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DEVELOPMENT OF TEST KIT FOR PROTEIN ALLERGENS IN NATURAL RUBBER LATEX AND RUBBER PRODUCTS

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โปรตีนแอลเลอเจนในน้ำยางธรรมชาติมีผลทำให้คนที่ใช้ผลิตภัณฑ์ยางที่มีภูมิไวเกินเกิดอาการแพ้ประเภท 1 ที่มีอันตรายถึงชีวิตได้ ดังนั้นการผลิตน้ำยางข้นโปรตีนแอลเลอเจนต่ำ และการพัฒนาชุดทดสอบโปรตีนแอลเลอเจนในน้ำยางธรรมชาติและผลิตภัณฑ์จึงเป็นวัตถุประสงค์ของการวิจัยนี้ วิธีการที่ใช้ผลิตน้ำยางข้นโปรตีนแอลเลอเจนต่ำคือการขจัดโปรตีนด้วยเอนไซม์แอลคาเลส เมื่อศึกษาสมบัติทางเทคนิค ISO 2004 ของน้ำยางข้นโปรตีนต่ำ (DPCL) ที่ผลิตได้เปรียบเทียบกับน้ำยางข้นควบคุม พบว่า DPCL มีปริมาณกรดไขมันระเหยได้ 0.079 % ลดลงจากน้ำยางข้นควบคุม 50% และมีเวลาความคงตัวต่อแรงกล (1486 วินาที) สูงกว่าน้ำยางข้นควบคุม (799 วินาที) นอกจากนี้ปริมาณโปรตีนวิเคราะห์โดยปริมาณไนโตรเจนทั้งหมด คือ 0.07$\pm$ 0.07 % ลดลงจากน้ำยางข้นควบคุม 70 % และมีปริมาณโปรตีนที่ละลายน้ำได้ (WEP) 17 $\pm$ 12 ไมโครกรัมต่อกรัมยาง ลดลงจากน้ำยางข้นควบคุม 95 % โดยโปรตีนที่ละลายน้ำได้ของ DPCL ไม่มีโปรตีนในช่วงขนาดโมเลกุล 14.4- 97 kDa ที่เป็นโปรตีนแอลเลอเจน สำหรับการพัฒนาชุดทดสอบโปรตีนแอลเลอเจนในน้ำยางธรรมชาติและผลิตภัณฑ์ยางโดยใช้โปรตีนจากซีรัมยางเป็นโปรตีนมาตรฐาน เปรียบเทียบกับการใช้โปรตีนจากซีรัมของวัว (BSA) และโปรตีนจากไข่ขาว (Ovalbumin) โดยวิธี modified Lowry และ Bradford พบว่าปริมาณโปรตีนที่วัดได้จากกราฟมาตรฐานของโปรตีนจากซีรัมยางให้ผลใกล้เคียงกับปริมาณโปรตีนที่วัดได้จากกราฟมาตรฐานของโอวัลบูมิน และ BSA เมื่อแยกโปรตีนจากซีรัมยางพบว่าโปรตีนจากซีรัมยางที่มีขนาดโมเลกุล 30 kDa เป็นโปรตีนหลัก และมีโปรตีนขนาด 20.1- 14.4 kDa และ 4.7 kDa ในพีคที่ 2 และ 3 ตามลำดับ เมื่อนำโปรตีนจากซีรัมยางไปใช้เป็นแอลเลอเจนมาตรฐาน เทียบกับแอลเลอเจนทางการค้าในวิธีสะกิดผิวหนัง (SPT) ในกลุ่มตัวอย่าง 112 คน แบ่งเป็น 2 กลุ่ม 1) กลุ่มบุคลากรที่ทำงานในโรงพยาบาลที่ถูกกระตุ้นด้วยถุงมือยาง 71 คน 2) กลุ่มผู้ป่วยภูมิแพ้ทั่วไป 41 คนที่คลินิคผิวหนัง พบว่ากลุ่มบุคลากรที่ทำงานในโรงพยาบาลที่ใช้ถุงมือยางให้ผลเป็นบวกกับสารแอลเลอเจนทางการค้า 19.7 % และให้ผลเป็นบวกกับโปรตีนแอลเลอเจนจากซีรัมยาง 46.5 % ขณะที่กลุ่มผู้ป่วยภูมิแพ้ทั่วไปให้ผลเป็นบวกกับสารแอลเลอเจนทางการค้า 9.8 % และให้ผลเป็นบวกกับโปรตีนแอลเลอเจนจากซีรัมยาง 15.5 % ผลสรุปจากงานวิจัยนี้คือโปรตีนจากซีรัมยางที่ใช้เป็นโปรตีนแอลเลอเจนมาตรฐานมีประสิทธิภาพในการให้ผลการศึกษาความชุกต่อการแพ้ยางโดยวิธีสะกิดผิวหนังสูงกว่าสารแอลเลอเจนทางการค้าในกลุ่มบุคลากรที่ทำงานในโรงพยาบาล ส่วนกลุ่มผู้ป่วยภูมิแพ้ทั่วไปโปรตีนจากซีรัมยางก็ให้ผลบวกสูงกว่า นอกจากนี้พบว่าการลดปริมาณโปรตีนในน้ำยางข้นและยางดิบ สามารถลดความชุกในการแพ้ยางของกลุ่มบุคลากรที่ทำงานในโรงพยาบาลเหลือเพียง 1- 3 % เมื่อเปรียบเทียบกับน้ำยางข้นควบคุม (8- 10 %) โดยสรุปชุดทดสอบโปรตีนแอลเลอเจนที่พัฒนาขึ้นนี้สามารถตรวจสอบความชุกของการแพ้ยางในกลุ่มบุคลากรเสี่ยงได้ และสามารถใช้ประเมินคุณภาพของผลิตภัณฑ์ยางโปรตีนต่ำได้ในระดับหนึ่ง

ภาควิชา.....ชีวเคมี..... ลายมือชื่อนิสิต.....

สาขาวิชา....ชีวเคมี..... ลายมืออาจารย์ที่ปรึกษา.....

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KEY WORD :NATURAL RUBBER LATEX ALLERGY/ WATER EXTRACTABLE PROTEIN/ LATEX PROTEIN ALLERGENS/ SKIN PRICK TEST

PAWIN NGAMLERT: DEVELOPMENT OF TEST KIT FOR PROTEIN ALLERGENS IN NATURAL RUBBER LATEX AND RUBBER PRODUCTS. THESIS ADVISOR: ASSOC. PROF. JARIYA BOONJAWAT, Ph.D. THESIS CO-ADVISOR: ASSOC. PROF. CHYAGRIT SIRI-UPATHUM. 127 pp. ISBN 974-17-2715-1

Protein allergens in natural rubber latex (NRL) and NRL products may cause life threatening allergenic hypersensitivity type I in hypersensitive users. Hence the objectives of this research are to develop a new grade of concentrated latex with low allergenic proteins and to develop the test kit for protein allergen determination in natural rubber latex and rubber products. Alcalase enzyme was used in the production of deproteinized concentrated latex (DPCL). The physical properties of DPCL obtained from this research meets with ISO 2004 specifications, with especially low volatile fatty acid (VFA) of 0.079 % or 50 % of control latex, and mechanical stability time, MST (1486 s) higher than control concentrated latex (799 s). The consistent low protein quality of DPCL were evident by 0.07 ± 0.07 % total nitrogen content and 17 ± 12 μg/g extractable protein (WEP) content, which were 70 % and 95 % reduction from control respectively. There was no WEP in the molecular weight (Mw) range of 14- 97 kDa or no major allergens. The test kit for latex protein allergens was developed by using latex serum proteins as standard protein comparing with BSA and ovalbumin in the total protein determination by modified Lowry and Bradford methods. Latex serum proteins showed similar protein quantities evaluated from the standard calibration graphs of BSA and ovalbumin. The Mw of latex serum proteins studied by Sephadex G- 75 column and SDS- PAGE showed the first dominant protein peak (P1) at 30 kDa, followed by the second peak at 20.1 – 14.4 kDa and P3 at 4.7 kDa respectively. The latex serum proteins were used as standard latex protein allergens in skin prick test compared to commercial latex allergens in 112 volunteers which were separated into 2 groups: 1) atopic health care workers who had been sensitized by latex gloves and 2) general atopic patients at the Dermatological Department. The results of SPT showed 19.7 % positive to commercial latex allergens and 46.5 % positive to latex serum proteins in health care workers. In general atopic patients, positive SPT 9.8 % and 15.5 % were observed with commercial and latex serum protein allergens developed in this research. In addition, deproteinization of latex and solid rubber can reduce the prevalence of latex allergy to 1- 3 %, while control latex showed 8- 10 % positive in sensitized health care workers. In conclusion the developed protein allergen test kit can be used to study the prevalence of latex allergy in high risk groups and to evaluate the quality of low- protein rubber products to some extent.

Department……Biochemistry…….. Student's signature……..…

Field of study….Biochemistry…...…… Advisor's signature……..

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ABBREVIATIONS

AL	Ammoniated latex
ASTM	American Society for Testing and Materials
CL	Control concentrated latex
DPCL	Deproteinized concentrated latex
DPNR	Deproteinized natural rubber
DRC	Dry rubber content
FDA	Food and Drug Administration
HA	High- ammoniated latex
kDa	kilodalton
LA	Low- ammoniated latex
mm	millimeter
MST	Mechanical stability time
NR	Natural rubber
NRL	Natural rubber latex
NSS	Normal saline solution
phr	part per hundred rubber
ppm	part per million
RRIM	Rubber Research Institute of Malaysia
SAPNR	Saponified rubber
SDBS	Sodium dodecyl benzene sulfonate
SPT	Skin prick test
STR	Standard Thai rubber
TMTD	Tetramethyl thiuram disulfide
TSC	Total solid content
VFA	Volatile fatty acid
WEP	Water extractable protein
μg	microgram

CHAPTER I
INTRODUCTION

1.1 Utilization of Natural rubber latex

Natural rubber latex (NRL) was known as a source of rubber for hundred years. The Indians of Central and South America knew about rubber as early as the 11th century, but it was not until the French scientist Charles de la Condamine visited South America (1736- 44) that the first sample was sent back to Europe (Allen, 1987). The first modern use for substance was discovered in 1818 by a British medical student named James Syme. He used it to waterproof cloth to make the first raincoat. In the mid- 19th century Charles Goodyear discovered sulfur- vulcanization process and Thomas Hancock had proposed using liquid latex, as an alternative to coagulated rubber ('dry rubber') (Allen *et al*., 1986). In 1882 John Boyd Dunlop was granted a patent for his pneumatic tire. As the demand for tires began to deplete natural rubber supplies, NR plantations were established out of South America. By the early 1900s, various countries sought ways to improve rubber compounding and vulcanization procedures. In 1910 natural rubber consumption increased rapidly and caused the increase of rubber plantation areas of rubber trees in South East Asia including Thailand.

NRL is harvested from the rubber tree, it then undergoes a complex coagulation and vulcanization process involving the addition of various chemical substances which are termed compounding ingredients (Hamann, 1993). Latex gloves gained popularity during the 1980's. They are excellent protective barriers against human immunodeficiency virus (HIV), the virus that causes acquired immune deficiency syndrome (AIDS). Gloves and condoms made from NRL are also extremely durable. They can be stretched to over 5 times their original length without tearing. The snug fit is important for surgeons and dentists. NRL is the most common source material for production of surgical or medical gloves including dental products, bandages, medical tape, and dental cofferdams. Besides gloves, other medical devices made from NR include, some catheters, protective sheets, enema tips, hemodialysis equipment, blood pressure cuffs and ventilator equipment. Several other household goods contain NRL such as carpet backing, condom, underwear, rubber band, and eraser etc. (Loos, 1998),

because of its excellent tensile strength and high elongation at break as well as its good barrier property due to its film ability (Palosuo *et al*., 1997).

In 2001 Thailand produced 2,283,878 tons of natural rubber of which only 253,105 tons were used by the local rubber industry and the rest was exported (Thai Rubber Research Institute, 2002).

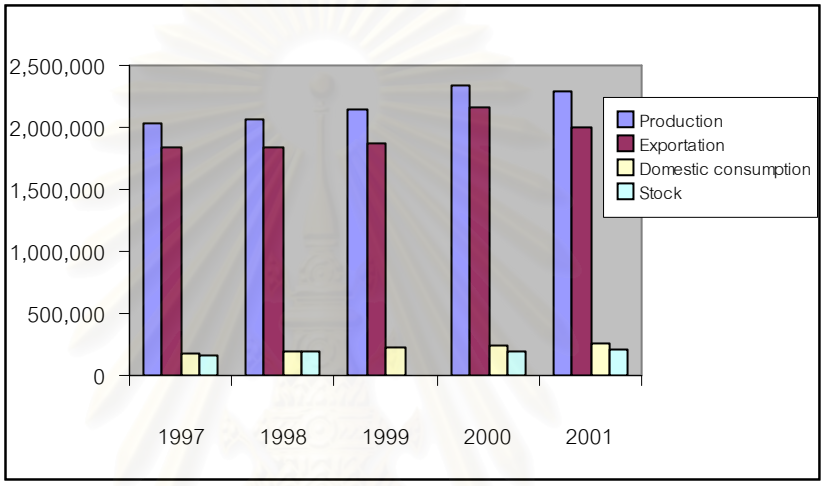

Figure 1.1 Thailand Natural rubber production, exportation, domestic consumption and stock

Natural rubber products from latex have been used widely for over a hundred years. This is attributed to the superior-processing behavior and high physical strength of rubber. There are several products made from latex which can be divided into two groups. The first is NRL made from concentrated latex such as glove, condom, tip catheters, endotracheal tubing latex balloon, baby bottle nipples, and dental cofferdams. The second is NRL made from solid rubber such as tire, shoes, adhesives, elastic rubber, and medical products. Figure 1.2 shows the overall picture of concentrated latex and other solid rubber production scheme in Thailand.

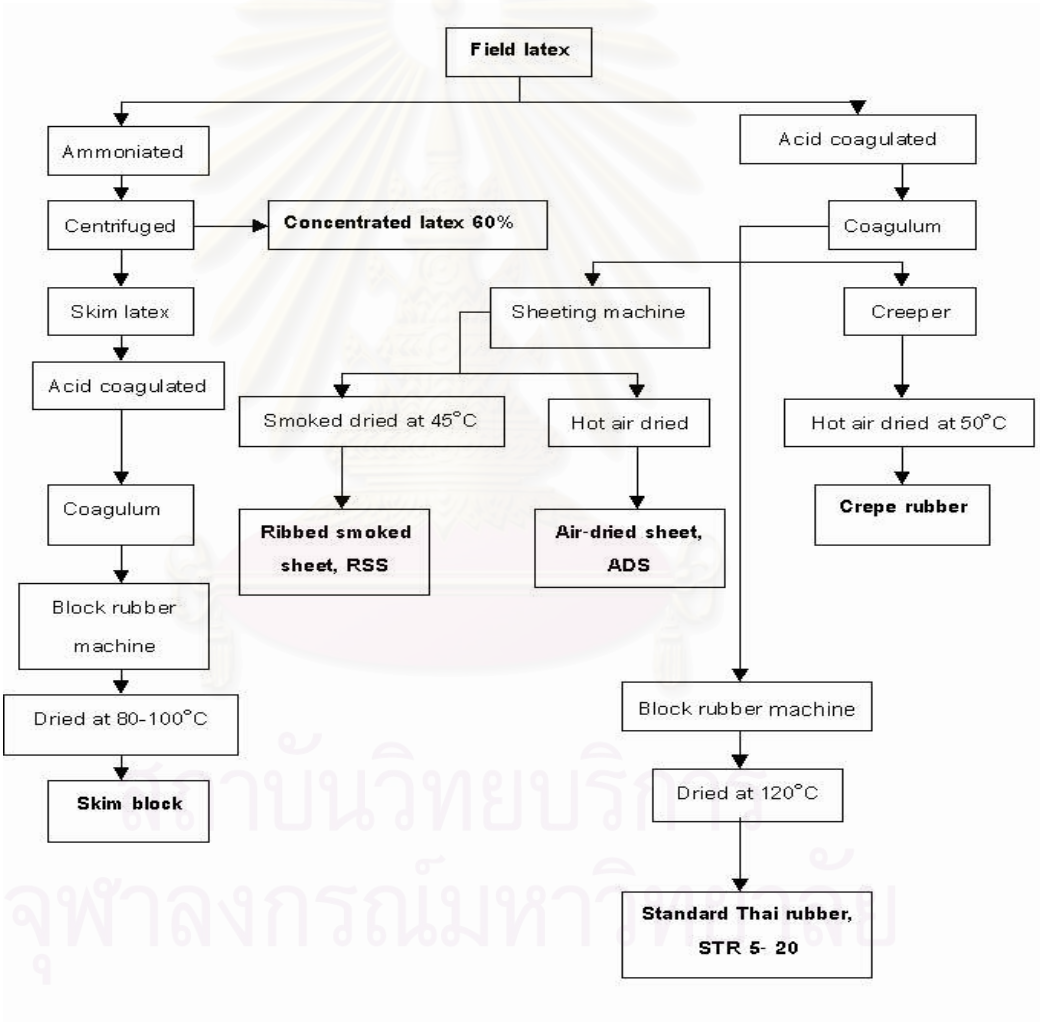

Figure 1.2 Natural rubber production process in Thailand

(From Para rubber Bulletin, Thailand 7, 1986)

Most latex gloves are made by a process of dipping ceramic or aluminum formers (or molds) into a solution of latex compound. The formers are pre-coated with a solution of coagulant to 'hold' the latex on them. The 'wet' gloves are dried and cured in a heated oven. Powdering by cornstarch to improve doning is done on line. For household gloves cotton flocks may be applied to the inner surface to make them non-tacky and silky smooth. They may be double dipped with a different colored latex compound to add thickness and variations to the design and appearance. Formers may also be textured or patterned to lend a better grip or control during usage of the gloves. The presence of sulfur in medical gloves may pose a problem for their disposal by incineration. Use of radiation or peroxide prevulcanized latex would help to overcome this pollution problem. Research is continuing to overcome whatever limitation NR latex gloves may have in improving further their green image.

1.2 Natural rubber latex

1.2.1 Tapping of natural rubber latex

Latex is a natural substance harvested from the rubber tree *Hevea brasiliensis* or Para rubber tree by a process called “tapping”. The rubber trees are usually 6- 7 years old with the stem- perimeter not less than 50 cm at the height 150 cm above the ground. The sap from this tree (natural rubber latex) is a translucent cloudy and milky white liquid which undergoes acid-coagulation to solid elastic rubber in 4- 6 hours at room temperature. Inside a rubber tree, the latex flows in vessels found in the thin layer closet to the cambium, a region of a tree trunk where rapid cell division and growth occurs. The latex vessels spiral up the trunk, forming a right- handle helix. Fresh latex has the density of 0.975- 0.98 gdL^{-1} with the pH of 6.5- 7.0. To harvest the latex, a worker shaves off a slanted strip of bark halfway around the tree about 2- 3 millimeters. Tapping is repeated every 2 days and ceased on the 3^{rd} day. When the last scars created by the cuts is about 1.2 meters above the ground, the other side of the tree is tapped in a similar fashion while the first side renews itself.

1.2.2 Composition of Natural rubber latex

Fresh latex is a polydisperse system in which negatively charged particles of various types are suspended in an ambient serum (C-serum). The two main particulate phases contained in latex are rubber particles (Figure 1.3) constituting 30-45% which are spherical droplets of hydrocarbon enclosed in a fine phospholipoprotein envelope (Jacob *et al*., 1992) suspended in serum and lutoid particles (10-20%) which contain several enzymes. The third type, on a quantum basis, is the Frey-Wyssling complexes (Frey-Wyssling, 1929) which contain pigments such as carotenoids.

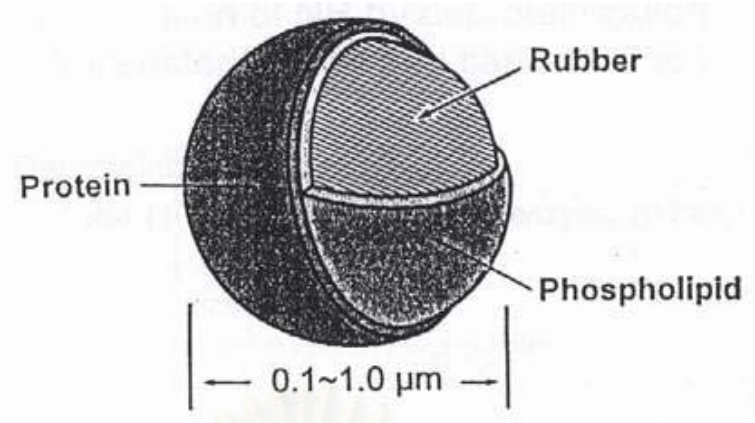

Figure 1.3 Presumed structure of rubber particle

The rubber particles contain polymers of cis-1,4 polyisoprene coated with a hydrophilic layer of proteins, lipid and phospholipids (Vandenplas, 1995).

The composition of the fresh latex is rather complex due to its origin and the relative proportions of certain constituents (e.g. proteins and minerals),which can show important variations depending on many factors (clone, season, tapping system). The same is true for its rubber content, which is normally between 34 and 38%. A typical composition is as shown in Table 1.1.

Table 1.1 Composition of fresh fieldlatex (Fong, 1992 and Kevin, 1999)

Constituents	Percent (w/v)
Total Solids Content	25.00- 45.00
Dry Rubber Content	23.00- 42.00
Amino Acids and N-Bases	0.30- 0.40
Neutral lipids	0.40- 1.00
Proteins	1.00- 1.80
Phospholipids	0.50- 0.60
Inositols-Carbohydrates	1.00- 2.00
Salts (mainly K,P and Mg)	0.40- 0.60
Water	48.50- 71. 80

When the latex is coagulated by 2% formic acid and dried, it contains the dry rubber content (DRC) about 25-45 % w/v which depending on the season of tapping, clonal and other factors. The latex contains the total solids higher than the dry rubber content of about 2-5 % (w/w). The difference between TSC and DRC is non-rubber portion, which made up mainly of 2-3% protein and phospholipid, 1%fatty acids, 0.4% carbohydrates, and 0.3% inorganic salts (Table 1.2).

Table 1.2 Composition of acid coagulated NR

Constituents	Percent (w/w)
Rubber hydrocarbons	93.70%
Neutral lipids	2.40%
Glycolipids, Phospholipids	1.00%
Proteins	2.10%
Carbohydrates	0.40%
Inorganic constituents	0.20%
Others	0.10%

1.2.3 Proteins in Fresh field latex

The total protein content of fresh field latex is about 1.0- 1.80%. They are distributed in three major fractions; the rubber phase (27%), the C- serum (48%) and within the lutoid or the B- serum (25%). The proteins in the rubber fraction are mainly associated with the interfacial layer surrounding the rubber particles. They are mostly water insoluble and are only extractable by detergents or organic solvents such as sodium dodecyl sulfate (SDS) and chloroform- methanol. The protein adsorbed on the rubber particles have not been studied in much details due to the difficulties of removing them from the rubber particles (Fong, 1992). There is however a small fraction of the rubber particle proteins (1 mg/g rubber) which are water soluble and extractable by ammonia. They are largely anionic proteins with pI between pH 3.5- 6.0 and molecular weight of 14.0 and 24.0 kDa. The major proportion of water soluble proteins in fresh latex is derived from the B- and C- sera. They consist of variety of both anionic and cationic proteins with pI ranging from pH 3.5- 9.5; majorities are anionic proteins with molecular weights between 14.0 to 66.0 kDa. A few low molecular proteins (< 14 kDa) are also observed. Hevein, a 5.0 kDa (pI 4.7) B- serum protein could be one of them. Proteins do not confer any significant advantages to the properties of NRL (Shin *et al*., 1974). The presence of proteins in the natural rubber have been reported to have resulted in some undesirable properties such as poor creep and stress- relaxation, reduces modulus and increasing sensitivity to water and moisture.

1.3 Latex allergy

It had been recognized that rubber products can induce contact dermatitis from Type IV allergy or delayed type allergy due to the effect of glove occlusion namely accelerator which is, particularly the thiuram group. In 1979 the first case of immediate contact urticaria on the hands after the use of latex household gloves was reported (Nutter, 1979).

1.3.1 Definition of antigen and allergen

An antigen is a foreign chemical or biological molecule that can sensitize immunological reactions in animals namely increasing sensitized lymphocytes and increasing specific antibody.

An allergen is any antigen that causes allergy. The term is used to describe either the antigenic molecule itself or its source, such as pollen grain, animal dander, insect venom, food product or other natural products. Hypersensitivity and sensitivity are often used as synonyms for allergy. Immediate hypersensitivity and delayed hypersensitivity are the term formerly used to define antibody- mediated allergy and T lymphocyte- mediated allergy, respectively (Coombs and Gell, 1963).

1.3.2 Definition of allergy

Coombs and Gell (1963) analyzed the clinical manifestations and proposed for definition and mechanisms of Type I- IV allergic reactions caused by allergens (cited by Amos, 1976).

Type I: This first type corresponds to immediate hypersensitivity and is brought about the antigen- antibody reaction at the surface of mast cells and basophils, resulting in the release of pharmacologically active mediators. Clinically, type I hypersensitivity is expressed as systemic anaphylaxis or as a local weal and flare reaction

Type II: The damage associates with type two is mainly complement- mediated cytolysis, brought about antibody reacting with antigens on the surface of cells and fixing complement. The antigenic determinants can be part of the cell surface such as blood group antigens, or may become fixed to the cell surface so that the cell is

damaged indirectly. The latter is the mechanism for antigen formation in hemolytic anemia by penicillin.

Type III: The damage results from the deposition of antigen- antibody complexes. The clinical signs and symptoms produced depend on the target organ in which the complexes are deposited, but the organs which are most often affected, are the kidney and the skin. Serum sickness is also included in this category of allergy.

Type IV: This reaction corresponds to reactions termed originally 'delayed hypersensitivity'. No serum antibodies are involved in this type: the tissue damage is brought about by the direct interaction of antigen with specifically sensitized (allergized) lymphocytes.

1.3.3 Latex allergy caused by NRL products

Allergic reactions to natural rubber latex have increased during the past 10 years, especially in many health care workers who have high exposure to latex allergens both by skin contact and by inhalation of latex particles from powdered gloves (Nielson *et al*., 2000). The Type I (immediate- type hypersensitivity) and Type IV (delayed type hypersensitivity)- allergies are relevant in glove use.

