Results

Fresh natural rubber (NR) latex contains a vast number of polypeptides which are potential allergens. These proteins have been reported in all 3 major fractions obtained during ultracentrifugation of fresh *Hevea* latex (Moir, 1959), namely: (1) a white creamy layer at the top which contains virtually of the rubber particles; (2) a translucent fluid call C-serum; and (3) a bottom fraction containing organelles, including the lutoids and Frey-Wyssling. The rubber phase, C-serum and B-serum contain 27%, 48% and 25% respectively, of the total protein content (Tata, 1980a).

On processing the fresh field latex into high ammoniated (HA) latex concentrate, the protein content was reduced from 30-50 mg/g rubber to 16-26 mg/g rubber and distributed in two main fractions; the rubber phase and the serum phase (Hasma, 1994). The rubber particle proteins remain qualitatively similar to those isolated from fresh latex with 14 and 24 kDa as the major proteins. However, a fewer number of proteins was present in the HA latex serum as compared to a great variety of them in fresh latex serum (Hasma, 1992). The serum proteins were subjected to further change upon compounding and heating of the compounded latex (Hasma and Amir-Hashim, 1997). Finally, in contrast to fresh NR latex which contains more than 35 IgE-binding polypeptides, finished latex products and gloves appeared to contain a limited number of allergen peptides (Nel and Gujuluva, 1998). Thus, although about 10 proteins have been identified to be potential allergens in latex (Palosuo, 1996; Nieto, *et al.*, 1998), it is questionable whether all of them could survive the

stringent manufacturing processes and remain as part of an extractable proteins in latex products. Information regarding the status of allergenic proteins in latex products is presently incomplete. About 25% of latex allergens is still missing in the commercial test kit for detection of IgE-antilatex (latex-specific IgE) used in clinical latex allergy diagnosis (Hamilton *et al.*, 2002). Most of latex allergens identified thus so far were from rubber particles, B- and C-serum. An investigation on bottom fraction membrane (BFM) as another source of protein allergens in latex system has, however, been overlooked. A previous study by our group had proposed an essential role of lutoid membrane lectin in coagulum formation between RP and lectin containing lutoid membrane (Piyaporn, 2001). The affinity binding of lutoid lectin towards glycoprotein on rubber particles leading to formation of rubber particle aggregates was clearly demonstrated. Thus, to view the allergenic proteins of NR latex that also remain as part of an extractable proteins in latex products, one should pay attention to the BFM proteins entrapped with RP or final latex product.

In order to present a complete picture of NR proteins and their involvements in latex allergy, the scope of study in this thesis is, therefore, focused on the BFM proteins in term of their allergenicity as compared to those reported in RP, B- and C-serum. An investigative study on BFM as an original site that give rise to the most persistent proteins found in ultra-low protein gloves was also performed.

Accordingly, the first part of the experimental results was dealt with isolation of BFM proteins and characterizing their allergenicity. The second part was related to alkaline stability characterization of the BFM proteins as

compared to those from RP, B- and C-serum. The third part was focused on the alternations of latex composition and protein composition of latex concentrate upon processing of field latex into latex concentrate. The final part was related to protein composition in latex gloves and the source of immunogenic glove proteins.

Part 1. Bottom fraction membrane (BFM) proteins and their allergenicity

1.1 Preparation of BFM and protein extraction

Aqueous B-serum and other colloidal substances within bottom fraction organelles (lutoid) were initially removed after membrane rupture (bursting). In order to rupture the membrane, the bottom fraction was either immersed in excess distilled water (method A), distilled water containing 0.7% ammonium hydroxide (method B) or buffer containing antioxidants and protease inhibitors (method C). The isolated BFM was further washed with isotonic buffer to remove the contaminated residual B- and C-sera. The intrinsic proteins of washed BFM were extracted by using buffer containing 0.2% Triton X-100 or the mixture of chloroform-methanol (2:1, v/v).

Fig. 15 showed SDS-PAGE of different compositions of BFM proteins and proteolipids, dependent on bursting method employed for BFM isolation and extracting conditions. Chloroform-methanol extracted BFM (method C) gave proteolipids consisting of thick protein bands appeared at 17 kDa and triplet bands around 30-35 kDa (lane 6). Whereas under other methods, the BFM proteolipids appeared as thick bands at 17 kDa (method B, lane 1) and at 17 and 35 kDa (method A, lane 2). More numerous BFM protein bands were observed under Triton X-100 than chloroform-methanol extraction. BFM obtained under method A and extracted with 0.2% Triton X-100 gave highest number of bands with molecular masses around 17, 20, 22, 33, 35 and 55kDa (lane 4). This is in contrast to those derived from alkaline bursting where only a few fainted bands observed (lane 3).

These distinct detergent and lipid-soluble proteins found in the BFM should, therefore, be another attractive source of hydrophobic latex proteins in studying latex allergens.

Fig. 16 showed different protein patterns of 3 major protein fractions obtained from ultracentrifuged fresh latex; the rubber particles, B-serum, C-serum as compared to those of isolated BFM, under SDS-PAGE. The C-serum showed most numerous bands ranging from 14 kDa up to >94 kDa and mostly above 40 kDa (lane 5). The B-serum has fewer in number mostly below 40 kDa with distinct bands at 14, 17, 22, 23, 30-35 kDa (lane 4). The BFM obtained after lipid solvent and detergent extractions (lane 2) showed distinct protein bands of similar masses, around 17 kDa and 30-35 kDa, to those observed in the B-serum. Two major unique proteins of rubber particles were detected at 14 kDa and 24 kDa, respectively (lane 1).

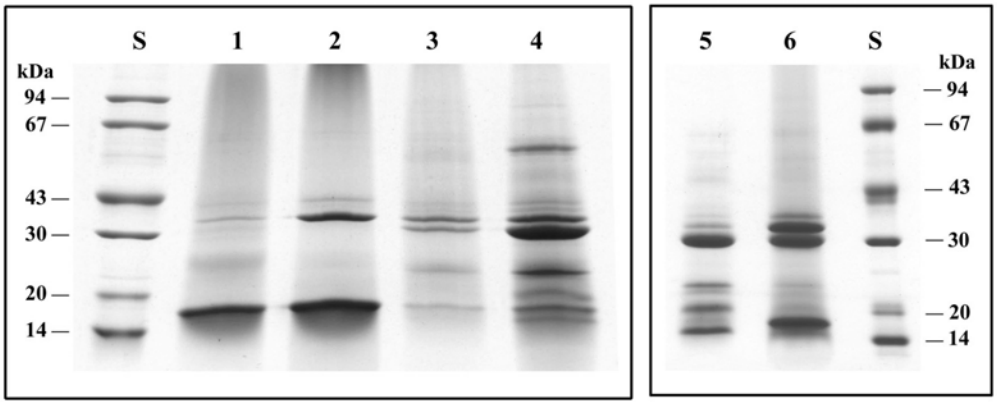

Fig. 15 SDS-PAGE (7-15% gradient gel) of BFM proteins obtained from various BFM preparative and extraction methods, as indicate.

S = Molecular weight markers

1 =Proteolipids from BFM prepared by immersing the BF in ammonia solution

2 = Proteolipids from BFM prepared by immersing the BF in distilled water

3 = Triton X-100-extracted proteins from BFM prepared by immersing the BF in ammonia solution

4 = Triton X-100-extracted proteins from BFM prepared by immersing the BF in distilled water

5 = Triton X-100- extracted proteins from BFM prepared by immersing the BF in buffer containing antioxidants and protease inhibitors

6 = Proteolipids from BFM prepared by immersing the BF in buffer containing antioxidants and protease inhibitors

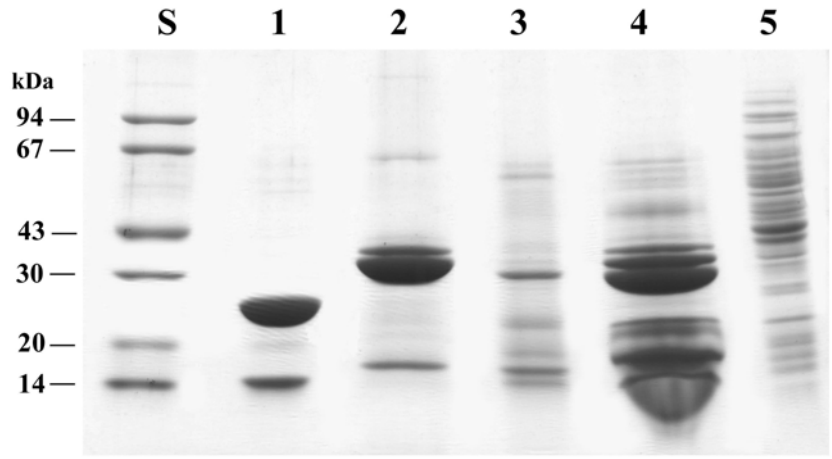

Fig. 16 SDS-PAGE (7-15% gradient gel) of proteins from various fractions of fresh latex.

S = Molecular weight markers

1 = RP proteins

2 = BFM proteolipids

3 = Triton X-100- extracted BFM proteins

4 = B-serum proteins

5 = C-serum proteins

1.2 Reactivities between IgE and fractionated fresh latex proteins

A Western-immunoblot was performed to compare allergenicity among fractionated fresh latex proteins by using IgE antibodies present in the sera of patients with latex allergy (positive clinical history and skin prick test) obtained from Siriraj Hospital (S1 and S6), Songklanagarind Hospital (P3 and P6) and a cord blood serum pool.

By using horseradish peroxidase (HRP) as the enzyme conjugate for immunological detection on blotted membrane, IgE antibodies in all four sera were found to be reactive to both B-serum and BFM. Only three out of four were reactive to the C-serum proteins while only one positive serum found with the rubber particle membrane proteins (Fig. 17).

The immunoblots also revealed a presence of some common allergenic protein bands that were recognized by the IgE antibodies in the tested sera. The most frequently observed bands among four individual BFM blot were in the region of 17, 22, 30, 33, 35 and 45 kDa. Similar to the BFM, bands in the region of 17,22, 30, 33, 35 and 45 kDa were also observed among the B-serum blots. Fewer positive bands were observed in C-serum blots and 43, 55 and 67 kDa being most frequent.

