36.8350
32	1.0211	3.8822E-01	38.0194

Table D5 Polysaccharide content in bottom precipitate

Temperature, °C	Product Dry Weight, g	Polysaccharide Content, g (as Glucose)	% Polysaccharide per dry weight
7	0.8317	NA	NA
15	0.6722	1.6129E-01	23.9937
32	0.8360	2.4229E-01	28.9825

Table D6 Polysaccharide content in total precipitate

Temperature, °C	Product Dry Weight, g	Polysaccharide Content, g (as Glucose)	% Polysaccharide per dry weight
7	1.7403	NA	NA
15	1.6001	5.0308E-01	31.4404
32	1.8571	6.3051E-01	33.9513

APPENDIX E

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

เจ้าของตัวอย่าง รัตน์ฉัตร ฉันทวงศ์วุฒิ งานเลขที่ 13/PE2400/2547

ใบส่งตัวอย่างรหัส 470274 ฉบับที่ 24/CHNS/2547 จำนวน 6 ตัวอย่าง

ตัวอย่าง polysaccharide จากว่านหางจระเข้

วันที่วิเคราะห์ 24-ก.พ.-47

เครื่องมือวิเคราะห์ CHNS/O Analyzer (Perkin Elmer PE2400 SeriesII)

วิธีวิเคราะห์ Gaseous products freed by pyrolysis in high-purity oxygen and and were chromatographically separated by frontal analysis with quantitatively detected by thermal conductivity detector.

ชื่อตัวอย่าง	%C	%H	%N	
A	36.177	5.983	1.591	
	35.954	5.938	1.489	
	36.066	**5.961**	**1.540**	4°C บน
B	30.878	5.639	0.994	
	30.647	5.395	1.028	
	30.763	**5.517**	**1.011**	4°C ล่าง
C	36.040	6.698	2.197	
	35.828	6.393	1.829	
	35.934	**6.546**	**2.013**	45°C บน
D	29.572	4.051	1.200	
	29.441	4.010	1.363	
	29.507	**4.031**	**1.282**	45°C ล่าง
E	32.164	4.724	1.374	
	32.418	4.454	1.519	
	32.291	**4.589**	**1.447**	-32°C บน
F	32.433	3.575	1.047	
	32.290	3.982	0.996	
	32.362	**3.779**	**1.022**	-32°C ล่าง

อัมพร อึ้งปกรณ์แก้ว ผู้วิเคราะห์

ส่งงาน 24/2/2004 9:34

รับคืน [illegible]

เจ้าของตัวอย่าง นางสาวรัตน์ฉัตร ฉันทวงศ์วุฒิ

เครื่องมือวิเคราะห์ Kjeldahl (KD-02) ยี่ห้อ TECATOR

วิธีวิเคราะห์ Macro Kjeldahl

ผลวิเคราะห์

ชื่อตัวอย่าง	น้ำหนักตัวอย่าง (g)	% N
A-1	0.999	1.69
A-2	0.844	1.96
B-1	1.023	1.17
B-2	0.791	1.27
C-1	1.404	1.59
C-2	1.333	1.55
D-1	0.571	0.48
D-2	0.617	0.42

สถาบันวิทยบริการ
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APPENDIX F

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Appendix F

Phytochemistry

Phytochemistry deals with the chemistry of plant metabolites and their derivatives. The metabolic system of a plant may be regarded as being constituted of regulated processes within which biochemical conversions and mass transfer take place. Our understanding in this field has advanced to a stage in which definite metabolic processes, biosynthetic pathways and their interconnection are distinguished and studied in the context of their function and genetic control.

The metabolic performance of living organisms can be distinguished into primary metabolism and secondary metabolism. Primary metabolism is associated with fundamental life processes common to all plants. It comprises processes such as photosynthesis, pentose cycle, glycolysis, the citric acid cycle, electron transport, phosphorylation and energy regulation and management. Primary metabolites are produced and converted molecular entities, that are needed in anabolic pathways to build, maintain and reproduce the living cell. In catabolic pathways, primary metabolites (and food products) provide the chemical energy and precursors for biosynthesis.

Primary and secondary metabolism are interconnected in the sense that the biosynthesis of accumulating secondary metabolites can be traced back to ubiquitous primary metabolites. However, in contrast to primary metabolites, secondary metabolites represent features that can be expressed in terms of ecological, taxonomic and biochemical differentiation and diversity. The biosynthesis and accumulation of secondary metabolites provide a basis for biochemical systematics and chemosystematics. In addition, the wide molecular

diversity of secondary metabolites throughout the plant kingdom represents an extremely rich biogenic resource for the discovery of novel drugs and for developing innovative drugs. Not only do plant species yield raw material for useful compounds; the molecular biology and biochemistry provide pointers for rational drug development.

Primary and secondary metabolites can be classified on the basis of their chemical structure into much the same categories of chemical compounds: carbohydrates, lipids, amino acids, peptides, proteins, enzymes, purine, and pyrimidine derivatives. Within such compound classes, secondary metabolites generally show greater individuality and diversity in their molecular structure than primary metabolites. On the other hand, certain compound classes appear to be extraordinarily rich in secondary metabolites. Examples are the structurally diverse groups of alkaloids, phenolics, acetogenins and terpenoids. Ubiquitary primary metabolites belonging to these compound classes seem to be restricted to only a limited number of key compounds functioning as biosynthetic precursors.