1.3.3.1 Delayed- type allergy (Type IV, T-cell- mediated)

The sensitizing agents were mercaptobenzothiazole, carbamate and *p*- phenylenediamine. In the delayed hypersensitivity, the induction and sensitization stages include development of immunized T- lymphocytes able to recognize the antigen, which take about seven days. Thereafter, exposure of the sensitized person to that antigen elicits the cutaneous reaction, which peaks in approximately 48 hours.

The rubber product which most often causes dermatitis is gloves. Rubber gloves are used in food industry, the household and especially in the hospital. Often they are used because of dermatitis of another type, which started on the hands. After that a secondary sensitization to rubber chemicals may occur. Other common forms of contact are via boots, shoes, tubes, handles, tires, fingerstalls, undergarments, protectors on fingers, bands, packings, adhesive tapes, condoms and condom urinals.

1.3.3.2 Type I Immediate- type allergy (caused by latex proteins)

This type of latex allergy occurs within an hour of exposure to natural rubber products. The spectrum of latex- related allergic reaction ranges from mild urticaria, to rhinitis, bronchospasm and anaphylactic reactions, which can cause death. Immediate latex hypersensitivity has different immunologic mechanisms, mediated by IgE. Interaction between IgE and antigen elicits massive local release of histamine and arachidonic acid metabolites, and consequently increased vascular permeability to the antigen, which cause systemic intermediate reactions. The antigens in the IgE- mediate response are latex proteins or polypeptide.

Type I allergy to natural rubber latex and its products can be an important health problem for latex- exposed individuals such as health care workers (Liss *et al*., 1997), spina bifida children (Niggeman *et al*., 1998) and multi- operation patients. The first case of an immediate allergy to NRL was reported in 1927 by Stern who described severe generalized urticaria caused by a rubber dental prosthesis. In 1979, Nutter reported the first glove related cause of this type, contact urticaria. The first report of anaphylactic shock, in this case caused by surgical gloves, came from Finland in 1984 (Turjanmaa *et al*., 1984). Anaphylactic shock can be described as follows: exposure to protein allergens in certain individuals lead to a response in their immune system which brings about a condition of hypersensitivity known as *anaphylaxis* (Greek *ana*: against; *phulaxis*: protection). On subsequent exposure, the interaction of the allergen and antibody formed in the sensitizing phase culminates through a complex mechanism in a systemic release of histamine and other amines. The main effect of histamine in the circulation is to dilate the peripheral vessels, with a consequent severe fall in blood pressure and anaphylactic shock. Among the effects seen are: speeding of the heart rate, breathing difficulty, urticarias, and fluid release in the tissues and unconsciousness. Between 1979 and 1986 Morales *et al* (1989) studied six patients with anaphylactic shock caused by glove and balloon contact and published a strong evidence that proteins in the articles were responsible. They also made the interesting observation that simple washing of the gloves (conditions unspecified) allowed the sensitized patients to wear them without any problems. Sensitization to latex, however, is also frequently observed beyond the risk groups (health care workers, spina bifida children and multi

operation patients). In a group of 1,000 blood donors 6.5% were found to have IgE antibodies against latex (Ownby *et al*., 1996) and 25% of 493 patients consecutively attending an allergy clinic were sensitized to latex (Ruëff., 1998).

1.3.4 Prevalence of latex hypersensitivity

A person with multiallergen atopic history appears to increase the risk of specific latex hypersensitivity as, of cause, does frequent latex exposure (Gonzalez, 1992). Sussman *et al*. (1991) found that 57% of sensitized to latex gloves had a background of rhinitis, bronchial asthma, eczema, or food allergy. A series of serologic studies indicates that tropical fruits, vegetables, tobacco, grass pollen and latex contain latex cross-reactive allergens (Alenius *et al*., 1996; Brehler *et al*., 1997; Breiteneder *et al*., 1998; Chen *et al*., 1998; Hänninen *et al*., 1999; Mahler *et al*., 2000).

Prevalence of latex hypersensitivity in the general population is probably less than 2% (Table 1.3). Studies using radio allergosorbent test (RAST) with serum samples from blood donors indicate higher rates of sensitization may be due to different test method or probably because health care workers, Known to be at risk for latex allergy, are more likely to donate blood.

Table 1.3 Prevalence of latex hypersensitivity in atopic and general patients, health care workers and blood donors

Population	Sample size	Diagnosis procedure	% Positive	Author(s)
Consecutive allergy clinic patients	130	Scratch	0.8	Turjanmaa, 1987
Allergy clinic patients without risk factors	272	SPT	0.4	Moneret- Vautrin *et al*., 1993
Consecutive preoperative patients	800	SPT	0.13	Turjanmaa, 1994
Blood donors	1000	RAST	6.5	Ownby *et al*., 1994
Hospital and Dental staff	202	SPT	3.5	Wrangsjo *et al*., 1994
Atopic patients seen for annual check- up	195	SPT/ RAST	5.6/ 8.6	Porri *et al*., 1995
Non- atopic patients seen for annual check- up	170	SPT/ RAST	1.2/ 2.3	Porri *et al*., 1995
Blood donors	1436	RAST	7.9	Merrett *et al*., 1995
Blood donors	352	EAST	4.5	Harncharoen, 1996
Emergency medical providers	41	SPT	9.8	Safadi *et al*., 1996
Health care workers	224/ 405	SPT/ Quiz	3.13/ 12.4	Teeraratkul *et al*., 1997
Hospital employees	135	SPT	8.2	Kibby *et al*., 1997
Anaethesiology staff	101	SPT	15.8	Konrad *et al*., 1997
Hospital employees	1326	SPT	12.1	Liss *et al*., 1997
Dental students	131	SPT	10	Danne *et al*., 1997
Nurse ward/ ITC	140	SPT	22	Douglas *et al*., 1997
Latex glove manufactory workers	583	SPT	1.7	Chaiear *et al*., 2000
Latex tappers	475	SPT	1.3	Chaiear *et al*., 2000
College students	144	SPT	0	Chaiear *et al*., 2000

(Adapted from Warshaw *et al*., 1998)

* SPT: Skin prick test; RAST: Radioallergosorbent test

EAST: Enzyme allergosorbent test Quiz: Questionnaires

1.3.5 Latex antigen identification

Several methods to detect natural rubber proteins have been developed and tested (Alenius *et al*., 1991; Jones *et al*., 1994; Yunginger *et al*., 1994; Swanson *et al*., 1995; Beezhold *et al*., 1996). The total protein assays such as Bradford, Lowry or bicinchoninic acid protein assays have been used to estimate protein concentration in glove samples but they are not specific to the protein allergens. These assays are subjected to error because a variety of chemicals in latex products interfere in these tests (Beezhold, 1993). The Lowry method can be improved by the use of a precipitation step to remove these interfering substances (Yeang *et al*., 1993). Biologically a more relevant way to quantify latex proteins is to use immunchemical methods. Several types of immunochemical assays such as Radio allergosorbent test (RAST), Enzyme link immunosorbent assay (ELISA) and Western blot, are being employed in several countries for natural rubber protein determinations, and comparison of these methods has been reported (Beezhold *et al*., 1996).

In immunoblotting, latex- allergic patient sera show IgE antibodies binding to several NRL allergens, the most important of which appear to proteins with approximate molecular weights of 14, 20, 27, 30, 36, 45 and 75 kDa (Slater and Chhabra, 1992; Alenius *et al*., 1993; 1994). At present, important allergens have been characterized in NRL, but knowledge about the allergens and their concentration in manufactured NRL products such as gloves is scanty. Nine NRL allergens, eight of which have a nomenclature designation on the official Nomenclature List, are described in Table 1.4.

Table 1.4 Names, structure and clinical importance of NRL allergens viewed by Ylitalo (2000).

Systematic / conventional name of the allergen	MW (kDa)	Function as NR allergen and structural homology	Sensitized patients	Authors
Hev b 1/ Rubber elongation factor	14.6	Major allergen in multioperated children, in HCW Tightly bound to rubber particles	1) 67%SB(ELISA), 81%SB(EAST) 2) 10%A+C(ELISA), 52%HCW(EAST)	Alenius *et al*., 1996a Chen *et al*., 1997b
Hev b 2/ β-1,3- glucanase	36	Defence- related protein, homology to several plant β-1,3- glucosidase	21%A+C(IB)	Alenius *et al*., 1995a
Hev b 3/ 23 kD Rubber particle protein	23	Major allergen in children with spina bifida homology to Hev b 1	1) 80%SB(IB), 76%SB(ELISA) 2) 20% HCW(ELISA)	Alenius *et al*., 1993 Lu *et al*., 1995
Hev b 4/ Microhelix protein complex	50- 57	Significance as NRL allergen unknown	-	Sunderasan *et al*., 1995
Hev b 5/ Acidic C- serum protein	16	Major allergen in children with spina bifida and HCW homology to kiwi fruit protein	1) 52%HCW(IB) 2) 92%HCW and 56%SB(RAST)	Akasawa *et al*., 1996 Slater *et al*., 1996
Hev b 6.01/ Prohevein	20	Major NRL allergen	1) 69%A+C, 84%HCW, 48%SB(ELISA)	Alenius *et al*.,1996c Banerjee *et al*., 1997
Hev b 6.02/ Hevein, prohevein N- domain	4.7	Major NRL allergen, homology to several chitin- binding proteins in plants	2) 56%A+C, 88%HCW, 56%SB(ELISA), 75%HCW, 27%SB(EAST)	Alenius *et al*.,1996c Banerjee *et al*., 1997 Chen *et al*., 1997a
Hev b 6.03/ Prohevein C- domain	14	Homology to wound- inducible proteins (WIN 1 in potato)	21%A+C, 3) 40%HCW, 28%SB(ELISA)	Alenius *et al*., 1996c Banerjee *et al*., 1997
Hev b 7/ Patatin- like protein	46	Homology to patatin storage proteins in potato and tomato	23%HCW(IB)	Beezhold *et al*., 1994; 1996
Hev b 8/ Profilin	14	Significance as NRL allergen unknown	11%HCW(IB)	Vallier *et al*., 1995
Hevamine, Chitinase	30	Minor allergen	3%A+C(IB)	Alenius *et al*., 1995a

HCW: Health care workers SB: Spina bifida IB: Immunoblotting

A: Adults C: Children EAST: Enzyme allergosorbent test

The immunoblotting studies show that IgE from sera of latex- allergic patients binds heterogeneously to many different proteins ranging from 4- 200 kDa as summarized in Table 1.5.

Table 1.5 Identified latex antigens by IgE- immunoblotting

Author(s)	≤ 5- 25 kDa	26- 50 kDa	> 50 kDa
Turjanmaa *et al*., 1988	25	30	
Morales *et al*., 1989	10, 24	35	100
Turjanmaa and Reunala, 1989	3, 10		
Turjanmaa *et al*., 1990	10		
Alenius *et al*., 1991	4, 14, 21		70
Alenius *et al*., 1992	14, 21	29	53
Chambeyron *et al*., 1992	10, 15, 18, 20, 25	30, 35	60
Fuchhs and Wahl, 1992		28	
Jaeger *et al*., 1992	14	30, 45	
Slater and Chhabra, 1992	14, 20		
Tomazic *et al*., 1992	4, 20(AL)		200(NAL)
Czuppon *et al*., 1993	14.6		58(tetramer of 14.6)
Alenius *et al*., 1994	14, 20	27	
Slater and Trybul, 1994	14.3	26.7, 46	
Alenius *et al*., 1995	14, 20, 24	30, 36, 46	
Chiu *et al*., 1997	14, 18, 23, 25		
Eriksen *et al*., 1997	14, 21	30, 35, 44	
Nieto *et al*., 1997	11, 12, 13	27, 32	
Yeang and Ward, 1997	22, 23		
Posch *et al*., 1998	4.7, 6.02, 14.6		
Guillermo *et al*., 2000	7, 14, 17	27, 30, 33	

(Adapted from Warshaw *et al*., 1998)

AL: Ammoniated latex NAL: Non- ammoniated latex

1.4 Removal of proteins from natural rubber latex

The removal of proteins from natural rubber latex has been formerly studied to improve technological and dynamic properties. There are major approaches for removal of proteins from natural rubber latex, which can be summarized as below:

1. Treatment by using surfactant or detergent by which proteins were eluted out from the surface of rubber particles (Yapa, 1984).
2. Chemical or alkaline treatment by immersing in sodium hydroxide solution for 24 hours by which proteins were hydrolyzed by chemical reaction such as saponification. The raw rubber obtained from this process was so called “Saponified rubber; SAPNR” (Ong, 1974).
3. Biochemical or enzymatic treatment by using proteolytic enzyme such as Papain, Alcalase, Trypsin, Superase etc., which proteins were hydrolyzed into small peptides and amino acids. These can be leached out easily. This method is the most suitable for removal of proteins due to its mild condition with little effect on rubber (Nadarajah *et. al*., 1973, Chin *et. al*., 1974, Ong, 1974).
4. A new process for the preparation of protein free latex has been developed. In this new process the radiation- vulcanised, centrifuged latex is subjected to dilution and then centrifuged. In the case of field latex, it is irradiated first and then centrifuged after dilution. The new process results in pre- vulcanized latex almost free from soluble proteins (Varghese *et. al*., 2000).

1.5 Properties of deproteinized natural rubber

Deproteinized natural rubber (DPNR) is very closely similar in physical and chemical characteristics to synthetic cis-1,4- polyisoprene. DPNR has better green strength and building tack than synthetic polyisoprene. DPNR shows excellent dynamic properties and improved stress relaxation behavior when compounded with rubber-soluble vulcanizing ingredient, particularly suitable for use in engineering applications. The properties of DPNR produced at the Rubber Research Institute of Malaysia (RRIM)Experiment Station (Subramaniam *et al.*, 1993) were shown in Table 1.6.

Table 1.6 Properties of DPNR produced at RRIM

Properties	DPNR	High ammonia latex
Total solid content (% w)	63.32	61.59
Dry rubber content (% wt))	61.51	60.04
Non rubber content (% w)	2.04	1.42
NH_3 (%)	0.66	0.69
Ash (% wt)	0.27	0.46
Volatile Fatty acid (%)	0.01	0.02
Nitrogen (% wt)	0.07- 0.09	0.2
KOH	0.30	0.69

1.5.1 The development of deproteinized natural rubber latex (DPNR)

There are several attempts to produce solid rubber product and concentrated latex with a very low protein content. This type of natural rubber has been known as 'Deproteinized natural rubber, DPNR' and 'Deproteinized concentrated latex, DPCL'. There are several methods used for deproteinization of natural rubber latex, which can be summarized in Table 1.7.

Table 1.7 Development in DPNR, DPCL, DP skim rubber production

Raw material	Deproteinized procedure	Product(s)	Author(s)
Skim latex	Sodium hydroxide	DP Skim rubber	Firestone Tyre Rubber Co,1955
Field latex	Dioctyl- octyl sodium sulfursuccinate	Solid rubber	John, 1971
Skim latex	Sodium sulfosuccinate and calcium chloride	DP Skim rubber	John and Sin, 1973
Field latex	Papain	Solid rubber	Nadarajah *et al*., 1973
Concentrated latex	Superase	Solid rubber	Chin and Smith, 1974
Skim latex	Trypsin	DP Skim rubber	Ong, 1974
Skim crumbs	Sodium hydroxide and oxalic acid	DP Skim rubber	Ong, 1974
Field latex	Papain / $NH_2OH.HCl$	Low nitrogen content and constant viscosity DPNR	Yapa, 1975
Clarified latex	Superase or Alcalase	DPNR	Chang *et al*., 1977
Clarified latex	Superase/ $NH_2OH.HCl$	DPNR	Chang *et al*., 1977
Field latex	Papain/ Alkaline treatment	DPNR	Yapa, 1977
Field latex	Papain/ Alkaline treatment/ $NH_2OH.HCl$	DPNR	Yapa, 1977
Skim latex	Papain	DP Skim rubber	Yapa *et al*., 1978

Raw material(s)	Deproteinized procedure	Product(s)	Author(s)
Field latex	Pineapple juice (Bromelain)	DPNR	Yapa *et al*., 1980
Field latex	Papain	DPNR	Yapa *et al*., 1984
Field latex / Concentrated latex	Papain or Alcalase	CV- DPNR	Visessanguan, 1992
Concentrated latex	Alcalase/ Sodium naphtenate	Solid rubber	Eng *et al*, 1992
HA latex	Alcalase/ Sodium dodecyl sulfate	DPNR	Eng *et al*, 1993
Field latex	Immobilized Papain on chitin	DPNR	Boonjawat *et al*., 1995
Field latex	Alcalase or Papain	CV- DPNR	Weeraphasuk, 1995
HA latex	Proteolytic enzyme	Highly DPNR	Nakade *et al*., 1997
Field latex	Enzymeatic, Transesterificationn and Saponification	DPCL, DPNR	Tangpakdee and Tanaka, 1997
Skim crumbs	Saponification	DP Skim rubber	Rungvichaniwat *et al*., 1998
Field latex, ammoniated latex, skim latex	Saponification	SAP-NR, SAP- AL, SAP-SK	Boonsuk, 1999
Field latex	Alcalase or Papain	DPCL	Klinpituksa *et al*, 1998
Field latex	Papain or Bromelain	DPCL	Adulyathum and Kongsilpa, 2000
Field latex	Papain/ microwave energy	DPNR	Chianrungsang, 2000

(Adapted from Weeraphasuk, 1995)

DPNR: Deproteinized natural rubber DPCL: Deproteinized concentrated latex

CV- DPNR: Constant viscosity deproteinized natural rubber

SAP- AL: Saponified ammoniated latex SAP- L: Saponified latex

SAP- SK: Saponified skim rubber

1.6 Diagnosis of Natural rubber latex allergy

A detailed history of allergy symptoms and clinical examination are always important for diagnosing NRL allergy. The patients may have only mild symptoms which easily pass unnoticed or may be confused with other allergies. In addition, patients sensitized to NRL can also be asymptomatic (Yassin *et al*., 1992; Hadjiliadis *et al*., 1995; Sussman and Beezhold, 1995; Nieto *et al*., 1996). It should be noted that at present there are no diagnotic test methods for NRL allergy which are 100% sensitive and specific.

1.6.1 Skin prick test (SPT)

Many previous studies have used SPT in diagnosing NRL allergy (Wrangsjö *et al*., 1988; Moneret- Vautrin *et al*., 1993; Turjanmaa *et al*., 1995; Hadjiliadis *et al*., 1995; De Swert *et al*., 1997; Blanco *et al*, 1998). Because of the lack of standardized commercial NRL, glove extract and crude NRL with or without ammonia have been used in skin prick testing. The sensitivities and specificities of different SPT reagents are given in Table 1.8.

Table 1.8 Sensitivities and specificities of different SPT reagents

SPT reagents	Sensitivity Adult*/ Children with spina bifida**	Specificity Adult*/ children with spina bifida**
Glove extract^	84%[3]- 92%[1]/ 100%	100%[1,3]/ 95%
Stallergenes	88%[1]- 93%[2]/ 64%	100%[2,3]/ 91%
ALK	54%[1]- 90%[3]/ NK	100%[3]/ NK
Bencard	92%[1]/ NK	100%[1]/NK

* Turjanmaa *et al*., 1994[1], 1997[2], Blanco *et al*., 1998[3]

**De Swert *et al*., 1997 ^ Triflex® glove

ALK: Commercial reagent (ALK a/s, Hørsholm, Denmark)

NK: not known

Skin prick testing has been used safely for years by many researchers in the screening and diagnosis of NRL allergy (Turjanmaa *et al*., 1996; Hadjiliadis *et al*., 1995). Prick test technique is regarded safer than intradermal testing and one peaked SPT lancet with shoulders can be recommended over multipeaked lancet (Turjanmaa *et al*., 1995). If there is still a need to use in- house SPT reagents, the allergen concentration of the NRL source material should be known in order to avoid adverse reactions or as well as false negative test results.

1.6.2 Latex RAST and other measuring IgE antibodies

IgE antibodies to NRL in the patients' serum have been most often measureed by the CAP RAST method (Pharmacia, Uppsala, Sweden). Latex RAST seems to detect well highly allergic patients, but this test has been negative even in NRL- allergic patients with anaphylactic reactions (Axelsson *et al*., 1998; Leynadier and Dry, 1991; Jäger *et al*., 1992). Moreover, the sensitivity and specificity of latex RAST have not been clarified in the NRL- allergic children who have no history of multiple operation (*Sorva et al*., 1995).

IgE antibodies can also be measured by ELISA method. In one study, which included also patients with spina bifida, in- house ELISA method showed a 87%sensitivity and 66% specificity (Kelly *et al*., 1993).

There is some evidence that the manufacture of glove may change the NRL allergens or even produce neo- allergens (Makinen- Kiljunen *et al*., 1992).

1.6.2 Challenge tests

1.6.3.1 Use test

A use test with NRL glove is needed to confirm the NRL allergy diagnosis when there is discrepancy between clinical history and the SPT and/ or RAST are positive. The NRL glove use test should be performed with a highly allergic glove brand (Turjanmaa *et al*., 1996; Baur *et al*., 1998). It should be started with a finger piece of a glove because the use test with the whole glove on eczematous skin can cause anaphylaxis (Turjanmaa and Reunala, 1988). If no wheals occur with a finger piece, the test is continued with a whole glove. To increase allergen penetration into the skin from

the NRL gloves during challenge, Hamilton *et al.* (1997) first punctured the skin of the hands with needle, and then applied the NRL gloves.

1.6.3.2 Inhalation tests

When rhinitis or asthma is suspected to be caused by the NRL products, an inhalation challenge can be performed to confirm the diagnosis. Bronchial provocation tests have been performed by inhaling nebulized glove extract or by handling and shaking powdered NRL gloves in a special challenge room. A nasal provocation test has been performed by applying NRL glove powder on a cotton swab to the nasal mucosa for 5 min. Allergic response is detected by anterior rhinoscopy, rhinomanometry and measurement of nasal secretions (Kujala *et al.*, 1995).

In Thailand there are several researchers who studied NRL allergic reaction. Harncharoen (1996) has developed the immunoassay to detect protein allergens in rubber. He found that the atopic patients had 2.6 time higher risk than the healthy ones. By using the latex- specific IgE positive sera to identify the molecular weight of protein allergens. The identified- allergens were 14, 18, 25.5, 30, 38 and 52 kDa.

Bhuvanath and Pornpetsuk (1999) have detected protein allergens in rubber glove and allergic reaction in guinea pigs by Latex ELISA of Antigen Protein (LEAP) assay. They found that ten of twenty- four gloves had protein allergens, especially surgical gloves which are the highest allergen- contained and house- hold gloves which are the lowest allergen- contained. They also found that there were no correlation between protein allergens and protein quantities determined by modified Lowry method.

Sri- Akajunt *et al*. (1999) studied natural aeroallergen exposure in rubber plantation workers and glove manufacturers in Thailand and health care workers in a UK hospital. Personnel sampling was conducted using a 25 mm polytetrafluroethylene (PTFE) into an IOM sampling at 2 L/ min. NRL aeroallergens were measured by inhibition assay with NRL- specific IgE antibodies from NRL- sensitized people. They found that the highest geometric mean (GM) NRL aeroallergen concentration was found in the glove manufacturing factories (7.3 $\mu g/ m^3$), followed by the rubber plantations (2.4 $\mu g/ m^3$) and

the UK hospital (0.46 μg/ m^3). The GM NRL aeroallergen for these tasks were in the range of 12.90 to 17.80 μg/ m^3.

1.7 The rationale and purposes of this study

In 2000 Thailand ranked at the first place of the world natural rubber supplier. But the raw rubber do not cost as much as it's end products such as tires, footwears, gloves and condoms.

Latex gloves and condoms gained popularity during the 1980's. They are excellent protective barrier against HIV, the virus that causes AIDS. Latex gloves are also extremely durable. This type of thin film latex product can be stretched to over 5 times its origin length without tearing. The snug fit is important to surgeons and dentists. These health professionals must have maintained tactile senses and manual dexterity during procedures.

Since in the late 1980's, latex allergy has been recognized as a major problem in Europe and North America, especially in individuals such as healthcare workers who use latex gloves everyday and in patients heavily exposed to latex gloves for example, children with spina bifida, the patients who undergo multiple surgical interventions for congenital neurological or urological abnormalities, atopic individuals, housekeeping personnel and other glove- wearing persons.

The aims of this research are to promote the production of purified natural rubber latex for more value added low protein products and to develop the test kit for protein allergens in natural rubber latex and its products. It is expected that, firstly better quality concentrated latex developed in this research should replace ordinary concentrated latex and secondly the standard latex protein allergen test kit for diagnosing latex allergy in health care workers and protein allergen in NR products, which can decrease cost of protein allergens determination both for hospital and industrial sector.

The objectives of this research are:

1. To produce deproteinized concentrated latex by Alcalase and study physical properties of deproteinized concentrated latex compare to control concentrated latex
2. To extract water extractable protein (WEP) from concentrated latex to use as standard proteins for protein determination procedures
3. To study molecular weight distribution of WEP in deproteinized concentrated latex compare to control concentrated latex
4. To separate latex serum protein in the range of 4- 60 kDa by Gel- filtration to use those proteins as standard proteins for sodium dodecyl poly-acrylamide gel (SDS- PAGE)
5. To provide a standard latex serum proteins kit for SPT from latex serum proteins and column purified serum latex proteins

CHAPTER II
MATERIALS AND METHODS

2.1 Biological materials

2.1.1 Natural rubber latex

Two types of natural rubber latex were used in this research;

Ammoniated latex from mixed rubber clones were obtained from Rayong Bangkok Rubber Co., Ltd., was preserved with 0.3% (v/v) ammonia and 0.025% (w/v) Tetramethyl thiuram disulfide (TMTD)/ ZnO.

Skim latex was the serum fraction after centrifugation of ammoniated latex containing about 4- 6 % dry rubber content (DRC), kindly provided by Rayong Bangkok Rubber Co., Ltd.

2.1.2 Alcalase

Alcalase (Purafect, 4000 E) was purchased from Lion Co. Ltd.

2.2 Chemicals

Most chemicals used in this investigation were analytical or reagent grade.

Acetone was purchased from Scharlua.

Acrylamide and N, N'- methylene- bis- acrylamide were products of Sigma.

Ammonium persulfate was product of BIO- RAD.

Copper sulfate was purchased from Fluka.

Selenium powder, Folin reagent, boric acid potassium hydroxide, sodium hydroxide, methanol, *di*- sodium hydrogen phosphate anhydrous GR, Triton X- 100 were products of Merck.