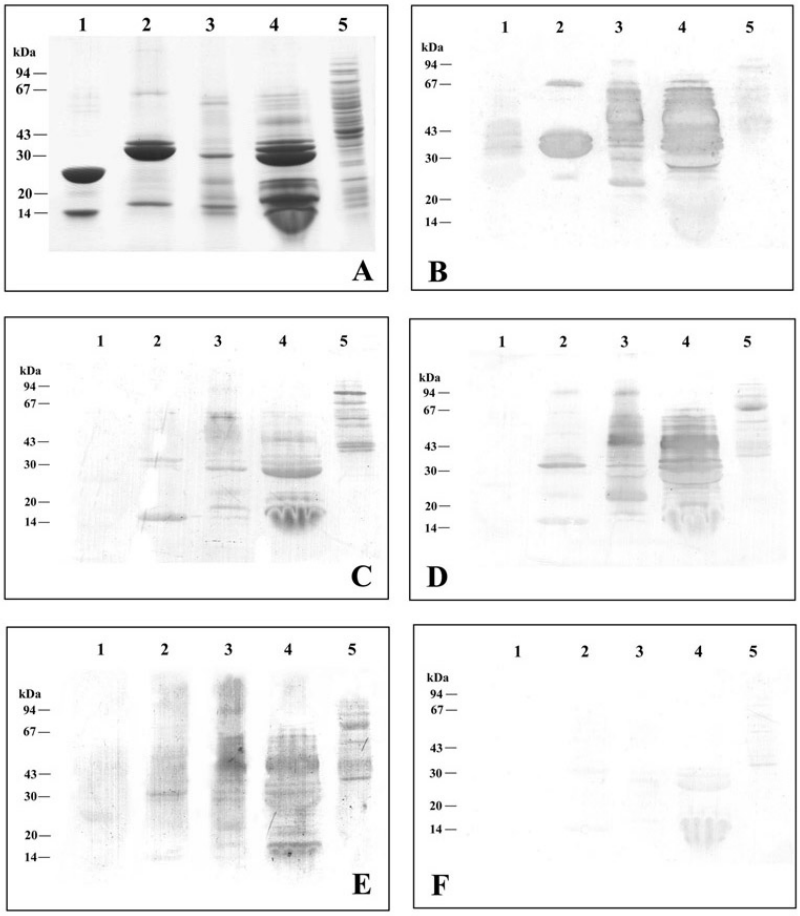

Fig. 17 SDS-PAGE (7-15% gradient gel) and immunoblotting of proteins from various fractions of fresh latex.

1= RP proteins, 2 = BFM proteolipids, 3 = Triton X-100-extracted BFM proteins, 4 = B-serum proteins, 5 = C-serum proteins

A : Coomassie Blue staining

B-F: Nitrocellulose blots of a replicate gel after incubations with respective sera from patients with latex allergy (B, C, D and E) and pool cord blood serum (F) and followed by visual staining with goat anti-human IgE conjugated with peroxidase.

The binding of IgE to BFM proteins was further studied on more individual serum of patients with latex allergy. Sixteen individual sera of patients with latex allergy (Table 4 and 5), five sera from Siriraj Hospital (S2-S6) and eleven sera from Songklanagarind Hospital (P1-P11), three negative control sera of individuals with negative skin prick tests (N1-N3) and cord blood serum pool from Songklanagarind Hospital were used in this study.

The SDS-PAGE-IgE-Immunoblotting of BFM proteins, using sixteen individual sera of patients with latex allergy, demonstrated the presence of IgE binding proteins (allergens) in the BFM with notable different response among individuals (Fig. 18).

By using HRP as the enzyme conjugate for immunological detection on the BFM blotted membrane, patient's sera from Siriraj Hospital (Fig. 18, lane S2-S6) revealed IgE-reactive BFM proteins in the region of 17, 22, 30, 43, 55, 58, and 94 kDa whereas fewer reactive bands including at 14, 17, 35, 43, and 55 kDa observed on patient's sera from Songklanagarind Hospital (Fig. 18, lane P1-P11).

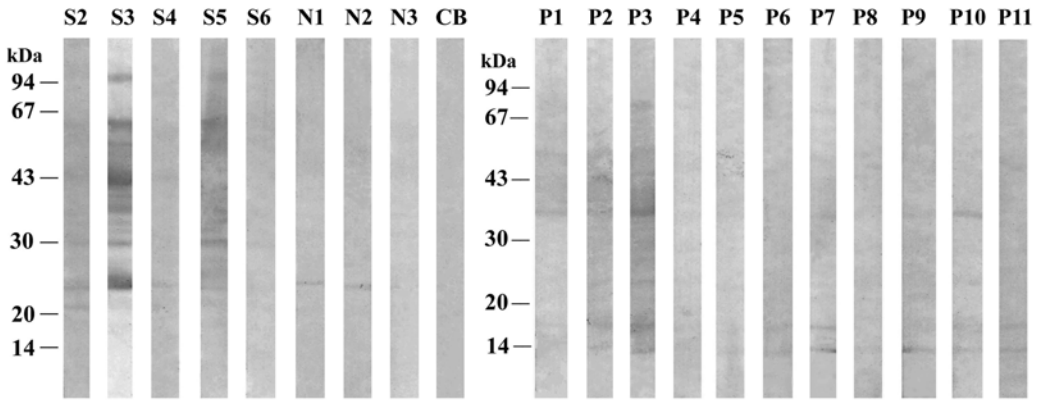

Fig. 18 SDS-PAGE immunoblots of BFM proteins, using sera from patients with positive SPT to fresh latex and glove extracts and horseradish peroxidase as the enzyme conjugate for immunological detection on blotted membranes.

S2-S6 : Patients'sera obtained from Siriraj Hospital

P1-P11: Patients'sera obtained from Songklanagarind Hospital

N1-N3 : Sera from individuals with negative SPT

CB : Pool serum of cord blood

1.3 Lutoidic membrane proteins

The bottom fraction obtained from centrifuged fresh latex is not only composed of mainly lutoid particles but also contains some minor Frey-Wyssling particles. Hence, the BFM proteins do not represent solely on lutoidic membrane. To identify lutoidic membrance proteins, pure lutoid particles was separated from fresh latex bottom fraction by sucrose density gradient centrifugation, as described under Materials and Methods (Figure 11).

After bursting of the lutoid particles in distilled water and removed of contaminated B-serum by washing in isotonic buffer, the lutoidic membrane was extracted with 0.2% Triton X-100 or chloroform-methanol mixture.

Fig. 19 showed the protein compositions of lutoidic membrane (LM) and BFM as analysed by SDS-PAGE, under reduced and non-reduced conditions. Under Triton X-100 extraction, both reduced and non-reduced proteins from LM and BFM contained common protein bands in the regions of 14, 17, 18, 20, 22, 29 and 33 kDa (lane 1-4). Some thick bands of LM and BFM proteins, especially in the region of 17, 29 and 33 kDa were more prominantly detected under the reduced than non-reduced condition. For the proteolipids of LM, both under reduced and non-reduced SDS-PAGE, one major prominent protein band at 17 kDa and another sharp band at 33 kDa (lane 6 and 7) were observed. However, an additional band at 41 kDa was detected in the case of BFM (lane 5 and 6).

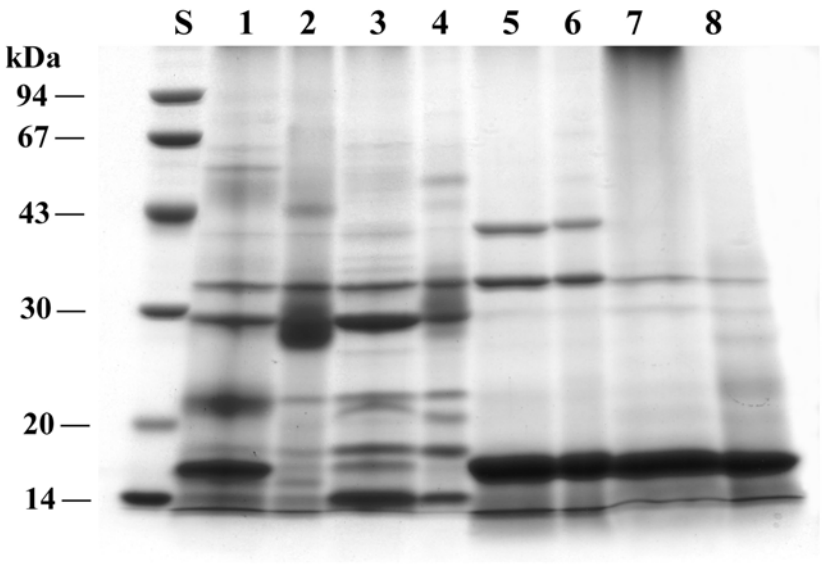

Fig. 19 SDS-PAGE (12% gel) of BFM and LM proteins under reduced and non-reduced condition.

S = Molecular weight markers

1 = Triton X-100-extracted BFM proteins, reduced condition

2 = Triton X-100-extracted BFM proteins, non-reduced condition

3 = Triton X-100-extracted LM proteins, reduced condition

4 = Triton X-100-extracted LM proteins, non-reduced condition

5 = BFM proteolipids, reduced condition

6 = BFM proteolipids, non-reduced condition

7 = LM proteolipids, reduced condition

8 = LM proteolipids, non-reduced condition

1.4 Reactivities between IgE and lutoidic membrane proteins

Two serum sources from patients with latex allergy were used to react with LM proteins in immunoblotting. But, instead of using peroxidase as the enzyme conjugate for immunological detection as previously employed in the BFM immunoblots, the reactivities of IgE and LM proteins were enhanced by the reaction of biotin-streptavidin with alkaline phosphatase as the enzyme conjugate.

The SDS-PAGE-IgE-immunoblotting of LM proteins, using seventeen individual sera of patients with latex allergy, indicated the presence of allergens in LM, similar but more pronounced than those of BFM (Fig. 20). The most frequent bands observed in the regions of 17, 22, 29, 35, 43, 58, 65 and 94 kDa. The 17 kDa and 29 kDa were found to be recognized by IgE in all sera used.

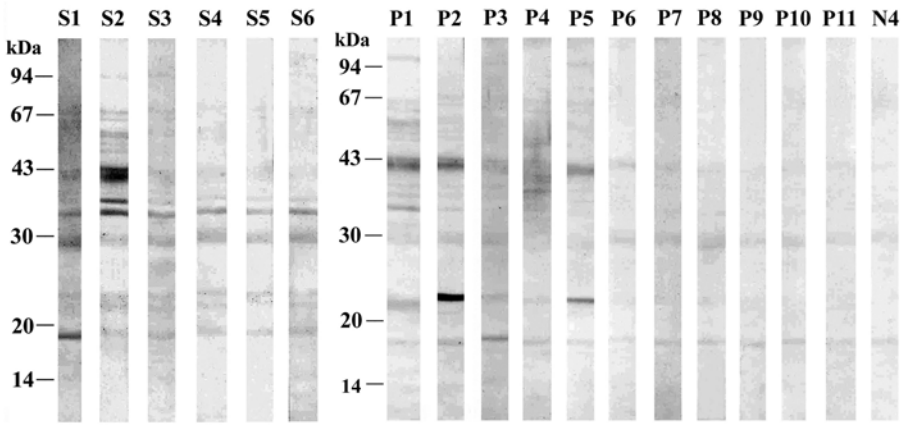

Fig. 20 SDS-PAGE immunoblots of LM proteins using sera from patients with positive SPT to fresh latex and glove extracts and alkaline phosphatase-streptavidin as the enzyme conjugate for immunological detection on blotted membranes.