Most of the plant compounds that have been found to be medicinally useful and interesting tend to be secondary metabolites. Nonetheless, the discussion of compound classes that follows has been arranged according to chemical structure classes usually clustered as such.

F.1 Carbohydrates

The first products plants produce by photosynthesis are carbohydrates. They are formed from water and carbon dioxide and can be grouped into sugars and polysaccharides. The sugars are either monosaccharides such as glucose and fructose, or oligosaccharides containing up to 5 or 6 monosaccharide units. Monosaccharides are classified according to the number of carbon atoms they contain; thus, trioses, tetroses, pentoses, hexoses, and heptoses are C_3 to C_7 compounds. The polysaccharides are macromolecules, containing a large number of monosaccharide residues.

Carbohydrates constitute a large portion of plant biomass, e.g. cellulose as part of the cellular framework, and starch as a food reserve.

Sugars can unite with a wide variety of compounds to form glycosides, increasing the water solubility of the compounds. Glycosides vary in chemical structure and pharmacological activity due to their aglycone component.

In addition to their use as bulking agents in pharmaceuticals, carbohydrates have recently been recognized to have useful pharmacological properties. Several polysaccharides exhibit immunomodulatory, antitumour, anticoagulant (e.g. heparin), hypoglycaemic or antiviral activities. The various carbohydrate products traded include fibre, cellulose and its derivatives, starch (glucose polymers) and its derivatives, dextrins, fructans (fructose polymers; e.g. inulin), algenic acids, agar and gums.

F.2 Lipids

F.2.1 Vegetable oils

Vegetable oils are major sources of β-sitosterol, which is a steroid drug precursor. One vegetable oil, obtained from groundnut, yields lecithins, which are used to enhance food digestibility. Lecithins are also used in pharmaceutical formulations. Recently, some vegetable oils have been found to be rich in γ-linolenic acid (see Figure F1), which is the precursor of prostaglandins, leukotrienes and thromboxanes. All these compounds are involved in platelet aggregation and inflammatory processes. Only members of *Onagraceae*, *Saxifragaceae* and *Boraginaceae* contain γ-linolenic acid.

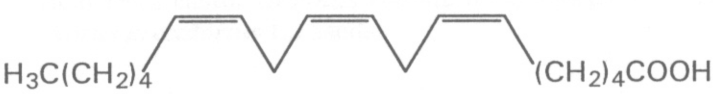

Figure F1 Structure of γ-linolenic acid

Vegetable oils are significant in both the food and pharmaceutical industries. Some are used as solvents for lipid-soluble drugs such as vitamins and antibiotics. Others, e.g. almond oil and olive oil, are used in cosmetics. Castor oil is well known for its purgative activity, but has fallen out of favour because of its unpleasant taste.

F.2.2 Acetogenins

Acetogenins are long-chain aliphatic compounds with 35 – 39 carbon atoms, ending with a γ-lactone, most often unsaturated and cyclized into one or two tetrahydrofuran rings that may or may not be adjacent. They are characteristic of *Annonaceae* (e.g. *Annona*, *Goniothalamus*, *Rollinia* and *Uvaria*). The potential application of acetogenins is linked to their antitumour (e.g. asimicin, bullatacine), antibacterial (e.g. cherimolin) and insecticidal (e.g. asimicin, annonin, annonacin) properties. See Figure F2 for the structure of annonacin, as an example of an acetogenin.

Figure F2 Structure of annonacin

F.3 Amino acids and their derivatives

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Amino acids are constituents of peptides, proteins and enzymes, but also the precursors of a large variety of secondary metabolites including alkaloids and phenolic compounds, which are both discussed separately.

F.3.1 Amino acids

The function of amino acids is not only for protein synthesis; they are also considered to be a form of nitrogen storage (e.g. cannovanine,

hemoarginine) and a germination inhibitor. The few studies of the pharmacological activities of amino acids include reports of curcubitine being used as taeniacide. Many toxic amino acids have been identified; examples include β-(γ-L-glutamylamino)proprionitrile and γ-N-oxalyl-L-α,β-diaminoproapionic acid which are responsible for the toxicity of grass pea (*Lathyrus sativus* L.) that brings about osteolathyrism and neurolathyrism in livestock, and mimosine from *Leucaena* inhibiting protein and nucleic acid synthesis which results in livestock losing appetite and weight, and their growth being inhibited.

F.3.2 Cyanogenic glycosides

Cyanogenic glycosides are compounds derived from amino acids. Hydrolysis of these compounds by enzymes or acids yields hydrocyanic acid, a toxic principle. Biosynthetically, the aglycones of cyanogenic glycosides are derived from L-amino acids. Cyanogenic glycosides are prevalent in the families *Rosaceae, Leguminosae, Gramineae, Araceae, Euphorbiaceae* and *Passifloraceae*. Examples are linamarin, amygdalin and prunasin.