Sulfuric acid and acetic acid were purchased from J.T. Baker.

Sodium metabisulfite was a product from Mallinckrodt.

Potassium sulfate anhydrous was purchased from May and Baker.

Sephadex G- 75 was a product of Pharmacia.

Sodium dihydrogen orthophosphate was a product of CARLO ERBRA.

Sodium alginate and Brilliant Blue R 250 were purchased from Sigma.

Sodium chloride was a product from UNIVAR.

Normal saline solution was kindly provided from the Division of Dermatology, Department of Medicine, Chulalongkorn University.

Sodium dodecyl benzene sulfonate (SDBS) was industrial grade purchased from Lion Co. Ltd.

2.3 Apparatus

2.3.1 Apparatus in the Department of Biochemistry

pH meter model PHM-83 autocal, Radiometer, Denmark

Centrifuge Type H-11 N, Kokusan Ensinki Co., Ltd., Japan

Centrifuge, refrigerated centrifuge model J- 21 C, Beckman Instrument Inc, USA.

Centrifuge, microcentrifuge high speed model MC- 15 A, Tomy Seiko Co. Ltd., Japan

Incubator shaker model G 76D, New Brunswick Scientific Co., Inc. Edison, N. J. USA.

Magnetic stirrer and heater model IKAMA® GRH, Jane & Kunkel Gmbh & Co. KG, Japan

UV- Visible spectrophotometer model UV- 240, Shimadzu, Japan

Freeze dryer (Lyophilizer), Eyela Tokyo Rikakikai Co. Ltd., Japan

2.3.2 Apparatus kindly provided by National Research Council of Thailand

Elisa Reader, Multiskan Ex 200- 240 v, Labsystems,

Total nitrogen analyzer, Gerhardt, Germany

Gel filtration set from Bio- RAD included Econo pump model EP- 1, Econo system rack, Econo fraction collector model 2110, USA.

SDS- PAGE, Hoefer mini VE set from Amersham Pharmacia, Sweden

Freeze dryer (Lyophilizer), Labconco Lyph 1 L, Labconco Corporation, USA.

2.3.3 Apparatus kindly provided by Rayong Bangkok rubber Co. Ltd.,

Centrifuge, (Alfa Laval, 7000xg) and 200- liter latex storage tank

2.4 Assay of Alcalase specific activity

The enzyme activity was determined by measuring the absorbance of tyrosine (A280) liberated from casein substrate. This procedure was modified from Richardson and Te Whaiti's method (1978). By dissolving Alcalase in 0.05 M Tris- HCl buffer pH 7.6 at the concentration of 0.02 g/ 100 ml and 0.1 ml of this enzyme solution was diluted with 0.9 ml Tris- HCl before preincubation at 45 ^{o}C in a shaking water bath. At zero time, 1 ml of preincubated 0.5% casein substrate solution was added into the enzyme solution and incubated for exactly 20 minutes at 45 ^{o}C, then the reaction was stopped by adding 2 ml of 10 % trichloroacetic acid (TCA) solution and kept for another 30 minutes. Only clear solution was removed by centrifugation at 2500x g and measured for the optical density (OD) at 280 nm wavelength in a spectrophotometer. Blank of test was prepared by adding 2 ml of 10 % TCA in enzyme solution before adding 1 ml of casein substrate solution and incubated at the same condition as sample. Tyrosine standard curve, showing relationship between absorbance at 280 nm and concentration of tyrosine, was prepared by dissolving 0.01 g tyrosine in 100 ml distilled water and then diluted to various concentrations (20- 160 μg/ ml). Alcalase specific activity was reported in casein digestion unit (CDU) according to the following calculation:

$$\text{Alcalase specific activity (CDU/ mg)} = \frac{\text{net } OD_{280} \times 4 \text{ (ml)}}{\text{Slope} \times w \text{ (mg)} \times 20 \text{ (min)}}$$

In which net OD_{280} is the difference between OD_{280} of sample and blank tube; 4 is the total volume, in ml, of the final incubation; slope is the slope of tyrosine standard curve; w is the weight in mg of original enzyme preparation in 0.1 ml aliquot of test solution added to the incubation mixture and 20 is the incubation time in minute. By definition 1 casein digestion unit (CDU) was defined as 1 μg of tyrosine liberated from casein digestion by enzyme 1 mg in one minute under the condition of the assay.

2.5 Deproteinization of natural rubber latex

Treatment of latex by Alcalase was performed with field latex which stabilized and preserved with 0.3 % (v/ v) NH_3 and 0.025 % (v/ v) TMTD/ ZnO. The ammoniated latex was determined total solid content (TSC), dry rubber content (DRC), volatile fatty acid (VFA), NH_3 and Mg content and then added diammoniumphosphate (DAP) to reduce Mg content to 50 ppm, maximum. The DAP- ammoniated latex was deproteinized with Alacalase solution that contained 0.08% Alcalase, 0.3 % NH_3, 0.25% (w/ v) sodium dodecyl benzene sulfonate (SDBS), 0.025 % (v/ v) Triton X100 and 0.002 % (w/ v) sodium metabisulfite then left at room temperature for 12 hours. After that, the latex was centrifuged at 7,000 x g and then adjusted to 60 % DRC and 0.6 % NH_3 as high ammonia deproteinized concentrated latex. The reaction size started at 300 ml of DAP- ammoniated latex and then scaled up to 200- 400 L of DAP- ammoniated latex.

2.6 Testing of concentrated latex specification (ISO- 2004- 1979 (E))

2.6.1 Determination of total solid content (TSC)

Weighed the empty petridish and record the empty pretidish weight. An aliquot of 5 g of latex was pipetted into a petridish and dried in an oven at 60 °C for 10-12 hours. Dried rubber in pretidish was weighed and calculated TSC content by the equation below.

$$\% \text{ TSC} = W_1 / W_0 \times 100$$

Where W_1 = weight of the dry rubber in pretidish (g)

W_0 = weight of the latex after subtraction the weight of pretidish (g)

2.6.2 Determination of dry rubber content (DRC)

An aliquot of 5 ml of latex was pipetted into a petridish and coagulated with 5% acetic acid in ethyl alcohol. After complete coagulation occurred, the coagulum was then removed, washed with water, creped and dried in an oven at 60 °C for 10-12 hours. Dried coagulum was weight and calculated DRC content by the equation below.

$$\% \text{ DRC} = W_1 / W_0 \times 100$$

Where W_1 = weight of the dry rubber (g) W_0 = weight of the latex taken (g)

2.6.3 Determination of non rubber content (NR)

Non rubber content was calculated by the equation below.

TSC- DRC = (%) NR

2.6.4 Determination of volatile fatty acid (VFA)

Weighed 50 g of latex in a stainless beaker and added 50 ml of 30% $(NH_4)_2SO_4$. Heat on water bath at the temperature of 100 ^{o}C and squeeze for the serum. Filtrated the serum through Whatman number 1 filter paper. Pipetted 25 ml of serum into a flask containing 5 ml of 50% H_2SO_4. Passed steam through the steam jacketed distillation apparatus for at least 15 minutes with steam outlet open, introduce into the innertube 10 ml of acidified serum by pipette. Place a 100 ml graduated cylinder under the tip of condenser to obtain 100 ml of distilled serum. Degassed for 3 minutes and titrated with standard 0.01 N $Ba(OH)_2$ using phenolphthalene as indicator. The % VFA was calculated by the equation below.

$$\text{Volatile fatty acid, VFA(\%)} = \frac{[67.32 \times N \times V]}{m \times TSC} \times 50 + \frac{m(100 - DRC)}{100 \times P}$$

Where N: Normal of $Ba(OH)_2$ m: weight of latex (g)

V: Volume (ml) of $Ba(OH)_2$ used in titration TSC: % TSC of latex

P: Serum density = 1.02 megagram/ m^3 DRC: % DRC of latex

2.6.5 Determination of alkalinity (NH_3)

Weighed 10 g of latex into the beaker diluted with 200 ml distilled water and added non- ionic stabilizer such as Teric 16A. Titrated with standard 0.1 N $(NH_4)_2SO_4$ with methyl red indicated. Calculated in g NH_3 per 100 g latex.

2.6.6 Determination of Mg content (%Mg)

Weighed 10 g of latex into the beaker, added 10 ml of distilled water and 5 ml of 25% (v/v) acetic acid. Squeezed for the serum and poured on to the 80 mesh filter. Pipetted filtrated 10 ml serum into 50 ml beaker adjusted pH to 10.5 by NH4Cl or NH4OH then pipetted 4 ml of 4% KCN into the serum. Added 0.1 g of Erichrome Black T into the

serum and the serum would turn violet. Titrated with 0.05 M EDTA. End point was the violet color turned blue.

Calculation: the Mg content was expressed in percent (w/w)

$$\text{Percentage Mg} = \frac{24.32 \times B \times D \times 10{,}000}{1{,}000 \quad 10 \quad C}$$

Where B: EDTA factor = $\frac{\text{burette reading}}{(v)} \times \frac{(M)\ \text{EDTA}}{(M)}$

C: Value of solid in 10 g of latex (g) $C = \frac{A \times TSC}{100}$; A: Weight of latex (g)

D: Total volume of serum in sample (ml); D = (A- C)+ 15

(15, 10 ml of water added + 5 ml of 25% acetic acid)

24.32 = Mw of Mg

2.6.7 Determination of mechanical stability time (MST; second)

Adjusted % DRC of concentrated latex to 55 % TSC with NH_3. Weighed 80 g of latex, warmed to 35 ^{o}C and spun with Klaxon machine at 14,000 rpm. Determined clotting time of rubber particles by dipping stirring rod into latex and dropped in water.

2.6.8 Determination of potassium hydroxide (KOH)

Added formaldehyde solution into 50 % TSC latex and diluted to 30 % TSC with water and titrated with standard KOH indicated end- point by pH meter. Plotted graph of 1) pH or 2) dpH/ dV or 3) d^2pH/ dv^2. V is volume of KOH at end- point.

2.6.9 Determination of nitrogen content (RRIM, 1992)

This specification is the rubber specification added to this research because of the requirement from glove manufacturers. The concentrated latex sample was dried as described on Methods 2.4.2. Rubber specimen was weighed accurately about 0.1-0.2 g into a micro Kjeldahl tube and 0.65 g of catalyst mixture (K_2SO_4 : Cu_2SO_4 .5HO : SeO; 30:4:1) and 2.5 ml of concentrated sulfuric acid were added. The mixture was boiled gently in the digestion unit until the solution becomes clear green or colorless with no yellow tint. Cool the digest and transfer to distillation unit followed by three washing with

distilled water, then add indicators which is the mixture of methyl red and bromocresol green into the receiving conical flask. Add about 10 ml of 67% sodium hydroxide solution to the distillation vessel, and pass steam through the distillation apparatus until the volume of distillate in the receiving flask reach 150 ml which take about 5 minutes. Immediately titrate the distillate with standardized 0.01N H_2SO_4. Blank can be prepared by adding all the reagents but omitting the sample.

Calculation: Total nitrogen content was calculated as follows:

$$\% \text{ Total nitrogen} = \frac{(V1-V2) \times M \times 1.4}{W}$$

Where V1= Volume of blank (ml)

V2= Volume of titrant (ml)

M= concentration of H_2SO_4 (N)

W= weight of sample (g)

2.7 Preparation of latex proteins

2.7.1 Extraction of water extractable proteins in solid rubber (ASTM D 5712- 99)

The concentrated latex was poured onto the 20x 20 cm square plate then air-dried at room temperature for 8-12 hours. A piece of latex film (DPCL and its control) was cut from each sample with 1x 1 cm square size, weighed and transferred to a 200 ml flask and added 10 ml of distilled water per gram of specimen. The flask was sealed with sealing film; extraction was at 37 °C and shook for 15 seconds after adding the water and again at 60 and 120 minutes. The extracted solution was filtered through filter paper (Whatman no.1) followed by centrifugation at 2,000 x g for 5 minutes. The filtrated-solution was lyophilized (Method 2.7.2).

The total protein concentrations in these samples were determined by modified Lowry method.

2.7.2 Lyophilization

The filtrated-solution was aliquated in plastic tube, frozen at –80 °C, then lyophilized for 48 hours. Random sampling of lyophilized protein was resuspended with distilled water and assayed for water extractable protein (Method 2.8).

2.7.3 Acetone precipitation

The 200- ml latex serum was added with 1,000 ml of cold acetone (-20 °C) and vortex. After incubation at –20 °C for 10 minutes the latex serum proteins were centrifuged at 8,000 x g for 5 minutes. The supernatant was removed and air-dried pellet was resuspended in 10- 20 volume of buffer solution or distilled water.

2.7.4 Latex protein extracts for skin prick test

The samples were weighted, cut into small strips (1 x 1 cm square) and then extracted in physiologic normal saline solution (NSS) at a 1:5 weight by volume for 15 minutes, at room temperature. The extracts, along with histamine phosphate; positive control and NSS; negative control were used for skin prick testing. The result was defined as that with a wheal equal to or greater than that of a positive control.

2.8 Determination of water extractable proteins by modified Lowry method (ASTM D 5712- 99)

The lyophilized protein samples (2.7.2) were resuspended with distilled water. The reaction was carried out with 160- 200 μl of 0.1 N sodium hydroxide and 2.5- 40 μl of protein solutions, then added 75 μl of alkaline copper sulfate, Reagent D, into each well of a flat bottom, 96-well, polystyrene microtiter plate, mixed and allowed to stand for 15 minutes at room temperature. The reaction was then added with 25 μl of dilute Folin solution, Reagent B, mixed thoroughly and allowed for 15 minutes at room temperature. Protein levels were evaluated against standard protein, ovalbumin, using a microplate reader (Multiskan Ex, Labstytems) at 750 nm wavelength.

2.9 Determination of water extractable protein by Bradford method (Bradford, 1976)

The lyophilized proteins were resuspensed with distilled water. Protein solutions were pipetted (maximum 100 μl) into a test tube, then added with 10 mM Phosphate buffer saline (150 mM NaCl) to make a total volume of 100 μl. One ml of Bradford working buffer was added and mixed thoroughly. The reaction mixture was read for optical density at 595 nm wavelength after 2 minutes but before 1 hour. Protein levels were evaluated against standard protein, bovine serum albumin.

2.10 Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was carried out according to Slater et al. (1990), with a slight modification. Polyaclyamide gel at 15% was used as separating gel and 3% of the gel was used as stacking gel. Tris glycine (25 mM Tris, 192 mM glycine) buffer pH 8.3 containing 0.25% w/v SDS was used as electrode buffer. Sample to be analyzed, was dissolved in Tris buffer, containing 60 mM Tris, 2% w/v SDS, 25% v/v glycerol, 14.4 mM 2-mercaptoethanol, and 0.1% w/v bromophenol blue, and boiled for 5 minutes prior to application to the gel. The electrophoresis was carried out at constant current of 15 mA, on a Mini-Protein (Hoefer mini VE) from cathode toward anode. When the electrophoresis was completed, the gel was stained with Coomassie blue R-250.

2.11 Sephadex G- 75 column chromatography

Sephadex G- 75 column (2.24x 118 cm) was equilibrated with the buffer containing 10 mM phosphate and 100 mM NaCl at 25 oC for 5 column volumes at a flow rate of 20 ml/ hr to allow stabilization of bed volume of the column. An aliquot of the crude latex serum protein in 10 mM phosphate buffer saline (150 nM NaCl) obtained from acetone precipitation (Method in 2.5.3) was applied to the column and separated latex serum proteins were then eluted with the buffer containing 10 mM PBS (100 mM NaCl). Fractions of 2 ml were collected using a fraction collector. The elution profile was monitored for protein by the optical density observed at 280 nm wavelength.

The elution volume (V_e) of the separated latex serum proteins were compared with standard molecular weight maker proteins; bovine serum albumin (BSA; 66 kDa), ovalbumin (45 kDa), chymotrypsinogen A (27 kDa) and cytochrome C (12.3 kDa). Blue dextran 2000 and potassium dichromate were used to determine the position of void volume (V_o) and the total bed volume (V_t), respectively. The partition coefficient (K_{av}) values for each standard marker protein calculated from (V_e- V_o)/ (V_t- V_o) were plotted against log molecular weight of each protein on semi- logarithmic paper to obtain a calibration curve. The K_{av} of the separated latex serum proteins were calculated in the same way and used to determined the native molecular weight from the calibration curve.

2.12 Allergen detection by Skin Prick test (SPT)

Skin testing was performed by use of an epicutaneous method on forearms, using extracts made from control concentrated latex film, deproteinized concentrated latex film and saponified natural rubber (SAP- NR) prepared from ammoniated crumb. These samples were weighted, cut into small strips (1 x 1 cm^2) and then extracted in physiologic normal saline solution (NSS) at a 1:5 weight by volume for 15 minutes, at room temperature. There were 2 latex allergen solutions used in this research, the commercial latex allergen (Stallergenes, France) and the latex serum proteins. The latex serum proteins were prepared by method 2.7.3. The extracts, along with histamine phosphate; positive control and NSS; negative control were used for skin prick testing. The result was defined as that with a wheal equal to or greater than that of a positive control.

CHAPTER III
RESULTS

3.1 Deproteinization of natural rubber latex by Alcalase

3.1.1 Alcalase specific activity

The specific activity of commercial Alcalase used in this research is 0.28 CDU/ mg as determined according to Methods 2.4.

3.1.2 Development of deproteinization process

In the conventional way field latex was stabilized and preserved with 0.3 % (v/ v) NH_3 and 0.025 % (v/ v) tetramethyl thiuram disulfide (TMTD)/ ZnO. The ammoniated latex was determined total solid content (TSC), Dry rubber content (DRC), Volatile fatty acid (VFA), NH_3 and Mg content before adding diammoniumhydrogenphosphate (DAP) to precipitate Mg for 12 hours to reduce Mg content to 50 ppm, maximum. The DAP- ammoniated latex was then centrifuged at 7000 xg. The concentrated latex obtained was adjusted % DRC and % NH_3 to 60 % DRC and 0.6% NH_3 as high ammonia commercial concentrated latex. There were 2 deproteinization processes in this research.

3.1.2.1 The first development of deproteinized process (DP1)

The conditions for deproteinization of the DAP- ammoniated latex by Alcalase can be summarized as follows: field latex with the reaction volume of 300 ml was deproteinized with Alacalase solution that contained 0.1 phr Alcalase (0.28 CDU/ mg), 0.3 % NH_3, 0.25% (w/ v) sodium dodecyl benzene sulfonate (SDBS), 0.025 % (v/ v) Triton X100 and 0.002 % (w/ v) sodium metabisulfite at room temperature for 12 hours. After that, the latex was centrifuged at 7,000 x g and then adjusted to 60 % DRC and 0.6 % NH_3 as high ammonia deproteinized concentrated latex (HA- DPCL). The yield of DPCL starting from 300 ml of field latex was 37.5 %. However when the batch scale was increased to 200 L under the same conditions the yield of DPCL was 26 % and the mechanical stability time (MST) was only 368 s which was lower than the ISO 2004 specifications (Table 3.1).

3.2.1.2 The second development of deprotienization process (DP2)

Due to the low MST of DPCL, stabilizers were added to increase MST in DP2. After incubation of latex with Alcalase for 12 hours, the deproteinized latex was stabilized with 0.2 phr of KOH and 1% ammonium alginate and then centrifuged at 7,000 x g. The latex was adjusted to 60% DRC and 0.6 % NH_3 then added ammonium laurate to 0.35% (v/v). The quality of DPCL obtained was tested according to ISO 2004 specifications. The yield of DPCL of was 37% and the MST increased as shown in Table 3.1.

The conventional process for production of concentrate latex 60% and the developed deproteinized concentrated latex process were summarized in Figure 3.1.

Ammoniated latex 20- 35 % DRC,
0.3 % NH^3, 0.025 TMTD/ ZnO

Mg^2+ precipitated with DAP

Conventional Process	Deproteinization Process 1 (DP 1)	Deproteinization Process 2 (DP 2)
Centrifuged 7,000 x g	0.08 % Alcalase (0.28 CDU/ mg)	0.08 % Alcalase (0.28 CDU/ mg)
% DRC adjusted and NH_3 added to make 60 % DRC and 0.6 % NH_3	0.3% NH_3, 0.1 % SDBS	0.3% NH_3, 0.1 % SDBS
	0.025 % Triton X 100, 0.0025 Sodiummetabisulfite	0.025 % Triton X 100, 0.0025 Sodiummetabisulfite
	Incubated for 12 hours	Incubated for 12 hours
	Centrifuged 7,000 x g	Added 0.2 phr KOH, 1 % ammonium alginate
	% DRC adjusted and NH_3 added to make 60 % DRC and 0.6 % NH_3	Centrifuged 7,000 x g
		Ammonium laurated added to 0.35 % (w/ v)
		% DRC adjusted and NH_3 added to make 60 % DRC and 0.6 % NH_3
Concentrated latex 60 % Quality Analysis for ISO 2004- 1979 (E)		

Figure 3.1 Comparison of concentrated latex production processes

3.2 Quality of concentrated latex

The properties of concentrated latices produced according to the developed process exhibited good quality based on ISO 2004 specifications in terms of percent non- rubber impurities (non- rubber content, KOH, Mg, volatile fatty acid) and the stability of rubber latex (%NH_3, MST). The low-protein properties of DPCL produced in this research were verified firstly by % total nitrogen content normally used by standard block rubber factory, and secondly by total water extractable protein (WEP) content used for gloves qualification currently used by the US Food and Drug Administration, FDA (1998), which states that the rubber product should contain 50 micrograms or less of total water extractable protein per gram product.

The properties of control concentrated latex and DPCL from the second deprotinization process indicated that the first developed deproteinization process and the second developed of deproteinized process followed the ISO 2004 specified as shown in Table 3.1.

Table 3.1 Physical properties of control concentrated latex, deproteinized concentrated latex

Physical properties	ISO 2004 Specification	DP1		DP 2											
		Production date (1/ 07/ 00)		Production date (27/ 07/ 00)		Production Date (3/ 08/ 00)		Production date (19/ 09/ 00)		Production date (25/ 09/ 00)		Production date (1/ 10/ 00)		Production date (3/ 03/ 00)	
		Control	DPCL	Control	DPCL	Control	DPCL	Control	DPCL	Control	DPCL	Control	DPCL	Control	DPCL
(%)Total solid content, (TSC)	min. 61.5 %	61.70	62.90	63.35	60.11	62.96	63.13	62.72	64.41	64.19	61.10	62.62	62.08	61.72	67.70
(%)Dry rubber content, (DRC)	min. 60.0 %	60.05	61.70	62.26	59.60	61.96	62.01	61.77	63.79	63.30	60.90	61.39	61.24	60.27	66.52
(%)Non rubber content, (NRC)	max. 2.0 %	0.15	1.20	1.09	0.50	1.04	1.12	0.95	0.62	0.89	0.20	1.23	0.84	1.45	1.18
(%)Ammonia (HA- L)	min. 0.60 %	0.67	0.77	0.60	0.60	0.61	0.74	0.89	0.73	0.67	0.66	0.61	0.60	0.58	0.85
(%)KOH	max. 1.0 %	0.70	0.48	0.47	0.56	0.33	0.47	0.49	0.56	0.25	0.37	0.62	0.51	0.48	0.56
(%)Volatile fatty acid (VFA)	max. 0.20 %	0.10	0.05	0.08	0.09	0.04	0.08	0.10	0.12	0.05	0.09	0.05	0.09	0.03	0.02
Magnesium content (ppm)	max. 50 ppm	44.53	39.78	31.83	31.80	28.03	29.49	36.30	50.00	28.15	29.09	38.15	29.09	28.37	20.31
Mechanical stability time, MST (second)	min. 650 sec.	690	368	650	984	990	4363	890	660	900	2400	780	650	880	690

DPCL: Deproteinized concentrated latex

The deproteinized concentrated latex was carried out at the batch scale of 200-400 L to obtain enough specimens for testing of concentrated latex properties. Table 3.2 shows that deproteinized concentrated latex (DPCL) have lower amount of volatile fatty acid (0.079±0.035) which indicated for lower contamination of microorganisms. The deproteinized concentrated latex also have higher MST except the DPCL lot number (1/ 07/ 00), which was from the first developed procedure of deproteinization without addition of stabilizers 0.2 phr of KOH, 1% ammonium alginate and 0.35 % ammonium laurate. However there is no significant difference in other physical properties namely %TSC, % DRC, % NH_3, contaminants (non- rubber content, % KOH, % Mg) (Table 3.2).

Table 3.2 Comparison of physical properties in control concentrated latex and DPCL

Physical properties	ISO 2400 Specifications	Control (n= 7)	DPCL (n= 7)	% Difference from control
(%)Total solid content, (TSC)	min. 61.50 %	62.75 ± 0.88	63.06 ± 2.48	0.5
(%)Dry rubber content, (DRC)	min. 60.0 %	61.57 ± 1.13	62.25 ± 2.27	1
(%)Non rubber content, (NRC)	max. 2.00 %	0.97 ± 0.41	0.81 ± 0.39	-16
(%)Ammonia (HA- L)	min. 0.60 %	0.68 ± 0.11	0.71 ± 0.09	4
(%)KOH	max. 1.00 %	0.48 ± 0.15	0.50 ± 0.07	4
Volatile fatty acid (VFA)	max. 0.20 %	0.16 ± 0.17 [a]	0.079 ± 0.04 [b]	-50
Magnesium content (ppm)	max. 50.00 ppm	33.62 ± 6.31	32.79 ± 6.85	-2.5
Mechanical stability time, MST (second)	min. 650 sec.	799 ± 129.24 [b]	1486±1453.69 [a]	86

DPCL: Deproteinized concentrated latex

Significant difference of physical properties between control concentrated latex and deproteinized concentrated latex are marked by different letter (a, b) analyzed by t- test.

3.3 Effect of deproteinization on nitrogen content of solid deproteinized rubber

Nitrogen content in natural latex rubber has a correlation with protein content. The total nitrogen content in the solid rubber film prepared from control concentrated latex and deproteiniized concentrated latex (DPCL) were determined according to Methods 2.6.9.