S1-S6 = Patients'sera obtained from Siriraj Hospital

P1-P11 = Patients'sera obtained from Sonklanagarind Hospital

N4 = Serum from individual with negative SPT

Part 2. Alkaline stability study

Natural rubber (NR) products especially latex gloves are produced from preserved high ammoniated (HA) latex concentrate. Thus, the extractable residual proteins in latex gloves that induce allergenic reaction in human beings should also be derived from the native NR latex protein material.

In the preparation of latex concentrate, ammoniation is recommended to be carried out as early as possible, right after field latex collection. Such practice efficiently inhibits bacterial growth in latex which would otherwise bring about an increase in the volatile fatty acid contents. However, ammoniation can lead to rupturing of membrane bound latex organelles, namely lutoids, and Frey-Wyssling. The fluid organelles'contents (B-serum) are discharged into the latex serum upon bursting. The organelles membrane are left as amorphous hydrophobia materials, readily for aggregating/entrapping with other hydrophobic rubber particles in the process.

When HA latex concentrate was centrifuged, the latex fractionated into only two layers; the rubber phase and the serum phase. The result obtained was different from those of ultracentrifuged fresh latex where 3 distinct layers of rubber, C-serum and bottom fraction usually observed. According to the ammoniation effects on membrane rupture and hydrophobic interactions/ aggregations as mentioned above, it is expected that the less dense or bottom side of rubber layer is more highly associated with the debris of membrane fragments derived from organelles in the bottom fraction of ultracentrifuged fresh latex whereas aqueous serum phase are mainly consisted of C-serum and liberated B-serum. Hence, to view the protein of NR latex concentrate that gives

rise to allergy, one should also consider to the liberated BFM proteins in addition to the soluble B- and C-serum proteins.

For better understanding on fresh latex sites of the alkaline-stable proteins remaining in HA latex concentrate, alkaline stabilities among native proteins intrinsic to RP, B-serum, C-serum and BFM as well as a mixture of non-rubber components (C-serum and bottom fraction) were compared by separately subjected to alkaline treatment at various time durations. Moreover, for the potential important of BFM proteins as latex allergens, the charge property and heat stability of alkaline treated BFM proteins were also investigated.

2.1 Alkaline-stable proteins in fractionated fresh latex

2.1.1 Rubber particle proteins

The rubber particle (RP) proteins are those proteins associated within interfacial layer surrounding the RP. They consisted of two tighly bound proteins with molecular weights of 14 and 24 kDa as shown in SDS-PAGE profile (Fig. 16, lane 1 and Fig. 21, lane 1).

The time-course study on alkaline destabilization (alkaline hydrolysis) of freshly prepared RP proteins revealed 24 kDa protein to be more alkaline-sensitive than the 14 kDa protein, as shown in SDS-PAGE profile (Fig. 21). The amount of released 24 kDa protein found in the alkaline medium and remaining on RP were inversely proportional to the alkalinization time (Fig. 21, lane 2-5 and 6-9). Moreover, the released 24 kDa but not the 14 kDa was completely degraded after 120 days storage whereas the 14 kDa still detected (Fig. 21, lane 5). Similarly, the longer the alkaline treatment, the lesser the intact

24 kDa but not 14 kDa protein remaining on the RP (Fig. 21, lane 6-9). However, after 120 days of incubation, both 14 and 24 kDa proteins were still detectable on the RP (Fig. 21, lane 9). This result suggests that bound RP proteins are more alkaline resistant than the released ones.

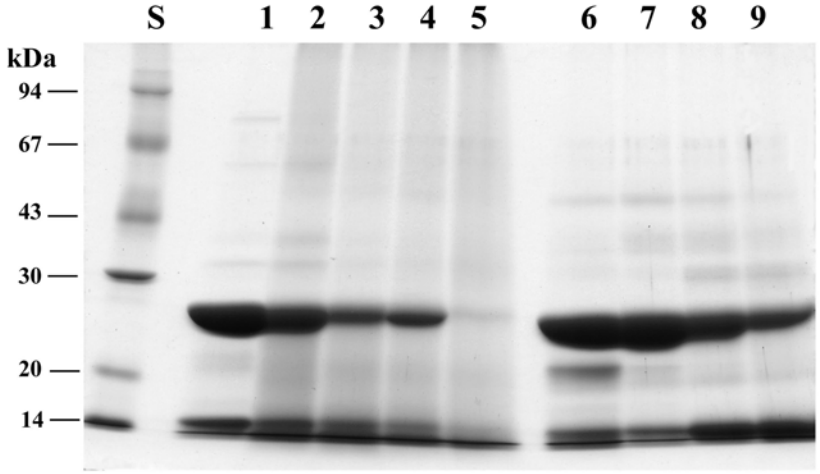

Fig. 21 SDS-PAGE (12% gel) of rubber particle proteins upon alkaline treatments.

S = Molecular weight markers

1 = Proteins of freshly prepared rubber particles

2, 3, 4 and 5 = Released rubber particle proteins in alkaline solution after 15, 30, 60 and 120 days of alkaline suspension, respectively

6, 7, 8 and 9 = Remaining intact proteins of the rubber particles after suspending in alkaline solution for 15, 30, 60, 120 and 180 days, respectively

2.1.2 C-serum proteins

As revealed by SDS-PAGE profile, the number of native proteins found in aqueous C-serum was more numerous than those observed in RP (Fig. 21), B-serum (Fig. 23) and BFM (Fig. 24), respectively. The molecular weight (MW) of C-serum proteins ranges from 14 kDa to >94 kDa with majority above 30 kDa. Upon alkaline treatments, a rapid degradation of the soluble C-serum proteins was observed (Fig. 22, lane 2-6). After 15 day alkalinization, most of the C-serum proteins, except for the 34 kDa protein, were completely degraded. However, the 34 kDa protein still remained relatively stable up to 60 days (Fig. 22, lane 2-5). The result suggests 34 kDa as an alkaline-stable C-serum protein.

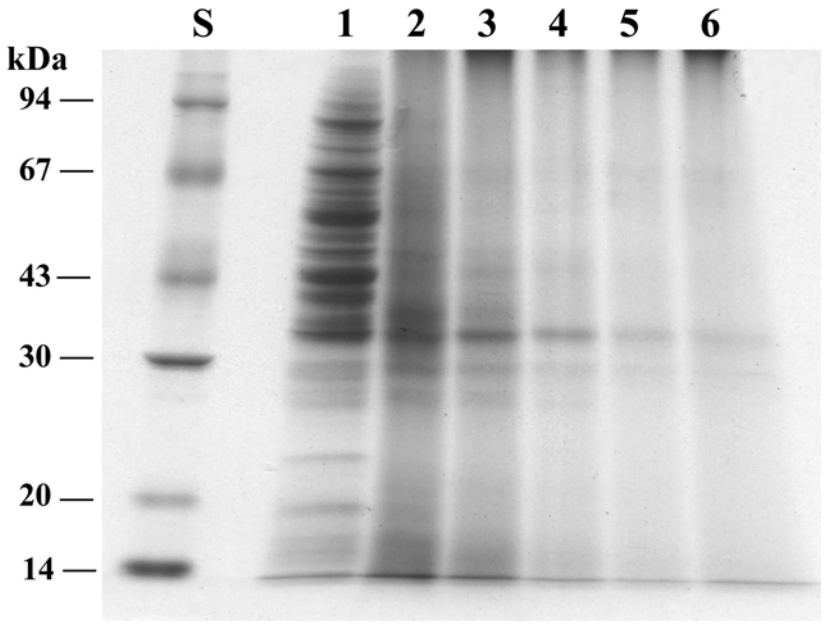

Fig. 22 SDS-PAGE (12% gel) of C-serum proteins upon various alkaline treatment durations.

S = Molecular weight markers

1 = Proteins of freshly prepared C-serum

2, 3, 4, 5 and 6 = Remaining C-serum proteins after alkaline treatment for 15, 30, 60, 120 and 180 days, respectively

2.1.3 B-serum proteins

SDS-PAGE profile analyses (Fig. 23) showed the native proteins in B-serum consisted of three major protein bands with molecular weight of 14, 30 and 35 kDa, four minor bands of <14, 20, 24, 37 kDa and minute fainted bands of 18, 23, 27, 48, 60 and 70 kDa. The major proteins of 14 and 30 kDa were found to be very alkaline-stable and remained detectable throughout the treatment up to 180 days. However, almost all the minor proteins, including 35 and 37 kDa, were less stable undergone complete degradation and hardly detectable after 120 days. This suggests that there are mainly only two major alkaline-stable B-serum proteins (14 and 30 kDa) as possible allergen candidates in the HA latex concentrate.

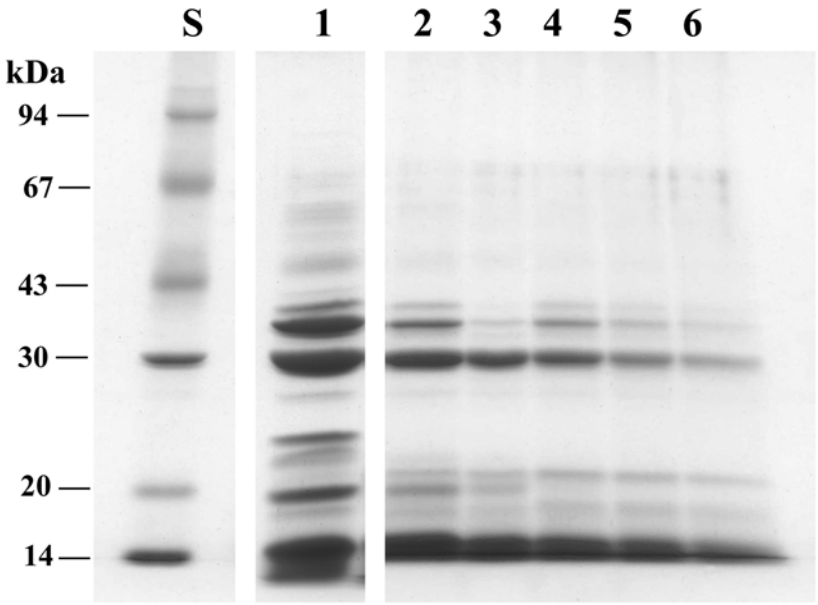

Fig. 23 SDS-PAGE (12% gel) of B-serum proteins upon various alkaline treatment durations.

S = Molecular weight markers

1 = Proteins of freshly prepared B-serum

2, 3, 4, 5 and 6 = Remaining B-serum proteins after alkaline treatment for 15, 30, 60, 120 and 180 days, respectively

2.1.4 Bottom fraction membrane proteins and their proteolipids

The alkaline destabilization (alkaline hydrolysis) of freshly prepared BFM and the BFM whitish proteolipid-fluff were carried out in a solution containing 0.7% ammonia for 15, 30 and 60 days. The proteins that were released into the solution media and those still remained with BFM and the BFM proteolipid-fluff were correspondingly separated and subjected to SDS-PAGE profile analyses (Fig. 24 and 27) and IgE-immunoblotting analyses (Fig. 25 and 28).

