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Latex Allergy and Recent Developments in Deproteinisation of Natural Rubber Latex

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Natural rubber latex is used in a large number of useful products like gloves and condoms. Due to the threat of AIDS, hepatitis and other infectious diseases, the worldwide use of materials like gloves and condoms have increased. Allergy produced by latex proteins present in these materials has been reported. The issue of latex protein allergy is reviewed, giving emphasis to the types of allergy and ways to measure the amount of protein present in natural rubber latex. Proteins can be removed effectively from latex products, either in the latex stage itself or from the products. Emphasis has been given to the different ways in which proteins present in latex products can be reduced.

Key words: latex allergy; deproteinisation; NR latex: gloves; condoms: latex proteins: latex products

Natural Rubber Latex (NRL) is a white or slightly yellowish opaque liquid with a specific gravity in the range of 0.96 – 0.98 and having variable viscosity. Latex as such is a complex biochemical system. Obviously, chemical changes occur shortly after the latex leaves the tree. Latex coagulates within a few hours, which is, termed spontaneous coagulation. The time required for coagulation depends upon the ambient temperature and upon the colloidal stability of latex. In a later stage, another process called putrefaction occurs with the development of bad odour. The composition of natural rubber latex is given in *Table 1*.

Rubber particles in the latex are strongly protected in suspension by a film of absorbed protein, natural lipid and phospholipid. The solids in freshly tapped latex are distributed into three phases, *i.e.* rubber, aqueous, and lutoid. The rubber phase typically contains 96% of rubber hydrocarbon, 1% proteins and 2% lipids. The presumed structure of a natural rubber particle is given in *Figure 1*. There are also trace amounts of cations, mainly Mg^{2+}, K^{+} and Ca^{2+}. The lipids associated with the rubber particles comprise sterols, sterol esters, fats, waxes and phospholipids. The phospholipids are adsorbed on the surface of the rubber particles. The three phases of fresh NR latex separated by ultracentrifugation are shown in *Figure 2*. The aqueous phase, which is sometimes referred to as C-serum, is a dilute aqueous solution with a density slightly over 1.0 g/cm^3. The serum contains water and various non-rubber solids. Nitrogenous solids include 23.4% ammonium salts and 23.6%

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TABLE 1. COMPOSITION OF NATURAL RUBBER LATEX

Component	Amount (%)
Rubber	30–40
Protein	1–1.5
Resin	1–2.5
Sugar	1
Ash	Less than 1
Water	55–60

proteins and amino-acids. The sugars include quebrachitol (22.7%), glucose (4.2%), fructose (2%) and galactose (1%). The bottom fraction normally comprised of lutoid particles.

Materials, which provide antioxidative action to rubber in latex, have been identified. The most active substances are the lecithins, amino-acids and tocopherols. Certain particles called Frey-Wyssling particles are present in latex. They have intense yellow colouration due to the presence of carotenoid pigments. The slight yellow colour of latex is due to these particles.

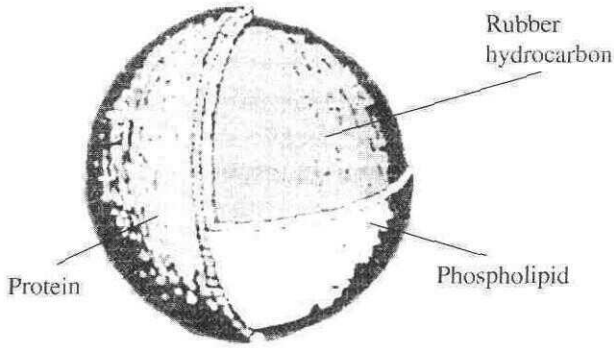
Natural Rubber Latex is used in a wide variety of products that come in contact with human body. In health care field, latex products include gloves, catheters, condoms and hundreds of different medical devices. NRL has wide applications owing to its colloidal stability, low Permeation to body fluids *etc*. In this review Allergy problems due to the presence of proteins in latex are discussed, giving special attention to the methods of eliminating these problems.

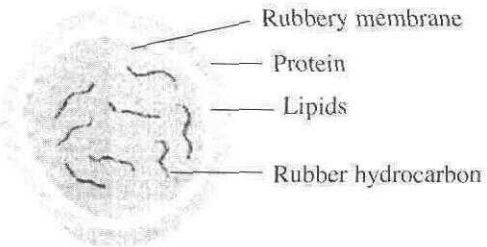
PROTEIN ALLERGY AND LATEX PRODUCTS

It has been established for several decades that contact with articles manufactured from natural rubber may produce dermatitic conditions in certain individuals. These skin allergies are caused not by rubber itself, but additives such as accelerators and antioxidants.

However, in the last decade there have been increasing reports of another kind of allergy to dipped rubber products (latex products). Proteins native to *Hevea* latex, and not chemicals added and during manufacture cause this type of allergy. There is no doubt that this is an important new allergy, especially among those more exposed to latex goods (*e.g.* health-care workers) than the general population. This discussion includes primarily the nature of the allergy, its history and the evidence implicating latex proteins as the allergens. Secondly, the discussion aims at the factors known to control the allergen/protein content of dipped goods and the measures which can be taken to reduce or eliminate these substances.

The clinical condition of latex allergy is relatively light in most cases but it sometimes develops to general urticaria and anaphylaxis. Scientific investigations on the substances responsible for latex allergy are in progress in many countries. It is now known that proteins in the raw natural rubber and their derivatives that survived to the final product are responsible for the latex allergy[1]. The protein content of natural rubber varies with different lots and





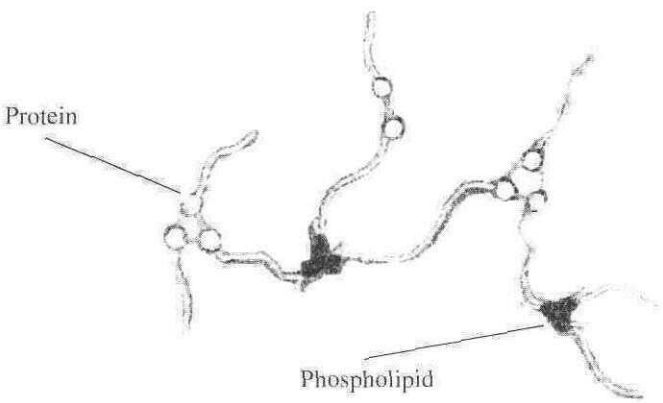


Figure 1. Presumed structure of natural rubber particle.

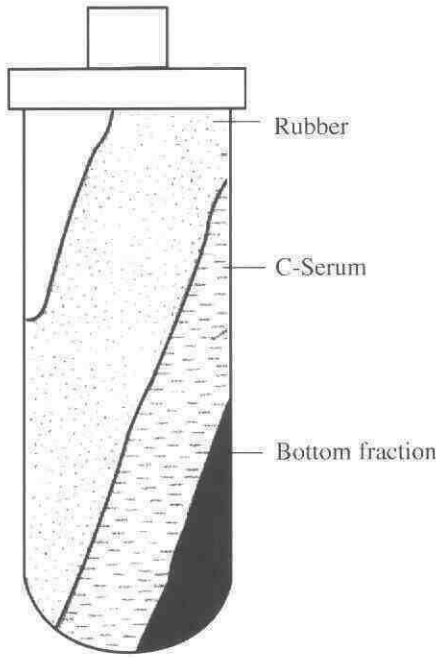


Figure 2. The principal ultracentrifuged fractions of fresh latex.

production methods[1]. In most researchers' experience, latex proteins is readily elutable from latex gloves, condoms and catheters under mild, physiologic conditions such as a neutral saline buffer at 37°C for 1 h–2 h. Analytical studies have shown that the protein content of latex is about 1% by weight of latex, part of which is on the surface of the rubber while the reminder is distributed between the aqueous serum phase of the latex and in the lutoids[2,3].

Hevea latex allergens include soluble and insoluble proteins[4]. It should be noted that while the total amount of proteins present in natural rubber latex is relatively constant, the amount of proteins present in natural rubber latex products is highly variable. A high content of extractable protein in latex gloves is generally associated with positive allergic responses in latex hypersensitive persons[5]. Low extractable protein content on the other hand tend to show weak or in some cases no positive response[5]. So far, no threshold level has been established[4]. One of the notable features of latex allergy is the cross reactivity of latex allergic patients. Yagami *et al.* [6–14] have hypothesized that plant pathogenesis or defense related proteins are the latex allergens and the cross reactive antigens. Through the verification of this hypothesis they were able to identify several latex antigens recognised by IgE antibodies in latex allergic patients. They are included in the official registration of latex allergens, given in *Table 2*.

Classification of Latex Allergy

Latex allergy involves more than one rubber antigen; at least six distinct allergens have been

found to bind to the antibody (IgE), which is involved in allergic reactions. The clinical symptoms associated with reactions to latex may be divided into three categories.

Contact urticaria (Type I). Type I allergies are sometimes referred to as 'immediate allergies' because the response on exposure to the allergen is fast. Individuals with Type I allergy to latex goods are in a state of hypersensitivity brought about by repeated contact with latex proteins and have antibodies to these proteins in their blood. At some point, further exposure to these protein antigens (allergens) can cause immediate response such as contact urticaria or anaphylactic shock. More detailed information, especially in the clinical aspects of the problem, can be found in the excellent reviews of Levy[15] and Tomazic and others[16].

Contact urticaria (also known as hives), is an eczematous reaction characterised by the formation of weals or flares at the contact site, with itching or stinging, the effect usually disappearing within 0.5 h–2 h. In the case of anaphylactic shock, the state of hypersensitivity is known as anaphylaxis (Greek: ana–against; phylaxis–protection). The shock reaction is characterised by a severe fall in blood pressure, difficulty in breathing, speeding heart rate and unconsciousness. Some other effects seen are conjunctivitis, rhinitis, and local or general urticaria. These effects are directly due to the release of histamine and other substances from cells of the immune systems affected by the interaction of the allergens and their antibodies. A more detailed account can be found in Bier and co-workers[17].

Systemic reactions include non-local reactions such as generalised urticaria or itching, rhino-conjunctivitis, asthma as well as multiple presentations of anaphylaxis (*e.g.* hypertension, shock, respiratory failure *etc.*). From the immunologist's point of view, Type IV and SR are generally considered to be the result of activation of two different arms of the immune system and therefore are quite distinct[18].