Table 3.3 Nitrogen content in different lots of DPCL comparing to its control

(%)Nitrogen content 0.07- 0.09 % maximum	Deprotenized process 1		Deproteinzed process 2											
	Lot no. (1/ 07/ 00)		Lot no. (27/ 07/ 00)		Lot no. (3/ 08/ 00)		Lot no. (19/ 09/ 00)		Lot no. (25/ 09/ 00)		Lot no. (1/ 10/ 00)		Lot no. (3/ 03/ 01)	
	control	DPCL	control	DPCL	control	DPCL	Control	DPCL	control	DPCL	control	DPCL	control	DPCL
	0.19	0.04	0.31	0.11	0.24	0.19	0.16	0.12	0.23	0.027	0.23	0.02	0.22	0.01

DPCL: Deproteinized concentrated latex

Table 3.3 shows that % nitrogen content of all deproteinized rubbers decreased from their control rubbers. Some lots gave good result in decreasing % nitrogen content such as DPCL lot no. (25/ 09/ 00), (1/ 10/ 00) and (3/ 03/ 01).

Table 3.4 Comparison of the average nitrogen content in control natural rubber and DPCL

(%) Nitrogen content, 0.07- 0.09 % max.	% N of control (n= 7)	% N of DPCL (n= 7)	% reduction from control
	0.23 ± 0.05^{a}	0.07 ± 0.07^{b}	70

DPCL: Deproteinized concentrated latex

Table 3.4 shows significant decrease of 70% nitrogen content in the solid rubber film between control solid rubber and deproteinized solid rubber as marked by different letter (a, b) analyzed by t- test at 95 % confidence. It is noted that all these DPCL were at the scale of 200- 400 L, which is closed to production scale.

3.4 Effect of deproteinization on water extractable protein of DPCL- film

The concerns about latex allergy or hypersensitivities associated with NRL have led to the development of a number of protein assays. These standardized tests are designed to measure the protein levels of CL and gloves made from CL. The one of those is modified Lowry method. The method to extract water extractable protein was done by pouring DPCL on to a plate with 20 x 20 cm square. The raw air- dried specimen prepared from concentrated latex is called CL- film where the sample prepared from deproteinized concentrated latex is called DPCL- film. Each sample was cut into small pieces about 1x 1 cm square and about 1 g of rubber sample was extracted with 10 volume of distilled water at 37 ^{o}C for 2 hours (Methods 2.7.1). The water extractable protein can be determined by modified Lowry method (Methods 2.8). This method involved determination of protein in the presence and the absence of copper sulfate ($CuSO_4$). Figure 3. 2 shows optical density at 750 nm (OD_{750}) wavelength of the standard ovalbumin that determined by modified Lowry method in the presence and the absence of $CuSO_4$. The OD_{750} in the presence of $CuSO_4$ is higher than the OD_{750} in the absence of

$CuSO_4$. The high value is due to $CuSO_4$. Thus water extractable protein was evaluated from standard graph after subtracting the OD_{750} in the absence of $CuSO_4$.

These results suggest that the amount of water extractable protein, which was determined by modified Lowry method, can be interfered by divalent cation contaminants, resulting in the high absorbance without protein presence. Therefore to obtain the correct result, cation should be removed by dialyzing sample or precipitating protein by acid such as trichloroacetic acid and phosphotungstic acid (ASTM; D- 5712-99, PRIM; MS 1392) before determination by modified Lowry method.

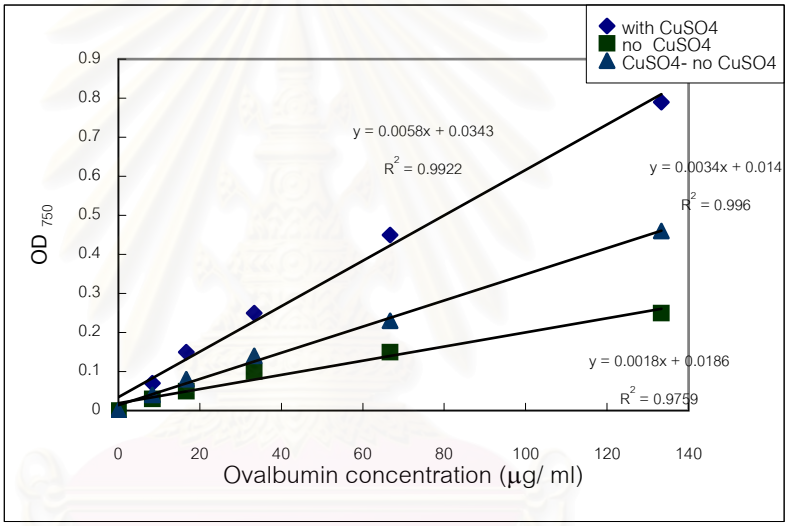

Figure 3.2 Effect of $CuSO_4$ on standard curve of ovalbumin measured by modified Lowry method.

The water extractable protein (WEP) of control commercial concentrated latex film from the conventional process, DPCL film of DPCL from the first development of deproteinized process and the second development of deproteinized process were shown in Table 3.5.

Table 3.5 Water extractable protein extracted from different lots of CL and DPCL- film

WEP content FDA recommended 50 μg / g rubber product	Deprotenized process 1		Deproteinized process 2											
	Lot no. (1/ 07/ 00)		Lot no. (27/ 07/ 00)		Lot no. (3/ 08/ 00)		Lot no. (19/ 09/ 00)		Lot no. (25/ 09/ 00)		Lot no. (1/ 10/ 00)		Lot no. (3/ 03/ 01)	
	control	DPCL-film	Control	DPCL-film	control	DPCL-film	Control	DPCL-film	control	DPCL-film	control	DPCL-film	control	DPC-film
	196	25	59	20	218	38	840	14	150	11	610	<2	213	12

DPCL- film: Deproteinized concentrated latex film

FDA: Food and Drug Administration

Table 3.5 shows that water extractable protein of all deproteinized concentrated latex film are decreased from their control concentrated latex film. All of them show WEP content less than the permitted water extractable protein level of 50 μg / g. Four groups of deproteinized concentrated latex [lot no. (19/09/00), (25/09/00), (1/10/00) and (3/03/01)] show WEP content less than 20 μg / g which is the recommended protein content for surgical gloves. Table 3.6 shows the variability of WEP content in control concentrated latex and deproteinized concentrated latex.

Table 3.6 Comparison of the average water extractable protein content in control concentrated latex film and deproteinized concentrated latex film

Protein content recommended by FDA, 50 μg / g product	Control (n= 7)	DPCL- film (n= 7)	% reduction from control
	326 ± 285 [a] □□g/ g	17 ± 12 [b] □g/ g	95

[a] and [b] are marked for significant difference analyzed by t- test at 95 % confidence. DPCL- film: Deproteinized concentrated latex

The DPCL lot number (25/ 09/ 00) with the interesting specifications for glove factory then underwent glove process (Family Glove, Thailand, 2001) as deproteinized household gloves (DP- HG) and irradiation process (Department of Nuclear Technology, Faculty of Engineering, Chulalongkorn University, 2000) as irradiated deproteinized concentrated latex (DPCL). The effect of glove process and irradiation process on water extractable protein in DP- HG and DPCL film is shown in Table 3.7.

Table 3.7 Effect of deproteinization on water extractable protein in DPCL products

Protein content permitted by FDA, 50 μg / g product	Control latex film (25/ 09/ 00)	DPCL film (25/09/00)	Irradiated DPCL film (25/09/00)	Control HG	DP- HG
	150	11	470	365	1898

DPCL film : Deproteinized concentrated latex film

Control HG : Control household glove

DP- HG : Deproteinized household glove

Table 3.7 shows that water extractable protein (WEP) content determined by modified Lowry method in irradiated DPCL film and DP- HG were drastically increased. These results suggest that manufacturing process such as gamma irradiation and vulcanization may have some effects on insoluble proteins associated with the rubber and resulted in the increasing WEP content of irradiated rubber. WEP content in control glove shows the same values as control latex film (Table 3.7), but DP- HG shows surprisingly high WEP content. Both glove samples were processed under the same condition. The household gloves were coated with cotton flocks to the inner surface to make them non- tacky and silky smooth. There were double dipped with a different colored latex compound to add thickness and variations to the designs and appearance. These steps may result in increasing solubility of protein from DP- HG. The OD_{750} observed may be a real increase in WEP of short poly peptide in DP- HG.

3.5 Molecular weight distribution of water extractable protein analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS- PAGE)

Figure 3.3 shows separation of water extractable protein by sodium dodecyl sulfate gel electrophoresis (SDS- PAGE) before and after Alcalase deproteinization of ammoniated field latex in comparison with standard molecular weight markers (lane 1, 8 and Appendix 5). Three lots of WEP prepared from control concentrated latex film (CL- film) show typical variation in protein bands such as WEP from CL lot number (1/ 10/ 00) and (3/ 08/ 00) display several protein bands in the range of 30- 14.4 and lower molecular weight (Mw) than the 14.4 kDa molecular weight marker. Two clear bands of proteins at 14.4 and about 30 kDa were observed (lane 2 and 3). CL- film from CL lot number (3/ 08/ 00) also displays a clear protein band of Mw at 66 kDa. CL- film from CL lot number (27/ 07/ 00) displays five clear band of proteins with molecular weight of 13.2, 13.9, 14.4,18.5 and 28 kDa (lane 5) and two slightly higher Mw than the 30 kDa. Deproteinized concentrated latex film (DPCL- film) from DPCL lot number (1/ 10/ 00) and (3/ 08/ 00) display slightly smeared band of proteins (lane 6 and 7). Latex serum proteins, the side product of concentrated latex lot number (3/ 08/ 00), (lane 4) display different bands of protein from CL- film. The bands are 16.3 and the lower Mw than the 14.4 kDa.

Figure 3.4 shows separation of WEP by SDS- PAGE from CL- film, DPCL- film, irradiated DPCL- film, household glove, and DP- household glove in comparison with standard molecular weight marker (lane 1, 8 and Appendix 8). CL- film of CL lot no (19/ 09/00, 25/ 09/ 00 lane 5, 6) show 2 clear bands of protein at Mw of 45 and 30 kDa. It also shows extended smeared bands of proteins below 45, and below 20 kDa. While DPCL- film of DPCL lot number (25/ 09/ 00) in lane 7 shows extended smeared bands without any clear band of protein. Household glove (lane 2) shows a faint band of protein at about 66 kDa. This protein is usually found in NRL products such as gloves and condoms (Makinen- Kiljunen *et al*., 1992; Guillermo *et al*., 2000). While DP- household glove (lane 3) and irradiated DPCL film of DPCL lot number (25/ 09/ 00) (lane 4) display slightly smeared band of proteins. This result indicates that the over detected values of water extractable

protein from irradiated deproteinized concentrated latex and deproteinized household glove by modified Lowry method may come from small peptides produced from irradiation pre- vulcanized process and chemicals interfered from glove process.

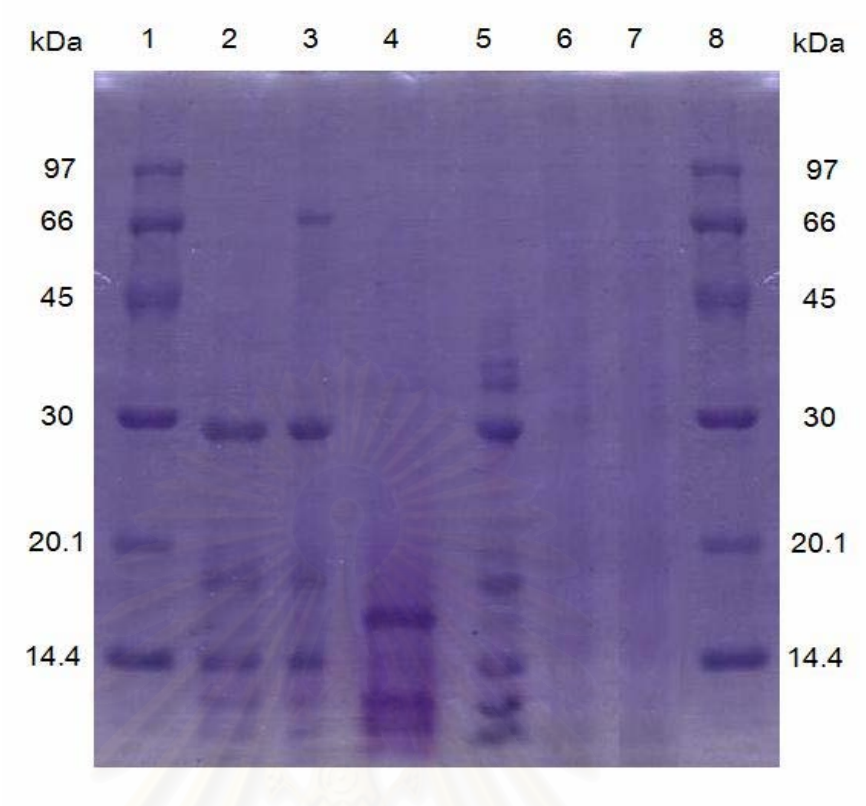

Figure 3.3 Sodium dodecyl sulfate polycrylamide gel electrophoresis of control concentrated latex and deproteinized concentrated latex

Lane 1, 8 Standard molecular weight makers (Phosphorylase B 97 kDa, Bovine serum albumin 66 kDa, Ovabumin 66 kDa, Carbonic anhydrase 30 kDa, Trypsin inhibitor 20.1,α- Lactalbumin 14.4 kDa)

Lane 2 Control concentrated latex lot number (1/10/00)

Lane 3 Control concentrated latex lot number (3/08/00)

Lane 4 Latex serum protein (3/08/00)

Lane 5 Control concentrated latex lot number (27/07/00)

Lane 6 Deproteinized concentrated latex lot number (1/10/00)

Lane 7 Deproteinized concentrated latex lot number (3/08/00)

Each well was loaded with 60 μg protein (measured by modified Lowry method).

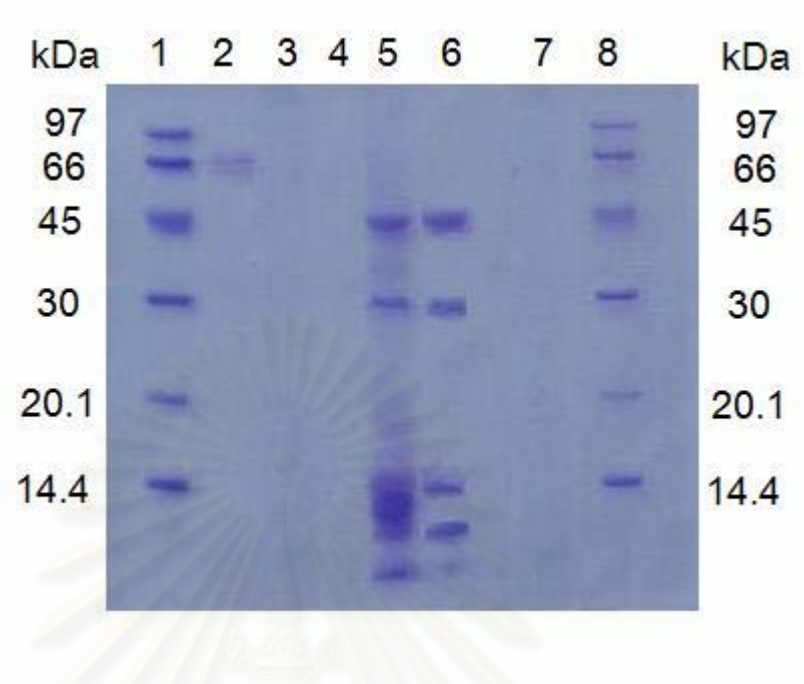

Figure 3.4 Sodium dodecyl sulfate polycrylamide gel electrophoresis of control concentrated latex, deproteinized concentrated latex and vulcanized products

Lane 1, 8 Standard molecular weight makers (Phosphorylase B 97 kDa, Bovine serum albumin 66 kDa, Ovabumin 66 kDa, Carbonic anhydrase 30 kDa, Trypsin inhibitor 20.1,α- Lactalbumin 14.4 kDa)

Lane 2 Household glove

Lane 3 Deproteinized household glove

Lane 4 Irradiated deproteinized concentrated latex (25/09/00)

Lane 5 Control concentrated latex lot number (19/09/00)

Lane 6 Control concentrated latex lot number (25/09/00)

Lane 7 Deproteinized concentrated latex lot number (25/09/00)

Each well was loaded with 60 μg protein (measured by modified Lowry method).

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3.6 Utilization of latex serum protein as standard protein in protein determination methods

Since only small amount of WEP were obtained from rubber film and not enough for using in one set of protein detremination, latex serum proteins which contained various protein bands and higher protein concentration were used instead.

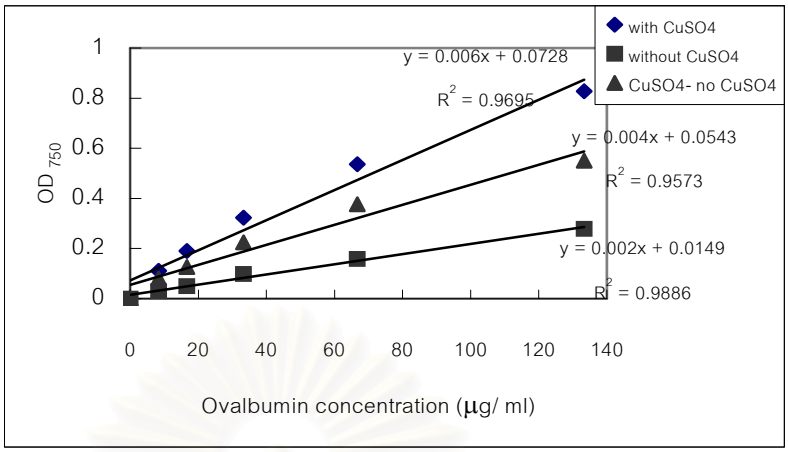
In view of the latex protein allergy problem related to NRL products, especially gloves, there is a need to evaluate the allergic potential of these products. However a universally standardized test for doing so is still lacking. Many different methods are being used in various laboratories. Table 3.9 shows optical density at 280 nm wavelength of the stock solution 1 mg/ ml of bovine serum albumin, ovalbumin and latex serum protein.

Table 3.9 OD_{280} of different proteins at 1 mg/ ml concentration

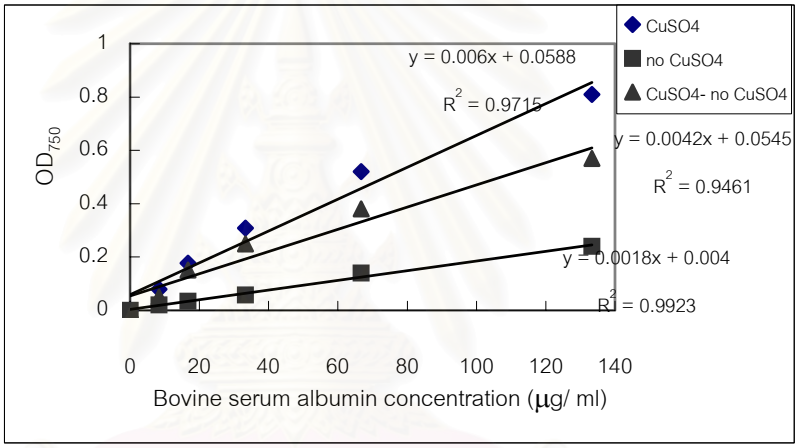
Protein	OD280
Bovine serum albumin	0.58
Ovalbumin	0.66
Latex serum protein	3.60

3.6.1 Modified Lowry method

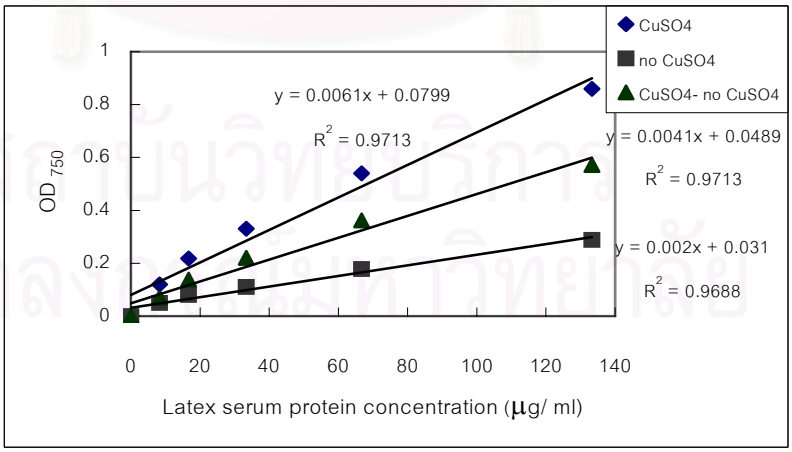
The methods most commonly adopted in NRL manufacturing countries are the RRIM test (MS 1392) and the ASTM test (D- 5712- 99) both based on modified Lowry method. The modified Lowry method is currently the preferred methods because of its sensitivity (5- 100 μg/ ml), reproducibility and simplicity, although these tests are non-specific with reference to the allergens. The standard protein used in the modified Lowry method is ovalbumin. This research requires utilization of latex serum protein as standard protein instead of ovalbumin for determination of NRL proteins and NRL products' protein. The standard curve of ovalbumin measured by modified Lowry method compared with the calibration curve of latex serum protein measured by modified Lowry method is shown in Figure 3.5.



(a)



(b)



(c)

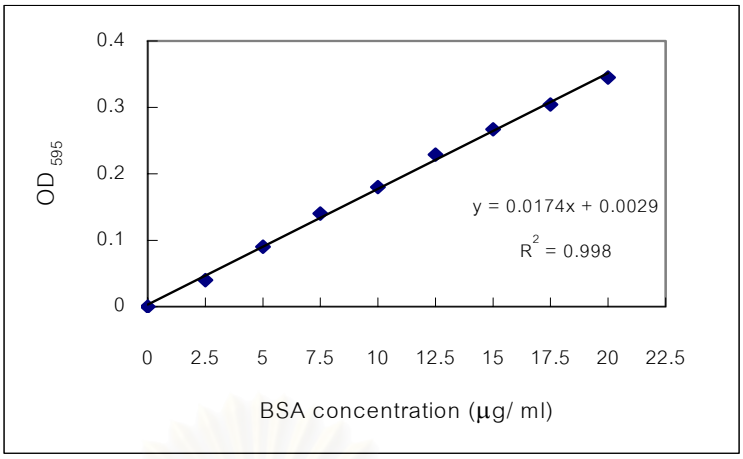
Figure 3.5 Comparison of standard graphs of (a) ovalbumin, (b) bovine serum albumin and (c) latex serum proteins measured by modified Lowry method

Figure 3.5 shows quantities of proteins evaluated from three standard graphs of ovalbumin, bovine serum albumin and latex serum proteins. At the same OD_{750} the quantities of protein evaluated from each standard graph gave approximately the same quantities. For example at OD_{750} = 0.40, the protein evaluated from any standard graph of either ovalbumin, BSA or latex serum protein is 80 μg (Figure 3.5 a, and c).

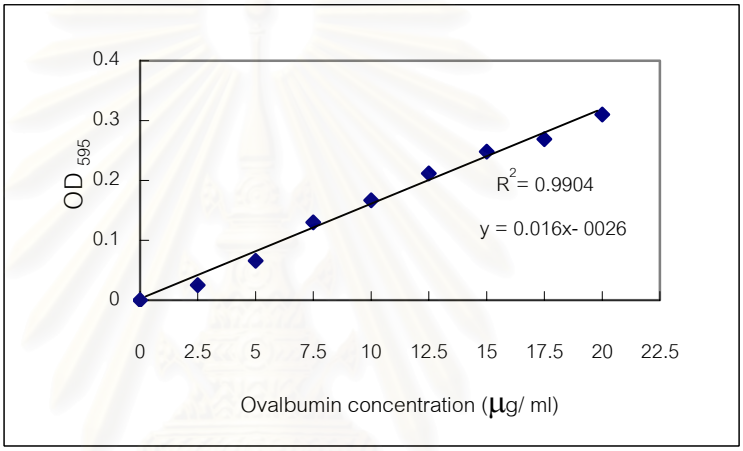
3.6.2 Bradford method (Bradford, 1976)

This method utilizes the binding of Coomassie® Brilliant Blue G 250 Dye to proteins. The dye has both a blue and a red form. When this dye binds to proteins, the red form is converted to the blue form and the absorption maximum of the dye shifts from 465 to 595 nm. Standard proteins used in the method is normally bovine serum albumin (BSA). The Bradford method has accurate determinations of samples containing 2.5 – 20 μg protein. When ovalbumin or latex serum protein was utilized as standard protein instead of BSA, there is no significant difference among three standard graphs of BSA, ovalbumin and latex serum protein as shown in Figure 3.6.

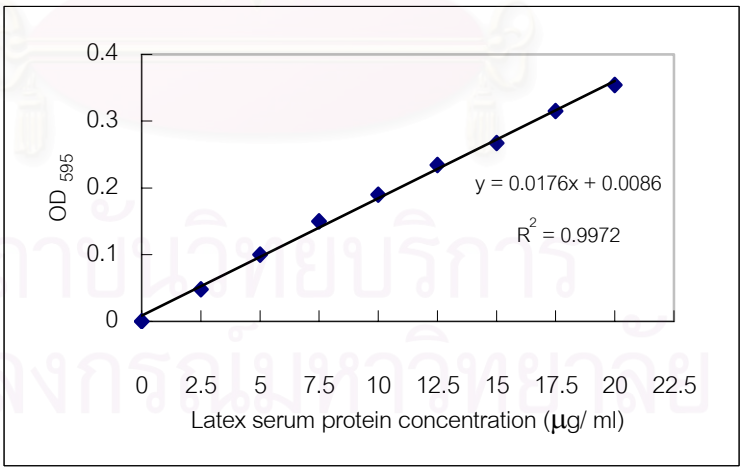
All these results indicate that latex serum protein can be used as standard protein in the total protein determination either by modified Lowry or Bradford method.



(a)



(b)



(c)

Figure 3.6 Comparison of standard curves of (a) bovine serum albumin, (b) ovalbumin and (c) latex serum proteins measured by Bradford method

3.7 Separation of latex serum protein by Sephadex G- 75 column and molecular weight distribution

One milliliter of crude serum latex protein was prepared for purification. The 1- ml latex serum protein was prepared from skim latex. The skim latex was added with 50% sulfuric acid to coagulate the rubber from latex serum. The rubber obtained from this process was called skim rubber (SK- R). The clear yellow latex serum from this process should have pH in the range of 5- 6. The latex serum was precipitated with 5 volume of cold acetone then stirred at 4 ^{o}C for 12 hours. The precipitated latex serum protein was dissolved in 10 mM phosphate buffer saline (150 mM NaCl), pH 7.4. The protein was loaded on to Sephadex G- 75 column. The fraction was eluted by 10 mM phosphate buffer saline (100 mM NaCl), pH 7.4. It was found that the latex serum protein was separated to three peaks, the first peak at fraction 70- 92, the second peak at fraction 95- 101 and the third peak at fraction 122- 139 (Figure 3.7). The molecular weight of the separated latex serum proteins were determined from molecular weight calibration curve (Figure 3.8) obtained from chromatography of standard proteins on Sephadex G- 75 column (section 2.11). The separated latex serum proteins were found to have molecular weights of 29, 12 and 4.7 kDa. The molecular weights of the separated latex serum proteins were also determined by SDS- polyacrylamide gel electrophoresis (Figure 3.9 and 3.10). From the mobility in SDS- PAGE, the molecular weight of the separated latex serum proteins was 30 kDa, the other two bands of proteins were extended smeared bands below 20.1- 14.4 kDa (lane 4) and the extended smeared band below 14.4 kDa (lane 5).