Examination of SDS-PAGE protein profile, Triton X-100 extract of freshly prepared BFM was found to comprise predominantly of 17, 22, 30 and 58 kDa proteins (Fig. 24, lane 7). The minor bands are proteins of 20, 33 and 35 kDa. Upon incubation of BFM in 0.7% ammonia for 15 days, most of the BFM proteins, especially the ones around 30-35 kDa, were released into the aqueous alkaline medium whereas only minor portion of 17 and 30-35 kDa proteins left remaining with the BFM (Fig. 24, lane 1 and 2). A rapid disappearance of very alkaline-sensitive proteins were noted on the 25 and 58 kDa found in native BFM proteins. Upon further alkaline treatments (30 and 60 days storage), the released 17 kDa protein was completely degraded /disappeared and those of 30-35 kDa became less visible. An appearance of additional faint protein band of 22 kDa was, however, noticed.

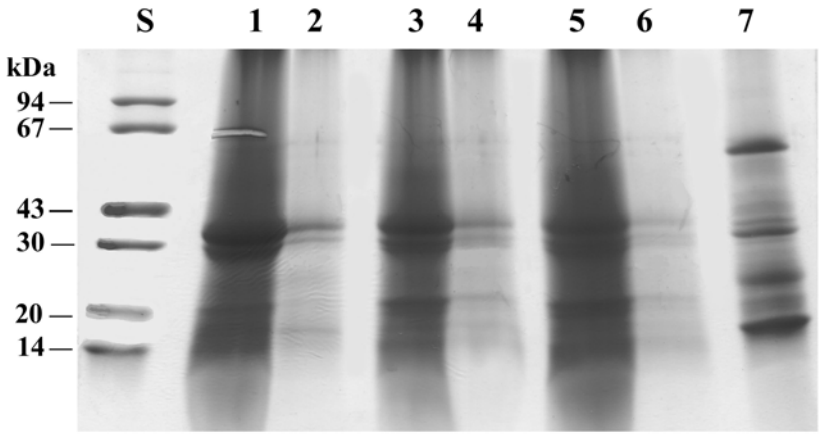

Fig. 24 SDS-PAGE (7-15% gradient gel) of BFM proteins upon various durations of alkaline hydrolysis.

S = Molecular weight markers

1, 3 and 5 = Released BFM proteins found in the alkaline solution after 15, 30 and 60 days of alkaline suspension, respectively

2, 4 and 6 = Remaining proteins in the sedimented BFM after 15, 30 and 60 days of alkaline suspension, respectively

7 = Proteins extracted from freshly prepared BFM

The fate of alkalinized BFM proteins was also followed by SDS-PAGE immunoblotting analyses, using rabbit polyclonal antibodies against the 17 and 30-35 kDa proteins (Fig. 25). The 17 kDa proteins was revealed in both the solution and sedimented BFM after 15 days-alkalinization. Upon further storage, the released 17 kDa protein became more sensitive to alkaline degradation and mostly degraded after 60 days storage (Fig. 25A). On the other hand, an intense broad protein band around 35 kDa was remained detectable in both the solution and sedimented BFM fractions throughout treatment periods up to 60 days storage (Fig. 25B). This result indicate the 17 and 35 kDa proteins as alkaline-stable proteins and the 35 kDa being more stable.

The effects of alkaline treatments on the released BFM proteins obtained after subjected to serial acetone fractionation was further analysed under SDS-PAGE (Fig. 26). At 15 day-alkalinization, most the released BFM proteins were found to be intact as sharp protein bands in all acetone fractionated fractions (Fig. 26A). However, further alkaline treatment (60 days storage), higher degree of degradation on the released BFM proteins was noted in all fractions as smeared/diffused bands (Fig. 26B).

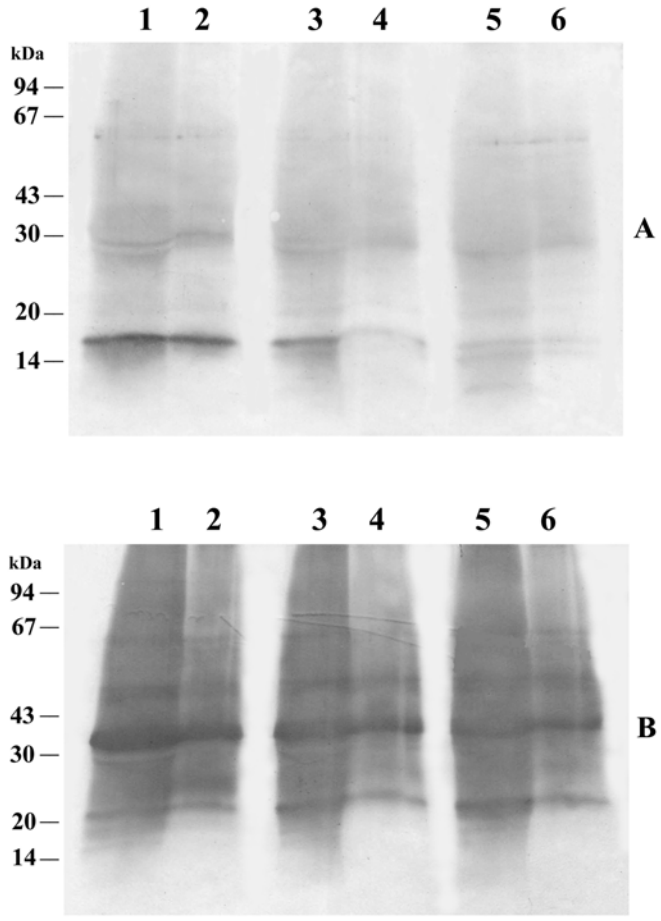

Fig. 25 Immunoblots of alkaline-stable BFM proteins, using rabbit anti- serum against 17 kDa (A) and 30-35 kDa BFM proteins (B).

1, 3 and 5 = Released proteins found in the alkaline solution after 15, 30 and 60 days of alkaline suspension, respectively

2, 4 and 6 = Remaining proteins in the sedimented BFM after 15, 30 and 60 days of alkaline suspension, respectively

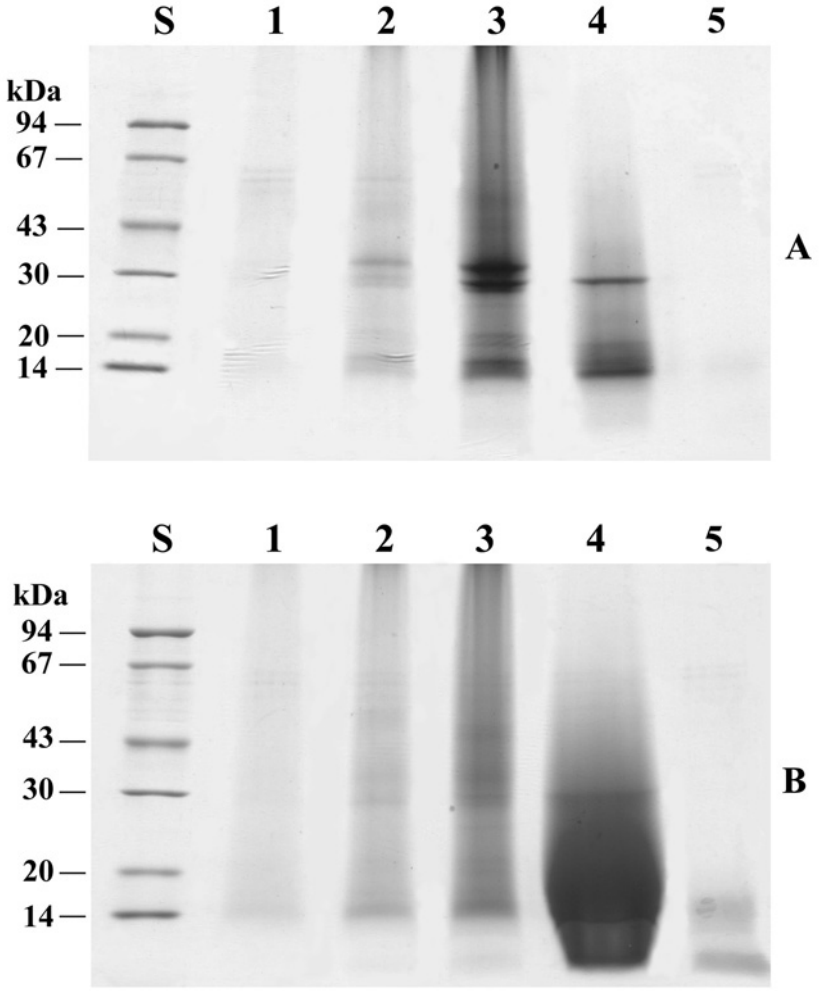

Fig. 26 SDS-PAGE (12% gel) of released BFM proteins found in the alkaline solution after alkaline treatment for 15 days (A) and 60 days (B)

S = Molecular weight markers

1, 2, 3, 4 and 5 = Proteins obtained after acetone fractionation, ranging from 0-20%, 20-40%, 40-60%, 60-80% and 80-95% of saturation, respectively

In the case of BFM proteolipids, the SDS extract of freshly prepared BFM fluff as analysed by SDS-PAGE, contained major protein bands of 33-35 and minor bands of 17 and 65 kDa (Fig. 27, lane 1). A much higher level of 30-35 kDa proteins was obtained when lipid solvent was used instead of the non-ionic detergent in the extraction procedure (Fig. 27, lane 1 and Fig. 24, lane 7). After the BFM fluff was suspended in ammonia solution for 15 days, most of the proteins still retained with the insoluble fluff (Fig. 27, lane 5) and only a minute amount being liberated or released into the solution (Fig. 27, lane 2). The protein pattern of 15-day-old alkaline treated fluff was different from that of the untreated control. The 15-day-old alkaline treated fluff showed the additional protein bands of 22, 30, 43, and 52 kDa (Fig. 27, lane 5). Upon further alkaline treatment, more fluff proteins were found to be continuously liberated into the solution (Fig. 27, lane 2-4). The staining pattern of the liberated proteins was similar to those found remaining in the 15-day storage fluff. Moreover, the intact 22, 30-35, 52 and 65 kDa proteins were still observable in the remaining fluff even after 60 days storage. The results thus indicated the presence of highly alkaline-stable proteins of 22, 30-35, 52 and 65 kDa in the BFM fluff.