F.3.3 Sulphur-containing compounds

The sulphur-containing compounds of pharmaceutical significance are allein, allicin, ajoene and other related compounds isolated from garlic. Allicin and ajoene (the latter is a condensation product of allicin) exhibit many biological activities, including antihypercholesterolaemic, antiplatelet aggregation, antihypertensive, fibrinolytic and antifungal activities. Recently, diallyl cysteine, an odourless active ingredient of garlic, was found to be biosynthesizable.

F.3.4 Lectins

Lectins are proteins or glycoproteins that are able to bind with the carbohydrate moiety on cell membranes in a specific and reversible fashion,

without displaying enzymatic activity. Most lectins in higher plants are located in seeds. They are commonly found in legumes such as groundnut, soya bean and common bean.

Some lectins have the ability to agglutinate red blood cells of a specific blood group. These lectins are referred as phytohaemagglutinin. The haemagglutination activity is important in immunological studies. Some lectins are toxic, e.g. ricin from castor (*Ricinus communis* L.) seeds and abrin from jequirity bean (*Abrus precatorius* L.) seeds.

F.3.5 Enzymes

Plant-derived enzymes used as drugs include papain and bromelein. Both are proteolytic enzymes useful as an anti-inflammatory drug. Ficin has similar properties.

F.4 Alkaloids

It is not easy to define the term 'alkaloid' precisely, since there is no sharp border between alkaloids and naturally occurring complex amines. At present, the term is used for plant-derived compounds containing one or more nitrogen atoms (usually in a heterocyclic ring), and usually having a marked physiological action on humans or animals. The term 'proto-alkaloids' or 'pseudo-alkaloids' is sometimes applied to compounds that lack one or more of the properties of the typical alkaloids, e.g. the nitrogen in a heterocyclic ring system; examples include mescaline and ephedrine. To avoid problems with this common definition of alkaloids, some authors propose a more narrow definition: an alkaloid is a cyclic organic compound containing nitrogen in a negative oxidation state, which has limited distribution in living organisms.

Based on their chemical structures, alkaloids are divided into several subgroups: non-heterocyclic alkaloids, and heterocyclic alkaloids which are again divided into 12 major groups according to their basic ring structure. Figure

F3 shows some examples; mescaline is an example of a non-heterocyclic or pseudo-alkaloid, tetrandrine of a bisbenzylisoquinoline alkaloid and solasodine of a triterpene alkaloid. Free alkaloids are soluble in organic solvents such as ether or chloroform. Alkaloids will furthermore react with acids to form water-soluble salts. There are a few exceptions to this general rule. In certain alkaloids, e.g. in ricinine, the lone pair of electrons on the nitrogen atom can be protonated. Another example is berberine, a quaternary ammonium alkaloid; the free base is already water-soluble. Physically, most alkaloids exist in solid form, but some are liquid, e.g. nicotine.

Alkaloids in plants are believed to be waste products and a nitrogen source. They are thought to play a role in plant protection and germination, and to be plant growth stimulants. Alkaloids are more common in dicotyledons than in monocotyledons; families rich in them are *Amaryllidaceae, Liliaceae* s.l., *Apocynaceae, Berberidaceae, Leguminosae, Papaveraceae, Ranunculaceae, Rubiaceae and Solanaceae.*

Many alkaloids are pharmaceutically significant, e.g. morphine as a narcotic analgesic, codeine in the treatment of coughs, colchicines in the treatment of gout, quinine as an antimalarial, quinidine as an anti-arrythmic and L-hyoscyamine (in the form of its racemic mixture known as atropine) as anti-spasmodic and for pupil dilation.