Nutter[19] obtained the first piece of evidence that latex itself could cause contact urticaria in 1979. Subsequent work implicated latex proteins and the immune system in the urticaria[15,16,20,21]. The first report of anaphylactic shock from latex products (surgical gloves) came from Finland[22] in 1984. Contact urticaria and anaphylactic reaction following exposure to a wide range of dipped goods have been described in many papers[15,16].

In 1990 a few fatalities were reported in the US following anaphylactic shock during examinations involving barium enemas. It was established that the latex proteins in the devices used for enema administration[23,24] caused deaths. These events raised awareness of the potential severity of immediate type allergic reactions to latex goods, and led to the involvement of the Food and Drug Administration (FDA).

Another kind of systemic reaction, which has been seen is an asthmatic response to the crosslinked cornstarch used to lubricate gloves. The latex proteins can be adsorbed on the starch particles and become airborne[25–27]. There is evidence that asthma-like symptoms can be caused by specific latex proteins on airborne glove powder[27]. It is possible that some cases of asthma and dermatitis ascribed to cornstarch may actually have been induced by latex proteins on the starch[16]. Talc has also been found to adsorb latex protein[28].

It is apparent that exposure to latex dipped goods can have severe consequences in

certain sensitised individuals However it is important to keep a sense of perspective The effects described important though they are in the case of sensitised individuals, are rare when viewed against the enormous usage of latex products throughout the world It must also be borne in mind that rubber is an excellent barrier against hepatitis, HIV[29] and other viruses (despite claims to the contrary[30]) and the spread of AIDS Seen in this light, it can be justifiably claimed that the Type I allergy is a relatively minor problem

Classical dermatitis (Type IV) This includes rash, usually of eczematous or keratonic form, confined to skin This type of allergy is also called contact eczema This allergy is caused by direct contact with rubber articles and irritation appears 48 h after the exposure This type of allergy is mainly due to the chemical additives in rubber such as accelerators and antioxidants *etc*

Prevalence of Allergy to Latex Products[15,16]

Since the importance of the latex allergy problem has only been recognised recently, there has been little epidemiological research on this subject The first studies were in Europe in 1987 and 1988[20,1] In addition to prolonged exposure to dipped articles genetic predisposition is a determining factor in the development of the allergy Atopic individuals (those with a constitutional tendency to develop allergies) who have an elevated level of Immunoglobulin E antibodies (IgE) and who have acquired other allergies are at much higher risk of developing Type I allergy to dipped goods

Identification of Latex Protein Allergens

The Western Blot Analysis qualitatively assesses the molecular weight distribution of antigenic protein in latex extracts by separating the proteins on a SDS-PAGE (sodium dodecylsulphate polyacrylamide gel electrophoresis) transferring them to nitrocellulose and then reacting the blot using anti-latex antibody By using methods which fractionate proteins according to molecular weight [SDS-PAGE, GPC (gel-permeation chromatography)] at least ten allergenic proteins have been identified (though this depends how they are measured) by various groups working on fresh ammoniated latex concentrate and extracts of gloves and condoms This is only a small fraction of the number of proteins in latex, as it exists in the tree Allergens were found with molecular weight ranging from 2 kD–100 kD and in this group, protein of molecular weight 14 kD and 21 kD have been suggested as major antigens for allergy to latex[32,33] It is not yet clear whether some of these allergens are sub-units of higher molecular weight proteins The major identified proteins related to latex[34–54] are given in *Table 2*

At least four potential reasons, which may interact can be advanced for the present problem They are

- Greater use of gloves/condoms as a result of AIDS, hepatitis, *etc*
- Changes in manufacturing procedures as a consequence of greater rate of output (less-thorough leaching and other changes)
- Clonal/seasonal/environmental differences in protein composition of latex and
- Failure to diagnose correctly in earlier cases

Greater use of gloves and condoms, both in terms of frequency and contact time, could cause a greater proportion of individuals to become sensitised The fact that modern dipping lines run faster, to meet increasing demand could have led, through insufficient leaching, to a general increase in protein levels

TABLE 2. OFFICIAL REGISTRATION OF IDENTIFIED PROTEIN ALLERGENS

Name	Trivial name	Predicted physiological roles
Hev b 1	Rubber elongation factor	Rubber biosynthesis
Hev b 2	Beta-1,3-glucanases	Defense-related protein
Hev b 3	Small rubber particle protein	Rubber biosynthesis
Hev b 4	Microhelix component	Defense related protein
Hev b 5	Acidic latex protein	?
Hev b 6.01	Hevein preprotein	
Hev b 6.02	Hevein	Defense related protein
Hev b 6.03	Prohevein C-terminal domain	(Latex coagulation)
Hev b 7.01	Patatin-like proteins	Latex protein allergen
Hev b 7.02		(Inhibitor of rubber biosynthesis)
Hev b 8	Latex profilin	Structural protein
Hev b 9	Latex enolase	?
Hev b 10	Mn-superoxide dismutase	?
Hev b 11w	Class I endochitinase	Defense related protein
Hev b 12	Lipid transfer protein	Defense related protein

of particular proteins in the finished articles. There appears to be no published experimental evidence on this point. Possibly other changes in production methods may continue.

Though clonal differences in latex proteins do exist, there is no reason to pick this as the explanation. Many clones have been used for a long time and clonal lattices are pooled in the routine production of concentrate. Nevertheless, there is a possibility of clonal input and, given the time and money, it would obviously be possible to pin this down. It has been claimed elsewhere, that there has been no work bearing on seasonal/environmental differences in the protein composition of latex, though marked seasonal changes in other latex components definitely take place[55]. The reason for the failure to diagnose earlier clearly does not help understanding the cause of the problem.

EXTRACTABLE PROTEIN CONTENT

The fraction of the total protein in latex goods which is readily extracted in aqueous media under suitably defined conditions and which contains the allergens, is often referred to as 'extractable protein' (EP). There is now a considerable knowledge of this and the salient features are discussed below.

EP which appears rapidly in the liquid medium when a dipped article is extracted at room temperature or 35°C in water, physiological saline, or saline buffered to meet natural pH with phosphate. This initial phase is followed by a much slower release (*Figure 3*)[56,57]. This indicates that there is a soluble protein fraction. This is a somewhat curious fact, considering those dipped articles are subject to drying conditions

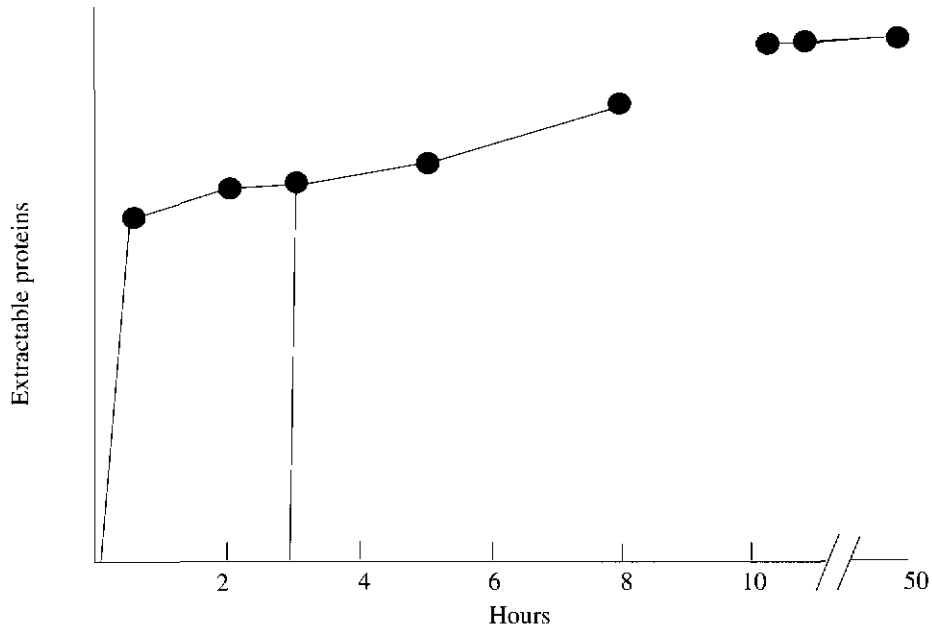


Figure 3. Curve showing extractable protein content with respect to time (Reprinted from Yeang and Faridah[62]).

(temperature/time) under which many proteins readily precipitate. It has been found that the proteins in the sera of HA Latex, and prevulcanised latex (MR Revultex®) show no sign of precipitation even after heating for 0.5 h at 100°C. At the same time, proteins in the serum (about neutral pH) from fresh latex coagulate within a couple of minutes at this temperature. It has been shown that the non-precipitation is partly attributable to the relatively high pH and/or presence of ammonia in the concentrate[58].

The protein in a dipped film or article comprises a major fraction. which is already tightly bound to the rubber particles as they exist in the concentrate and another arising from the aqueous serum (very roughly 60% and 40% respectively). It has been estimated that even in poorly leached articles, EP is about 4% of the total protein at most (*Table 3*)[56]; *i.e* a great deal of the serum-derived protein remains within the film and therefore has the potential to migrate during storage.

The EP is at, or near, the surface of the article. The ready elution of the protein described earlier, itself is evidence of this and the results of other experiments lead to the same conclusion[56]. Presumably, the EP is squeezed out of the latex gel as the rubber particles coalesce during drying/ vulcanisation.

It has been reported that the amount of EP can vary greatly between different types of dipped articles, brands of the same article, and within a particular brand[16,28,59,60]. These variations cannot be accounted for, by differences in the levels of the soluble proteins in latex concentrates, because those differences are much smaller[28]; they arise from variations in

TABLE 3 COMPARISON OF TOTAL NITROGEN AND EXTRACTABLE PROTEIN LEVELS [56]

Sample [a]	Total nitrogen (%)[c]	Calculated protein (mg/g) [b]	Extractable protein (mg/kg)[c]
Gloves A	0.38 (12, 15.3%)	24	685 (6, 7 5%)
Gloves B	0.40 (5, 7.5%)	25	230 (3, 6.0%)
Gloves C	0.22 (5, 6.3%)	14	70 (3, 5.1%)
Low non-protein nitrogen film	0.19 (4, 0.65%)	12	Not determined

[a] Gloves A and B are different brands made from standard latex concentrate, Gloves C. from double centrifuged concentrate

[b] Assuming all nitrogen derives from protein = N x 6.25

[c] Figures in brackets are the number of determinations and the coefficients of variation

manufacturing procedure[61]. The number of proteins and the range of molecular weights can also differ considerably from brand to brand[59,61].