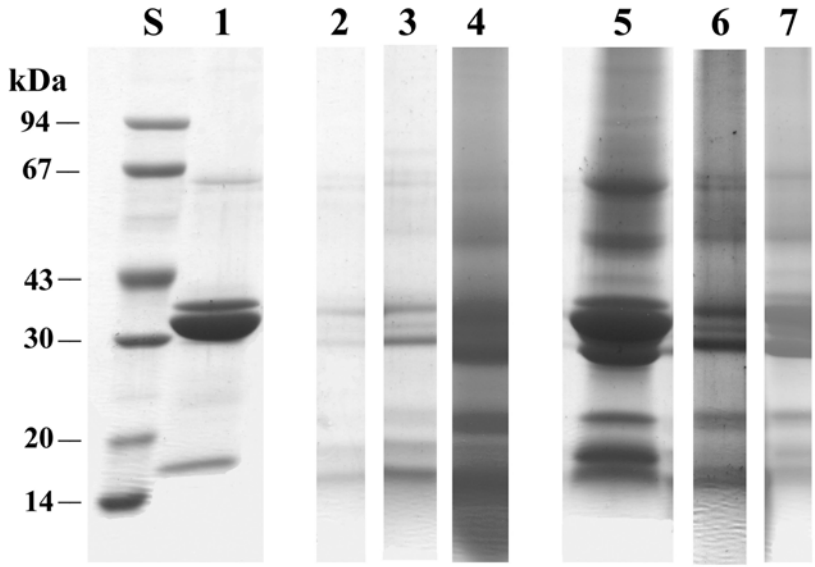

Fig. 27 SDS-PAGE (7-15% gradient gel) of proteolipids from ammonia-treated BFM fluff.

S = Molecular weight markers

1 = Proteolipids from freshly prepared BFM fluff

2, 4 and 6 = Released proteolipids found in the solution after 15, 30 and 60 days of alkaline suspension, respectively

3, 5 and 7 = Proteolipids found in the remaining insoluble fluff portion after 15, 30 and 60 days of alkaline suspension, respectively

Degradation rate and the fate of the released proteins was followed and analysed by immunoblotting with rabbit polyclonal antibodies against 17 and 30-35 kDa proteins (Fig. 28). The released 17 and 30-35 kDa proteins were observed as both intact and partially degraded forms after 15 days storage. However, total degradation of the released proteins was observed and revealed as smeared or diffused bands after 60 day-alkaline treatment. This study thus suggested the BFM proteins that associated with fluff are mainly the alkaline-stable ones.

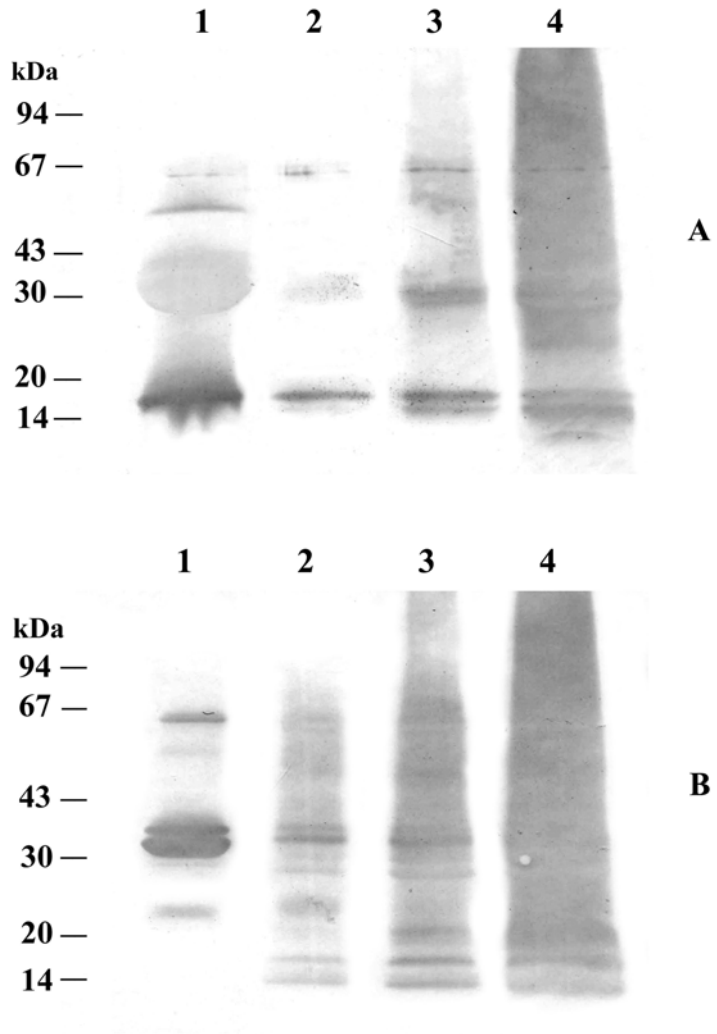

Fig. 28 Immunoblots of proteolipids from alkaline treated BFM fluff, using rabbit antiserum against 17 kDa (A) and 30-35 kDa BFM proteins (B).

1 = Proteolipids of native BFM fluff

2, 3 and 4 = Released proteolipids found in the alkaline solution after 15, 30 and 60 days of alkaline suspension, respectively

2.2 Alkaline-stable proteins in the mixture of C-serum and bottom fraction

Alkaline stability studies on fractionated fresh latex proteins indicated that the proteins of B-serum and BFM are more alkaline-stable than the C-serum ones. However, some of those alkaline-stable proteins found especially in B-serum and BFM, may derived from the same origin. In order to investigate this possibility, a rubber fraction-free latex containing C-serum and the intact bottom fraction was treated with ammonia and stored for 15, 30, 60 and 120 days. Under this condition, the sedimented BFM proteins, suspended in the aqueous mixture of the B- and C-sera, was gradually released its intrinsic proteins into the alkaline solution medium.

Accordingly, most of the protein bands in the alkaline solution medium during the treatments were similar to those of intrinsic BFM proteins. The exceptions were on the 40 and 50 kDa proteins which might originate from C-serum (Fig. 29).

By using Tricine-SDS-PAGE as a means for resolving the small MW of proteins, it was found that some soluble proteins but not the remaining insoluble BFM proteins were hydrolysed into small peptides with MW lower than 14 kDa (Fig. 30). Many protein bands >14 kDa observed among the soluble proteins under various alkaline treatment periods were similarly detected in the Triton X-100 extracted native BFM proteins (Fig. 30, lane 9). This result suggested that most of the alkaline soluble proteins found in the solution media were probably liberated from the BFM.

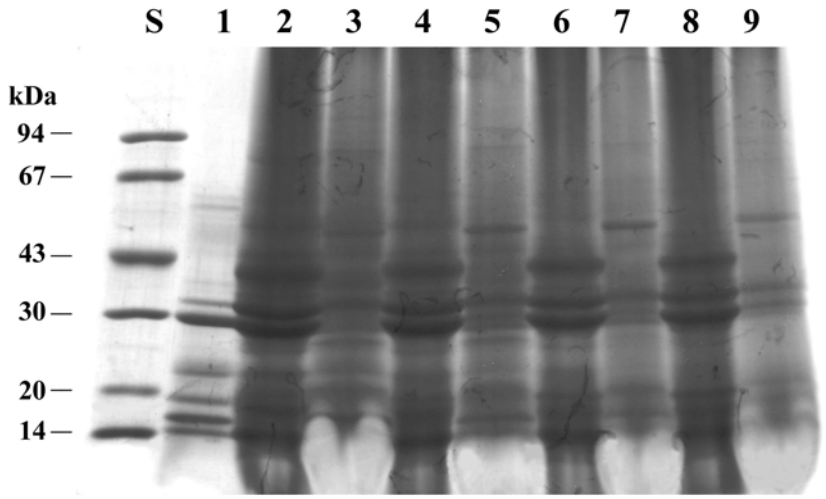

Fig. 29 SDS-PAGE (7-15% gradient gel) of proteins obtained after alkaline treatment on mixture of C-serum and bottom fraction.

S = Molecular weight markers

1 = Proteins extracted from freshly prepared BFM

2, 4, 6 and 8 = Soluble proteins in the solution media after alkaline treatment for 15, 30, 60 and 120 days

3, 5, 7 and 9 = Remaining insoluble proteins in the sedimented BFM after alkaline treatment for 15, 30, 60 and 120 days, respectively.

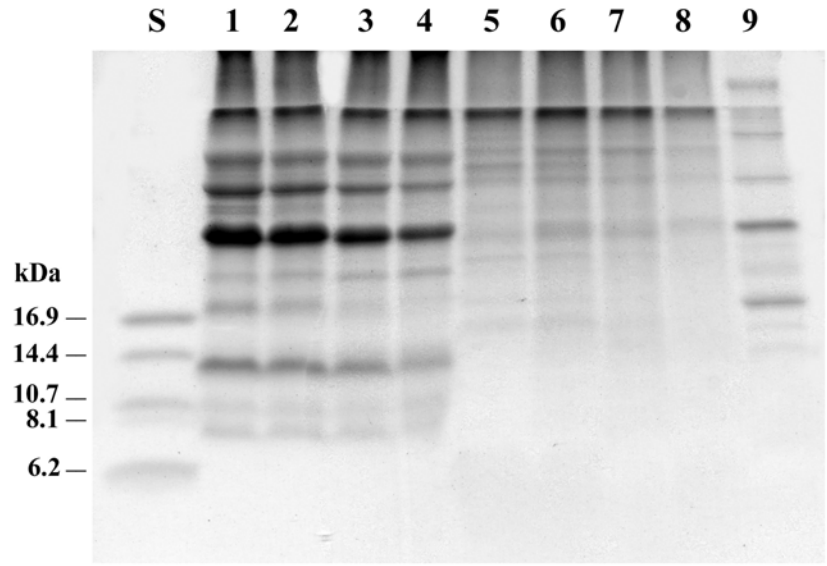

Fig. 30 Tricine-SDS-PAGE of proteins obtained after alkaline treatment on mixture of C-serum and bottom fraction.

S = Molecular weight markers

1, 2, 3 and 4 = Soluble proteins in the solution media after akaline treatment for 15, 30, 60 and 120 days, respectively

5, 6, 7 and 8 = Remaining insoluble proteins in the sedimented BFM after alkaline treatment for 15, 30, 60, and 120 days, respectively

9 = Proteins extracted from freshly prepared BFM

SDS-PAGE immunoblotting by using rabbit polyclonal antiserum against two major BFM proteins (17 and 30-35 kDa proteins) demonstrated the presence of BFM alkaline-stable (17 and 30-35 kDa) components in the solution media (Fig. 31, A and B). The BFM protein at MW of 17 and 30-35 kDa were observed in both solution media and sedimented BFM up to 60 and 120 days, respectively. This result is very similar to those obtained on alkaline treated BFM as shown in Fig. 25. Hence, the alkaline stable 17 kDa and 30-35 kDa proteins observed in the mixture contained both C-serum and bottom fraction were derived from the BFM.

Similarly, SDS-PAGE immunoblotting by using serum IgE positive to BFM proteins also revealed the presence of allergenic BFM proteins in the alkaline solution media and some were stable up to 120 days (Fig. 32). On this immunoblot, the IgE-reactive bands were mainly observed in the solution media in the regions of 17, 22, 30, 33, 45, 55 and >94 kDa (lane 3, 5, 7 and 9). All the IgE-reactive proteins were also detectable in both Triton X-100 extract and proteolipids of the BFM.