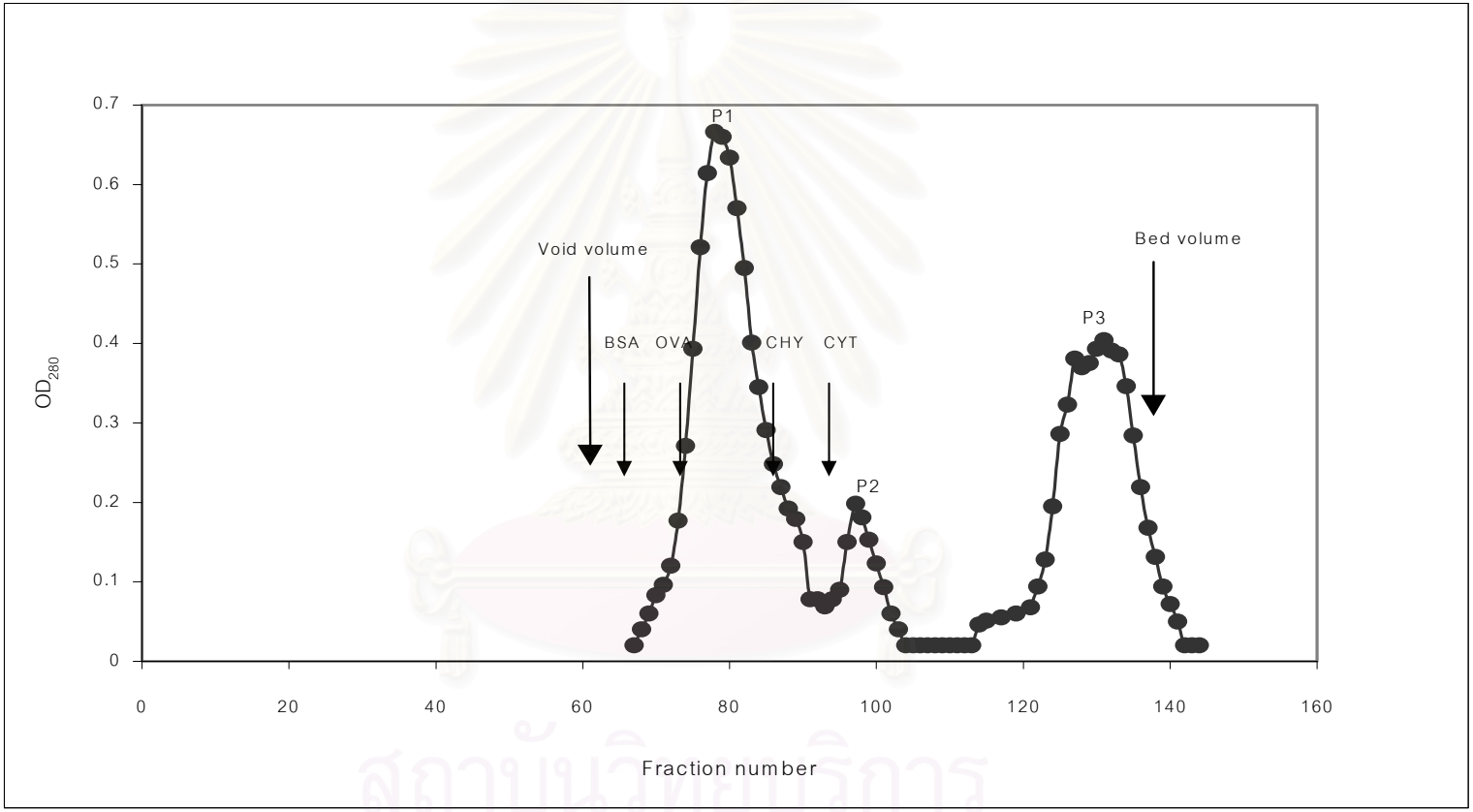


Figure 3.7 Chromatogram of latex serum proteins on Shepadex G- 75 column

BSA: Bovine serum albumin 66 kDa OVA: Ovalbumin 45 kDa

CHY: Chymotrypsinogen A 27 kDa CYT: Cytochrome C 12.3 kDa

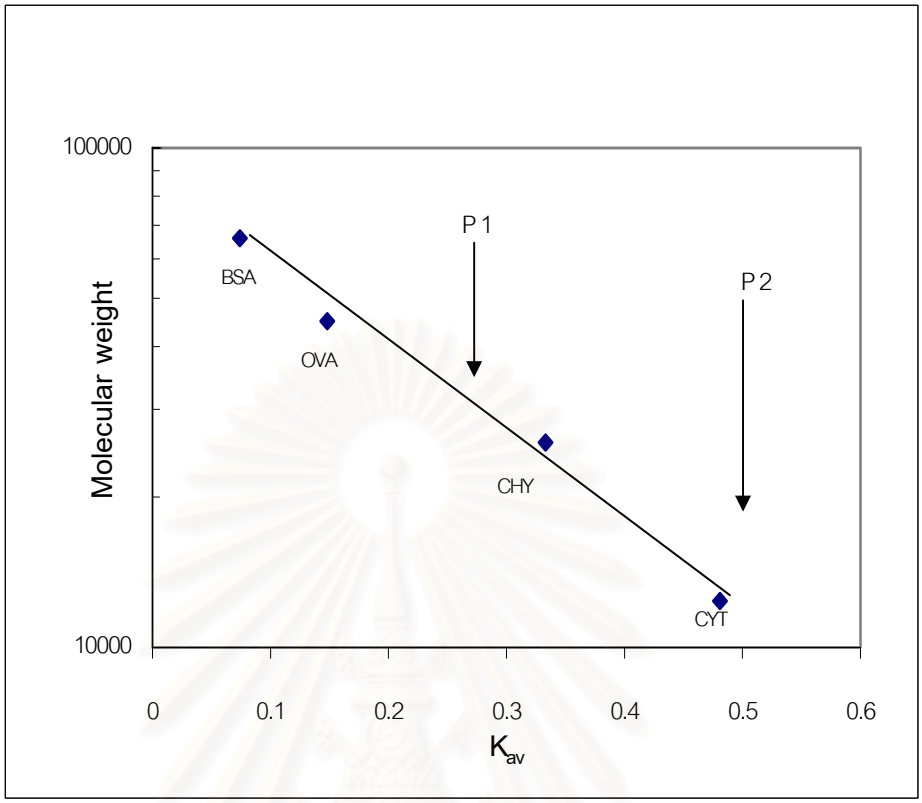


Figure 3.8 Calibration curve for molecular weight of separated latex serum proteins determined by gel filtration chromatography on Shephadex G- 75 column

BSA:	Bovine serum albumin	(Mw 66 kDa)
OVA:	Ovalbumin	(Mw 45 kDa)
CHY:	Chymotrypsinogen A	(Mw 27 kDa)
CYT:	Cytochrome C	(Mw 12.3 kDa)

P3 has K_{av} of 0.93 which did not appear on the calibration curve

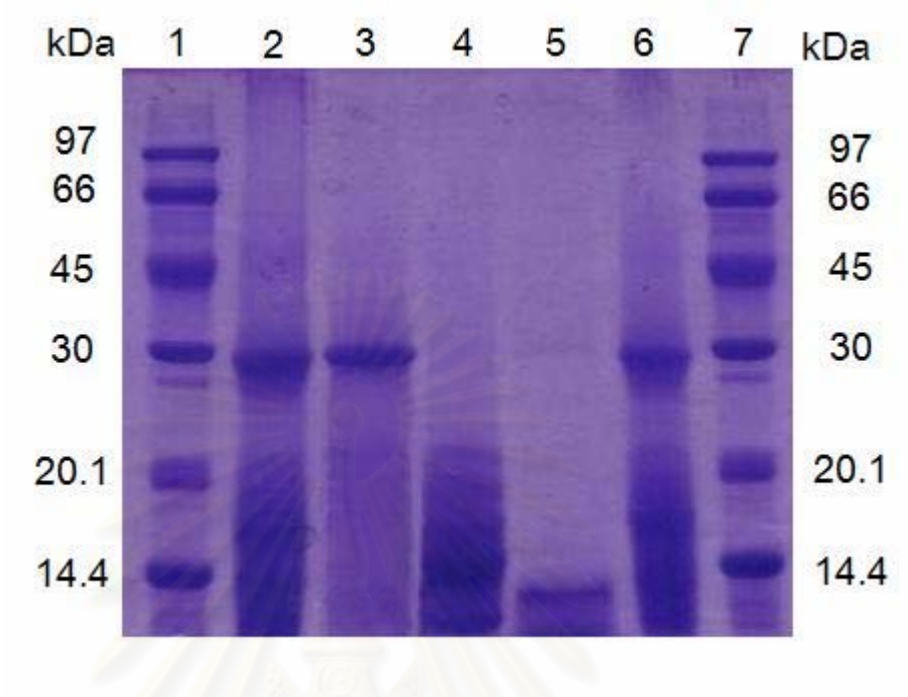


Figure 3.9 Sodium dodecyl sulfate polycrylamide gel electrophoresis of separated latex serum proteins

Lane 1, 7	Standard molecular weight makers (Phosphorylase B 97 kDa, Bovine serum albumin 66 kDa, Ovabumin 66 kDa, Carbonic anhydrase 30 kDa, Trypsin inhibitor 20.1,α- Lactalbumin 14.4 kDa)
Lane 2, 6	Crude latex serum protein
Lane 3	Separated latex serum protein 1 (30 kDa)
Lane 4	Separated latex serum protein 2 (20.1- 14.4 kDa)
Lane 5	Separated latex serum protein 3 (below 14.4 kDa)

Each well was loaded with 60 μg protein (measured by modified Lowry method).

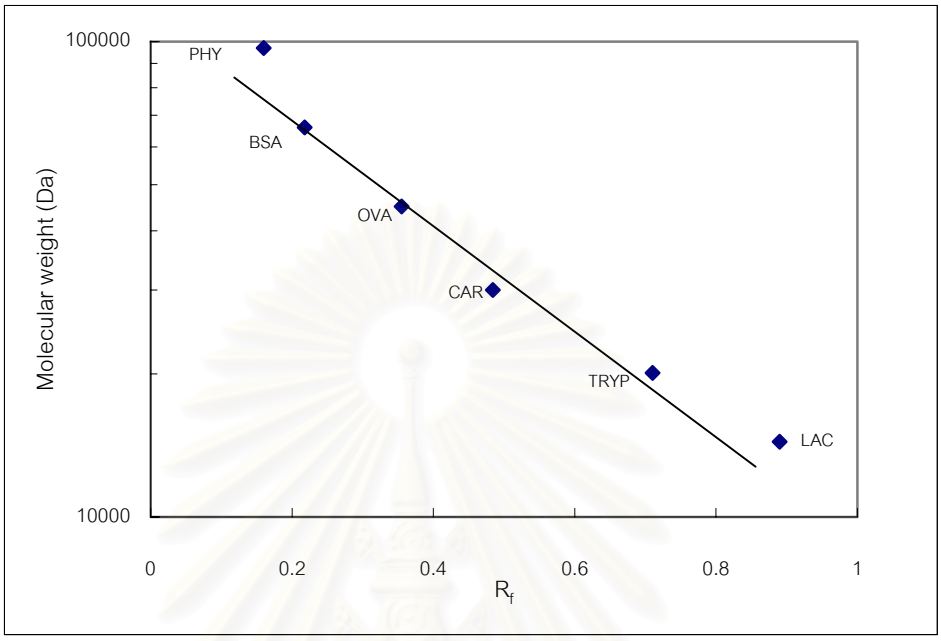


Figure 3. 10 Calibration curve for molecular weight of separated latex serum proteins on SDS- polyacrylamide gel electrophoresis

PHOS:	Phosphorylase B	97 kDa,
BSA:	Bovine serum albumin	66 kDa,
OVA:	Ovabumin	66 kDa,
CAR:	Carbonic anhydrase	30 kDa,
TRYP:	Trypsin inhibitor	20.1 kDa
LAC:	α- Lactalbumin	14.4 kDa

3.8 Utilization of latex serum protein as standard protein allergens in skin prick test (SPT)

The Human Right and Ethics Committee of the Division of Dermatology, Department of Medicine, Faculty of Medicine, Chulalongkorn Hospital approved this study. There were 112 people who had volunteered for skin prick test (SPT) that were separated into 2 groups; 71 health care workers (13 males, 58 females) who had been sensitized with latex gloves and 41 atopic patients (10 males and 31 females) who had been general atopic patients. The SPT was kindly conducted by Assoc. Prof., Dr. Porntip Puvabanditsin. The latex serum protein was prepared by cold acetone precipitation by Method 2.5.3 and dissolved in 0.85 % normal saline solution. The others were prepared followed Method 2.5.4. Table 3.9 shows the list of test solutions and total protein concentration and Table 3.10 shows scoring relative to histamine wheal.

Table 3.9 List of test solutions in SPT

Test solution	Protein concentration (μg/ ml)
Histamine (1000 μg/ ml)	0
Commercial allergen (Stallergene, Extraits Allergeniques, France)	40,000
Latex serum protein	4,000
CL- film 1	196
CL- film 2	840
CL- film 3	218
Normal saline solution (0.85 % NaCl)	0
DPCL- film	11
SAPNR 1	15.45
SAPNR 2	17

Protein concentration determined by modified Lowry method.

3.10 Scoring relative to histamine wheal

SPT results	Definition
++++Strong positive	with ≥ 9x 9 mm square wheal size
+++ Clear positive	with ≥ 5x 5 mm square wheal size
++ Positive	with ≥ 3x 3 mm square wheal size
+, - Negative	with < 3x 3 mm square wheal size

Table 3.11 Skin prick test results

Volunteer	Skin Prick Results																					
	CL- film1		CL- film2		CL- film3		% pos.	DPCL-film 1		SAPNR1		SAPNR2		% pos.	NSS		Histamine		Stallergenes		Latex serum protein	
	neg.	pos.	neg.	pos.	neg.	pos.		neg.	pos.	neg.	pos.	Neg.	Pos.		Neg.	Pos.	neg.	pos.	neg.	pos.	Neg.	Pos.
Health care workers																						
1) Male 13	11	2	12	1	12	1		13	0	13	0	13	0		13	0	0	13	9	4	9	4
2) Female 58	53	5	53	5	53	5		57	1	56	2	56	2		58	0	0	58	48	10	29	29
Sub Total 1	64	7	65	6	65	6		70	1	69	2	69	2		71	0	0	71	57	14	38	33
% positive		9.86		8.45		8.45	8.92		1.41		2.82		2.82	2.35		0		100		19.72		46.48
Atopic patients																						
1) Male 10	10	0	10	0	10	0		9	1	9	1	9	1		10	0	0	10	8	2	5	5
2) Female 31	30	1	31	0	31	0		31	0	30	1	31	0		31	0	0	31	29	2	25	6
Sub total 2	40	1	41	0	41	0		40	1	39	2	40	1		41	0	0	41	37	4	30	11
% positive		2.43		0		0	0.81		2.43		4.88		2.43	3.25		0		100		9.76		15.49
Total	104	8	106	6	106	6		110	2	106	6	109	3		112	0	0	112	94	18	68	44

CL-film1: Control concentrated latex film (27/07/00), CL-film 2: Control concentrated latex film (19/09/00), CL-film3: Control conc. latex film (3/08/00)

DPCL- film: Deproteinized latex film (25/09/00), SAPNR1: Saponified rubber1, SAPNR2: Saponified rubber2, NSS: Normal saline solution

Table 3.11 shows the skin prick test results that the health care workers who were sensitized with latex gloves give 19.72 % positive SPT results with commercial latex protein allergens (Stallergenes, France) and 46.48 % positive results with latex serum protein prepared from latex serum. The general atopic patients give 9.76 % positive SPT results with commercial allergen and 15.49 % positive SPT results with latex serum protein prepared from latex serum.

CHAPTER IV
DISCUSSION

4.1 Deproteinization of natural rubber latex

In this research Alcalase (Purafect, 4000 E) was used to hydrolyze proteins in the natural rubber latex. The first development of deproteinization of field latex with 0.3% NH_3 and 0.025 % TMTD/ ZnO processed under conditons of 0.08 % Alcalase (0.28 CDU/ mg), 0.1 % SDBS, 0.3 % NH3, 0.025 % Triton X100 and 0.002% sodiummetabisulfite. The DPCL obtained from the first deproteinization process (DP1) had low nitrogen content and low water extractable protein, but still had low mechanical stability time. Since MST was correlated with stability and storage time of concentrated latex, in order to solve this inferior effect of the first deproteinization process, stabilizers including KOH and ammonium alginate were added to the latex before centrifugation and ammonium laurate was added to increase MST after centrifugation. The DPCL obtained from the second deproteinzation process (DP2) had higher MST than DPCL from the first deproteinization process. However, there was no significant difference in others physical properties between DPCL from DP1, DPCL from DP2 and their control concentrated latex. The best conditions for deproteinized field latex in this research were summarized in Figure 4.1.

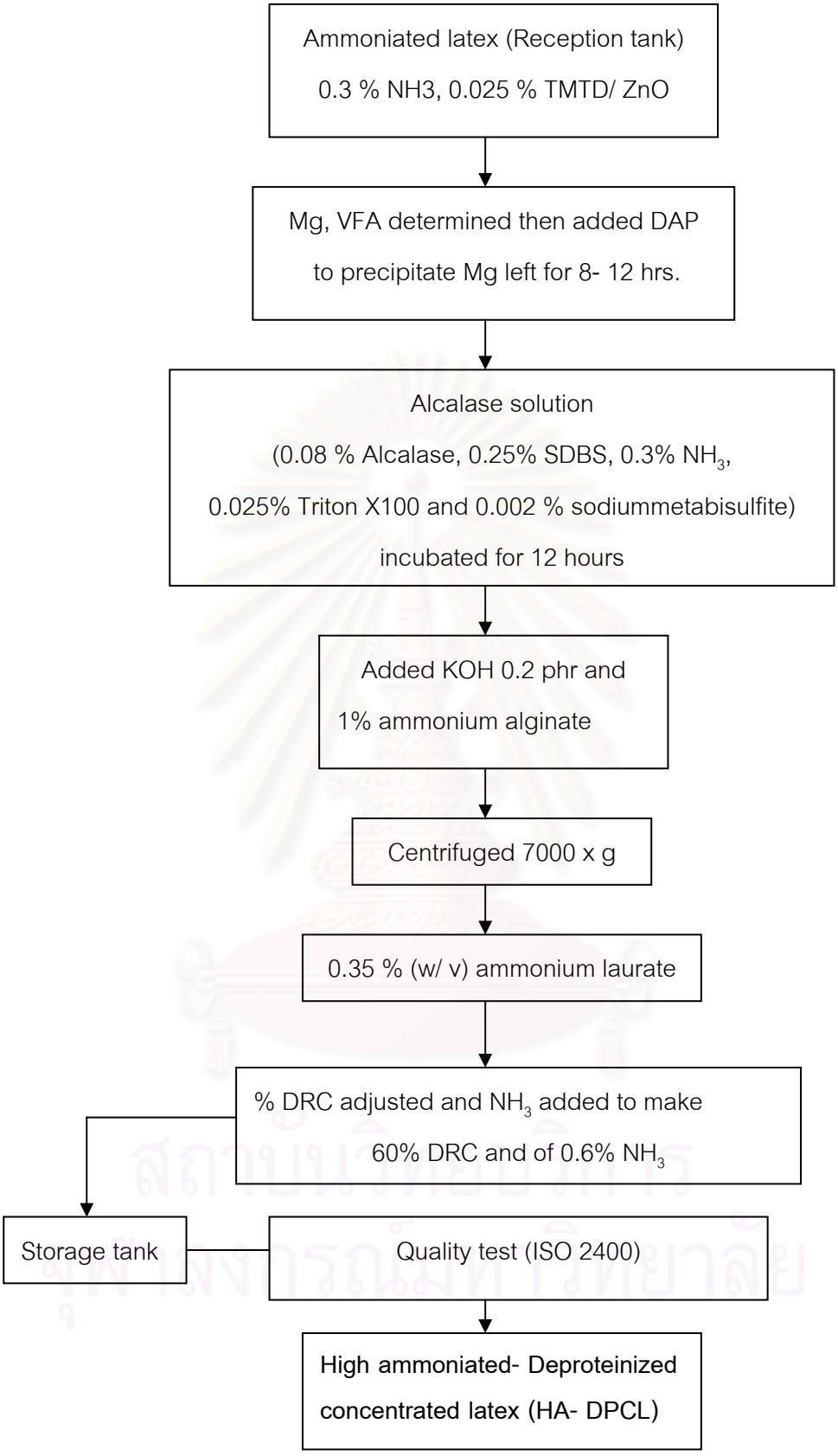


Figure 4.1 The conditions for filed latex deproteinization by Alcalase

4.2 Effect of deproteinization on nitrogen content and water extractable protein in concentrated latex

The deproteinization process developed in this research has produced deproteinized concentrated latex (DPCL). The total nitrogen content (Standard Thai Rubber, STR specification), which indicates the percentage of total protein per gram rubber, decreased about 70 % from the control rubbers. The total nitrogen content in DPCL is 0.07 $\pm$ 0.07 %N, which is more similar to standard DPNR latex concentrate proposed by RRIM (Ghazaly, 1994). It was found that contaminants such as magnesium may affected to the nitrogen content. If DPCL were prepared from field latex with high Mg content the DPCL obtained would still have high nitrogen content though deproteinized with Alcalase. This problem could be decreased by selecting low Mg content latex for the process of concentrated latex or precipitation of Mg by diammonium hydrogenphosphate before the process of concentrated latex.

From the determination of water extractable protein (WEP) by modified Lowry method, it was found that WEP in control concentrated latex were varied in the wide range (326 $\pm$ 285 μg/ g). With the deproteinization process developed in this research, seven lots of the DPCL show much lower WEP 17 $\pm$ 12 μg/ g. The average WEP of DPCL decreased, about 95 % from their control concentrated latex. The narrow variation of water extractable protein in latex is the quality that many glove factories require as raw materials for production. However when DPCL (lot no. 25/09/00, WEP = 11 μg/ g) was used in radiation pre-vulcanized process and sulfur vulcanized process to form household glove, increasing WEP was detected by modified Lowry method. Yatim, (1993) also reported similar result in sulfur vulcanized gloves from concentrated latex. The protein solubility increased during the process could be abated by washing process used during manufacturing to remove or denature natural water soluble proteins and remove adverse materials (Subramaniam *et. al.*, 1993, Aziz, 1993; Yatim, 1993). Varghese *et. al.*, (2000) suggested that the radiation- vulcanized, centrifuged latex was subjected to dilution and then centrifuged. In the case of field latex, it was irradiated first and then centrifuged after dilution.

4.3 Molecular weight distribution (MWD) of WEP by SDS- PAGE

From SDS- PAGE (Figure 3.3) concentrated latex film (CL- film) displays several protein bands in the range of 45- 14.4 kDa and lower molecular weight than the 14.4 kDa while deproteinized concentrated latex film displays extended smeared bands without any clear band of protein. Figure 3.4 household gloves obtained from DPCL underwent the manufacturing process displays a different band of protein at about 66 kDa. This 66 kDa was previously reported by Makinen- Kiljunen *et. al*. (1992) in NRL products such as gloves and condoms. Guillermo *et al*. (2000) also reported new protein band at 69 kDa in NRL products that were not present in CL, and can become a neoallergen. DP- household glove and irradiated DPCL film from DPCL display slightly smeared band of proteins. These results indicated that the over- detected value of WEP from irradiated deproteinized concentrated latex and deproteinized household glove by modified Lowry method may come from small peptides produced from irradiation pre- vulcanized process and chemicals interfered from glove sulfur- vulcanization process. It can also explain why high protein values determined by modified Lowry method may not correspond with allergenicity.

4.4 Utilization of latex serum proteins as standard proteins instead of ovalbumin and BSA in protein determination methods

From the comparative determination of protein by modified Lowry method. Figure 3.5 a, b and c indicate that any of standard proteins: ovalbumin, BSA or latex serum proteins can be used to calibrate the standard curve. The latex serum proteins are also equivalent to BSA and ovalbumin in the Bradford method to determine total protein quantity of latex (Figure 3.6 a, b and c).

The advantage of using latex serum proteins as standard proteins is the potential to use the same stock solution to identify the presence of latex protein allergens by SDS- PAGE.

4.5 Separation of the latex serum proteins by Sephadex G- 75 column and MWD on SDS- PAGE

From the chromatogram of standard proteins and latex serum proteins on Sephadex G- 75 column. It was found that the latex serum proteins were separated to 3 broads peaks (Figure 3.7). The molecular weight (Mw) of the separated latex serum proteins were then determined from the molecular weight calibration curve. The separated latex serum proteins were found to have molecular weight of 29, 12 and 4.7 kDa. The SDS- PAGE shows that the Peak I of separated latex serum proteins have molecular weight of 30 kDa. The other 2 peaks of proteins separated from Sephadex G-75 column were extended smeared bands below 20.1-14.4 kDa and the band below the 14.4 kDa (Figure 3.9 and 3.10).

From Sephadex G- 75 chromatogram and SDS- PAGE, it shows that the latex serum protein used in test kit should contain most of the importance protein allergens namely Hev b1 (14.6 kDa), Hev b5 (16 kDa), Hev b6.01/ Prohevein (20 kDa), Hev b6.02/ Hevein (4.7 kDa), Hev b6.03/ Prohevein C- domain (14 kDa), Hev b8 (14 kDa) and Hevamine/ Chitinase (30 kDa).