It has been found that the amount of EP on the inner surface of gloves is much greater than on the outside[62] (*Table 4*). Asymmetric distribution of EP was first described by Dalrymple and Audley[56] but their findings were subsequently shown to be partly in error. A result with one brand of gloves, which apparently showed EP higher on the outside, was later found to be ascribable to material (s), which interfered with the Bradford assay for protein[63]. Another pair of gloves examined by these workers did have the usual distribution[56]. It is likely that the asymmetry in the location of EP results from transport of protein (and other solutes) away from the dipping former, as water preferentially evaporates from the surface in contact with air. This will usually become the inner surface, since in most cases the gloves are inverted on stripping. The distribution of EP in gloves which have not been turned inside out on removal from the former, does not appear to have been studied.

The amount of proteins extractable from dipped films (and presumably dipped products) depends on the drying temperature; films dried at lower temperatures have low EP[64] (*Figure 4*).

As with other solutes[65], proteins are more readily extracted from pre-vulcanised films than post-vulcanised ones. This is probably because the rubber particles in the latter are more fused to form a relatively less permeable sheet (*Table 5*)[57]. The limiting case of this would be a cast film from unvulcanised concentrate.

Storage studies on dipped films (one year in the dark at room temperature). exhibit no changes of EP in two cases, four cases of slight increase and considerable increase in one (post-vulcanised)[28]. The increases are presumably a result of migration from within the films, and/or changes in film structure

TABLE 4 EXTRACTABLE PROTEIN (EP) ON INNER AND OUTER SURFACES (PALM AREA) OF EXAMINATION GLOVE[62]

	Inner	Outer
No of brands of gloves	12	12
Mean EP (mg cm ^{2})	7 83	0 33
C V	54 5	51 5
Range EP (mg cm ^{2})	1 52–15 14	0 11–0 76

Mean inner/outer ratio is 26 7
(EP measured by Lowry method)

TABLE 5 EFFECT OF VULCANISATION SYSTEM ON EXTRACTABLE PROTEIN CONTENT OF DIPPED FILMS

System	Formulation	Storage time days[a]	Extractable protein (mg/kg)
Prevulcanised	4	1	190
	4	11	320
	5	3	380
	5	5	380
Post vulcanised	6	3	150
	7	1	175
	7	7	185
Radiation vulcanised	–	–	700[b]

[a] Time elapsed between preparing compound and dipping film
[b] Wet-gel leached 2 min at 60°C
(EP measured by Bradford method)

during storage (*Table 6*) With the exception of the one finding the results are encouraging, considering that latex goods are sometimes stored for considerable periods before being used

It has been well established that the EP content of latex products varies according to the processing conditions during manufacturing Currently, extensive studies are being carried out in different countries to identify the various allergenic proteins Unfortunately, much of such information is still lacking except that these allergenic proteins can be detected in their different molecular weight fractions or bands depending on experimental techniques

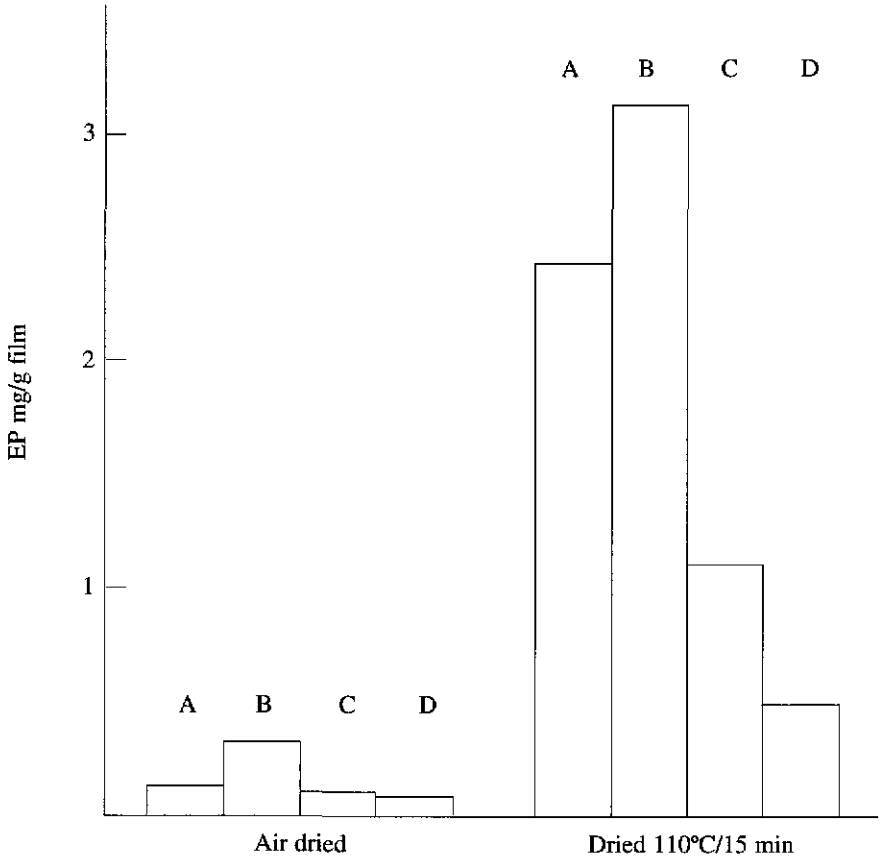


Figure 4. Effect of drying temperature on extractable protein content of dipped films.
A, C–Compound matured at room temperature for 5 days; B, D–Prevulcanised for 2 h at 70°C, all the films are air-dried; A, B–Unleached films; C, D–Wet-gel leached, 5 min (Redrawn from Reference 64).

used[66]. Furthermore, it is not clear if the allergic response is related to the quantity of total EP, although some direct relationship has been implied in the case of latex films[67].

TESTS FOR PROTEINS IN LATEX

The different methods available to quantitate protein levels can be classified into chemical and immunological. Each of these methods are discussed below.

Chemical Methods

There are numerous laboratory methods available to study proteins and to quantitate protein levels in solutions. The most commonly used assays include Kjeldahl, Lowry, Bradford, Biuret, Ninhydrin, and Ultraviolet extinction methods. There are advantages and disadvantages to each of these methods. The chemical methods that have been used for latex proteins are summarised below.

TABLE 6. EXTRACTABLE PROTEIN LEVELS (EP) IN DIPPED FILMS BEFORE AND AFTER STORAGE FOR CA. 1 YEAR IN THE DARK AT ROOM TEMPERATURE

Film	EP (mg/kg film[a])	
	Original	*After storage (1 year)*
Prevulc. A (1)	25	40
Prevulc. A (2)	<30	35
Prevulc. B	35	35
Post-Vulc. A	Not measurable	Not measurable
Post-Vulc. B	35	100
MR Revultex (1)	<30	30
MR Revultex (2)	<30	50

[a] To nearest 5 mg/kg

Kjeldahl method. It is a widely used assay to measure total nitrogen levels. The method is accurate and reproducible, but is time consuming and more importantly not sensitive enough for low protein concentrations. The sample is digested by boiling with concentrated sulphuric acid in the presence of sodium sulphate and selenium catalyst. The digestion converts all the organic nitrogen to ammonia, which is trapped as ammonium sulphate. Ammonia released by the addition of excess sodium hydroxide is collected in boric acid and titrated with standard hydrochloric acid. Although this method is precise and reproducible for the determination of nitrogen, presence of nitrogen in contaminants like DNA make the estimation more complicated. In practice nitrogen content of proteins is generally assumed to be 16% by weight. This method is now not widely used.

Bradford method. Protein can be estimated by the method proposed by Bradford[68]. Read and Northcote[69], and Darymple and Audley[70] have suggested slight modifications. Coomassie Brilliant Blue G is used for this method and sample: dye volume is in the ratio 1:9. Absorbance at 595 mm is determined in plastic cuvettes 10 min after mixing. Linear calibration curves are run with each batch of assays using suitable diluted aqueous phase (serum) of high ammonia (HA) concentrate, prepared by centrifuging at 145 000 r.p.m. for a maximum for 1 h. Protein content is measured by nitrogen determination after dialyses and alkali treatment. The serum can be stored frozen and fresh dilution used for each calibration. Bradford method is similar to the so-called dye binding method.

Biuret method. This involves a strong alkaline copper reagent, which produces a purple colouration with protein. The principle reason why it is not widely used is due to its low sensitivity; several milligrams of sample must be sacrificed for a reliable measurement. The method gives an accurate measurement since there is little variation in colour yield from protein to protein. This is because the copper reagent reacts with the peptide chain rather than side groups. Ammonia interferes by complexing with copper, so ammonium sulphate fractions do not give accurate results. A greater sensitivity of the biuret reaction is achieved by observing the copper-protein

complex not at 540 nm, but at 310 nm in the near-ultraviolet. Unfortunately this method needs several blanks to subtract spurious absorption at this wavelength. So it has not been widely employed.

Lowry method. The most widely used protein assay is that of Lowry, Rosebrough, Farr, and Randall[71]. The reaction is dependent on pH, and is a combination of the copper reaction used in the biuret method and the reaction of the Folin-Ciocalteau reagent[72] with phenols such as tyrosine in proteins. This was found to give a strong dark blue colour with proteins. The Lowry test depends on a reaction that is not specific for latex proteins and requires a chemical reaction between apparent proteins and a chemical reagent. The original paper surveyed ranges of concentrations of reagents to determine the ideal values. Since then many modifications have been reported mainly to avoid interference by specific components. Unfortunately many of the compounds used in enzyme purification interfere with the reaction and different modifications are required to overcome each problem. Since it is a sensitive method giving a good colour with 0.1 mg/ml of protein or less, interfering compounds are often diluted out to levels where their effect is insignificant.

UV absorption. UV absorption has been employed for measuring protein ever since a method based on 260 nm and 280 nm absorption was developed, which would correct for nucleic acid and nucleotide content. This correction is very important with crude extracts, but becomes less relevant as protein purification progresses and interfering compounds as removed; a simple 280 nm reading is then sufficient. Proteins absorb UV at 280 nm solely because of tyrosine and tryptophan residues (unless they also contain UV-absorbing prosthetic groups). Since the content of these two amino-acids varies enormously, the extinction coefficient, usually expressed either as E 1% 280 or E 1 mg/mL 280, varies considerably. Most proteins fall in the range of (for 1 mg/mL) 0.4 –1.5, but extremes include some parvalbumins and related Ca^{2+} binding proteins (0.0) at one end and lysozyme (2.65) at the other end. Absorption at 280 nm gives only a rough idea of the actual protein content, except with pure proteins. If the extinction coefficient for the pure protein is accurately known, (this should be standardised against dry weight, or at least against 205 nm absorption—see below) then the reading at 280 nm provides an accurate measure of the protein content. Indeed, it is the most accurate method for a pure protein, since no manipulation other than appropriate dilution is required. Of course, the solvent must not absorb at 280 nm, if it does, an accurate blank must be taken. The method is non-destructive, so although a milligram or so is required for accurate determination. this can be returned to the bulk sample.