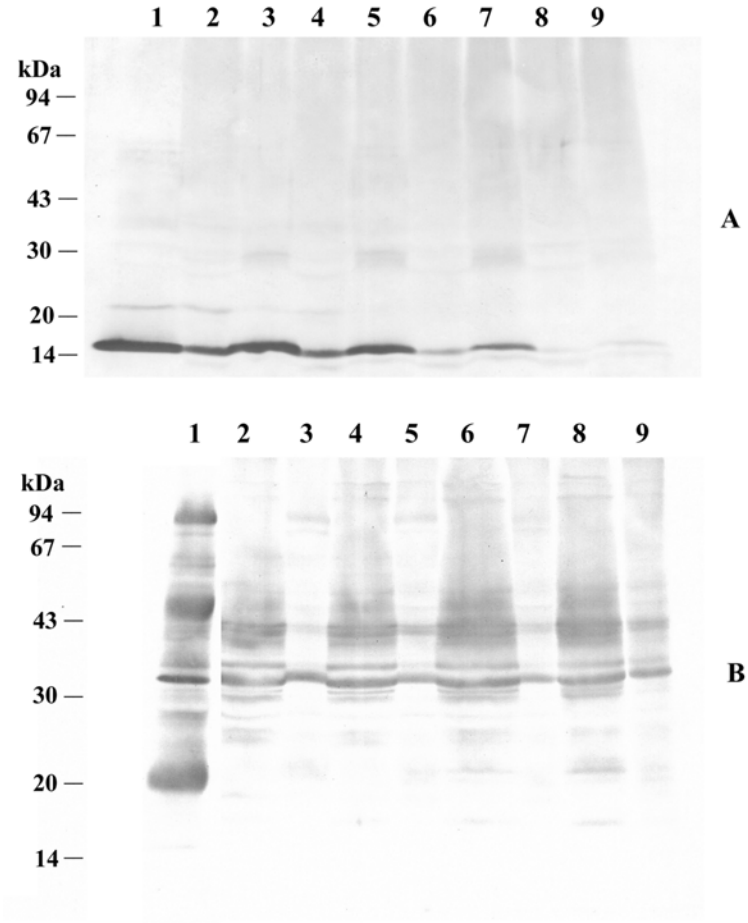

Fig. 31 Immunoblots of alkaline-stable proteins in mixture containing C-serum and bottom fraction, using rabbit antiserum against 17 kDa (A) and 30-35 kDa BFM proteins (B).

1 = Protein extract of freshly prepared BFM

2, 4, 6 and 8 = Soluble proteins in the solution media after alkaline treatment for 15, 30, 60, and 120 days, respectively

3, 5, 7 and 9 = Remaining insoluble proteins in the sedimented BFM after alkaline treatment for 15, 30, 60 and 120 days, respectively

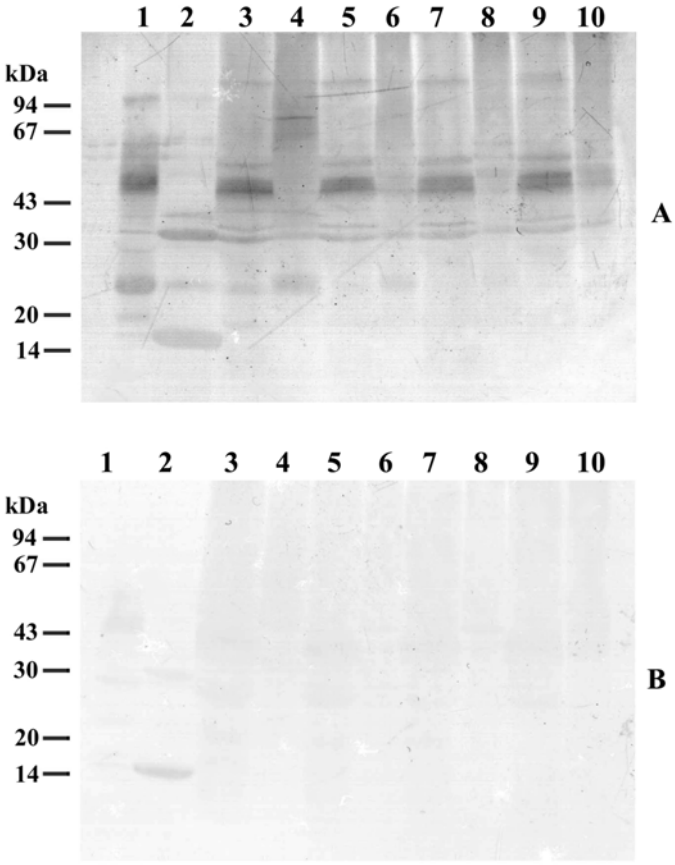

Fig. 32 Immunoblots of proteins obtained after alkaline treatment on a mixture containing C-serum and bottom fraction, using either serum from a patient with latex allergy showing positive SPT to BFM proteins (A) and normal subject (B).

1 = Proteins extracted from freshly prepared BFM

2 = Proteolipids from native BFM

3, 5, 7 and 9 = Soluble proteins in the solution media after alkaline treatment for 15, 30, 60 and 120 days, respectively

4, 6, 8 and10 = Remaining insoluble proteins in the sedimented BFM after alkaline treatment for 15, 30, 60 and 120 days, respectively

Common alkaline-stable proteins were revealed among samples containing either a mixture of non-rubber C-serum and bottom fractions or separately isolated B-serum, C-serum and BFM fractions as analysed by SDS-PAGE (Fig. 33A). Similar alkaline-stable protein patterns were commonly observed among all samples, except for the C-serum. This result suggests that the alkaline-stable proteins found in fractionated B- and C-sera are more or less the same as those found in the solution media of the alkaline-treated mixture containing C-serum and bottom fraction. However, as previously demonstrated in Fig. 31-32, most of the alkaline stable proteins remaining in the solution media of the mixture containing C-serum and bottom fraction were derived from the proteins released from suspended BFM portion. Therefore, the alkaline-stable proteins found in C- and B-serum should also represent the proteins released from the BFM of the bottom fraction during alternate freezing and thawing treatments. Moreover, the presence of BFM proteins in alkaline-treated B- and C-sera was also confirmed by immunoblot analyses using rabbit polyclonal antibodies against the 30-35 kDa of the BFM proteins (Fig. 33B).

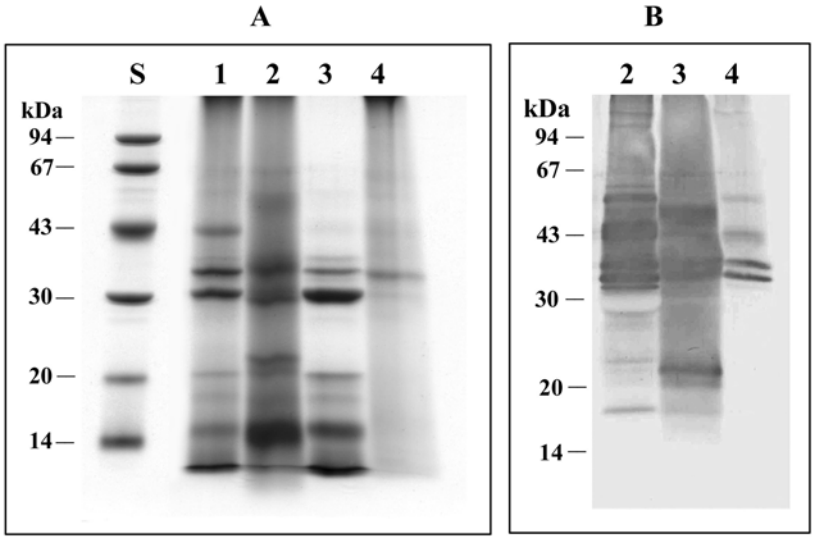

Fig. 33 SDS-PAGE (12% gel) of proteins obtained from 60-day alkaline treatments on either a mixture containing C-serum and bottom fraction or isolated C-serum, B-serum and BFM fractions.

S = Molecular weight markers

1 = Soluble proteins in the solution media of alkaline treated mixture containing C-serum and bottom fraction

2 = BFM proteins released into the ammonia solution

3 = Proteins from ammonia treated B-serum

4 = Proteins from ammonia treated C-serum

A: Coomassie Blue staining

B: Nitrocellulose blot of a replicate gel after incubation with rabbit antiserum against BFM proteins (30-35 kDa) and visual staining with goat anti-rabbit IgG conjugated with peroxidase.

2.3 Source of immunogenic alkaline-stable proteins

In this study, alkaline-stable proteins obtained from 60-day-old ammoniated latex were used as immunogens for raising antibodies in rabbits. The rabbit polyclonal antibodies against those corresponding alkaline-stable proteins were used to identify their local/native sites in various isolated fresh latex fractions.

Fig. 34 showed SDS-PAGE of the C-serum, rubber particle membrane, B-serum and BFM extracts and their Western- immunoblot using rabbit polyclonal antibodies against alkaline-stable proteins. Although numerous coomassie stained bands were on the C-serum, the immunoblot revealed only two predominant anti-alkaline-stable protein reactive bands at 27 and 40 kDa. On the other hand, several IgG reactive-bands were observed on the immunoblots of B-serum and BFM proteins covering a wide range of molecular weight. This result is in agreement with previously study on alkaline treated C-serum, B-serum and BFM which indicated higher number of alkaline-stable proteins in the B-serum and BFM than the C-serum (Fig. 22-24). Although the protein band pattern of B-serum was somewhat similar to those of BFM. However, there were significant differences in the degree of protein binding response towards IgG against alkaline-stable protein. The anti-alkaline-stable protein IgG recognized both 14 kDa and 24 kDa proteins in the rubber particle extract. This is in agreement with the result obtained from previous study on alkaline-treated rubber particles (Fig. 21).

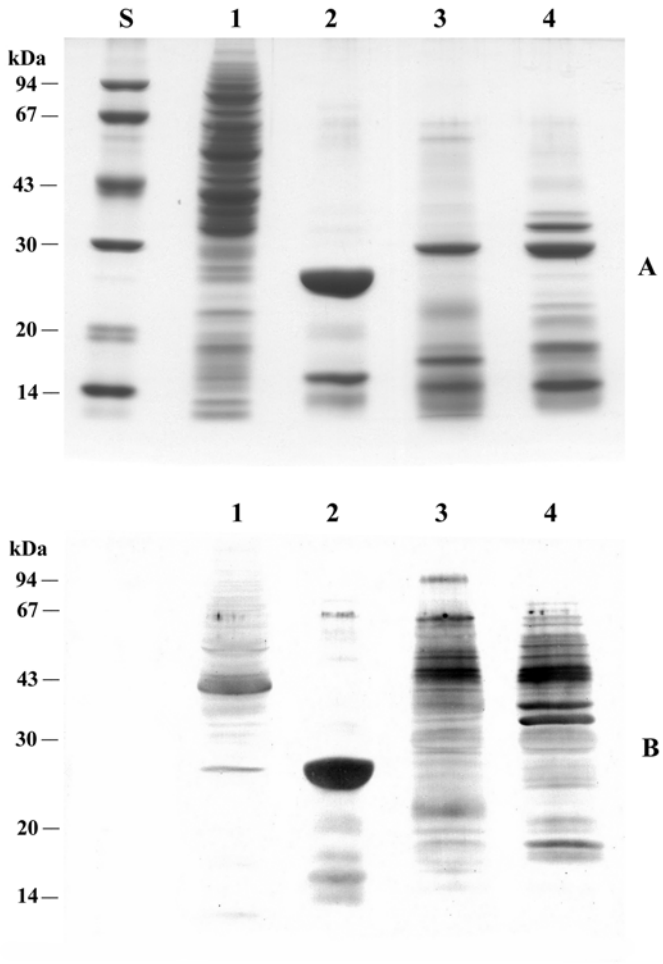

Fig. 34 SDS-PAGE (12% gel) and immunoblotting of proteins from various fraction of fresh latex.