It is noted that P2 and P3 contained all proteins in the Hevein family which are considered as major allergens (Alenius *et al*., 1995; 1996; Banerjee *et al*., 1997; Chen *et al*., 1997; Slater *et al*., 1996; Vallier *et al*., 1995). Hev b 2 (β- 1,3- glucanase), Hev b 4 (Microhelix protein complex) and Hev b 7 (Patatine- like protein) are the three allergens missing, however these 3 allergens have high homology to several plants and not very specific to *Hevea* latex (Alenius *et al*., 1995; Beezhold *et al*., 1994; 1996; Sunderasan *et al*., 1995).

4.6 Utilization of latex serum proteins as standard protein allergens in skin prick test (SPT)

The skin prick test (SPT) is known to be a rapid test of high sensitivity for IgE-mediated allergy. Besides being used to identify sensitized patients, it can also be used to detect the presence of protein allergens in latex products (Turjanmaa *et. al*.,1988; Turjanmaa *et. al*., 1988) . In this research there were 112 people who had volunteered for SPT that can be separated in 2 groups: 71 atopic health care workers (13 males and 58 females) who have been sensitized with latex gloves, and 41 atopic patients (10 males and 31 females) who had been general atopic patients. The ability to produce a wheal response in the volunteers was shown by the positive histamine wheals. The SPT results (Table 3.11) show that the atopic health care worker who have been sensitized with latex gloves give 46.5 % positive results with latex serum protein and 19.7 % positive results with commercial latex protein (Stallergenes, France). It is consistent with the fact that people who have been sensitized with latex proteins their immune system would produce IgE antibodies against latex antigens. The allergens in commercial latex allergens were shown in table 4.1 (Turjanmaa *et. al*., 1997) compared to latex serum protein. From the results of SPT in atopic health care workers from Table 3.11 and comparison of approximate types of protein allergens in commercial allergens and the latex serum allergens used in this research, which indicates that latex serum proteins have broader range of major allergens and therefore more potential in giving the positive SPT results.

Table 4.1 Approximate allergens including proteins in latex allergens

Latex allergen solution	Total water extractable protein (□g/ ml)	Approximate allergen (kDa)
Commercial latex allergens (Stallergenes, France)	4×10^4	14, 20, 27, 30, 45
Latex serum proteins	4×10^3	4.7, 14.4- 20.1, 30

It is noted that latex serum proteins gave negative SPT in 38 health care worker or 54 % of atopic health care workers, who had history of irritant contact dermatitis after wearing NRL gloves.

The reasons that these people showed negative SPT to latex serum proteins may be followings:

1. The skin irritation or hand eczema or contact dermatitis experienced by these group of health care workers may not be a true latex protein allergy. Contact dermatitis may be caused by exposure to chemicals resulted from repeated hand washing and drying or chemicals added to latex during harvesting, processing or manufacturing (Fregert, 1987;)
2. These people may have specific allergic reaction (IgE) to latex proteins with molecular weight higher than 30 kDa, which are scantly in this latetex serum protein allergens solution (Alenius *et al*., 1995; Beezhold *et al*., 1994; 1996; Sunderasan *et al*., 1995).
3. These people may have specific IgE to allergen occurred during the glove manufacturing process, not found in natural latex (Figure 3.4).

The atopic patients who have been general atopic patients (n = 41) showed 15.5 % positive results with latex serum proteins and 9.8 % positive results with commercial latex allergens (Stallergenes, France). These results can be explained by the cross-react phenomenon of latex allergens and other allergens such as tropical fruits, vegetables (Alenius *et. al*., 1996; Brehler *et. al*., 1997; Breiteneder & Scheiner, 1998) and grass pollen (Mahler *et. at*., 2000). The prevalence of latex hypersensitivity of 16 % in general atopic patients is rather high comparing to 5.6- 8.6 % reported by Porri *et al*. (1995), but comparable to 8- 22 % in hospital employee, reported by several authors (Douglas *et al*., 1997; Danne *et* al., 1997; Kibby *et al*., 1997; Konrad *et al*., 1997; Liss *et al*., 1997; Safadi *et al*., 1996; Teeraratkul *et al*., 1997).

CHAPTER V
CONCLUSION

1. Deproteinization of natural rubber latex by Alcalase has been optimized at 20-25 % DRC field latex with 0.08 % Alcalase (0.28 CDU/ mg), 0.1 % SDBS, 0.3 % NH_3, 0.025 % Triton X100 and 0.002% sodiummetabisulfite. The time of deproteinization is 12 hours. Before centrifugation 0.2 phr KOH and 1 % ammonium alginate were added as stabilizers, and ammoniam laurate is added after centrifugation. The MST observed from the DPCL (1486 second) is higher than control concentrated latex (799 second).
2. The DPCL obtained meets ISO 2004 specifications, and significantly shows low volatile fatty acid (0.079 % or 50 % reduction from its control concentrated latex), low nitrogen content (0.07 $\pm$ 0.07 % w/w or 70 % reduction from initial value), and low water extractable protein (17 $\pm$ 12 μg/ g rubber, 95 % reduction from initial).
3. Analysis of WEP molecular weight from DPCL by SDS- PAGE shows no clear bands of protein allergens in the range of 14.4- 97 kDa.
4. The WEP analysis of household gloves and radiation vulcanized sheets prepared from DPCL also shows no major protein allergen band, although the total protein amount assayed by modified Lowry seemed to be increasing as a result of increasing small peptides.
5. The latex serum proteins can be used as standard latex proteins for the protein determination test kit either by modified Lowry method or Bradford method and also SDS- PAGE.
6. Separation of latex serum proteins on Sephadex G- 75 column and SDS- PAGE indicated that the major latex serum proteins could be Hevamine/ Chitinase (30 kDa) and other most important latex protein allergens namely Hev b1 (14.6 kDa), Hev b5 (16 kDa), Hev b6.01/ Prohevein (20 kDa), Hev b6.02/ Hevein (4.7 kDa), Hev b6.03/ Prohevein C- domain (14 kDa), Hev b8 (14 kDa).

7. The comparison of SPT results between latex serum protein allergens in this research and commercial latex allergens (Stallergenes, France) in 71 atopic health care workers who have been sensitized with latex gloves shows 46.5% positive with latex serum proteins, higher than 17.9% positive with commercial latex allergens, which indicates that latex serum proteins allergens developed in this research have more potential to detect latex hypersensitivity by SPT. In 41 general atopic people only 15.5 % gave positive SPT with latex serum proteins, which is slightly higher than commercial latex allergens (9.8 %) indicating the high prevalence of latex allergy in general atopic patients, although less than the atopic health care workers.
8. Three sample of WEP from control latices show 8- 9 % SPT positive in this group of atopic health care workers (n= 71) whereas 3 samples of WEP from deproteinized rubber show only 1- 3 % positive SPT indicating that DPCL is significantly safer for the high risk group of health care workers. The general atopic patients (n= 41) show more or less the same rate of SPT positive for control rubbers and DPCL indicating the lower specificity of SPT test in general atopic groups, which may be due to cross reactivity of latex allergens with other sources of allergens such as protein from plants and animals.

REFFERENCES

Adulyathum, P. and Konsilpa, K., Use of Enzyme in Deproteinization of Latex for Latex Concentrate Process. Res Rep. Rubber Research Institue of Thailand. 2000.

Akasawa A., Hsieh, L., Lin, Y., Serum reactivities to latex proteins (Heavea brasiliensis). *J Allergy Clin Immunol*. 1995. **95**. 1196- 1205.

Alenius, H., Turjanmaa, K., Palosuo, T., Makinen- Kijunen S., Reunala, T., Surgical latex glove allergy: Characterization of rubber protein allergens by immunoblotting, *Int Arch Allergy Appl Immunol*. 1991. **96**. 376- 380.

Alenius, H., Palosuo, T, Kelly, K., Kuruv, V, Reunela, T., Makinen- Kiljunen, S., et. al., IgE reactivity to 14 kDa and 27 kDa natural rubber proteins in latex- allergic children with spina bifida and other congenital anomalies. *Int Arch Allergy Immunol*. 1993. **102**: 61- 66.

Alenius, H., Turjanmaa, K., Makinen-Kiljunen, S., Reunala, T., IgE immune response to rubber proteins in adult patients with latex allergy. *J Allergy Clin Immunol*. 1994. **93**: 859- 863.

Alenius, H., Kalkkinen, N., Lukka, M., Reunala, T., Turjanmaa, K., Makinen-Kiljunen, S., Yip, E. and Palosuo, T., Prohevein from the rubber tree (*Hevea brasiliensis*) is a major latex allergen., *Clin Exp Allergy*. 1995. **24**. 659-665.

Alenius, H., Makinen- Kiljunen, S, Ahlroth, M., Turjanmaa, K., Reunala, T & Palosuo, T., Crossreactivity between allergens in natural rubber latex and Banana studied by immunoblot inhibition. *Clin Exp Allergy*. 1996. **26**: 341- 348.

Allen, P. W., Naturak rubber: Aspects of a changing industry. IRC 86: Int Rub Conf, Sweden. p. 329. 1986.

Allen, P., W., Natural Rubber and the Synthetics. *Rubber Technology*. M. Morton. 3d ed. Crosby Lockwood. London. 1987.

Amir Hashim Mohd, Yatim, Effect of leaching on extractable protein content. Latex Protein and Glove Industry. Rubb. Rest. Inst., Malaysia. 51- 58. 1993.

Amos, H.E., Hypersensitivity mechanisms Responsible to tissue damage. Allergic drug reactions. 1 ed., Edward Arnold. UK. pp.9- 23. 1976.

ASTM standard test method for analysis of protein in natural rubber and its products. ASTM Designation: D 5712- 99. American Society for Testing and Materials. 1995.

Axelsson, I, Eriksson, M. and Wransjo, K., Anaphylaxis and angioederma due to rubber latex allergy in children. *Acta Paediatr Scand.* 1998. **77**: 314- 316.

Aziz, N.A.A., Chlorination of gloves. Latex proteins and glove industry. 59- 75. 1993.

Baur, X., Chen, Z., Allmer, H. and Raulf- Heimsoth, M., Results of wearing test with two different latex gloves with and without the use of skin protection cream. *Allergy*. 1998. **52**: 661- 664.

Banerjee, B., Wang, X., Kelly, K.J., Fink, J.N., Sussman, G.L., Kurup, V.P., IgE from Latex *Allergic* Patients Binds to Cloned and Expressed B-Cell Epitopes of Prohevein. *J Immunol*. 1997. **52**: 661- 664.

Beezhold, D.H., Measurement of latex proteins by chemical and immunological methods. Latex protein allergy: The present position. Hertford. UK: Rubber consultants. pp. 25- 31. 1993.

Beezhold, D.H., Sussman, G.L., Kostyal, D.A. and Chang, N.S., Identification of a 46-kd latex protein allergen in health care workers., *Clin Exp Immunol.* 1994. **98**. 408-413.

Beezhold, D.H., Sussman, G.L., Liss, G.M., & Chang, N.S., Latex allergy can induce clinical reactions to specific foods. *Clin Exp Allergy*. 1996. **26**: 416- 422.

Blanco, C., Carillo, T., Ortega, N., Alvarez, M., Dominguez, C., Castillo, R., Comparison of skin prick test and specific serum IgE determination for the diagnosis of latex allergy. *Clin Exp Allergy*. 1998. **28**: 971- 976.

Boonjawat, J., Chaopongpang, S., and Pitaksutheepong, C., Immobilization of papain on chitin for rubber latex deproteinization. Biopolymers and Bioproducts : Structure and Function and Applications. 22nd ed. FAOBMB Symposium. Bangkok. pp. 602-607. 1995.

Boonsook, S., Development of Natural rubber with low allergenic proteins by saponification process. Master's Thesis, Biochemistry, Chulalongkorn University. 1999.

Bradford, M.M., A rapid and sensitive method for quantification of microgram quantities of protein-dye-Binding. *Anal Biochem*. 1976. **72**. 248- 254.

Brehler, R., Theissen, U, Mohr, C & Luger, T., Latex fruit syndrome: Frequency cross-reacting IgE antibodies. *Allergy*. 1997. **52**: 404- 410.

Breitteneder, H & Scheiner, O., Molecular and immunological characteristic of latex allergen. *In Arch Allergy Immunol*. 1998. **116**: 83- 92.

Chambeyron, C., Dry, J.F., Leynadier, F., *et al*., Study of the allergenic fractions of latex. *Allergy*. 1992. **47**: 92- 97.

Chang, W.P., Lau, C.M. and Nambiar, J., Deproteinized natural rubber from field latex. Proceedings of PRIMN Planter's Conference. 295- 306. 1977.

Chaiear, N., Sadra, S., Jones, M., Cullinan, P., Foulds, I.S., Burge, P.S., Sensitization to natural rubber latex: Epidemiological study of workers exposed during tapping and gloves manufacture in Thailand. 2000.

Chen, Z., Pocsh, A., Cremer, R., Rauf-Heimsoth, M & Baur, X., Identification of hevein (Hev b6.02) in hevea latex as a major cross reacing allergen with avocado fruit patients with latex allergy. *J Allergy Clin Immunol*. 1998. **102**: 476- 481.

Chianrungsang, P., Deproteiniztion of natural by the coupling action of protease and microwave energy. Master's Thesis, Biotechnology, Chulalongkorn University. 2000.

Chiu, A.M., Murali, P.S., Fink, J.N., Kelly, K.J. and Kurup, V.P., Vrllulst immune response to purified latex proteins (abstract). *J Allergy Clin Immunol*. 1997. **95**: 154.

Coombs, R. R. A. and Gell P. G. H., *In Clinical Aspectsof immunology*. Ed. By P.G. Gell and R. R. A. Coombs. Blackwell scientific Publications, Oxford, 1953.

Czuppon, A.B., Chen, Z., Rennert, S., Engelke, T., Meyer, H.E., Heber, M. and Baur, X., The rubber elongation factor of rubber trees (*Hevea brasiliensis*) is the major allergen in latex. *J Allergy Clin Immunol.* 1993. **92**: 690- 697.

Danne, T., Weber, B., Niggemann, B., Wah, U., Prevalence of latex- specific IgE antibodies in atopic and non- atopic children with type I diabetes. *Diabetes Care*. 1997. 20(**4**): 476- 478.

De Swert, L.F.A., van Laer, K.M.I.A., Verpoorten, C.M.A., van Hoeyvekd, E.M., Cado, P., Stevens, E.A.M., Determination of independent risk factors and comparative analysis of diagnostic methods for immediate type latex allergy in spina bifida patients. *Clin Exp Allergy*. 1997. **27**: 1067- 1076.

Douglas, R., Morton, J., Czarny, D., O'Hehir, R.E., Prevalence of IgE- mediated allergy to latex in hospital nursing staff. *Aus N Z J Med*. 1997. **27**: 165- 169.

Downig, J.G., Dermatitis from rubber gloves. *J Med*. 1993. **208**: 196.

Eng, A.H., Tanaka, Y. et al., Sort communication FTIR studied on amino groups in purified *Hevea* rubber. *J Nat Rubb Res*. 1992. 7(**2**): 152- 155.

Eng, A.H., Tanaka, Y. et al.,Propertis of deproteinized natural rubber latex. Proc Int Rubb Tech Conf. 101- 110. 1993.

Firestone Tyre Rubber Company. Improvement in/ or relating to high grade rubber and method of making the same. Br Pat No 739: 750. 1955.

Frey-Wyssling, A., Microscopic investigations on the occurrence of resins in *Hevea* latex. Arch. v.d. Rubber. 13: 392. 1929.

Fong, C.S., Protein in natural rubber concentrated latex. *J nat Rubb*. 1992. 7(**2**) :102- 112.

Ghazaly, H.M., Properties of natural rubber low protein latex. Latex proteins and glove industry. 81- 91. 1993.

Gonzalez, E., Latex hypersensitivity: a new and unexpected problem. *Hospital practices*. 1992. **27**:137- 140.

Guillermo, H., Docena, Pablo, Beni'tez, Rita, Fern'andez, and Corlos A Fossati. Identification of allergenic Proteins in condoms by immunoenzymatic methods. *Annals of Allergy*. 2000. **85**: 77- 83.

Hadijiliadis D., Kan, K., Tarlo, S., Skin test responses to latex in an allergy and asthma clinic. *J Allergy Clin Immunol*. 1995. **96**: 431- 432.

Hänninen, AR., Mikkola, JH., Kalkkinen, N., Turjanmaa, K., Ylitalo, L., Reunala, T., Increased allergen production in turnip *(Brassica rapa)* by treatments activating defense mechanisms*. Allergy Clin Immunol* . 1999. **104**:194-201.

Hamann, C.P.Natural rubber latex protein sensitivity in review. *American J Cont Derm*. 1993. **4**: 4- 21.

Hamilton, R.G. and Atkinson N.F., Validation of the latex glove provocation procedure in latex- allergic subjects. *Ann Allergy Asthma Immunol*. 1997. **79**: 266- 272.

Harncharoen, K., Deveolpment of immunoassay for the detection of protein allergens in natural rubber products. Doctor's thesis, Philosophy in Biological Science, Graduate School, Chulalongkorn University. 1996.

Heese, A., Hintzenstern, J.V., Peters, K.P. et al., Allergic and irritant reactions to rubber gloves in medical health services. *J Ann Acad Dermatol*. 1995. **101**: 597.

Jacob, J.L., d'Auzac., and Prevot J.C., The composition of latex from *Hevea brasilliensis*. *Clin Rev Al*. 1992. **11**: 327- 337.

Jager, D., Kleinhans, D., Czuppon, A., Baur, X., Latex-specific proteins causing immediate-type cutaneous, nasal, bronchial and systemic reactions. *J Allergy Clin Immunol*. 1992. **89**:759- 768.

John, CK., Coagulatin of Hevea latex with surfactant an salt I: Development of the process and its effect on raw rubber properties. *J Rubb Inst Malaya*. 1971. **23**(2): 147.

Jones, R.T., Schepmann, D.L., Heilman, D.K., Yunginger, J.W., Porspective study of extractable latex allergen contents of disposable medical gloves. *Ann Allergy*. 1994. **73**: 321- 325.

Kelly, K., Kurup, V., Zacharisen, M., Resnick, A. and Frink, J., Skin and serologic testing in the diagnosis of latex allergy. *J Allergy Clin Immunol*. 1993. **91**: 1140- 1145.

Kevin, J.K., Latex allergy. **MD *slide presentation sample* Creative Educational** Options, LLC. 1999.

Klinpituksa, P., Chirong, Y., Wongboontarig, P., The production of deproteinized natural rubber from natural rubber latex by using Papain, Grant : Government Budget, Presented: 1st Academic Meeting, Prince of Songkla University, Pattani Campus, 1998.

Kujala, V., Pirila, T., Niinnimaki, A. Reijula, K.,Latex- induced allergic rhinitis in a laboratory nurse. *J Laryngol Otol*. 1995. **109**: 1094- 1096.

Latex proteins and gloves industry, Proc Int Rubb Technol Conf- workshop on latex proteins, Rubber Research Institute of Malaysia, Malaysia, 1993.

Leynadier, F. and Dry, J., Allergy to latex. (review) *Clin Rev Allergy*. 1991. **9**: 371- 7.

Liss, G.M., Sussman, G.L., Deal, K., *et. al*., Latex allergy : epidermiological study of 1351 hospitalworkers. *Occup Environ Med*. 1997. **54**: 415- 418.

Loos, K.A., Latex Allergy. University of Pacific Dental School. 1998.

Lowry, O.H., Rosebrough, N.J., Farr, A.l., and Randall, R.J., Measurement with the Folin phenol reagent. *J Biol Chem*, 1951. **193**: 265.

Lu, L.J., Kurup, V.O., Hoffman, D.R., Kelly, K.J., Murali, P.S., Fink, J.N., Characterization of a major latex allergen associated with hypersensitivity in spina bifida patients. *J Immunol*. 1995. **155**: 2721- 2728.

Mahler, V., Fischer, S., Fuchs, T., et al., Prevention of latex allergy by selection of low allergen gloves. *Clin Exp Allergy*. 2000. **30**: 509- 520.

Makinen- Kiljunen, Sorva, R. and Juntunen- Backman, K., Latex dummies as allergens. (letter) *Lancet*. 1992. **339**: 1068- 1069.

Moneret- Vautrin, D.A., Beaudouin, E., Widmer, S., Mouton, C., Kanny. G.,Prestat, F., Kohler, C., Feldman, L., Prospective study of risk factors in natural rubber latex hypersensitivity. *J Allergy Clin Immunol*. 1993. **92**: 668- 677.

Morales C., Basomba, A., Carreira, J., Sastre, A., Anaphylaxis produced by rubber gloves contact. Case report and immunological identification oft the antigens involved. *Clin Exp Allergy*. 1989. **19**: 425- 430.

Nadarajah, M., Yapa, P.A.J., Balasingham, C.G. and Kasinathan, S., The use of papain as biological coagulant for natural rubber latex. *J Rub Res Int (Malkaya)*. 1973. **23**: 76.

Nakade, S., Kuga, A., Hayashi, M. and Tanaka, Y., Highly purified natural rubber IV: Preparation and characteristics of gloves and condoms. *J Nat Rubb*. 1997. 12(**1**):33- 38.

Niggeman., B, Buck, D., Micheal, T & Wahn, U., Latex provocation tests in patients with spina bifida: who is at risk of becoming symptomatic. *J allergy Clin Immunol*. 1998. **102**: 665- 670.

Nielson, P.S., Nissen, D., Skov, P.S., Cieslsewicz *et al*., Assesment of IgE allergen specificity among latex- allergic health care workers: Review of IgE binding components of various latex extracts. Annals of Allergy, Asthma and Immunaology; Palatine. 2000.

Nieto, A., Estronell, S., Mazon, A. and Reig, C., Allergy to latex in spina bifida: A multivariate study of association factors in 100 consecutive patients. *J Allergy Clin Immunol*. 1996. **98**: 501- 507.

Nutter, A.F., Contact urticaria to rubber. *Br. J. Dermatology*. 1979. **101**: 597-598.

Ownby, D.R., Ownby, H.E., McCullogh, J., & Shafer, A.W., The prevalence of the anti latex IgE-antibodies in 1000 volunteer blood donors. *J Allergy Clin Immunol*. 1996. **97**: 1188- 1192.

Palosuo, T., Turjanmaa, K. and Reinikka- Railo, H., Allergen Content of Latex Gloves. A Market Surveillance Study of Medical Gloves Used in Finland in 1997. National Agency for Medicines (NAM). 1997. http://latexallergylinks.tripod.com/NAM.html

Palosuo T., Makinen- Kiljunen, S., Aleniue, H., Measurement of natural rubber latex allergen levels in medical gloves by allergen specific IgE- ELISA inhibition. RAST inhibition and skin prick test. *Allergy*. 1998. **53**: 59- 67.

Para rubber Bulletin. Thailand 7. 1986.

Porri, F., Pradal, M., Lemiere, C., Prevalence of anti- IgE antibodies in atopic and non-atopic subjects from the general population. *J Allergy Clin Immunol*. 1995. **95**:154.

Posch, A., Chen, Z., Raulf-Heimsoth, M. and Baur, X., Latex allergens. *Clin Exp Allergy*. 1998. **28**: 134- 140.

RRIM test methods for Standard Malaysian Rubbers. SMR bull no. 7 Rub Res Inst., Malaysia. 1992.

RRIM modified Lowry method for determination of extractable proteins in latex products. Latex Technology Division. Rubber Research Institute of Malaysia. 1994.

Rüeff, F., Thomas, P., ReiBig, G., & Pryzbilla, B., Natural rubber latex allergy in pateints noyt intensely exposed. *Allergy*. 1998. **53**: 445- 449.

Rungvichaniwat, D., Nithi- Uthai, B., Nithi- Uthai, P., The color and nitrogen content of skim rubber. The 7th seminar on elastomer. 1- 8. 1998.

Rypniewski, W.R., Osten, C.V.D., Oestergaard, P., Dauter, M. and Wilson, K.S., Alcalase. 1997.

Safadi, G.S., Corey, E.C., Taylor, J.S., Wagner, W.O, Pien, L.C., Meton, A.L., Latex hypersensitivity in emergency medical service providers, *Ann Allergy Asth Immunol*. 1996. **77**: 39- 42.

Slater, J.E., and Chahabra, S., Latex antigens. *J Allergy Clin Immunol*. 1989. **89**: 673-678.

Slater, J.E., Trybul, D.E., Affinity purification of latex antigens. *J Allergy Clin Immunol*. 1994. **93**: 644- 649.

Sorva, R., Makinen- Kiljunen, S., Suvilehto, K., Juntunen- Backman, K. and Hahhtela, T., Latex allergy in children with no known risk factor for latex sensitization. *Pediatr allergy Immunol*. 1995. **6**: 36- 38.

Subramaniam, A., Yip, E.K.P., N.G. and Mok, K.L., Extractable protein content of gloves from prevulcanized natural rubber latex. Latex proteins and glove industry. 76-80. 1993.

Sunderasan, E., Hamzah, S., Hamid, S., Ward, M.A., Yeang, H.Y. and Cardosa, M.J., Latex B-serum beta-1,3-glucanase (*Hev b* II) and a component of the microhelix (*Hev b* IV) are major latex allergens. *J Nat Rubb Res*. 1995 **10**: 82-99.

Sussman, G.L., Tarlo, S., Dolovich, J., The spectrum of IgE-mediated responses to latex. JAMA, **265**: 2844- 2847. 1991.

Sussman, G.L. and Beezhold, Allergy to latex rubber. *Ann Intern Med*. 1995. **122**: 43- 46.

Swanson, M.C., Bubak, M.E., Hunt, L.W., Quantification of occupational latex aeroallergens in medical center. *J Allergy Clin Immunaol*. 1994. **94**: 445- 451.

Swanson, M.C., Yunginger, J.W., Reed, C.E., Measurement of latex aeroallergens in nine dental offices. *J Allergy Clin Immunol*. 1995. **95**: 153- 158.

Tangpakdee, J. and Tanaka, Y., Purification of natural rubber. *J Nat Rubb Res*. 1997. 12(**2**): 112- 119.

Teeraratkul, A., Dangsuwan, T., Wittisuwannakul. R., Kerdsomnuk, S., et al., Epidemiology of latex allergy among healthcare personnel at Siriraj Hospital. Siriraj Hos Gaz. 1997. 49: 832- 845.

Thailand rubber statistics. Thai Rubber Research Institute. 30 (**4**). 2001.

Turjanmaa, K., Reunala, T., Tuimala, R. and Karkkainen, T., Severe IgE mediated allergy to surgical gloves. (abstract) *Allergy*.1984. 2: 35.