Absorption in the far-UV around the peptide band is used as a far more sensitive method, much less affected by the protein amino acid composition. On account of far-UV-light absorption by oxygen, it is not possible to measure at the peak of the peptide absorption at 192 nm using routine spectrophotometers. Measurements on the side of the band give sufficiently accurate results provided that the absorbance is kept down to no more than 0.5. The extinction coefficients for proteins (1 mg/mL solution) are approximately: 220 nm: 11; 215 nm: 15; 210 nm: 20; 206 nm: 29; 205 nm: 31; 200 nm: 45; peak at ~ 192 nm: 60. The value at 206 nm is included. This is the wavelength employed in far-UV monitors such as the LKB-Uvicord 3.

These values vary from protein to protein because aromatic and some other residues also add variable contributions in this range, and the

secondary structure (α helix, β sheet *etc*) has some influence on the shape and exact position of the peptide absorption peak By analysing possible variations, it can be shown that the most consistent value is obtained around 205 nm At this wavelength the extinction coefficients of proteins nearly all fall in the range 28 5–33 By making a correction for the tyrosine and tryptophan content (by determining the 280 nm absorption also) the extinction coefficient at 205 nm can be predicted with no more than 2% error The formula used is

$$E^{1}_{205}(\text{mg/mL}) = 27\ 0 + 120 \times A_{280}/A_{205}$$

Since 205 nm is close to the useful limits of routine spectrophotometers measurements at 205 nm require very clean cuvettes a relatively new deuterium lamp and buffers with minimal absorption Most salts absorb at this wavelength, about the only common anions that do not are sulphate and perchlorate One can use a weak phosphate buffer (5 mM, pH 7 0) in the presence of 50 mM sodium sulphate—this salt helps to avoid adsorption of protein to the cuvette The most convenient method is to zero the spectrophotometer with 3 mL of buffer, then mix in 1–10 μL of protein sample (containing between 2 and 50 mg/mL), and note the increase in absorption If the buffer composition of the protein sample is known, then the same amount of that buffer can be used to determine a blank Virtually all commonly used buffers absorb quite strongly at 205 nm, but because the dilution is of the order of a thousand-fold, the actual effect is usually very small For more dilute protein solutions, the buffer blank is relatively larger so use of the method may be impractical If less accurate estimates (± 10%) are sufficient then use of E^{1}_{205} (mg/mL)=31 is an adequate compromise For use of the 280/205 absorption method, the 280 nm determination must be done on a more concentrated sample and the appropriate factor used in the equation

Infra-red spectroscopy The amino groups Present in proteins can be well detected by infra red spectroscopy Studies have been reported[73] regarding the FTIR spectroscopic studies of NR latex The effectiveness of deproteinisation methods can be studied from peaks observed for N-H frequencies FTIR study reveales that non purified rubber shows weak transmittance bands at 1540 cm^{-1} and 3280 cm^{-1} which are characteristic vibrations of =N-C=O and =N-H, respectively[74] Upon successive surfactant washing or enzymatic deproteinisation followed by acid coagulation the intensities of these bands were significantly reduced[73] As the number of repeating units of peptide increases the relative intensity of the =N-H units of the peptide linkages which have a vibrating frequency at 3280 cm^{-1}, increases[75]

This brief summary of some of the more popular methods of protein measurement gives an idea of what is possible Each has its advantages and disadvantages The aim of enzyme purification is to get rid of as much protein as possible while retaining enzyme activity Relative advantages and disadvantages of some commonly employed methods of protein determination are given in *Table 7* The success of each step depends both on the retention of activity and the extent of improvement in specific activity (expressed in units per milligram protein)

Immunological Methods

RAST Assay Radio-immunosorbant assays (RAST) are sensitive techniques, which employ radioisotope labelled anti IgE to measure specific IgE antibody in patient sera The latex RAST is used to determine if specific IgE to the latex proteins is present and is semi-quantitative in determining the amount of IgE present The assay is used primarily as a diagnostic test for latex allergy

The RAST assay can also be performed as a competitive assay (RAST inhibition) to quantitate the amount of allergen in a latex extract In this assay soluble allergens in latex extracts compete for binding to latex specific IgE in pooled sera from latex allergic individuals When soluble allergens react with the IgE the antibody is prevented from binding to a solid phase latex allergen preparation The amount of inhibition reflects the level of soluble allergens in the extract The RAST inhibition assay is a very sensitive method to quantitate latex allergens It is, however, dependent upon the pool of individual sera as a source for latex specific IgE As described earlier, the individual IgE response to latex proteins is very heterogeneous and thus a large pool of individual sera is needed to ensure the inclusion of all of the relevant allergens In addition the assay requires special precaution because of the use of human sera and radioisotopes

LEAP assay The latex ELISA for allergenic proteins (LEAP) has proven to be a very useful tool for quantifying the level of antigenic protein in latex extracts[36] The ELISA assays are similar to RAST assays However, the source and quantity of the antisera can be controlled With increased awareness of protein allergy, procedures for testing extractable proteins have been developed by using the LEAP assay To measure antigenic protein levels in the extracts, various extraction parameters have to be evaluated The ELISA assay provides a sensitive method to measure not only total latex protein but also importantly immunologically reactive latex protein

The latex ELISA for antigenic protein (LEAP) is a technique in which latex proteins are immobilised by adsorption to plastic and reacted with rabbit anti-latex antisera (*Figure 5*) After washing, the plate is reacted with a second HRP-plated anti-rabbit IgG and finally a substrate is added which results in a colour change The amount of colour reaction produced is proportional to the amount of latex protein present The assay can easily detect latex proteins in extract solutions and is sensitive to 15 ng/mL This assay uses a preparation of latex proteins as an internal standard and compares the reactivity of the rabbit antisera with the latex protein standard to reactivity of the extracts The primary advantages of this assay are that it is very sensitive and it is specific for the latex proteins The enzyme-based assay does not utilise radioisotopes and is readily adaptable for routine use throughout the latex industry The range of the assay is between 15 2000 ng/mL, a level below the lowest levels available with the chemical methods Because of this sensitivity, the samples must be diluted, often 100-fold or more and interfering substances are diluted out

ELISA Inhibition *assay* The ELISA-Inhibition test for antigenic proteins measures latex antigens by using latex specific antibodies of the rabbit to recognise them ELISA inhibition is not the same as the LEAP test In the ELISA-Inhibition assay, a known amount of standardised latex protein is bound or absorbed to a solid support, (*e g* plastic wells of a microtiter plate) This approach reduces the variability and surfactant interference inherent in the LEAP assay because it relies on the binding of a standard preparation of NRL proteins to the plate and not an unknown glove extract Then a known solution of rabbit antiserum (latex specified IgG antibody) is added to the plate and mixed with one of a series of dilutions of the test glove extract Higher level of protein antigens in the test glove extract will inhibit the ability of the known quantity of IgG to bind with the known amount of standard latex protein on the plate Thus the use of the term ELISA-Inhibition The plate is

TABLE 7. ADVANTAGES AND DISADVANTAGES OF DIFFERENT METHODS EMPLOYED IN PROTEIN ESTIMATION

Method	Amount of protein needed (mg)	Destructive?	Variation of response with amino-acid composition	Comments
Biuret	0.5–5.0	Yes	Low	Caustic reagent; NH^{+4} interference; rapid colour
Lowry	0.05–0.5	Yes	Moderate	Slow colour development
Absorbance at 280 nm	0.05–2.0	No	Large	Interference by UV-absorbing materials; instantaneous
Absorbance at 205 nm	0.01–0.05	No	Low	Interference by UV-absorbing materials; instantaneous
Bradford method/dye binding	0.01–0.05	Yes	Moderate	Acid reagent; colour adsorbs to glassware; rapid colour formation

then washed to remove the unbound glove extract and IgG. The amount of IgG that did bind to the microtiter plate is measured with the second anti-IgG antibody that recognises it and produces a colour. The colour reaction product can then be detected and measured in a spectrophotometer: the lower the colour (greater inhibition of binding), the higher the level of standard latex protein in the test sample extract that was available to prevent IgG's binding to the standard latex protein coated plate. This method is sensitive and reproducible: nanogram quantities of latex antigens ($>1 \mu g/dm^2$) can be detected. Currently, the ELISA-Inhibition method is standardised as an *ASTM* standard test method.

The *ASTM D 6499-00* test method[76] covers an immunological method to determine the amount of antigenic protein in natural rubber and its products using rabbit antisera specific for NRL proteins. The immunoassay procedure quantitatively measures the level of antigenic latex proteins in solution using an inhibition format. Although this method detects antigenic proteins, it should not be considered as a measure of allergenic proteins. In this method, the latex device is extracted for 2 h in an aqueous buffer. The extract is recovered and the antigen levels are determined using ELISA inhibition technology[77].