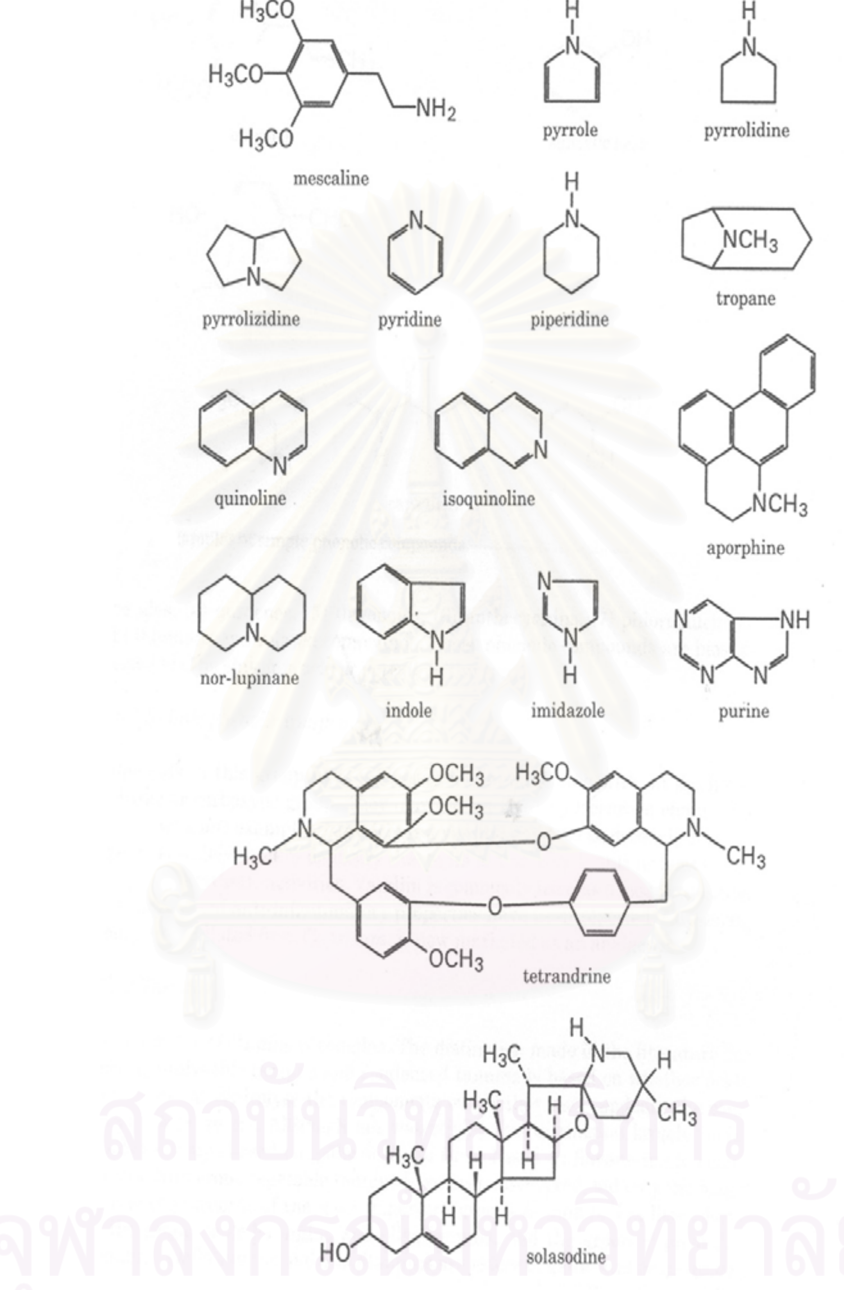

Figure F3 Alkaloids: basic structures and some examples

F.5 Phenols and phenolic glycosides

Phenols probably constitute the largest group of secondary plant metabolites. They range from simple structures with one aromatic ring to complex polymers such as tannins and lignins. Examples of phenolic classes of pharmaceutical interest are (1) simple phenolic compounds, (2) tannins, (3) coumarins and their glycosides, (4) quinines, (5) flavonoids, (6) anthocyanins, (7) phloroglucinols, and (8) lignans and related compounds. These phenolic compounds are biosynthesized via the shikimic acid or acetate pathways.

F.5.1 Simple phenolic compounds

Compounds in this group have a monocyclic aromatic ring with an alcoholic, aldehydic or carboxylic group. They may have a short hydrocarbon chain. Figure F4 shows some examples; capsaicin is a vanillyl amide of isodecenoic acid. Eugenol is widely used in dentistry due to its antibacterial, anti-inflammatory and local anaesthetic activities. Vanillin is commonly used as a food flavouring. For salicylic acid anti-inflammatory properties have been reported. Capsaicin, a compound isolated from *Capsicum*, is now marketed as an analgesic.

eugenol

salicylic acid

vanillin

capsaicin

Figure F4 Examples of simple phenolic compounds

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F.5.2 Tannins

The chemistry of tannins is complex. The distinction made in the literature between hydrolysable tannins and condensed tannins is based on whether acids or enzymes can hydrolyze the components or whether they condense the components to polymers. Although not watertight, this distinction largely corresponds to groups based on gallic acid and those based on flavane-related components. Numerous vegetable tannins have been discovered, but only the major tanning constituents of the most important groups of tannins are listed here, i.e. the group of gallotannins and ellagitannins, and the group of proanthocyanidins. Gallotannins and ellagitannins are esters of gallic acid or its dimmers digallic acid and ellagic acid with glucose and other polyols. Proanthocyanidins are oligomers of 3-flavanols (catechins) and 3,4-flavandiols (leucoanthocyanidins); see Figure F5 in which R=H or OH.

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Figure F5 Structures of some tannins

Tannins are able to react with proteins. On being treated with a tannin, a hide absorbs the stain and is protected against putrefaction, thereby being converted into leather.

Though tannins are widespread in plants, their role in plants is still unclear. They may be an effective defence against herbivores, but it is likely that their major role in evolution has been to protect plants against fungal and bacterial attack. The high concentrations of tannins in the non-living cells of many trees (heartwood, bark), which would otherwise readily succumb to saprophytes, have been cited in support of this hypothesis. Some authorities consider tannins to be waste products, and it has also been suggested that leaf tannins are active metabolites used in the growing tissues. However, tannins in different plant species probably have different functions.

Tannins are used against diarrhea and as antidotes in poisoning by heavy metals. Their use declined after the discovery of the hepatotoxic effect of absorbed tannic acid. Recent studies have reported that tannins have anti-cancer and anti-HIV activities.