Turjanmaa, K., & Reunala, T., Contact urticaria from rubber gloves. *Dermatol Clin*. 1988. 6(1).

Turjanmaa, K. and Reunala, T., Contact urticaria from rubber gloves. *Dermatol Clin*. 1988. 6: 47- 51.

Turjanmaa, K., Reulana, T. and Rasanen, L., Comparison of diagnosis in latex surgical glove contact urticaria. *Contact Derm*. 1988. 19: 241- 247.

Turjanmaa, K., Lauren, K., Makinen- Kiljulnen, S. and Reulana, T., Rubber contact urticaria: Allergenic properties if 19brands of latex gloves. *Contact Derm*. 1988. 19: 362- 367.

Turjanmaa, K., Alenius, H., Makinen- Kiljunen, S., Palosuo, T. and Reunala, T., Commercial skin prick test preparations in the diagnosis of rubber latex allergy. (abstract) J Allergy Clin Immunol. 1994. 93: 299.

Turjanmaa, K., Makinen- Kiljunen, S., Alenius, H. and Palosuo, T., Natural rubber latex allergy- the Experience. In: Latex allergy. pp 71- 88. Ed. Frink J. Philadelphia. 1995.

Turjanmaa, K., Alenius, H., Makinen- Kiljunen, S., Palosuo, T. and Reunala, T., Natural rubber latex allergy. (review) *Allergy*, 1996. 51: 593- 602.

Turjanmaa, K., Palosuo, T. Alenius, H., Leynadier, F., Autengarden, J.-E., Andre', C., Sicard, H., Hrabina, H., Tran, T.X., Latex allergy diagnosis: *in vivo* and *in vitro* standardization of natural rubber extract. *Allergy*. 1997. 52: 41- 50.

Vandenplas, O., Delwiche, J.P., Evrard, G., Aimont, P., Vanderbrempt, X., Jamart, J., Delaunois, L., Prevalence of occupational asthma due to latex among hospital personnel. *Am J Res Critic Care Medicine*. 1995. 151: 54- 60.

Varghese, S., Katsumura, Y., Makuuchi, K., Yoshii, F., Effect of water soluble polymers on radiation vulcanized natural rubber latex. *Rubber Chem Tech*. 1999. 72: 308.

Vallier, P., Balland, S., Harf, R., Valenta, R. and Deviller, P., Identification of profilin as an IgE-binding component in latex from *Hevea brasiliensis*: clinical implications. *Clin Exp Allergy*. 1995. 25: 332- 339.

Visessanguan, W., Optimization of natural rubber latex deproteinization by enzymes. Master's Thesis, Chulalongkorn university. 1992.

Warshaw, E.M., Latex allergy. *Am. Acad. Dermatol*. 1998. 39 (1):1- 24.

Weeraphasuk, W., Effect of mixing conditions on Mooney viscosity of deproteinised rubber. Mater's thesis, Department of Chemical Technology, Chulongkorn University. 1995.

Wilson, H.T., Rubber glove dermatitis. *Br Med J*. 1960. **20**: 5191.

Wrangsjo, K., Osterman, K. and van Hage- Hamsten, M., Glove- related skin symtomps among operating theatre and dental care unit personnel. (I) Interview Investigation. *Contact Dermatitis*. 1994. **30**: 102- 107.

Wrangsjo, K.,

Yapa, P.A.J., The preparation and properties of low nitrogen constantly viscosity rubber. *J Rubb Inst Sri*. 1975. **52**: 1- 9.

Yapa, P.A.J., Enzyme deproteinization of Hevea latex I Preparation and properties of DPNR and viscosity stabilized DPNR. *J Rubb Inst Sri*. 1977. **54**:509- 519.

Yapa, P.A.J., Nadarajah, M., and Lioel, W.A., High quality low protein rubber from skim latex. IRRDB Symposium Kuala Lumpur (Preprint). 1978.

Yapa, P.A.J. and Yapa, S., Recent development in the manufacture of deproteinized natual rubber. Proc Int Rubb Conf. 2: 145- 306. 1984.

Yassin, M., Sanyurah, S., Lierl, M., Fisher, T., Oppenheimer, S., Cross, J., O'Brien, K. and Steinmetz, C., Evalution of latex allergy in patients with meningomyelocele. *Ann Allergy*. 1992. **69**: 207- 211.

Yatim, A.H.M., Effect of leaching on extractable protein content. Latex proteins and glove industry. 51- 58. 1993.

Yeang, H.Y.,Sunderasan, E., Bahri, A.R.S., et al., Study on the behavior and characteristuics of glove proteins. International rubber technology conference, Kuala Lumpur, Malaysia. 1993.

Yeang, H.Y., Ward, M.A., Zamri, A.S.M. Dennis, M.S. and Light, D.R., Amino acid sequence similarity of Hev b 3 to two previously reported 27- and 23-kDa latex proteins allergenic to spina bifida patients. *Allergy*. 1998. **53**: 513- 519.

Ylitalo, L., *Natural Rubber Latex Allergy in Children.* Doctoral dissertation. Acta 719. 52 p., append. 2000.

Yunginger, J.W., Joens, R.T., Fransway, A.F. et al., Extractable latex allergens and proteins in disposable medical gloves and other rubber products. *J Allergy Clin Immunol*. 1994. **93**: 836- 842.

APPENDICES

Appendix 1

Specific activity of Alcalase

1. Casein substrate solution

Disperse 1 g of Casein hammarsten (moisture free basis) in 50 ml of 0.05 M sodium phosphate solution, and heat at the temperature about 50 ^{o}C for 30 minutes with sometimes shaking. Cool at the room temperature with shaking. Adjust pH to 6 $\pm$ 0.1 by 0.05 M citric acid solution. Adjust volume to 100 ml by distilled water.

2. Buffer solution for the assay of Alcalase activity

2.1 0.05 M Tris- HCl buffer pH 7.6

Tris	6.057	g
H_2O	50	ml

(Adjust pH to 7.6 by 0.1 M HCl or 0.1 M NaOH and adjust volume to 100 ml by distilled water)

2.2 10% (w/v) Trichloroacetic acid

Trichloroacetic acid	10	g
H_2O	100	ml

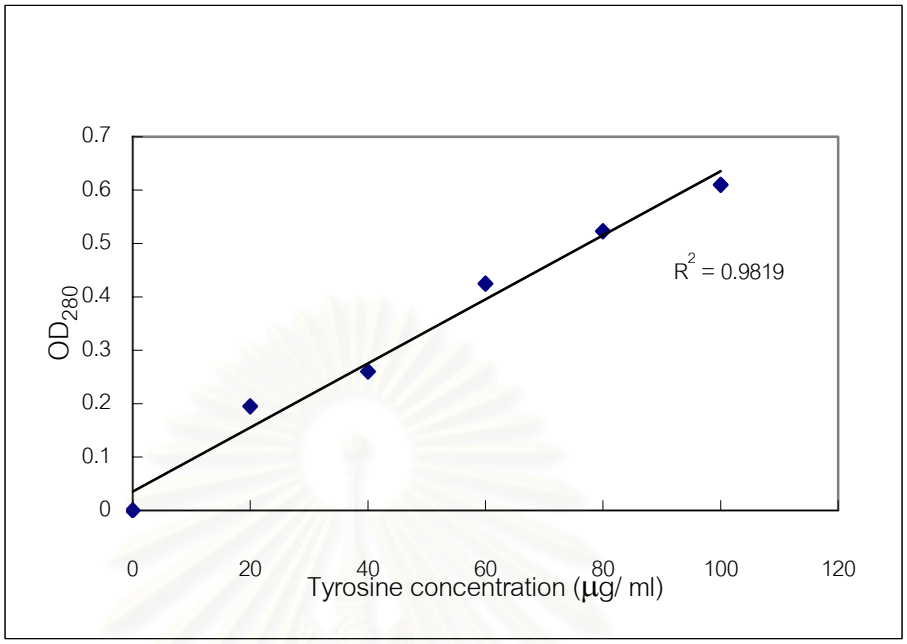


Figure A1 Tyrosine standard curve

Slope of tyrosine standard curve = 0.0065

Tyrosine concentration = OD_{280}/ slope

Appendix 2

Stock solution for preparation of deproteinized concentrated latex (DPCL)

1. 12% Alcalase

Alcalase (commercial grade) was weight about 120 g, added with 1 L of distilled water and mixed completely. Removed undissolved sediment by centrifugation at 8,000 x g for 30 minutes. Then obtained clear filtrated and kept this solution at 4 ^{o}C.

2. 50% Sodium dodecyl benzene sulfonate (SDBS)

SDBS	500	g

Adjusted volume to 1 L by distilled water

3. 10% Triton X100

Triton X100	100	ml

Adjusted volume to 1 L by distilled water

4. 5% Sodium metabisulfite

Sodium metabisulfite	50	g

Adjusted volume to 1 L by distilled water

5. 20% KOH

KOH	200	g

Adjusted volume to 1 L by distilled water

6. 1% Sodium alginate in 1% ammonia

Sodium alginate was weighted 10 g and dissolved in about 500 ml distilled water sodium alginate was then warmed on the hotplate until clearly dissolved. Added 50 ml of NH_3 solution (caution: this step must do in the hood). Adjusted volume to 1 L by distilled water.

Appendix 3

Determination of N content by Kjeldahl method

1. Effect of Alcalase deproteinization on % N

Production date	% Nitrogen control	% Nitrogen DPCL	% Nitrogen reduction
1/07/00	0.19±0.06	0.04±0.02	79
27/07/00	0.31±0.05	0.19±0.06	39
3/08/00	0.24±0.02	0.11±0.01	54
19/09/00	0.16±0.01	0.12±0.01	25
25/09/00	0.23±0.03	0.027±0.02	88
1/10/00	0.23±0.06	0.02±0.06	91
3/03/01	0.22±0.05	0.01±0.02	95

Appendix 4

Protein determination by modified Lowry method

1. Solution for modified Lowry method

1.1 With presence of $CuSO_4$

Solution C : 6 % w/v of sodium carbonate

Solution D : 1.5% w/v of copper sulfate in 3%w/v of sodium citrate

Reagent A: Alkali copper sulfate (10 parts of C: 0.2 part of D)

Reagent B: Diluted Folin Reagent

1.2 With absence of $CuSO_4$

Solution DC : 6 % w/v of sodium carbonate

Solution DD : 3%w/v of sodium citrate

Reagent A : (10 parts of DC: 0.2 part of DD)

Reagent B : Diluted Folin Reagent

2. Measurement water extractable protein with $CuSO_4$ by modified Lowry method

The reaction was carried out with 160- 200 μl of 0.1 N sodium hydroxide and 2.5- 40 μl of protein solutions, then added 75 μl of alkaline copper sulfate, Reagent A, (10 part of C and 0.2 part of D) into each well of a flat bottom, 96-well, polystyrene microtiter plate, mixed and allowed to stand for 15 minutes at room temperature. The reaction was then added with 25 μl of dilute Folin solution, Reagent B, mixed thoroughly and allowed for 15 minutes at room temperature. Protein levels were evaluated against standard protein, ovalbumin, using a microplate reader (Multiskan Ex, Labstytems) at 750 nm wavelength.

3. Measurement water extractable protein without $CuSO_4$ by modified Lowry method

The reaction was carried out with 160- 200 μl of 0.1 N sodium hydroxide and 2.5- 40 μl of protein solutions, then added 75 μl of Reagent A (10 part of DC and 0.2 part of DD), into each well of a flat bottom, 96-well, polystyrene microtiter plate, mixed and allowed to stand for 15 minutes at room temperature. The reaction was then added with 25 μl of dilute Folin solution, Reagent B, mixed thoroughly and allowed for 15 minutes at room temperature. Protein levels were evaluated against standard protein, ovalbumin, using a microplate reader (Multiskan Ex, Labstytems) at 750 nm wavelength.

The absorbance OD_{750} of protein sample

Sample	OD_{750}, $CuSO_4$	OD_{750}, no $CuSO_4$	OD_{750}, $CuSO_4$- no $CuSO_4$	Protein, μg/ well of titer plate	Protein, μg/ g rubber
CNR (1/07/00)	0.57	0.25	0.32	16	610
DPNR (1/07/00)	0.12	0.07	0.05	1	25
CNR (27/07/00)	0.33	0.17	0.16	7	59
DPNR(27/09/00)	0.29	0.15	0.14	3	20
CNR (3/08/00)	0.35	0.14	0.31	8	216
DPNR (3/08/00)	0.20	0.09	0.10	2	38
CNR (19/09/00)	0.62	0.24	0.38	21	840
DPNR(19/09/00)	0.17	0.07	0.10	2	14
CNR (25/09/00)	0.30	0.11	0.19	6	150
DPNR(25/09/00)	0.13	0.04	0.09	2	11
CNR (1/10/43)	0.58	0.23	0.35	18	610
DPNR(1/10/43)	0.10	0.03	0.07	-	2
CNR (3/03/01)	0.53	0.23	0.30	2.50	213
DPNR(3/03/01)	0.14	0.04	0.10	4.70	12

Calculation

C-AL: OD_{750} = 0.063, Protein evaluated from standard protein ovalbumin = 2.6 μg

Extraction: 0.8 g of C-AL / 8 ml of water then the solution was lyophilized and re-dissolved of 500 μl water

There fore water extractable protein = 2.6 x 500 / 50 = 26 μg

Total water extractable protein = 26 μg

= 26 / 8 = 3.25 μg/ml

= 32.5 μg/g

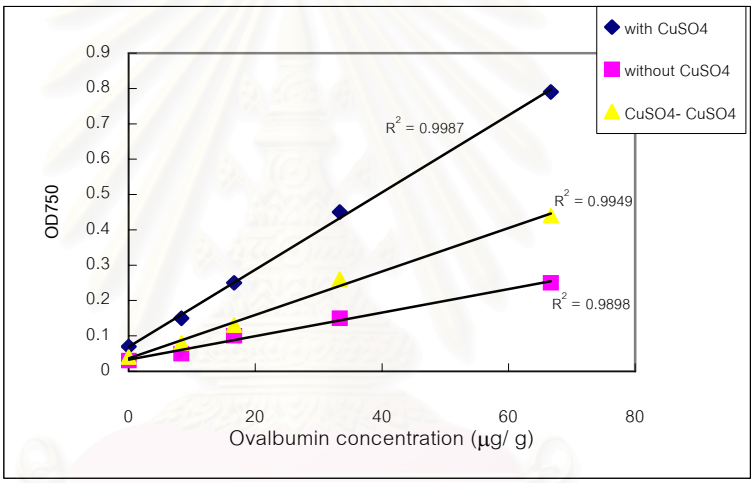


Figure A2 Standard curve of ovalbumin measured by modified Lowry method

Appendix 5

Protein determination by Bradford method

1. Solution for Bradford method

1.1 Bradford stock solution

95 % Ethanol	100 ml
88 % Phosphoric acid	200 ml
Serva Blue G	350 mg

Stable indefinitely at room temperature.

1.2 Bradford working buffer

H2O	425 ml
95 % Ethanol	15 ml
88 % Phosphoric acid	30 ml
Bradford stock solution	30 ml

Filter through Whatman No. 1 paper, store at room temperature in brown glass bottle. Usable for several weeks, but may need to refiltered.

1.3 Assay

1. Pipette protein solution (maximum 100 ml) into tube.
2. Add experiment buffer to make total volume of 100 μl.
3. Add 1 ml Bradford working buffer and vortex.
4. Read OD_{595} after 2 minutes but before 1 hour.

Appendix 6

Total cost in deproteinization by Alcalase

Items	Price/ unit (Baht)	Consumption /1 ton of amm. latex	Total cost (Baht)
Starting material ammoniated latex 200 L x 5 Days = 1 ton	20.5/ L	1 ton	20,500
Alcalase	650/ kg	1.05 kg	682.5
SDBS	60/ kg	3 kg	180
Sodiummetabisulfite	25/kg	0.28 kg	6.88
Titron X 100	100/ 1L	0.35 L	35
Alginic acid	2500/ kg	0.05 kg	125
KOH	82/kg	1 kg	82
Lauric acid	100/ kg	1.75 kg	175
Water	0.28	55	15.4

Total cost = 21,801.78 Baht

DPCL yield = 600 L

Cost of start asset = 21,801.78/ 600 = 36.34 Baht/ L

Appendix 7

Solution for SDS-PAGE

1. Tris glycine eletrode buffer

(25 mM Tris, 192 mM glycine)

Tris	3.0	g
Glycine	14.4	g
SDS	1.0	g
H_2O	1	L

2. Tris-HCl stock solution pH 8.8

(2 M Tris)

Tris	24.2	g
H_2O	100 ml	

(adjust pH to 8.8 with $HCl_{conc.}$ Or 0.1 M NaOH)

3. Tris-HCl stock solution pH 6.8

(1 M Tris)

Tris	12.2	g
H_2O	100 ml	

(adjust pH to 8.8 with HCl_{conc}. Or 0.1 M NaOH)

4. Sample buffer

Tris-HCl stock solution pH 6.8	0.6	ml
10 % SDS	2	ml
2-mercaptoethanol	0.5	ml
1% bromophenol blue	1	ml
H_2O	0.9	ml

5. Acrylamide stock (30%)

Acrylamide	29.2	g
Bis	0.8	g
H_2O	100	ml

6. Ammonium persulfate 0.1 g/ml

7. 15% Separating gel

Stock gel (30%)	10	ml
Stock buffer pH8.8	5	ml
H_2O	5	ml
Ammonium persulfate	100	µl
TEMED	10	µl

8. Stacking gel

Stock gel (30%)	1.34	ml
Stock buffer pH 6.8	2.0	ml
H_2O	4.6	ml
Ammonium persulfate	60	µl
TEMED	10	µl

7. Staining solution

Commassie Blue R-250	1.0	g
Methanol	450	ml
Glacial acetic acid	100	ml
H_2O	450	ml

8. Destain solution

Glacial acetic acid	100	ml
Methanol	100	ml
H_2O	800	ml

Appendix 8

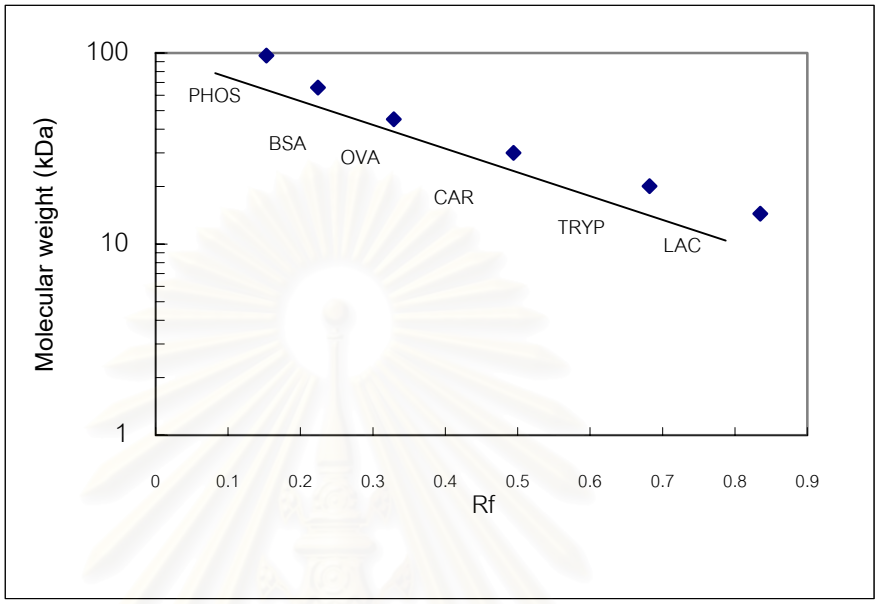


Figure: A3 Molecular weight markers calibration curve of SDS-PAGE

PHOS:	Phosphorylase B	97 kDa,
BSA:	Bovine serum albumin	66 kDa,
OVA:	Ovabumin	66 kDa,
CAR:	Carbonic anhydrase	30 kDa,
TRYP:	Trypsin inhibitor	20.1 kDa
LAC:	α- Lactalbumin	14.4 kDa

Appendix 9

Standard curve of each protein measured by modified Lowry method

Ovalbumin

Protein conc. (μg/ ml)	OD_{750} with $CuSO_4$	OD_{750} without $CuSO_4$	OD_{750} $CuSO_4$- no $CuSO_4$
0.00	0.00	0.00	0.00
8.33	0.11	0.03	0.08
16.67	0.19	0.05	0.13
33.33	0.32	0.10	0.22
66.67	0.54	0.16	0.38
133.33	0.83	0.28	0.55

Bovine serum albumin

Protein conc. (μg/ ml)	OD_{750} with $CuSO_4$	OD_{750} without $CuSO_4$	OD_{750} $CuSO_4$- no $CuSO_4$
0.00	0.00	0.00	0
8.33	0.15	0.06	0.09
16.67	0.18	0.03	0.15
33.33	0.31	0.06	0.25
66.67	0.54	0.14	0.40
133.33	0.72	0.26	0.46

Latex serum proteins

Protein conc. (μg/ ml)	OD_{750} with $CuSO_4$	OD_{750} without $CuSO_4$	OD_{750} $CuSO_4$- no $CuSO_4$
0.00	0.00	0.00	0.00
8.33	0.12	0.05	0.07
16.67	0.22	0.08	0.14
33.33	0.33	0.11	0.22
66.67	0.54	0.18	0.36
133.33	0.86	0.29	0.57

Appendix 10

Standard curve of each protein measured by Bradford method

Protein concentration (□g/ ml)	OD at 595 nm		
	BSA	Ovalbumin	Latex serum protein
0.00	0.00	0.00	0.00
2.50	0.04	0.03	0.05
5.00	0.09	0.06	0.10
7.50	0.14	0.14	0.15
10.00	0.18	0.17	0.19
12.50	0.23	0.21	0.23
15.00	0.27	0.25	0.27
17.50	0.30	0.28	0.32
20.00	0.35	0.30	0.35

Appendix 11

Skin prick test results

1. Group 1. 71 Atopic health care workers (13 males, 58 females)

Sex	Age (years)	Time work (years)	Usage (years)	1	2	3	S1	4	H	5	6	7	NSS
female	50	33	20	-	-	-	-	-	+	-	-	-	-
female	32	10	10	-	-	-	-	-	+	-	-	-	-
female	21	0	ND	-	-	-	-	+	+	-	-	-	-
female	36	17	17	+	+	+	+	+	+	-	-	-	-
female	31	6	6	-	-	-	-	+	+	-	-	-	-
female	48	27	31	-	-	-	-	-	+	-	-	-	-
female	50	29	29	-	-	-	-	-	+	-	-	-	-
female	22	1	1	-	-	-	-	-	+	-	-	-	-
female	32	8	ND	-	-	-	-	-	+	-	-	-	-
female	32	5	ND	-	-	-	-	+	+	-	-	-	-
female	42	19	ND	-	-	-	-	+	+	-	-	-	-
female	28	5	ND	-	-	-	-	-	+	-	-	-	-
female	30	10	10	-	-	-	-	-	+	-	-	-	-
female	32	5	ND	-	-	-	-	-	+	-	-	-	-
female	53	30	28	-	-	-	-	-	+	-	-	-	-
female	24	0	ND	-	-	+	+	+	+	+	-	-	-
female	39	16	6	-	-	-	-	+	+	-	-	-	-
female	57	25	25	-	-	-	-	+	+	-	-	-	-
male	25	4	4	-	-	-	-	-	+	-	-	-	-
male	20	0	ND	-	-	-	-	+	+	-	-	-	-
male	19	0	ND	-	-	-	-	-	+	-	-	-	-
female	23	0	3	-	-	-	-	-	+	-	-	-	-
female	21	0	3	-	-	-	-	+	+	-	-	-	-
female	22	0	3	-	-	-	-	+	+	-	-	-	-
female	22	3	3	-	-	-	+	+	+	-	-	-	-
female	39	16	6	-	-	-	+	+	+	-	-	-	-

Sex	Age (years)	Time work (years)	Usage(years)	1	2	3	S1	4	H	5	6	7	NSS
female	31	6	6	-	-	-	-	-	+	-	-	-	-
female	36	10	10	+	-	-	+	+	+	-	-	-	-
female	30	10	10	-	-	-	-	+	+	-	-	-	-
female	20	3	2	-	-	-	-	+	+	-	-	-	-
female	21	3	2	+	+	+	-	+	+	-	-	-	-
female	21	3	2	-	-	-	-	+	+	-	-	-	-
female	23	0	5	-	-	-	-	-	+	-	-	-	-
male	20	0	ND	-	-	-	-	-	+	-	-	-	-
female	53	28	30	+	-	+	+	+	+	+	+	+	-
female	25	3	3	-	-	-	-	-	+	-	-	-	-
female	41	17	ND	-	-	-	-	+	+	-	-	-	-
female	36	7	ND	-	-	-	-	+	+	-	-	-	-
female	45	23	ND	-	-	-	-	-	+	-	-	-	-
male	20	0	ND	-	-	-	-	+	+	-	-	-	-
female	20	0	ND	-	-	-	-	-	+	-	-	-	-
female	41	0	ND	-	-	-	-	+	+	-	-	-	-
female	21	0	ND	-	-	-	-	-	+	-	-	-	-
female	20	0	ND	-	-	-	-	-	+	-	-	-	-
female	27	5	ND	+	+	-	+	-	+	-	-	+	-
male	23	0	ND	+	+	+	+	+	+	-	-	-	-
female	23	5	ND	-	+	-	-	+	+	-	-	-	-
female	27	5	ND	-	-	-	+	+	+	-	+	-	-
female	41	17	ND	-	-	-	-	+	+	-	-	-	-
female	45	32	25	-	-	-	-	-	+	-	-	-	-
female	26	4	4	-	-	-	-	+	+	-	-	-	-
male	20	3	1	-	-	-	-	+	+	-	-	-	-
female	54	32	5	-	-	-	-	-	+	-	-	-	-
female	37	19	19	-	-	-	-	-	+	-	-	-	-

Sex	Age (years)	Time work (years)	Usage (years)	1	2	3	S1	4	H	5	6	7	NSS
female	22	5	5	-	-	-	-	-	+	-	-	-	-
female	47	ND	ND	-	-	-	-	-	+	-	-	-	-
female	27	3	3	-	-	-	-	-	+	-	-	-	-
female	50	ND	ND	-	-	-	+	+	+	-	-	-	-
female	54	ND	5	-	-	-		-	+	-	-	-	-
female	23	ND	2	-	-	-	-	-	+	-	-	-	-
male	22	ND	3	-	-	-	-	-	+	-	-	-	-
female	31	9	9	-	-	-	-	+	+	-	-	-	-
female	49	26	26	-	-	-	-	-	+	-	-	-	-
male	33	10	10	-	-	-	-	-	+	-	-	-	-
male	ND	ND	ND	-	-	-	-	+	+	-	-	-	-
female	59	30	20	-	-	-	+	+	+	-	-	-	-
female	26	5	5	-	-	-	-	-	+	-	-	-	-
male	19	0	ND	-	-	-	-	-	+	-	-	-	-
male	23	0	ND	+	+	+	+	+	+	-	-	-	-