Finger print assay. Czoppon *et al.*[78] have developed an assay to study the factors which

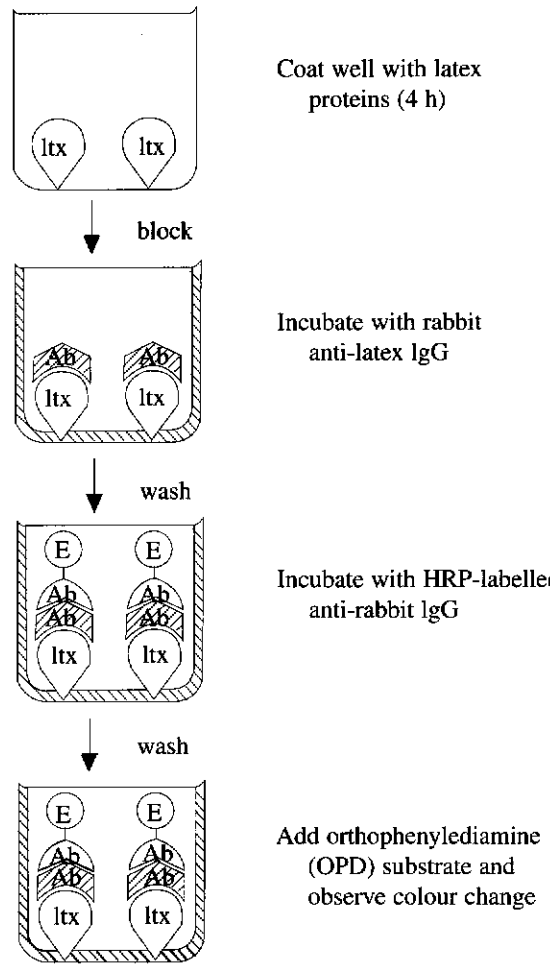


Figure 5. LEAP assay, indirect ELISA for latex protein concentration.

influence the direct transfer of protein allergens from latex gloves. This assay is a modification of a dot blot assay (*Figure 6*). This test is a sensitive method to specifically analyse the transfer of latex surface proteins. The assay is performed by touching a gloved finger to a nitrocellulose membrane. Proteins are transferred by contact of the latex to the membrane and then are identified by reactivity with the rabbit anti-latex antibody and a second enzyme-labeled antibody. The authors have used this assay to examine the parameters which influence the transfer of latex proteins to moist membranes. The data reveals that the proteins are transferred immediately upon contact and do not require time for solubilisation. Glove washing techniques failed to prevent the transfer of latex protein. As with extractable

proteins, different gloves vary dramatically in the amount of protein transferred by a short (3s) finger touch to the membrane. Furthermore, using the finger print assay, the authors observed that latex proteins are easily transferred to the hands of the wearer and that use of hand cream increases the amount of protein, which transfers to the skin of the wearer. These data demonstrate the ease with which latex proteins are transferred and emphasise the need to wash hands immediately after removal of gloves to prevent the spread of allergens and the inadvertent exposure of latex sensitive individuals.

Skin-prick test. The allergic responses to the allergens in sensitised persons can be easily measured using the skin-prick test. It is a simple and rapid test of high sensitivity for IgE-mediated allergy[79–81]. Besides being used for identifying sensitised patients, this test is also used for detecting the presence of protein allergens in some latex products[80,82,83]. The protocol involves the introduction of a small amount of the allergen into the skin usually on the forearm, by first placing a drop of the extract on the skin followed by lightly piercing through the drop with the tiny tip of a 1 mm sterile lancet with shoulder to prevent further penetration of the skin[83] The size of the wheal developed is measured 15 min after application. A negative control of the test solution (physiological saline) without any antigen and a positive control of histamine (Histamine hydrochloride 10 mg/ml) are always included in the test battery. The test solutions are prepared from non-prewashed latex of rubber samples cut into small pieces) at a concentration of 1 g/5 mL of physiological saline at room temperature for 15 min.

The test reactions or responses are evaluated in relation to the histamine wheal. Reaction size of twice that or more of the histamine denoted as 4+; same size as that of histamine is 3+ (a clear positive); at least one-half of that of histamine is 2+ (a weak positive). Smaller wheals are not considered to be positive.

PRODUCTION OF LATEX WITH LOW EXTRACTABLE PROTEIN CONTENT

There is little information available about the duration of exposure to and the amount of EP required to bring about sensitisation. On the other hand, the amount of EP required to trigger off an allergic response in sensitised subjects can be very small[84,85] and it is probably not practical and economical to produce latex goods with such low levels. However there is a general agreement that to manufacture goods with low EP is highly desirable, especially to prevent more people acquiring the allergy (*i.e.* becoming sensitised). The need for producing low protein latex lies in the following facts:

- To prevent latex products of excessively high protein content getting into the market and then sensitising further individuals.
- To ensure that medical devices are safe for use.
- Gloves of low protein content are likely to give low allergic response.

In general, the following methods are recommended to reduce extractable protein levels:

- Reduce protein levels in raw latex
- Modify formulation
- Use single non-allergic accelerators
- More leaching/washing of latex film
- Smaller batches of product to allow for adequate treatment.

The amount of EP present in NR latex and in latex after different treatments given by Ansell International is shown[86] in *Table 8.*

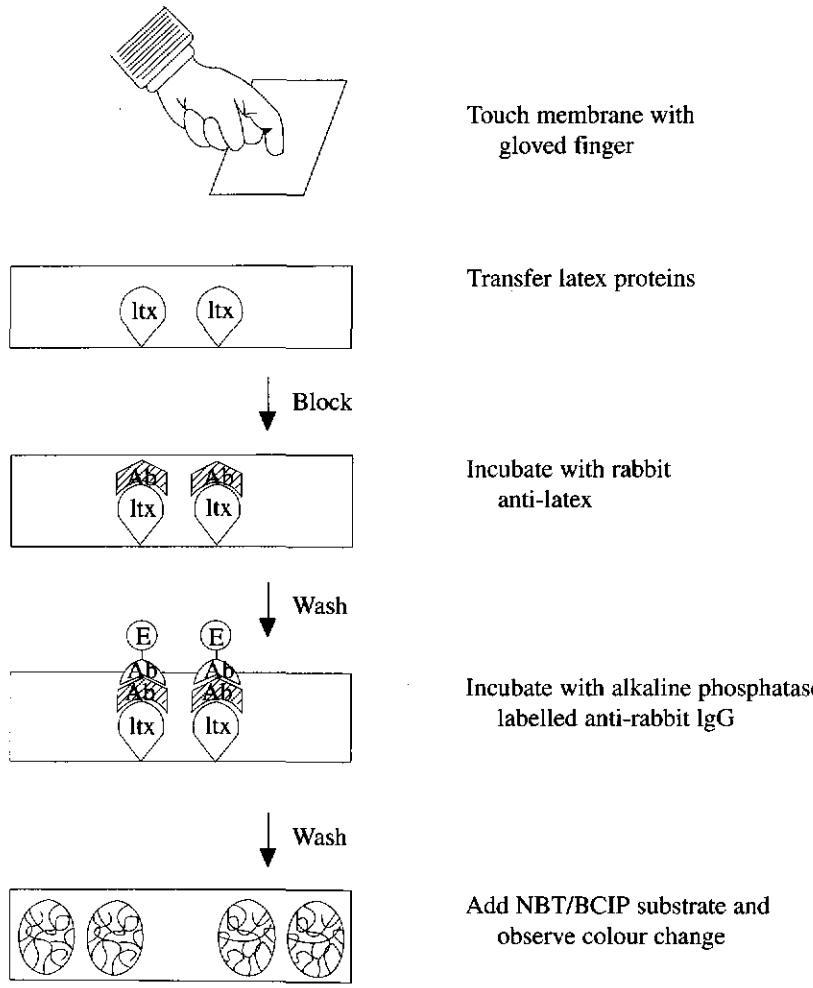


Figure 6. Finger print technique for tranfer of latex proteins.

MINIMISATION OF PROTEIN LEVELS

Extractable protein (EP) in natural rubber latex products can be minimised at two stages. First is the latex stage itself, in which the raw material is treated to reduce the amount of proteins. The second being the product stage in which the latex products are subjected to washing off in order to reduce the protein content. All the processes involved in these two operations are discussed below.

In the Latex Stage:

Substage and double-centrifuged latex. There are several methods available for concentration of field latex. Centrifugation

is the most important among them. Dilution of initially centrifuged latex and a further centrifugation reduces the soluble protein content of ammoniated concentrates. It would be expected that dipped films made from such lattices would have a lower EP than films made from the parent concentrate. This was observed for substage double-centrifuged latex, but the EP content was relatively high even after (static) leaching[35]. On account of these observations and the additional cost of production, there would not appear to be any advantages in using such lattices.

Deproteinisation by enzyme treatment. Studies on deproteinisation were started by mid-twentieth century. Attempts were made[87–89] to deproteinise *Hevea* Latex, using substantial number of proteolytic enzymes such as ficin, trysin, bromelan, and pepain. Cohin and Smith[91] reported somewhat promising results by using proteolytic enzyme of bacterial origin on ammoniated centrifuged latex. But Yapa's studies[92] did not support these findings. He stressed on the use of surfactants in centrifuged latex for treatment with enzymes in order to get reduced nitrogen value.

Certain drawbacks are reported for enzymatic method of deproteinisation. They are:

- Enzymatic hydrolysis of proteins under normal conditions removes only about 40%–50% of the latex proteins.
- Enzymes like superase and BPN (Bacterial Protease Novo) have broad specificity. Therefore, the type of peptide link age attacked can differ. Thus, deproteinised rubber obtained by this process may not show consistent behaviour.
- For obtaining reasonable nitrogen value of the deproteinised rubber (0.15% N2 and 0.15% ash content) by the enzyme treatment, the latex is diluted to 3% solid content. In this process PRI value of the deproteinised rubber becomes very low.
- Deproteinised rubber with low nitrogen content can be obtained by starting with skimmed concentrated latex while treating with enzyme and surfactants. In thismanner, the deprotenised rubber becomes uneconomical.

New developments in enzyme treated NRL has been made by Peralla[93]. In her study it is shown that the enzymatic process reduced the size of the stainable proteins from 10 kDa to 200 kDa range to 10 kDa or less. Her studies indicated that enzyme treated NRL is less immunogenic than control NRL and the proteolytic digestion of NRL associated proteins produced less allergic NR latex.

Nevertheless, enzymes so far are not an answer to the latex protein problem. Controlling enzymatic reactions, maintaining consistency of product and enveloping enough historical data still remain as the major obstacles. Enzymatic treatment promises a remedy to the latex protein problem, once some of the control issues are overcome.

Among the various proteins present in latex, alpha globulin has the highest concentration in fresh latex serum[94,95]. It gets absorbed on the surface of the rubber particles and is responsible for the colloidal stability. Works reported earlier[96,97] indicate that removal of alpha globulin from *Hevea* latex is the main task in order to get deproteinised latex.

Alpha globulin is insoluble in water. There are several types of proteins in alpha globulin. But those proteins are associated with carbohydrate (glycol type). Gupta[98] reported a method to solvate the proteins using glycerine. Mechanical

TABLE 8. REDUCING PROTEIN LEVELS WITH DIFFERENT PROCESSES

Methods	Amount (%)
Extractable protein in tree latex	100
After enzyme treatment	2
Preserved field latex	150
Centrifuged latex	13
Double centrifuged latex	8
Compounded latex (by solubilisation)	15
In-process leaching	10
Vulcanisation	15
Off-line leaching	4
Chlorination	0.3

properties obtained are claimed to be similar to that of Indian Standard Natural Rubber (ISNR-5). Nitrogen value and ash content were within international specification (*Table 9*).