F.5.3 Coumarins and their glycosides

Coumarins are benzo-α-pyrone derivatives that are common in plants both in a free state and as glycoside. They give a characteristic odour of new-mown hay and occur, for instance, in many *Leguminosae*. They are biosynthetically derived via the shikimic acid pathway. Figure F6 shows the structure of coumarin. Common derivatives are umbelliferone, herniarin, aesculetin, scopoletin, fraxin, and chicorin.

Figure F6 Structure of coumarin

The biological activities reported are spasmolytic, cytostatic, molluscicidal, antihistaminic and antifertility.

F.5.4 Quinones

Quinones are oxygen-containing compounds that are oxidized homologues of aromatic derivatives and are characterized by a 1,4-diketo-cyclohexa-2,5-diene pattern (paraquinones) or by a 1,2-diketo-cyclohexa-3,5-diene pattern (orthoquinones). In naturally occurring quinines, the dione is conjugated to an aromatic nucleus (benzoquinones) or to a condensed polycyclic aromatic system: naphthalene (naphthoquinones), anthracene (anthraquinones), 1,2-benzanthacene (anthracyclinones), naphthodianthrene (naphthodianthrone), pyrelene, phenanthrene and abietane-quinone. See Figure F7.

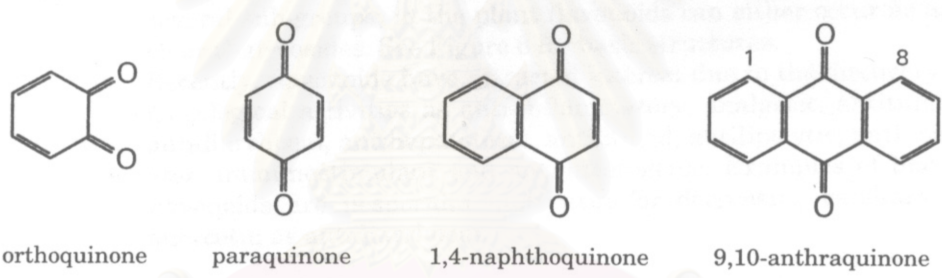

Figure F7 Basic structures of some quinines

Naphthoquinones and anthraquinones have some importance medicinally; see below.

Naphthoquinones

Naphthoquinones are yellow or orange pigments form plants. Most are 1,4-naphthoquinones; 1,2-naphthoquinones are rarely found. Hydroxyl and methyl substitutions at C-2 are common. Biosynthetically, the naphthoquinones are almost exclusively derived via the shikimic acid pathway.

The occurrence of naphthoquinones is limited in fungi and sporadic in Angiosperms. They are found in species of the families *Bignoniaceae, Ebenaceae, Droseraceae, Juglandaceae, Plumbaginaceae, Boraginaceae, Lythraceae, Proteaceae* and *Verbenaceae*.

The pharmaceutical significance of this group of quinines is limited. Plumbagin exhibits antibacterial and cytotoxic activities. Lawsone from henna (*Lawsonia inermis* L.) is a powerful fungicide and hair colourant.

Anthraquinones

Anthraquinones are characterized by the presence of phenolic and glycoside moieties, derived from anthracene, and have a variable degree of oxidation. They have a common double hydroxylation in the positions 1 and 8 (see Figure F7). The glycosidic linkage may be C- or O-bonding. The anthraquinones are mostly biosynthesized via the acetate pathway, although some examples may be derived via the shikimic acid pathway.

Anthraquinones are found in species of the families *Rubiaceae, Leguminosae, Polygonaceae, Rhamnaceae, Ericaceae, Euphorbiaceae, Lythraceae, Saxifragaceae, Scrophulariaceae* and *Verbenaceae*. In monocotyledons, they are found only in *Liliaceae* s.l.

Anthraquinones isolated from plants with laxative activity include sennosides, aloins and emodin. The therapeutic use of anthraquinones as laxatives is very well recognized. The products are sold commercially. Common medicinal plants which contain anthraquinones are *Senna* and *Aloe* species.

F.5.5 Flavonoids

Flavonoids are the compounds responsible for the colour of flowers, fruits and sometimes leaves. Some, such as chalcones and flavonols, are yellow. The name refers to the Latin word ‘flavus’, which means yellow. Some may

contribute to the colour by acting as co-pigment. Flavonoids protect the plant from UV-damaging effects and play a role in pollination by attracting animals by their colours.

The basic structure of flavonoids is 2-phenyl chromane or an Ar-C_3-Ar skeleton. Biosynthetically they are derived from a combination of the shikimic acid and acetate pathways. Small differences in basic substitution patterns give rise to several sub-groups; in the plant flavonoids can either occur as aglycones or as O- or C-glycosides. See Figure F8 for basic structures.

Recently, flavonoids have attracted interest due to the discovery of their pharmacological activities as anti-inflammatory, analgesic, antitumour, anti-HIV, antidiarrhoeal, antihepatotoxic, antifungal, antilipolytic, anti-oxidant, vasodilator, immunostimulant and anti-ulcerogenic. Examples of biologically active flavonoids are hesperidin and rutin for decreasing capillary fragility, and quercetin as antidiarrhoeal.