1: Control concentrated latex (27/07/00)

2: Control concentrated latex (19/09/00)

3: Control concentrated latex (3/08/00)

S1: Stallergenes, Commercial allergens

4: Latex serum proteins (18/06/01)

H: Histamine

5: Deproteinized concentrated latex (25/09/00)

6: Saponified crumb rubber

7: Saponified skim rubber

NSS: Normal saline solution

ND: Not detect

2. Group 2; 41 General atopic patients (10 males, 31 females)

Sex	Age (years)	1	2	3	S1	4	H	5	6	7	NSS
female	24	-	-	-	-	-	+	-	-	-	-
female	25	-	-	-	-	-	+	-	-	-	-
female	22	-	-	-	-	-	+	-	+	-	-
female	17	-	-	-	-	+	+	-	-	-	-
female	22	-	-	-	-	-	+	-	-	-	-
female	44	+	-	-	+	+	+	-	-	-	-
male	28	-	-	-	-	+	+	+	+	+	-
female	67	-	-	-	-	-	+	-	-	-	-
female	57	-	-	-	-	-	+	-	-	-	-
female	34	-	-	-	-	-	+	-	-	-	-
female	22	-	-	-	-	-	+	-	-	-	-
female	50	-	-	-	-	-	+	-	-	-	-
female	26	-	-	-	-	-	+	-	-	-	-
male	30	-	-	-	-	-	+	-	-	-	-
male	30	-	-	-	-	-	+	-	-	-	-
female	49	-	-	-	-	-	+	-	-	-	-
male	37	-	-	-	-	+	+	-	-	-	-
female	32	-	-	-	-	-	+	-	-	-	-
female	19	-	-	-	-	-	+	-	-	-	-
female	34	-	-	-	-	-	+	-	-	-	-
male	46	-	-	-	+	+	+	-	-	-	-
female	35	-	-	-	-	-	+	-	-	-	-
male	44	-	-	-	-	-	+	-	-	-	-
female	47	-	-	-	-	-	+	-	-	-	-
female	30	-	-	-	-	-	+	-	-	-	-
female	40	-	-	-	-	-	+	-	-	-	-
female	63	-	-	-	-	-	+	-	-	-	-
male	51	-	-	-	-	-	+	-	-	-	-
female	24	-	-	-	-	-	+	-	-	-	-

Sex	Age (years)	1	2	3	S1	4	H	5	6	7	NSS
male	41	-	-	-	-	-	+	-	-	-	-
male	49	-	-	-	-	-	+	-	-	-	-
female	32	-	-	-	-	+	+	-	-	-	-
female	30	-	-	-	-	-	+	-	-	-	-
female	48	-	-	-	+	+	+	-	-	-	-
female	45	-	-	-	-	-	+	-	-	-	-
female	18	-	-	-	-	+	+	-	-	-	-
female	51	-	-	-	-	+	+	-	-	-	-
female	22	-	-	-	-	-	+	-	-	-	-
female	25	-	-	-	+	+	+	-	-	-	-
female	54	-	-	-	-	-	+	-	-	-	-
male	40	-	-	-	-	+	+	-	-	-	-
female	31	-	-	-	-	-	+	-	-	-	-

1: Control concentrated latex (27/07/00)

2: Control concentrated latex (19/09/00)

3: Control concentrated latex (3/08/00)

S1: Stallergenes, Commercial allergens

4: Latex serum proteins (18/06/01)

H: Histamine

5: Deproteinized concentrated latex (25/09/00)

6: Saponified crumb rubber

7: Saponified skim rubber

NSS: Normal saline solution

ND: Not detect

Appendix 12

Statistical calculation:

Frequencies

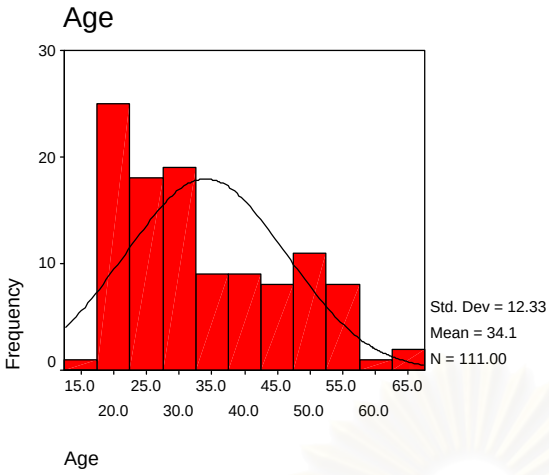
Statistics

Age

N	Valid	111
	Missing	1
Mean		34.08
Median		31.00
Range		50
Minimum		17
Maximum		67
Sum		3783

Age

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	17	1	.9	.9	.9
	18	1	.9	.9	1.8
	19	3	2.7	2.7	4.5
	20	7	6.3	6.3	10.8
	21	5	4.5	4.5	15.3
	22	9	8.0	8.1	23.4
	23	5	4.5	4.5	27.9
	24	3	2.7	2.7	30.6
	25	4	3.6	3.6	34.2
	26	3	2.7	2.7	36.9
	27	3	2.7	2.7	39.6
	28	2	1.8	1.8	41.4
	30	6	5.4	5.4	46.8
	31	4	3.6	3.6	50.5
	32	7	6.3	6.3	56.8
	33	1	.9	.9	57.7
	34	2	1.8	1.8	59.5
	35	1	.9	.9	60.4
	36	3	2.7	2.7	63.1
	37	2	1.8	1.8	64.9
	39	2	1.8	1.8	66.7
	40	2	1.8	1.8	68.5
	41	4	3.6	3.6	72.1
	42	1	.9	.9	73.0
	44	2	1.8	1.8	74.8
	45	3	2.7	2.7	77.5
	46	1	.9	.9	78.4
	47	2	1.8	1.8	80.2
	48	2	1.8	1.8	82.0
	49	3	2.7	2.7	84.7
	50	4	3.6	3.6	88.3
	51	2	1.8	1.8	90.1
	53	2	1.8	1.8	91.9
	54	3	2.7	2.7	94.6
	57	3	2.7	2.7	97.3
	59	1	.9	.9	98.2
	63	1	.9	.9	99.1
	67	1	.9	.9	100.0
	Total	111	99.1	100.0	
Missing	191	1	.9		
Total		112	100.0		



Frequencies

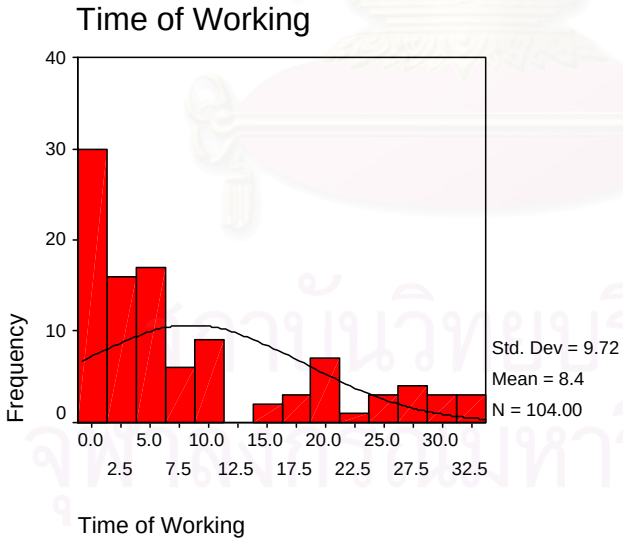
Statistics

Time of Working

N	Valid	104
	Missing	8
Mean		8.44
Median		5.00
Range		33
Minimum		0
Maximum		33
Sum		878

Time of Working

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	0	26	23.2	25.0	25.0
	1	4	3.6	3.8	28.8
	2	8	7.1	7.7	36.5
	3	8	7.1	7.7	44.2
	4	5	4.5	4.8	49.0
	5	9	8.0	8.7	57.7
	6	3	2.7	2.9	60.6
	7	2	1.8	1.9	62.5
	8	4	3.6	3.8	66.3
	9	2	1.8	1.9	68.3
	10	7	6.3	6.7	75.0
	16	2	1.8	1.9	76.9
	17	3	2.7	2.9	79.8
	19	2	1.8	1.9	81.7
	20	4	3.6	3.8	85.6
	21	1	.9	1.0	86.5
	23	1	.9	1.0	87.5
	25	2	1.8	1.9	89.4
	26	1	.9	1.0	90.4
	27	2	1.8	1.9	92.3
	28	2	1.8	1.9	94.2
	29	1	.9	1.0	95.2
	30	2	1.8	1.9	97.1
	32	2	1.8	1.9	99.0
	33	1	.9	1.0	100.0
	Total	104	92.9	100.0	
Missing	191	8	7.1		
Total		112	100.0		



Frequencies

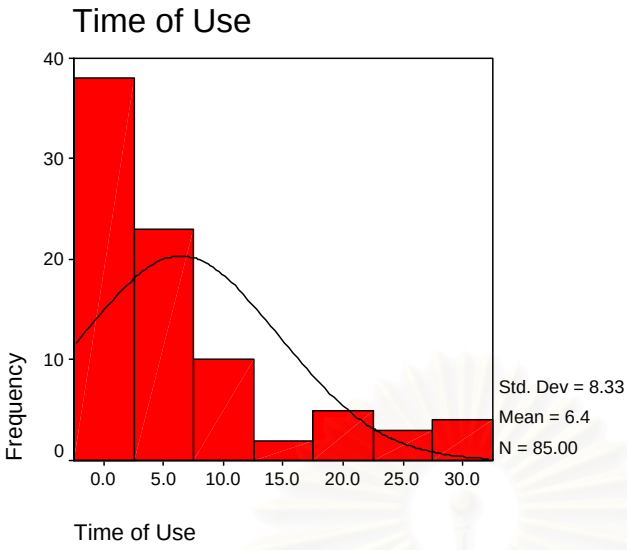
Statistics

Time of Use

N	Valid	85
	Missing	27
Mean		6.41
Median		3.00
Range		31
Minimum		0
Maximum		31
Sum		545

Time of Use

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	0	23	20.5	27.1	27.1
	1	7	6.3	8.2	35.3
	2	8	7.1	9.4	44.7
	3	8	7.1	9.4	54.1
	4	3	2.7	3.5	57.6
	5	7	6.3	8.2	65.9
	6	5	4.5	5.9	71.8
	8	1	.9	1.2	72.9
	9	2	1.8	2.4	75.3
	10	7	6.3	8.2	83.5
	15	1	.9	1.2	84.7
	17	1	.9	1.2	85.9
	19	1	.9	1.2	87.1
	20	4	3.6	4.7	91.8
	25	2	1.8	2.4	94.1
	26	1	.9	1.2	95.3
	28	1	.9	1.2	96.5
	29	1	.9	1.2	97.6
	30	1	.9	1.2	98.8
	31	1	.9	1.2	100.0
	Total	85	75.9	100.0	
Missing	191	27	24.1		
Total		112	100.0		



Frequencies

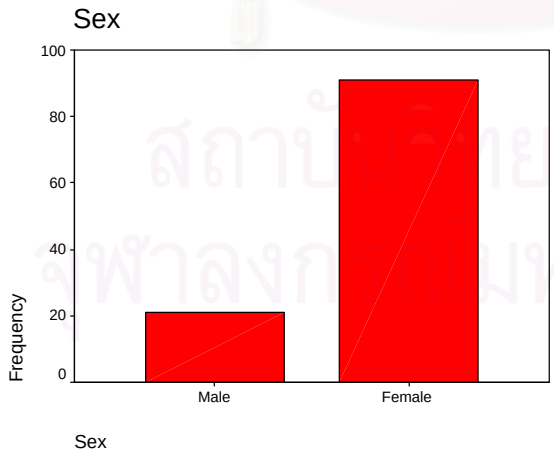
Statistics

Sex

N	Valid	112
	Missing	0

Sex

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Male	21	18.8	18.8	18.8
	Female	91	81.3	81.3	100.0
	Total	112	100.0	100.0	



Frequencies

Statistics

Occupation

N	Valid	112
	Missing	0

Occupation

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Atopic healthcare worker	71	63.4	63.4	63.4
	General atopic patients	41	36.6	36.6	100.0
	Total	112	100.0	100.0	

Crosstabs

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Occupation * Histamine	112	100.0%	0	.0%	112	100.0%

Occupation * Histamine Crosstabulation

Count

		Histamine		
		positive	negative	Total
Occupation	Atopic healthcare worker	10	61	71
	General atopic patients	2	39	41
Total		12	100	112

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	2.303[b]	1	.129		
Continuity Correction[a]	1.441	1	.230		
Likelihood Ratio	2.567	1	.109		
Fisher's Exact Test				.205	.112
Linear-by-Linear Association	2.282	1	.131		
N of Valid Cases	112				

a. Computed only for a 2x2 table

b. 1 cells (25.0%) have expected count less than 5. The minimum expected count is 4.39.

Symmetric Measures

		Value	Asymp. Std. Error[a]	Approx. T[b]	Approx. Sig.
Interval by Interval	Pearson's R	.143	.077	1.520	.131[c]
Ordinal by Ordinal	Spearman Correlation	.143	.077	1.520	.131[c]
N of Valid Cases		112			

a. Not assuming the null hypothesis.

b. Using the asymptotic standard error assuming the null hypothesis.

c. Based on normal approximation.

Correlations

Descriptive Statistics

	Mean	Std. Deviation	N
Occupation	1.37	.48	112
Histamine	1.89	.31	112

Correlations

		Occupation	Histamine
Occupation	Pearson Correlation	1.000	.143
	Sig. (2-tailed)	.	.131
	N	112	112
Histamine	Pearson Correlation	.143	1.000
	Sig. (2-tailed)	.131	.
	N	112	112

Crosstabs

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Occupation * Latex serum protein	112	100.0%	0	.0%	112	100.0%

Occupation * Latex serum protein Crosstabulation

Count

		Latex serum protein		
		positive	negative	Total
Occupation	Atopic healthcare worker	33	38	71
	General atopic patients	17	24	41
Total		50	62	112

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.265[b]	1	.607		
Continuity Correction[a]	.101	1	.751		
Likelihood Ratio	.265	1	.607		
Fisher's Exact Test				.694	.376
Linear-by-Linear Association	.262	1	.609		
N of Valid Cases	112				

a. Computed only for a 2x2 table

b. 0 cells (.0%) have expected count less than 5. The minimum expected count is 18.30.

Symmetric Measures

		Value	Asymp. Std. Error[a]	Approx. T[b]	Approx. Sig.
Interval by Interval	Pearson's R	.049	.094	.510	.611[c]
Ordinal by Ordinal	Spearman Correlation	.049	.094	.510	.611[c]
N of Valid Cases		112			

a. Not assuming the null hypothesis.

b. Using the asymptotic standard error assuming the null hypothesis.

c. Based on normal approximation.

Correlations

Descriptive Statistics

	Mean	Std. Deviation	N
Occupation	1.37	.48	112
Latex serum protein	1.55	.50	112

Correlations

		Occupation	Latex serum protein
Occupation	Pearson Correlation	1.000	.049
	Sig. (2-tailed)	.	.611
	N	112	112
Latex serum protein	Pearson Correlation	.049	1.000
	Sig. (2-tailed)	.611	.
	N	112	112

Crosstabs

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Occupation * Stallergene	112	100.0%	0	.0%	112	100.0%

Occupation * Stallergene Crosstabulation

Count

		Stallergene		
		positive	negative	Total
Occupation	Atopic healthcare worker	7	64	71
	General atopic patients	2	39	41
Total		9	103	112

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.873[b]	1	.350		
Continuity Correction[a]	.329	1	.566		
Likelihood Ratio	.936	1	.333		
Fisher's Exact Test				.482	.291
Linear-by-Linear Association	.865	1	.352		
N of Valid Cases	112				

a. Computed only for a 2x2 table

b. 1 cells (25.0%) have expected count less than 5. The minimum expected count is 3.29.

Symmetric Measures

		Value	Asymp. Std. Error[a]	Approx. T[b]	Approx. Sig.
Interval by Interval	Pearson's R	.088	.084	.929	.355[c]
Ordinal by Ordinal	Spearman Correlation	.088	.084	.929	.355[c]
N of Valid Cases		112			

a. Not assuming the null hypothesis.

b. Using the asymptotic standard error assuming the null hypothesis.

c. Based on normal approximation.

Correlations

Descriptive Statistics

	Mean	Std. Deviation	N
Occupation	1.37	.48	112
Stallergene	1.92	.27	112

Correlations

		Occupation	Stallergene
Occupation	Pearson Correlation	1.000	.088
	Sig. (2-tailed)	.	.355
	N	112	112
Stallergene	Pearson Correlation	.088	1.000
	Sig. (2-tailed)	.355	.
	N	112	112

Crosstabs

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Allergy History * Histamine	112	100.0%	0	.0%	112	100.0%

Allergy History * Histamine Crosstabulation

Count

		Histamine		
		positive	negative	Total
Allergy History	positive	7	70	77
	negative	5	30	35
Total		12	100	112

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.679[b]	1	.410		
Continuity Correction[a]	.244	1	.621		
Likelihood Ratio	.650	1	.420		
Fisher's Exact Test				.512	.302
Linear-by-Linear Association	.673	1	.412		
N of Valid Cases	112				

a. Computed only for a 2x2 table

b. 1 cells (25.0%) have expected count less than 5. The minimum expected count is 3.75.

Symmetric Measures

		Value	Asymp. Std. Error[a]	Approx. T[b]	Approx. Sig.
Interval by Interval	Pearson's R	-.078	.100	-.819	.415[c]
Ordinal by Ordinal	Spearman Correlation	-.078	.100	-.819	.415[c]
N of Valid Cases		112			

a. Not assuming the null hypothesis.

b. Using the asymptotic standard error assuming the null hypothesis.

c. Based on normal approximation.

Correlations

Descriptive Statistics

	Mean	Std. Deviation	N
Allergy History	1.31	.47	112
Histamine	1.89	.31	112

Correlations

		Allergy History	Histamine
Allergy History	Pearson Correlation	1.000	-.078
	Sig. (2-tailed)	.	.415
	N	112	112
Histamine	Pearson Correlation	-.078	1.000
	Sig. (2-tailed)	.415	.
	N	112	112

Crosstabs

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Allergy History * Latex serum protein	112	100.0%	0	.0%	112	100.0%

Allergy History * Latex serum protein Crosstabulation

Count

		Latex serum protein		
		positive	negative	Total
Allergy History	positive	39	38	77
	negative	11	24	35
Total		50	62	112

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	3.597[b]	1	.058		
Continuity Correction[a]	2.861	1	.091		
Likelihood Ratio	3.671	1	.055		
Fisher's Exact Test				.067	.044
Linear-by-Linear Association	3.565	1	.059		
N of Valid Cases	112				

a. Computed only for a 2x2 table

b. 0 cells (.0%) have expected count less than 5. The minimum expected count is 15.63.

Symmetric Measures

		Value	Asymp. Std. Error[a]	Approx. T[b]	Approx. Sig.
Interval by Interval	Pearson's R	.179	.091	1.911	.059[c]
Ordinal by Ordinal	Spearman Correlation	.179	.091	1.911	.059[c]
N of Valid Cases		112			

a. Not assuming the null hypothesis.

b. Using the asymptotic standard error assuming the null hypothesis.

c. Based on normal approximation.

Correlations

Descriptive Statistics

	Mean	Std. Deviation	N
Allergy History	1.31	.47	112
Latex serum protein	1.55	.50	112

Correlations

		Allergy History	Latex serum protein
Allergy History	Pearson Correlation	1.000	.179
	Sig. (2-tailed)	.	.059
	N	112	112
Latex serum protein	Pearson Correlation	.179	1.000
	Sig. (2-tailed)	.059	.
	N	112	112

Crosstabs

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Allergy History * Stallergene	112	100.0%	0	.0%	112	100.0%

Allergy History * Stallergene Crosstabulation

Count

		Stallergene		
		positive	negative	Total
Allergy History	positive	6	71	77
	negative	3	32	35
Total		9	103	112

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.020[b]	1	.888		
Continuity Correction[a]	.000	1	1.000		
Likelihood Ratio	.020	1	.889		
Fisher's Exact Test				1.000	.576
Linear-by-Linear Association	.020	1	.889		
N of Valid Cases	112				

a. Computed only for a 2x2 table

b. 1 cells (25.0%) have expected count less than 5. The minimum expected count is 2.81.

Symmetric Measures

		Value	Asymp. Std. Error[a]	Approx. T[b]	Approx. Sig.
Interval by Interval	Pearson's R	-.013	.096	-.139	.889[c]
Ordinal by Ordinal	Spearman Correlation	-.013	.096	-.139	.889[c]
N of Valid Cases		112			

a. Not assuming the null hypothesis.

b. Using the asymptotic standard error assuming the null hypothesis.

c. Based on normal approximation.

Correlations

Descriptive Statistics

	Mean	Std. Deviation	N
Allergy History	1.31	.47	112
Stallergene	1.92	.27	112

Correlations

		Allergy History	Stallergene
Allergy History	Pearson Correlation	1.000	-.013
	Sig. (2-tailed)	.	.889
	N	112	112
Stallergene	Pearson Correlation	-.013	1.000
	Sig. (2-tailed)	.889	.
	N	112	112

Crosstabs

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Allergy in Family * Histamine	112	100.0%	0	.0%	112	100.0%

Allergy in Family * Histamine Crosstabulation

Count

		Histamine		
		positive	negative	Total
Allergy in Family	positive	4	33	37
	negative	8	67	75
Total		12	100	112

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.001[b]	1	.981		
Continuity Correction[a]	.000	1	1.000		
Likelihood Ratio	.001	1	.982		
Fisher's Exact Test				1.000	.607
Linear-by-Linear Association	.001	1	.982		
N of Valid Cases	112				

a. Computed only for a 2x2 table

b. 1 cells (25.0%) have expected count less than 5. The minimum expected count is 3.96.

Symmetric Measures

		Value	Asymp. Std. Error[a]	Approx. T[b]	Approx. Sig.
Interval by Interval	Pearson's R	.002	.095	.023	.982[c]
Ordinal by Ordinal	Spearman Correlation	.002	.095	.023	.982[c]
N of Valid Cases		112			

a. Not assuming the null hypothesis.

b. Using the asymptotic standard error assuming the null hypothesis.

c. Based on normal approximation.

Correlations

Descriptive Statistics

	Mean	Std. Deviation	N
Allergy in Family	1.67	.47	112
Histamine	1.89	.31	112

Correlations

		Allergy in Family	Histamine
Allergy in Family	Pearson Correlation	1.000	.002
	Sig. (2-tailed)	.	.982
	N	112	112
Histamine	Pearson Correlation	.002	1.000
	Sig. (2-tailed)	.982	.
	N	112	112

Crosstabs

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Allergy in Family * Latex serum protein	112	100.0%	0	.0%	112	100.0%

Allergy in Family * Latex serum protein Crosstabulation

Count

		Latex serum protein		Total
		positive	negative	
Allergy in Family	positive	17	20	37
	negative	33	42	75
Total		50	62	112

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.038[b]	1	.846		
Continuity Correction[a]	.000	1	1.000		
Likelihood Ratio	.038	1	.846		
Fisher's Exact Test				1.000	.502
Linear-by-Linear Association	.038	1	.846		
N of Valid Cases	112				

a. Computed only for a 2x2 table

b. 0 cells (.0%) have expected count less than 5. The minimum expected count is 16.52.

Symmetric Measures

		Value	Asymp. Std. Error[a]	Approx. T[b]	Approx. Sig.
Interval by Interval	Pearson's R	.018	.095	.193	.847[c]
Ordinal by Ordinal	Spearman Correlation	.018	.095	.193	.847[c]
N of Valid Cases		112			

a. Not assuming the null hypothesis.

b. Using the asymptotic standard error assuming the null hypothesis.

c. Based on normal approximation.

Correlations

Descriptive Statistics

	Mean	Std. Deviation	N
Allergy in Family	1.67	.47	112
Latex serum protein	1.55	.50	112

Correlations

		Allergy in Family	Latex serum protein
Allergy in Family	Pearson Correlation	1.000	.018
	Sig. (2-tailed)	.	.847
	N	112	112
Latex serum protein	Pearson Correlation	.018	1.000
	Sig. (2-tailed)	.847	.
	N	112	112

Crosstabs

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Allergy in Family * Stallergene	112	100.0%	0	.0%	112	100.0%

Allergy in Family * Stallergene Crosstabulation

Count

		Stallergene		
		positive	negative	Total
Allergy in Family	positive	4	33	37
	negative	5	70	75
Total		9	103	112

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.576[b]	1	.448		
Continuity Correction[a]	.152	1	.697		
Likelihood Ratio	.552	1	.458		
Fisher's Exact Test				.475	.338
Linear-by-Linear Association	.571	1	.450		
N of Valid Cases	112				

a. Computed only for a 2x2 table

b. 1 cells (25.0%) have expected count less than 5. The minimum expected count is 2.97.

Symmetric Measures

		Value	Asymp. Std. Error[a]	Approx. T[b]	Approx. Sig.
Interval by Interval	Pearson's R	.072	.100	.754	.452[c]
Ordinal by Ordinal	Spearman Correlation	.072	.100	.754	.452[c]
N of Valid Cases		112			

a. Not assuming the null hypothesis.

b. Using the asymptotic standard error assuming the null hypothesis.

c. Based on normal approximation.

Correlations

Descriptive Statistics

	Mean	Std. Deviation	N
Allergy in Family	1.67	.47	112
Stallergene	1.92	.27	112

Allergy in	Pearson	Allergy	
	Sig. (2-	Famil .	Stallergen .452
	N	112	112
Stallergen	Pearson	.072	1.000
	Sig. (2-	.452	.
	N	112	112

BIOGRAPHY

Mr. Pawin Ngamlert was born on March 19, 1978. He graduated with the degree of Bachelor of Science in Chemistry from Srinakharinwirot University in 1999. He continued his study in the Master Program of Biochemistry, Faculty of Science at Chulalongkorn University