An enzymically produced low protein latex (Loprol) concentrate, has been developed at the Rubber Research Institute of Malaysia. Examination and surgical gloves with satisfactory physical properties were made from it[99]. *Table 10* shows that dipped films made from this latex have much reduced EP contents compared to controls made from untreated high ammonia latex, though so far there appear to be no published data on the allergy caused by articles manufactured from it. It seems unlikely for a variety of reasons (*e.g.* added cost, requirement for extra centrifuging capacity) that Loprol, or similar lattices which are commercially available, will become major materials for the production of gloves and other high volume articles. Other commercially available low protein lattices include Laptex® (Revertex), Selatex®, Startex® (Thailand) and Allotex® (Indonesia) *etc.*

A substantial amount of extractable protein can be removed from natural rubber prevulcanised latex by using either a double centrifuged latex concentrate or an additional recentrifugation stage during the production of prevulcanised latex[100]. Further reduction in the extractable protein content can be preferably achieved by dry film leaching of the latex products. A suitable combination of wet gel and dry gel leaching has been suggested by Ng *et al.*[101] to produce NR latex gloves with low extractable protein contents. They also reported that recentrifuged prevulcanised latex has low allergic responses. Ghazaly[102] reported that the use of a low protein latex concentrate is a viable process for large-scale production of gloves with residual amounts of extractable protein. The soluble protein in the latex is diminished by the use of multiple-centrifuged or enzyme (protease) treated latex[103–105].

However, the simplest method of protein reduction is to leach the gloves on the production line[103,104]. Further reduction is possible when dry film leaching is practiced or the leaching is carried out in a protease

TABLE 9. DEPROTEINISATION OF *HEVEA* LATEX BY DIFFERENT REAGENT

Sample	Concentration	Deproteinising agent	Nitrogen content (%)	Ash content (%)	Volatile matter (%)
Field latex	35%	–	0.43	0.3	0.5
Deproteinised latex	35%	50 mL glycerin	0.21	0.13	0.5
Conc latex	60%	–	0.32	0.3	1.0
Conc latex	60%	50 mL glycerin	0.19	0.15	1.0
Field latex	35%	50 mL ethylene glycol	0.28	0.18	0.5
Field latex	35%	50 mL ethanol	0.26	0 17	0.32

TABLE 10. VARIATION OF PROTEIN CONTENT AND TENSILE STRENGTH WITH DIFFERENT PROCESSING STEPS (MALAYSIAN FIELD LATEX)[119]

Process	Protein content (mg/g)		Tensile strenght (MPa)
	Cream phase	Serum phase	
Field latex (after centrifuging)	1.2	10.92	8.26
Field latex (after centrifuging and irradiation)	3.2	-	34.63
Field latex (after irradiation and centrifuging)	0.4	13.02	31.26
Field latex (after irradiation, centrifuging and dilution)	Not detected	13.85	29.86

Irradiation carried out at 20 kGy in the presence of n-BA
(Protein content measured by BCA method)

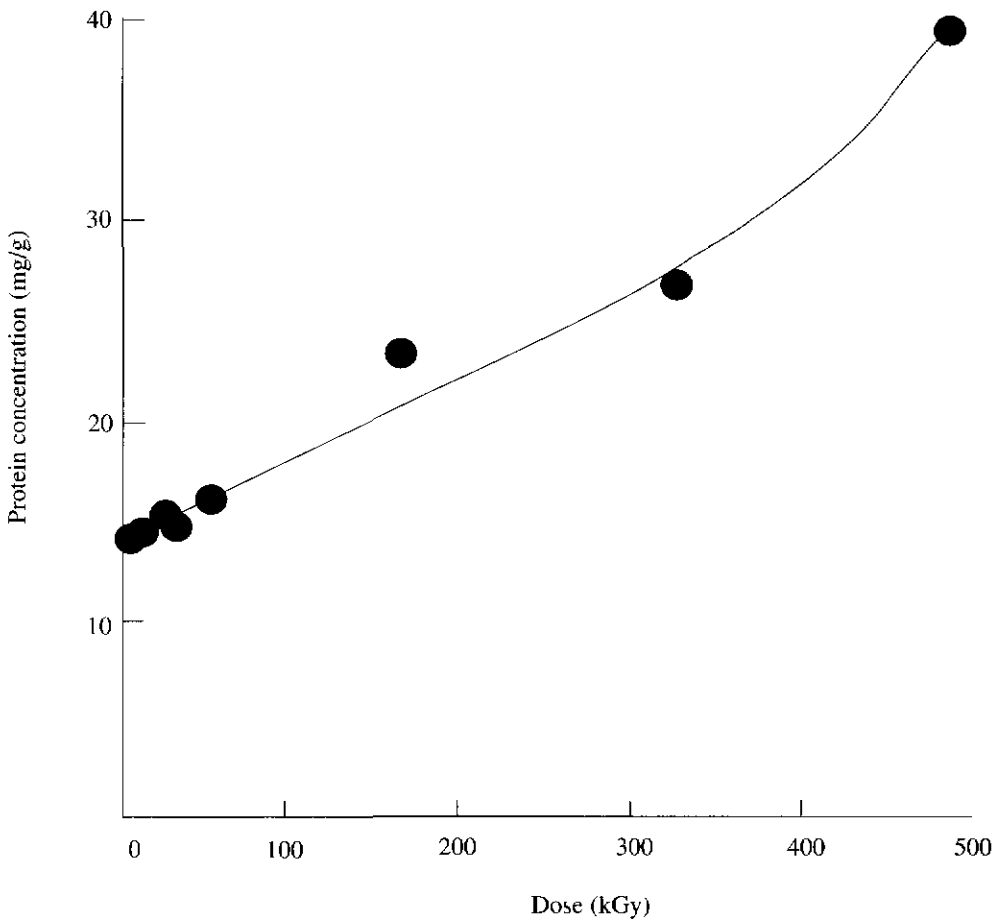
solution[105]. Highly deproteinised rubber was obtained by the enzymatic treatment followed by washing with surfactant[106]. Hasma *et al.*[107] reported that the proteins associated with rubber particles are mainly the 14 kDa protein with a minor amount of 24 kDa protein. A greater proportion of these proteins are tightly bound to the rubber particles and any influence of protein on the latex products would be attributed to these proteins. Cardosa *et al.*[108] reported that B-serum contributes more immunogenic polypeptides to the soluble protein elutable from natural rubber latex gloves than does C-serum.

Effect of radiation on deproteinisation. Natural rubber latex could be prevulcanised using γ-radiation. Since γ-radiation causes denaturing of latex proteins, it was expected that irradiated latex might not cause allergic reactions. However, the irradiated NR latex showed moderate allergic responses as evident from the PCA (passive cutaneous anaphylaxis) test in mice[109]. Makuuchi *et al.*[110] found that the ammonia extract of radiation vulcanised natural rubber latex (RVNRL) films contains seven proteins; some of them allergenic. Suitable methods for quantifying total soluble protein in NR latex products are, therefore, of great interest to the rubber industry. This arises from the fact that only part of the non-rubbers, the most important group of which is the proteins in the aqueous serum of the latex, has been removed during processing. The final quantity of extractable protein may depend upon variation in processing and manufacturing. This means that radiation and similar processes will change the soluble protein content of the latex. It is apparent that higher extractable protein contents are associated with allergic reactions in persons showing latex hypersensitivity[100]. The extractable protein content of natural rubber latex increase with radiation dose[110].

The mechanism of protein disintegration by radiation can be better understood from the distribution of protein in *Hevea* latex. There are several proteins present in natural rubber latex and the major ones are globulin, albumin and havein. Of these the protein albumin is hydrolysed by ammonia, which is used for the stabilisation of latex. The protein globulin is the major protein in ammoniated latex and field latex and is present both in the rubber phase and in the serum phase. It is reported that about 25% of the proteins, including the enzyme required for rubber biosynthesis, are bound to the latex particle surface[4,111]. This particle bound protein may undergo disintegration during irradiation.

The soluble protein in the field latex *vs* radiation dose is given in *Figure 7*. It is seen that the soluble protein content in natural rubber latex increases as γ-ray dose increased. This increase is sharp at high radiation dosage. This is due to the destructive effect of irradiation on latex proteins[110,112]. The major latex allergens bound to the rubber particle are 30, 35, 45 kD proteins[113]. These high molecular weight proteins[114] undergo disintegration with radiation, which results in low molecular weight proteins[110]. Probably this radiation-induced solubility of latex proteins might be the reason for the higher extractable protein content in the serum phase of the latex after irradiation. Studies have been reported saying that γ-radiation can result in protein disintegration, which might cause a reduction of Type I allergic reaction[112]. However processing of latex at higher radiation doses (160 kGy) is not advisable since the physical properties are found to be affected adversely due to the breaking down of polymer chains.

It is reported that the removal of proteins from the latex will affect the properties of rubber[115]. The mechanical stability of high



Firgue 7. Effect of radiation on protein concentration of NR field latex.

ammoniated latex (HA) is thought to be influenced by the proteins as well as the lipids[116,117]. Proteins have also been shown to influence the stress-strain and modulus of the vulcanised latex concentrate film[118]. Varghese *et al.*[119] have studied the effect of protein removal on mechanical properties of natural rubber with latex obtained from different sources. In their studies, authors have compared the extent of protein removal and change in tensile strength with Malaysian and Indonesian lattices. Their results are given in *Table 10* and *11*. The change in soluble protein content with different processing steps is similar in both types of lattices. The tensile strength values of the NR films are comparable with the conventional radiation vulcanised latex films. This suggests that removal of proteins does not change the mechanical properties of natural rubber. From their studies the authors have introduced a new process for the preparation of protein free

latex based on the radiation induced solubility of latex proteins. In this process, the radiation prevulcanised latex is subjected to dilution and then centrifuged and the field latex was irradiated first and centrifuged after dilution. The authors have claimed this method produces latex which is almost free from soluble proteins.

In the Product Stage:

Leaching Process. Leaching is the removal of hydrophilic materials from latex dipped products by washing them in water[120]. It is an essential process in the production of latex dipped products. The removal of excess calcium nitrate and water soluble non-rubber materials such as proteins and added compounding ingredients results in improvement of physical properties such as tensile strength and film clarity; provocation of surface blooms and reduction in water of latex dipped products. The effectiveness of the leaching process is critical in the determination of the overall quality of gloves produced.

There are basically two methods of leaching: wet-gel leaching, and dry-film leaching. The wet-gel leaching involves the washing of the wet-gel *i.e.* gelled deposit on the former, prior to drying and vulcanisation. Wet-gel leaching is usually carried out on line. In contrast, dry-film leaching consists washing of the dried, vulcanised latex product after removal from the former and is an off-line process. When complete removal of hydrophilic materials is required, dry-film leaching is carried for an extraction period of 16 h–48 h depending on the type of products made in the recommended practice.