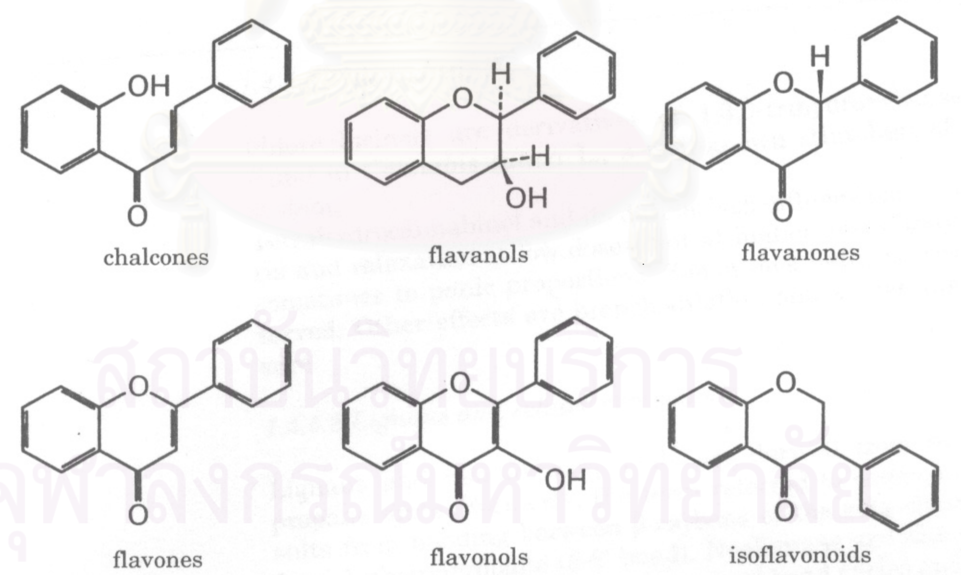

Figure F8 Basic structures of some flavonoids

F.5.6 Anthocyanins

Anthocyanins are the compounds responsible for the red, pink, mauve, purple, blue or violet colours of most flowers and fruits. These water-soluble pigments occur as glycosides (anthocyanins sensu stricto) and their aglycone (anthocyanidins). They are derived from the 2-phenyl benzopyrylium cation, more commonly referred to as the flavylium cation. Cyanin (see Figure F9) is an example of an anthocyanin.

Figure F9 Structure of cyanin (cyaniding-3,5-diglucoside), as an example of an anthocyanin

Anthocyanins are found in all Angiosperms, except for most species of the order *Caryophyllales*: only species of the families *Caryopyllaceae* and *Molluginaceae* contain them; in other families (e.g. *Chenopodiaceae*, *Cactaceae*), the pigmentation is due to betalains.

The application of anthocyanins is as food additive, e.g. in beverages, jams and confectionary products. The pharmacological activities are similar to flavonoids; for instance for decreasing capillary permeability and fragility, and as anti-oedema.

F.5.7 Phloroglucinols

Phloroglucinols are derivatives of 1,3,5-trihydroxybenzene, which e.g. are found in *Cannabis sativa* L., a well-known stimulant of the central nervous system.

Tetrahydrocannabinol and its derivatives influence behaviour, inducing euphoria and relaxation at low doses, but at higher doses, they may induce anxiety, sometimes to panic proportions. Sometimes hallucination and tinnitus are observed. Other effects are bronchodilation and a lowering of intra-ocular pressure.

F.5.8 Lignans and related compounds

Lignans and related compounds are derived from condensation of phenylpropane units. Formerly, the term referred to compounds whose skeleton results from bonding between β-carbons of the side chain of two units derived from 1-phenylpropane (8-8´ bond). Neolignans are also condensation products of phenylpropanoid units, but the actual bond varies and involves no more than one β-carbon (8-3´, 8-1´, 3-3´, 8-0-4´ for example). The term 'oligomers' is incorrect; designated lignans or neolignans result from the condensation of 2 – 5 phenylpropanoid units (e.g. sesquilignans and dilignans, lithospermic acid). Norlignans are probably specific to gymnosperms and have a C_{17} skeleton.

Lignans are substances deposited at the end of the formation of the primary and secondary cell walls. Chemically, they are polymers arising from copolymerization of alcohol with a p-hydroxycinnamic structure (p-hydroxycinnamyl, coniferyl or sinapyl alcohol). Lignins are always combined with polysaccharides.

The pharmacological activity of lignans is antitumour. Kadsurenone, a neolignan, exhibits anti-allergic and antirheumatic activity. The major application of lignins is as a precursor of vanillin, which is widely used in the food industry.

F.6 Terpenoids and steroids

Terpenoids and steroids are derived from isoprene (a 5-carbon unit), which is biosynthesized from acetate via mevalonic acid.

F.6.1 Monoterpenes

Monoterpenes are the simplest constituents in the terpene series and are C_{10} compounds. They arise from the head to tail coupling of two isoprene units. They are commonly found in essential oils. Iridoids and pyrethrins are included in this group. Examples of monoterpenes found in essential oils are shown in Figure F10.