S = Molecular weight markers, 1 = C-serum proteins, 2 = Rubber particle proteins, 3 = BFM proteins, 4 = B-serum proteins

A: Coomassie Blue staining

B: Nitrocellulose blot of a replicate gel after incubation with rabbit anti-ammoniated latex IgG and visual staining with goat anti-rabbit IgG conjugated with peroxidase.

2.4 Isoelectric focusing of alkaline-stable BFM proteins

The charge property of alkaline-stable BFM proteins released into solution medium after 60-day alkaline treatment was compared to those the native proteins extracted from freshly prepared BFM. Upon IEF polyacrylamide gel, there were fewer number of alkaline-stable proteins comparing to those extracted from freshly prepared BFM (Fig. 35). The alkaline-stable BFM proteins were found only in the acidic region with isoelectric points (pI) of 4.7 to 6.7 whereas the native BFM proteins showed significantly higher number of both anionic and cationic protein bands with pI ranging from 4.3 to 9.5.

2.5 Heat stability of alkaline-stable BFM proteins

A prevulcanization of compounded latex concentrate at 70° C for 2 hours is generally required before using as the dipping latex material. Hence, to study the heat stability of alkaline-stable BFM proteins similar to the prevulcanization process, the solution containing a portion of proteins released from the 60-day alkaline treated BFM was heated at 70° C for 2 hours.

Upon SDS-PAGE, the protein patterns obtained from the heated and unheated control samples were quite similar (Fig. 36). This indicated that the alkaline-stable proteins released from BFM are also tolerant to the heat required on the process of prevulcanization.

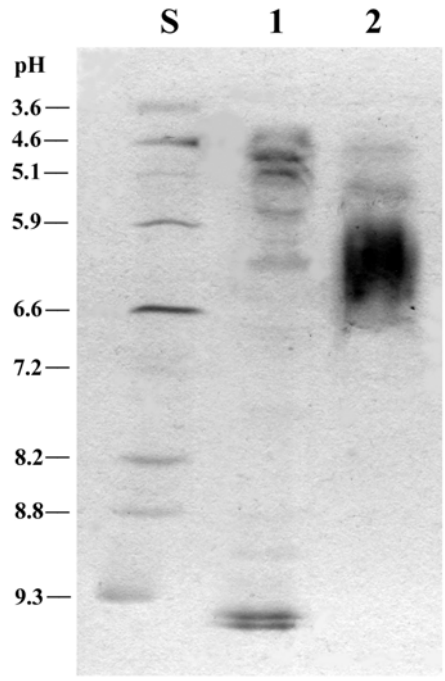

Fig. 35 IEF of alkaline treated and untreated control BFM proteins.

S = Standard marker pI

1 = Proteins extract of freshly prepared BFM

2 = Released BFM proteins found in the ammonia solution after alkaline treatment for 60 days

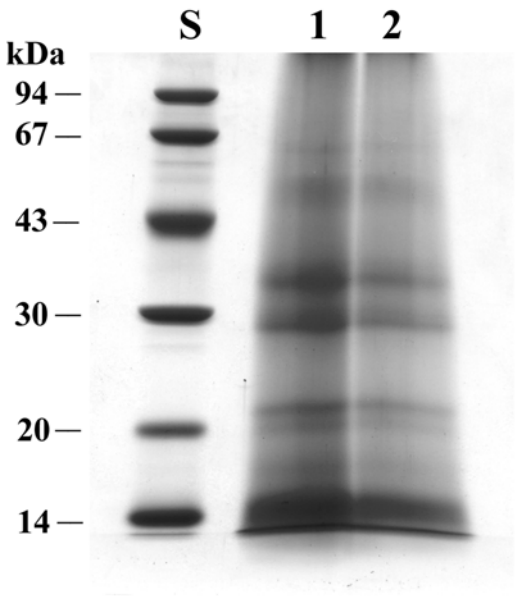

Fig. 36 SDS-PAGE (12% gel) of heated and unheated control of the released BFM proteins found in the ammonia solution after 60 days of alkaline suspension.

S = Molecular weight markers

1 = Control alkaline-stable BFM proteins

2 = Heated alkaline-stable BFM proteins

Part 3. Latex concentrate

Latex concentrate is processed from fresh field latex by initial addition of 0.4% ammonia and some secondary chemical preservatives to prevent latex coagulation. The ammoniated latex was then stored overnight in the settling tank to sediment heavy particulated materials or sludge before subjected to concentration by means of continuous flow centrifugation on the next day. After centrifugation, the level of ammonia in latex concentrate is adjusted to 0.7%. The high ammoniated (HA) latex concentrate is usually stored for about 1-2 months before using it as the raw material to produce dipping products such as glove, condom, and other medical devices etc. All processing steps certainly contribute to the changes in latex composition including the proteins of latex concentrate and its corresponding products.

3.1 Changes in latex composition upon field latex processing into latex concentrate

In this study, latex composition at different stages of latex concentrate preparation and the composition of sludge were separated by ultracentrifugation.

Fig. 37(A) represented fractionated fresh field latex (4 h after tapping) without any ammoniation. As expected, it consisted of 3 major fractions; rubber particles, C-serum and bottom fraction with the Frey-Wylssing (FW) particles beneath the uppermost layer. In contrast, the ultracentrifuged fresh field latex obtained 90 min after adding 0.4% ammonia together with secondary preservatives (ZnO_2 and TMTD, 0.1% w/w) lost only almost all of its bottom fraction. Only 2 major fractions; rubber particles and the translucent serum were

observed (Fig. 37B). Most of the Frey-Wylssing particles were still detectable on the upper bottom phase though some sedimented at the bottom of UC tube. Bursting of membrane bound organelles due to ammoniation led to rubber aggregate formation between certain portion of lutoid membrane and rubber particles and float to the top rubber layer (Piyaporn, 2001). The unaggregated membrane remained as suspended debris in the turbid serum and very small bottom fraction portion.

Upon storage of the ammoniated latex in the settling tank for an overnight, some sedimented sludge was observed at the bottom of the tank. At this stage, the latex collected from the upper part of the tank separated into rubber particles and serum fraction upon ultracentrifugation (Fig. 38A) whereas the latex from the lower part contained additional whitish sludge at the bottom of UC tube (Fig. 38B). It was also observed in this stage that the serum of latex located on upper part of the settling tank was less turbid than the latex serum obtained from previous shorter alkalinization period of 90 min (Fig. 37B). This evidence thus indicated that some of the free or unaggregated BFM membrane, originally seen in the turbid serum, sedimented into the bottom part of the settling tank during overnight storage.

After concentrating the latex by mean of continuous flow centrifugation, the concentrate and skim latex were separately obtained. Upon fractionation by ultracentrifugation, the latex concentrate consisted of 60% of rubber particles and 40% of very clear serum. The presence of FW particles on the upper phase of UC tube as in the case of field latex was not observed in latex concentrate

(Fig. 39A). A minute amount of rubber particles, C-serum and some sludge at the bottom of UC tube were observed on ultracentrifuged skim latex (Fig. 39B).

In addition to two main products obtained as latex concentrate and skim latex during centrifugation, a certain portion of latex serum (Fig. 14A) and sludge (Fig. 14B and 14C) were left remaining within the bowl casing of the centrifuge. As resolved by ultracentrifugation, the sludge consisted mainly of sedimented pellet, FW and rubber particles (Fig. 40A) whereas the remaining latex serum contained FW and rubber particles (Fig. 40B).

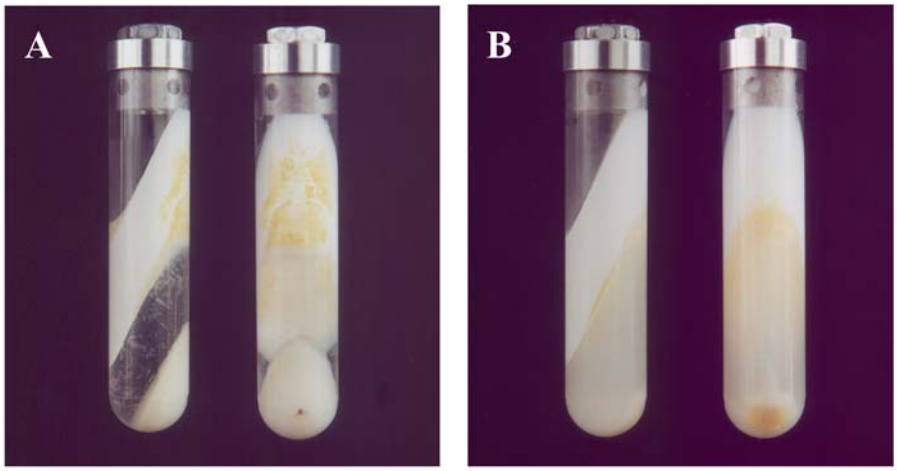

Fig. 37 Ultracentrifuged field latex before and after additions of ammonia and secondary preservatives.

A = Field latex (4 h after tapping) before ammoniation

B = Field latex separated 90 min after additions of 0.4% ammonia and secondary preservatives (ZnO_2 and TMTD)

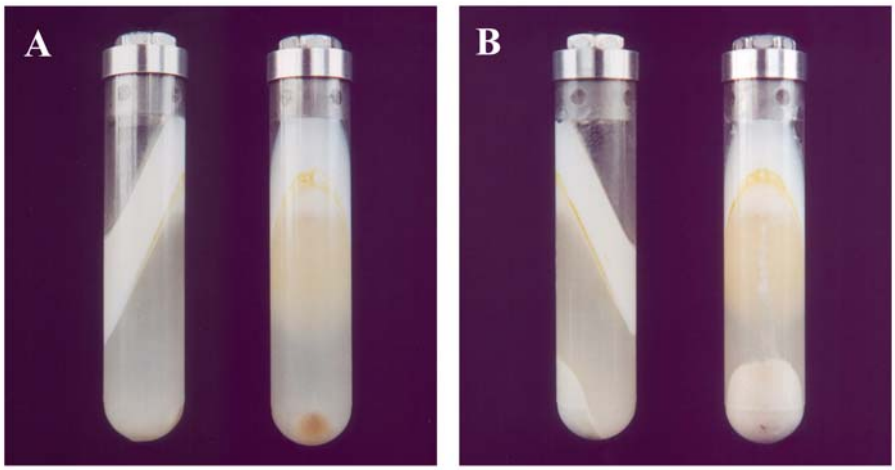

Fig. 38 Ultracentrifugation of ammoniated latex obtained after an overnight storage in the settling tank.