TABLE 11. VARIATION OF PROTEIN CONTENT AND TENSILE STRENGTH WITH DIFFERENT PROCESSING STEPS (INDONESIAN FIELD LATEX)[119]

Process	Protein content (mg/g)		Tensile strength (MPa)
	Cream phase	Serum phase	
Field latex (after centrifuging)	1.2	11.30	8.16
Field latex (after centrifuging and irradiation)	4.4	–	33.09
Field latex (after irradiation and centrifuging	0.60	13.86	31.43
Field latex (after irradiation, centrifuging and dilution	Not detected	14.01	29.91

Irradiation carried out at 20 kGy in presence of n-BA
(Protein content measured by BCA method)

TABLE 12. COMPARISON OF EXTRACTABLE PROTEIN LEVELS ON NR LATEX GLOVES BEFORE AND AFTER SLURRY DIPPING

Wet gel leaching time (min)	Extractable proteins (mg/g)		% Reduction
	Dip before drying	Dip after drying	
0	0.438	0.280	36
1	0.396	0.077	81
2	0.367	0.074	80
3	0.323	0.041	87
5	0.277	0.040	86

(Extractable protein contents measured using modified Lowry microassay and calibrated against Bovine serum Albumin.)

TABLE 13. EFFECT OF DRY-FILM LEACHING ON EXTRACTABLE PROTEIN OF GLOVES MADE FROM PREVULCANISED LATEX

Leaching time (min)	Extractable proteins (mg/g)	
	Without gel leaching	with gel leaching (50°C/5 min)
0	0.729	0.061
1	0.394	0.044
2	0.143	0.036
3	0.201	0.042
5	0.145	0.038
10	0.128	0.037

(Extractable protein contents measured using modified Lowry microassay and calibrated against bovine serum albumin)

In the production of latex examination gloves, wet gel leaching is often carried out for a period of several minutes, usually 1 min–10 min, in a continuous chain dipping line. The actual leaching time is very much dependent upon the design of the dipping unit. It was previously established that a substantial amount of water-soluble proteins are generated upon drying and vulcanisation of dipped product[64] and that the proteins are drawn towards the surface away from the former during this stage, giving rise to asymmetry of extractable protein distribution.[121,122] Any form of leaching or washing, including slurry dip,

TABLE 14. EFFECT OF ON-LINE SPRAYING IN ADDITION TO WET GEL LEACHING

Time of spraying (s)	Extractable proteins (mg/g)	
	PV 1	PV 2
0	0.189	0.159
10	0.095	0.105
30	0.085	0.070
60	0.070	0.075
120	0.060	0.056

Normal high-ammonia prevulcanised NR latex (PV)
Wet-gel leaching for 2 min at 50°C post-drying slurry dip, 10 s.
(Extractable protein contents measured using modified Lowry microassay and calibrated against bovine serum albumin)

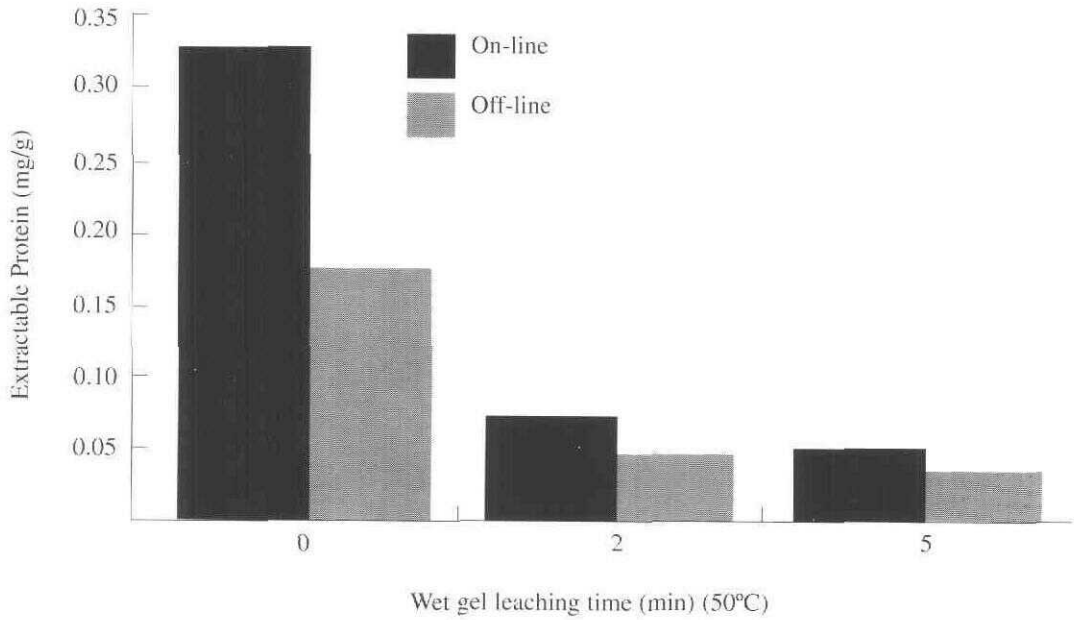


Figure 8. Comparison of on-line and off-line dry-film leaching (50°C/30 s) on EP (Redrawn from Reference 119).

TABLE 15. EXTRACTABLE PROTEIN CONTENTS OF LATEX GLOVES MADE FROM A RECENTRIFUGED PREVULCANISED LATEX (WET-GEL AND DRY-FILM LEACHING)

Time of dry-film leaching (min)	*Extractable proteins (mg/g)*
0	0.069
0.5	0.034
1	0.035
2	0.026
3	0.026

Wet-gel leaching: for 2 min at 50°C; with post-drying slurry dip, 10 s.
(Extractable protein contents measured using modified Lowry microassay and calibrated against bovine serum albumin.)

TABLE 16. EXTRACTABLE PROTEIN CONTENTS OF LATEX GLOVES MADE FROM RECENTRIFUGED PREVULCANISED LATEX

Time (s)	Extractable protein (mg/g)
0	0.062
10	0.029
30	0.029
60	0.029
120	0.024

(Extractable protein contents measured using modified Lowry microassay and calibrated against bovine serum albumin.)

after drying is therefore expected to further remove the extractable proteins.

Effect of slurry dip after drying. Ng *et al.*[101] have studied the effect of slurry dip after drying and a combination of wet gel leaching and dry film leaching in order to reduce the EP content in latex. It was found that a slurry dip immediately after drying gives much more effective reduction of the extractable protein content in latex gloves. A comparison of extractable protein values between slurry dips before and after drying clearly demonstrates this (*Table 12*). The slurry dip was carried out for a period of 10 s under agitation. However, it was noted the that protein level in the slurry tank increased as production went on. In such cases there is a possibility of redeposition of the soluble proteins onto the gloves, parti-cularly when the concentration has reached a sufficiently high level.

Combination of wet-gel leaching and dry-film leaching. In order to remove EP during production of latex gloves, it was found that a combination of wet-gel leaching

TABLE 17. EFFECT OF STRIPPING PROCEDURE ON EXTRACTABLE PROTEIN CONTENT OF LABORATORY-MADE GLOVES

Gloves	Leaching method (2 min at 60°C)	Strip method	Residual extractable protein (mg/kg)
1	Slowly stirred	Wet	15
2	Slowly strirred	Dry	290
3	Vigorously stirred	Wet	20
4	Vigorously stirred	Dry	255

TABLE 18. EFFECT OF HOT AIR AND STEAM ON EXTRACTABLE PROTEIN LEVELS OF DIPPED FILMS [62]

Treatment	Temperature (°C)	Time (min)	Residual extractable protein (%) Bradford	Lowry
		Post-vulcanised films[a]		
Air	120	60	69	51
Steam	120	60	20	10
		Prevulcanised films[b]		
Steam	120	60	61	60
Steam	120	60	BD[c]	BD
Steam	110	120	BD	–
Steam	110	60	BD	–
Steam	105	60	BD	–
Steam	105	30	BD	–

[a] Control, 415 mg/kg extractable protein = 100

[b] Control at 120°C, 580 mg/kg: at other temperatures, 520 mg/kg

[c] Barely detectable

and dry-film leaching is most desirable[101] (*Table 13*). In addition, the dry-film leaching can be carried out either on-line or off-line. The off-line leaching appears to be more effective as both surfaces of the gloves are involved (*Figure 8*). The authors have also investigated a combination of wet-gel leaching and direct water spraying after drying. They have observed that a spraying time of about 30 s could reduce the EP of gloves to below 0.1 mg/g for a normal prevulcanised NR latex (*Table 14*). It is known that water-soluble proteins could be generated during the compounding of latex and upon heating of a NR latex compound[120]. Studies were also conducted on re-centrifuged prevulcanised

TABLE 19. RESIDUAL PROTEIN IN DIPPED LATEX WITH AND WITHOUT COBALT SILICA, ANALYSED BY *ASTM D5712* TEST, GUTHRIE RESEARCH INSTITUTE, SAYRE, PA[130]

Rubber sample	Guthrie Research Test	Fumed silica (W%)	Proteins extracted *ASTM D 5712* (p.p.m.) .
017401-1	9335	0	105
109607-2	9814	1.0	<28
109607-3	9815	1.0	<28
109607-1	9813	2.5	<28
168901-1	9364	5.0	<28

TABLE 20. RESIDUAL ALLERGENIC PROTEIN IN DIPPED LATEX WITH AND WITHOUT COBOLT SILICA, ANALYSED BY LEAP TEST, GUTHRIE RESEARCH INSTITUTE, SAYRE, PA[130]

Rubber sample	Guthrie Research Test	Fumed silica (W%)	Allergenic protein (%)
149603-3	12253	0	26.8
149603-3	12253b	0	16.6
149603-3	12253c	0	15.3
109607-2	11652	1.0	<0.2
109607-2	11652b	1.0	<0.2
109607-2	11652c	1.0	<0.2

latex. It is possible through re-centrifugation of diluted prevulcanised NR latex to remove a large amount of water-soluble protein in the prevulcanised NR latex. Re-centrifuged prevulcanised NR latex is found to contain lower extractable protein content when compared to normal prevulcanised latex. (*Table 15*). The results of the study made by Ng *et al.*[101] indicate that EP content of latex gloves can be reduced by the use of re-centrifuged prevulcanised latex together with a combined protocol of either wet gel and dry-film leaching, (*Table 16*) or of wet gel leaching and direct on-line water spraying, (*Table 17*). A final low extractable protein content of 0.024 mg/g – 0.026 mg/g can be obtained.