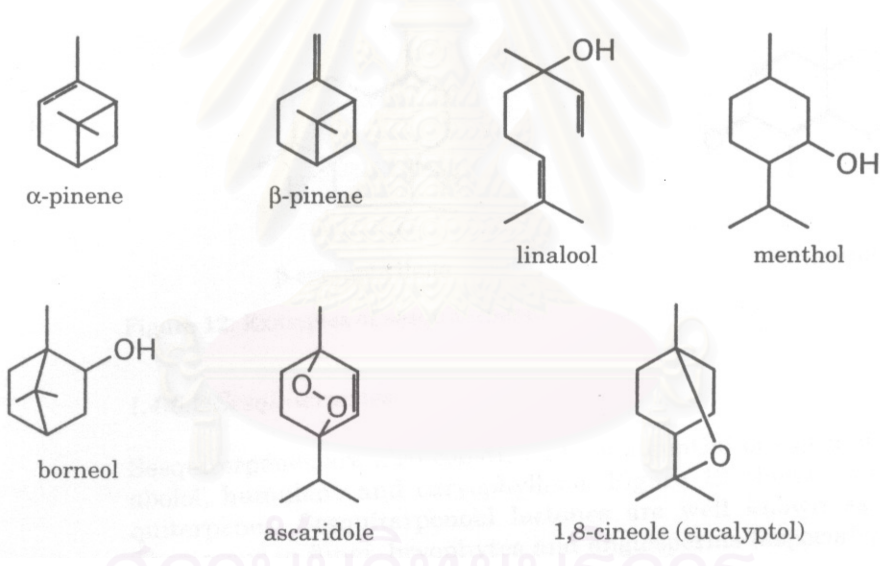

Figure F10 Examples of monoterpenes

Iridoids are monoterpenes characterized by a cyclepenta [C] pyranoid skeleton, also known as the iridane skeleton (cis-2-oxo-bicyclo-[4,3,0]-nonane). Secoiridoids, which arise from iridoids by cleavage of the 7,8-bond of the cyclopentane ring, are also included in the iridoids. Examples of secoiridoids are the bitter constituents of gentian, e.g. gentiopicroside, amarogentin and esters of sweroside and swertiamarin.

Pyrethrins are irregular monoterpenes arising from the non-classic coupling of isopentenyl pyrophosphate and dimethylallyl pyrophosphate. Some are found in essential oils. Figure F11 gives the basic structures of iridoids and secoiridoids and an example of pyrethrins.

Figure F11 Basic structures of iridoids and secoiridoids, and the structure of pyrithrin I as an example of pyrethrins

The pharmacological properties of iridoids are quite limited: the iridoid-containing drugs currently used do not yield any major active principle. However, there are reports on analgesic and anti-inflammatory activities of some iridoids, e.g. harpagoside. Pyrethrins are toxic for cold blooded animals such as fish, amphibians and insects. They are widely used as insecticides.

F.6.2 Sesquiterpenes

Sesquiterpenes are also constituents of essential oils of many plants, e.g. bis-abolol, humulene and caryophyllene. Figure F12 shows two examples of sesquiterpenes. Sesquiterpenoid lactones are well known as bitter principles. They occur in fungi, bryophytes and angiosperms (especially common in *Compositae*).

Sesquiterpenes posses a broad range of biological activities due to the α-methylene-γ-lactone moiety and epoxides. Their pharmacological activities are antibacterial, antifungal, anthelmintic, antimalarial and molluscicidal. Examples are santonin used as an anthelmintic and artemisinin as an antimalarial.

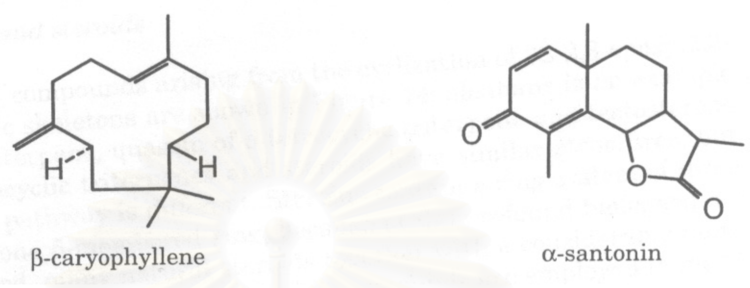

Figure F12 Examples of sesquiterpenes

F.6.3 Diterpenes

Diterpenes constitute a vast group of C_{20} compounds arising from the metabolism of 2E,6E,10E-geranylgeranyl pyrophosphate. They are present in some animals and plants; they are particularly abundant in the orders *Lamiales* and *Asterales*.

Diterpenes have some therapeutic applications. For instance, taxol (see Figure F13) and its derivatives from *Taxus* are anti-cancer drugs. Other examples are forskolin, with antihypertensive activity, zoapatanol, as an abortifacient, and stevioside, as a sweetening agent.