Direct evidence that leaching at the wet-gel stage or washing the finished products significantly reduces EP, has now been obtained by many groups of workers[35,40,64,123]. It was found that, by washing powder-free gloves three times, the contact with allergen was reduced several thousand times[124]. *Figures 9* and *10* show results obtained at MRPRA[35]. It can be seen that the temperature of the water and the duration of the leaching/washing are important in reducing EP. It was also found (at least with dipped films), that leaching with turbulent water was much more effective than with static water; soft water was generally more effective than hard (*Table 17*). As would be predicted, wet stripping of gloves was much

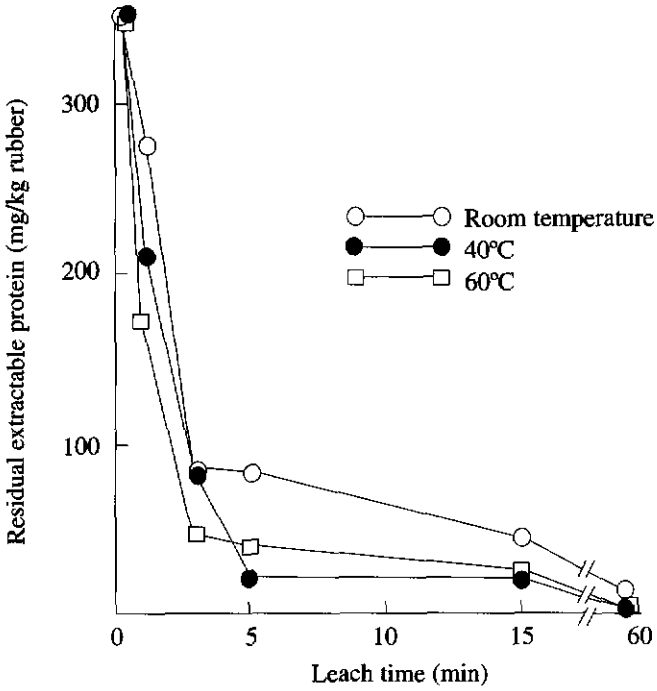


Figure 9. Effect of time and temperature on removal of extractable protein from dipped films by wet-gel leaching with agitation (Redrawn from Reference 56).

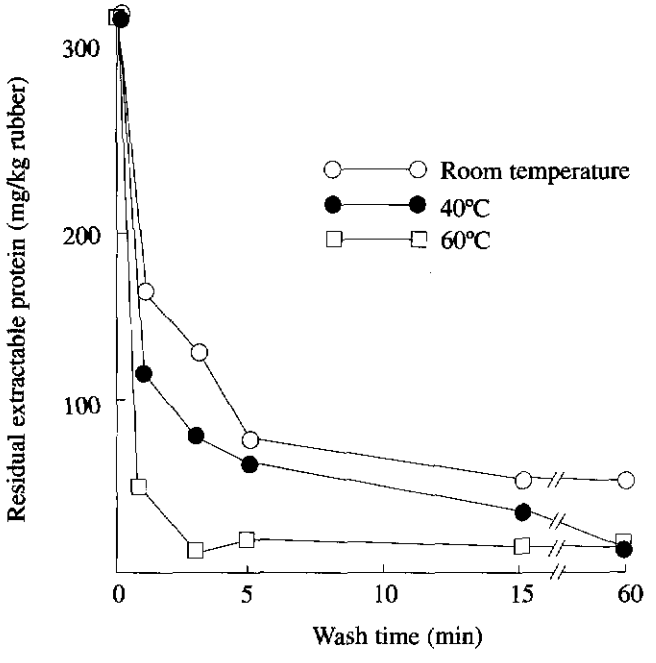


Figure 10. Effect of time and temperature on removal of extractable protein from dipped films by dry-film washing with agitation (Redrawn from Reference 56).

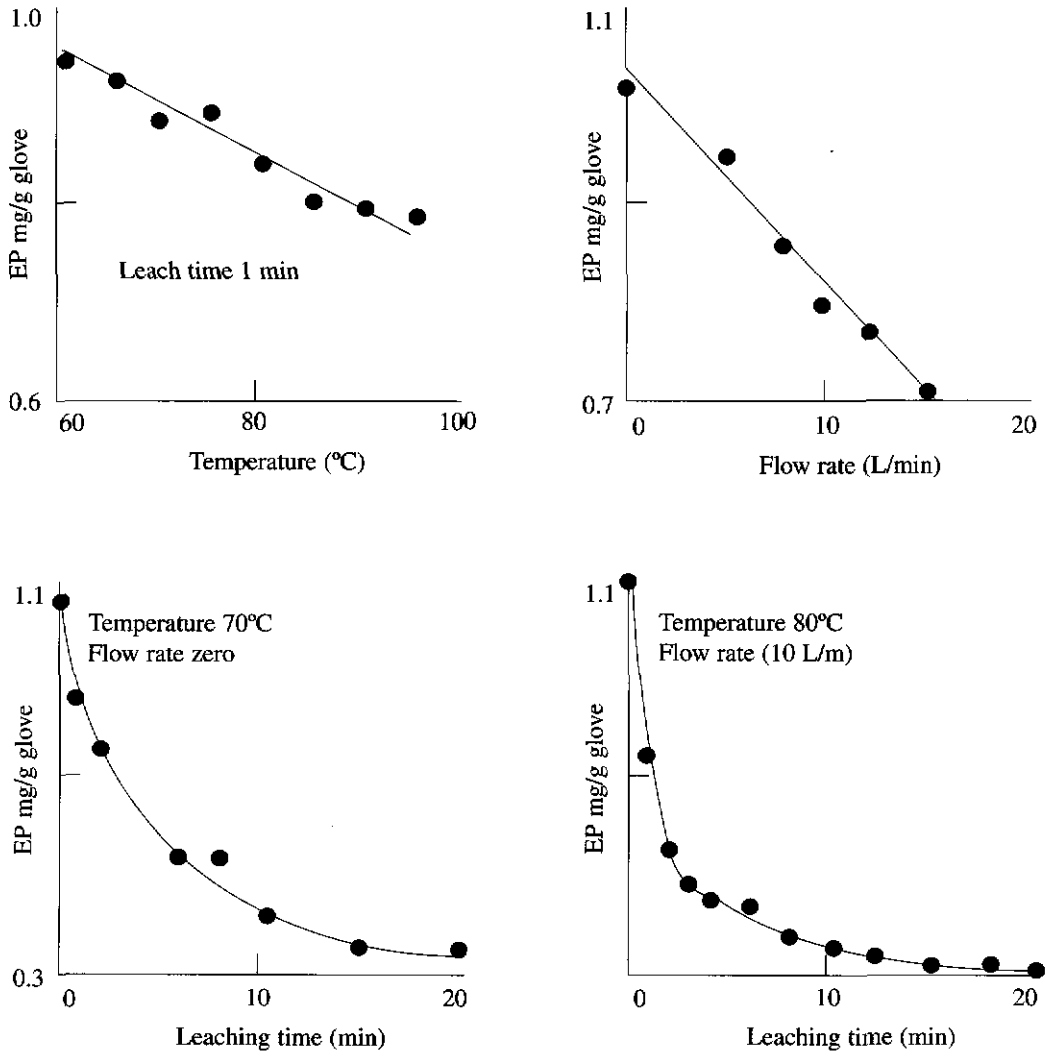


Figure 11. Factory study of variables affecting wet-gel leaching of extractable protein from examination gloves.

more effective than dry stripping in reducing EP; in this experiment the use of turbulent water made little difference[35] (*Table 17*). *Figure 11* shows the results of a study of variables influencing EP during wet-gel leaching in a glove plant.

Use of steam. It has been found that a combination of washing and autoclaving in steam is very effective in reducing both EP and responses to skin tests in sensitised subjects[125]. Other work has demonstrated that EP can be generally reduced under less severe conditions of autoclaving, but no dermatological tests were carried out[35] (*Table 18*). Steam autoclaving can affect physical properties of the products but this can be guarded against by appropriate formulation. It does not seem likely that autoclaving can be used on a large scale to produce articles of low allergenicity[35]. It is possible that the switch from steam-sterilised to disposable gloves has contributed to the spread of allergy by latex proteins.

Chlorination and associated processes. Chlorination, which is used to reduce surface friction and tack, markedly decreases EP [35,125,126]. It has been shown that it is not chlorination *per se*, but the washing procedures associated with it, which are largely instrumental in lowering the EP of chlorinated articles[35]. There appears to be little published detailed information on the allergenicity of chlorinated products, but treatment with chlorine would certainly chemically alter the protein. In a reference to work by Turjanmaa using skin-prick tests, it is stated[40] that only the chlorinated film was found to be non-allergenic. In other work, a chlorinated film was found to give a fairly high frequency of positive skin prick response[127]. However chlorinated gloves appear to be well tolerated in wear tests[127].

Recently, studies have been reported[130] where fumed silica is used as an additive in natural rubber latex for the production of allergy-free latex goods. The fumed silica attaches itself to the rubber particles and substitutes the proteins. The proteins are then easily removed. This is in contradiction to what was proposed earlier, where fumed silica attaches itself to the proteins on the surface of the latex particles, binding them there. The author claims that all this process can be performed on line, eliminating chlorination and /or extensive washing and handling of the products off-line[129]. The results obtained are given in *Tables 19* and *20*. Other methods for protein treatment include passing the latex through special ion-exchange resins[130] or altering the protein structure with protease enzymes[131].

CONCLUSIONS

Latex protein allergy has created health problems widely, particularly among health care workers. The different types of allergy, their causes and possible remedies are discussed in this review. It is said that people having once become allergic, have a greater chance of getting permanently allergic to latex products. Different methods to detect and estimate the protein level in latex products are discussed. Elimination of proteins from the NR latex can be done either in the latex stage itself or in the product stage. Use of double-centrifuged latex followed by enzymatic treatment can practically eliminate the allergy problem, but the process is quite expensive. Radiation vulcanisation of latex followed by dilution and centrifugation also is recommended but the poor mechanical property of the radiation-vulcanised latex is a problem that should be overcome. Leaching of the latex products, both on-line and off-line eliminates protein to a

considerable extent. The threshold amount of latex protein required to cause allergy is still unknown. Further research has to be conducted to identify the particular protein which is allergic to an individual. Also special attention is needed to determine whether any threshold limit exists in protein level between different individuals.

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