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Figure F13 Structure of taxol as an example of a diterpene

F.6.4 Triterpenes and steroids

Triterpenes are C_{30} compounds arising from the cyclization of 3S-2,3-epoxy,2,3-squalene. The basic skeletons are shown in Figure F14: oleanane is an example of a pentacyclic triterpene, quassin of a tetracyclic triterpene and testosterone of a steroid. Tetracyclic triterpenes and steroids have similar structures, but their biosynthetic pathway is different. Steroids contain a ring system of three 6-membered and one 5-membered ring; because of the profound biological activities encountered, many natural steroids together with a considerable number of synthetic and semi-synthetic steroidal compounds are employed in medicine (e.g. steroidal saponins, cardioactive glycosides, corticosteroid hormones, mammalian sex hormones).

The pharmaceutical applications of triterpenes and steroids are considerable. Cardiac glycosides have been used in medicine without replacement synthetic drugs. Saponins from ginseng and liquorice exhibit many therapeutic effects.

Figure F14 Basic structures of triterpenes and steroids

Saponins

Saponins constitute a vast group of glycosides which occur in many plants. They are characterized by their surfactant properties; they dissolve in water and, when shaken, form a foamy solution. Saponins are classified by their aglycone structure into triterpenoid and steroid saponins; most triterpenoid saponins are derivatives of one of the triterpenes oleanane, ursane and lupine, while steroid saponins generally posses the typical steroid skeleton enlarged with 2 extra rings E, a furan structure and F, a pyran structure, respectively. Examples of 2 aglycones are shown in Figure F15. In saponins, sugar and/or uronic acid residues are attached to the aglycones via the C-3 hydroxyl group.

Figure F15 Structures of two saponins (aglycone structure)

Most saponins have haemolytic properties and are toxic to cold blooded animals, especially fish. The steroidal saponins are important precursors for steroid drugs, including anti-inflammatory agents, androgens, oestrogens and progestins. Well-known steroid sapogenins are diosgenin from *Dioscorea*, hecogenin from *Agave* and smilagenin from *Smilax*.

Triterpene saponins exhibit various pharmacological activities: anti-inflammatory, molluscicidal, antitussive, expectorant, analgesic and cytotoxic. Examples include the ginsenosides, which are responsible for some of the pharmacological activity of ginseng, and the active triterpenoid saponins from liquorice.

Cardiac glycosides

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The aglycone part of cardiac glycosides is a tetracyclic steroid with an attached unsaturated lactone ring that may have 5 or 6 members. Cardiac glycosides are classified into two groups according to the lactone ring: the C_{23} cardenolides with an α,β-unsaturated d-γ-lactone (=butenolide), and the C_{24} bufadienolides with a di-unsaturated γ-lactone (=pentadienolide). The sugar moiety is normally attached via de C-3 hydroxyl group of the aglycone. The majority of the saccharides found in cardiac glycosides are highly specific. They are 2,6-dideocyhexoses, such as D-digitoxose, L-oleandrose or D-diginose. These sugars give a positive reaction with the Keller-killiani reagent.

Cardiac glycosides have been used as drugs for the treatment of cardiac insufficiency. An example is digitoxin from *Digitalis*, where the sugar moiety is attached to the aglycone digitoxigenin (see Figure F16) via the C-3 hydroxyl group.

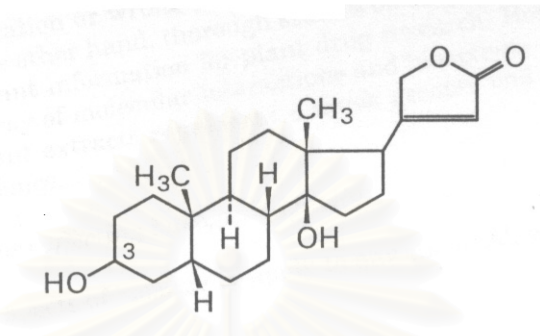

Figure F16 Structure of digitoxgenin

F.6.5 Carotenoids

Carotenoids contain 8 isoprene (C_{40}) units that are responsible for the yellow or orange colour of some vegetables and fruits. Among these compounds, the hydrocarbons are collectively referred to as carotenes and the hydroxylated derivatives as xanthophylls. Carotenoids are either acyclic (e.g. lycopene) or comprise one or two pentacyclic or hexacyclic rings at one end or the other (e.g. β,ψ,-carotene), or at both ends (e.g. β,β-carotene).

Carotenoids become interesting agents after the discovery of a negative correlation between the plasma concentration of β-carotene and the prevalence of certain forms of cancer. Some doctors prescribe β-carotene for cancer patients. Furthermore, in the intestine β-carotenes are converted to retinol (vitamin A). They can be used for the treatment of photosensitization, retinal disease and glaucoma. Carotenoids are also safe colouring agents for food and cosmetics.

BIOGRAPHY

Ms. Ratchat Chantawongvuti was born on February 10th, 1977 in Bangkok. She finished her secondary school from Mater Dei School in March, 1995. After that, she studied in the major of Chemical Engineering in Faculty of Engineering at Chulalongkorn University. She continued her further study in Master's degree in Chemical Engineering at Chulalongkorn University. She participated in the Biochemical Engineering Laboratories and achieved her Master's degree in April, 2004.

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