A = Ammoniated latex collected from the upper part of settling tank

B = Ammoniated latex collected from the bottom part of settling tank

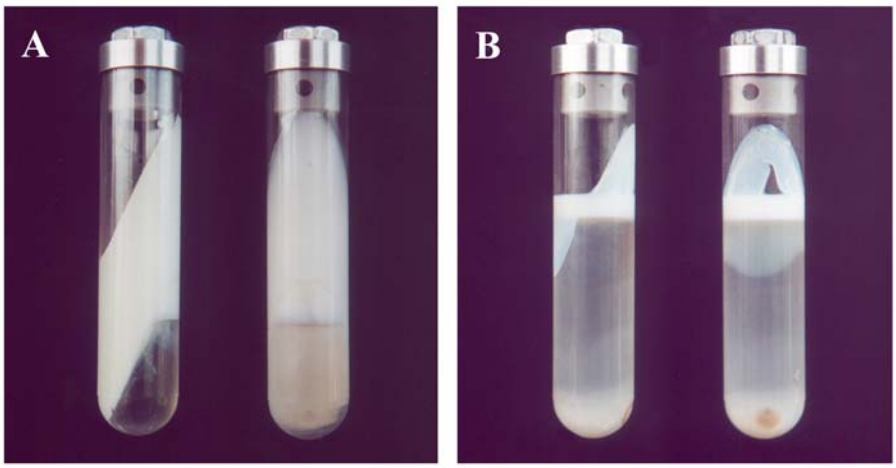

Fig. 39 Ultracentrifugation of latex concentrate and skim latex.

A = Latex concentrate

B = Skim latex

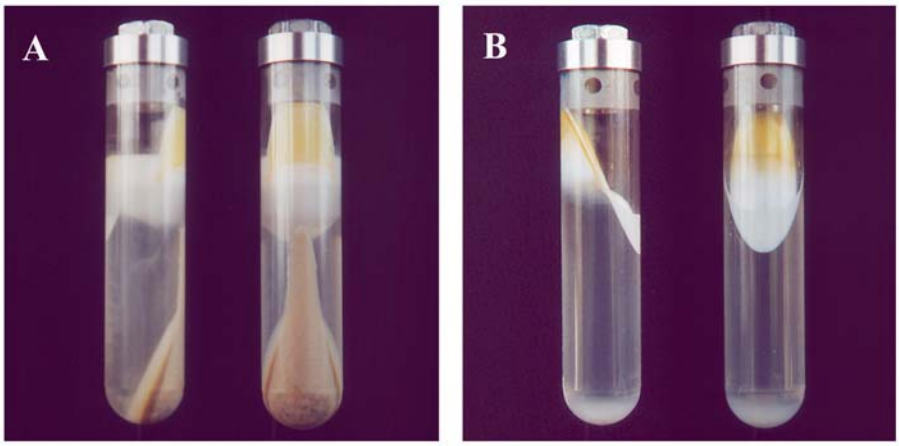

Fig. 40 Ultracentrifugation of latex sludge and remaining serum found within the centrifuge bowl.

A = Remaining sludge on the upper part of bowl casing

(diluted with one time of isotonic buffer)

B = Remaining serum found within the centrifuge bowl

3.2 Proteins of HA latex concentrate

Proteins of HA latex concentrate are distributed between two main fractions, the serum fraction and the rubber fraction.

SDS-PAGE of the proteins extracts obtained from HA latex concentrate under different storage period revealed gradual disappearance of proteins in the serum and rubber fractions along the alkaline aging period (Fig. 41).

In the serum fraction, the 4-day-old latex concentrate showed the predominant bands with MW of >14, 14, 20, 30, 33, 35, 43 and 55 kDa (Fig. 41, lane 1). The 37-day-old latex concentrate also still revealed less predominant bands but similar to the 4-day-old latex concentrate (Fig. 41, lane 2). For longer storage of HA latex concentrate (101 and 145-day-old), almost all of the sharp serum protein bands turned into faint or smeared bands (Fig. 41, lane 3 and 4). Moreover, it was noticed that the serum protein patterns obtained from 4-day-old latex concentrate were somewhat similar to the soluble and insoluble protein obtained after the alkaline treatment on the C-serum and bottom fraction mixture (Fig. 29 and 33,A). This evidence thus indicates that, except the rubber particle proteins, all other latex concentrate serum proteins were originated from BFM.

In rubber fraction of latex concentrate, stored at either periods, a prominent band at 14 kDa and less prominent bands at 20, 22, 28 and 33-35 kDa were always present (Fig. 41, lane 5-8). The presence of the 14 kDa, which is native big (less dense) rubber particle, as a very thick band in SDS-PAGE profile indicated that zone1 rubber particles are the major rubber component in latex concentrate. The appearance of the less predominant bands in rubber fraction at MW of 20, 22, 28, and 30-35 kDa also indicated the presence of BFM proteins

associated with rubber particles via aggregation which could take place at the stage of ammoniation.

SDS-PAGE and immunoblotting using rabbit antiserum against two main BFM proteins (17 and 30-35 kDa) demonstrated the heavily presence of the BFM proteins in the latex concentrate serum and only slightly present in the rubber fraction (Fig. 42). The bands in the region of 33 and 35 kDa were highly observed in the latex concentrate serum and rubber fraction under all storage times (Fig. 42A) whereas the 17 kDa band was only slightly observed in the serum and rubber fraction of 4-day-old latex concentrate (Fig. 42B).

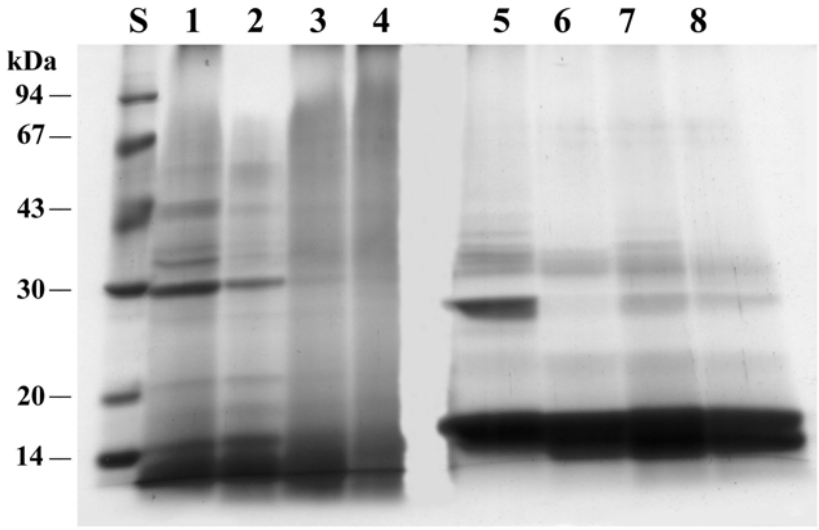

Fig. 41 SDS-PAGE (12% gel) of proteins isolated from the serum and rubber fractions of HA latex concentrate.

S = Molecular weight markers

1, 2, 3 and 4 = Serum proteins from 4, 37, 101 and 145-day-old latex concentrate

5, 6, 7 and 8 = SDS-extracted proteins of RP isolated from 4, 37, 101 and 145-day-old latex concentrate

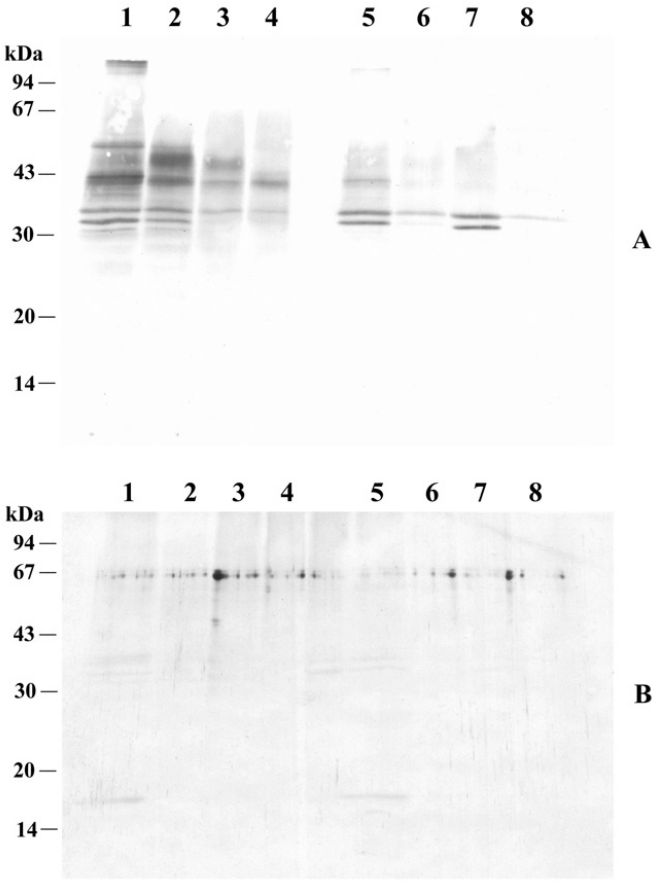

Fig. 42 SDS-PAGE and immunoblots of proteins obtained from HA latex concentrate, using rabbit antiserum against 30-35 kDa (A) and 17 kDa BFM protein (B).

1, 2, 3 and 4 = Serum proteins from 4, 37, 101 and 145-day-old latex concentrate

5, 6, 7 and 8 = SDS-extracted proteins of RP from 4, 37, 101 and 145-day-old latex concentrate

3.3 Sludge protein content

During processing of field latex into latex concentrate, sludge formation was usually observed at the bottom of settling tank prior to latex concentration and also within the bowl casing of the centrifuge during the machine operation.

Fig. 43 showed SDS-PAGE of Triton X-100 extracted sludge proteins from various sites collected during processing of latex concentrate. Similarity in protein patterns were observed between BFM and sludge proteins on the SDS-PAGE profile. This indicated that BFM is the major component of sludge that sedimented at the bottom of the settling tank and remained within the bowl casing of the centrifuge. The remaining non-rubber-aggregating BFM portion in the serum of ammoniated latex is expected to be the main source of BFM found in the sludge (Fig. 37B and 38B).

Proteolipids recovered from sludge chloroform-methanol extraction were somewhat similar to those from freshly prepared BFM (Fig. 44). The unwashed sludge revealed more of the 30-35 kDa than the 17 kDa protein (Fig. 44, lane 5 and 6) whereas the washed sludge consisted mainly of 17 kDa protein (lane 1-4).

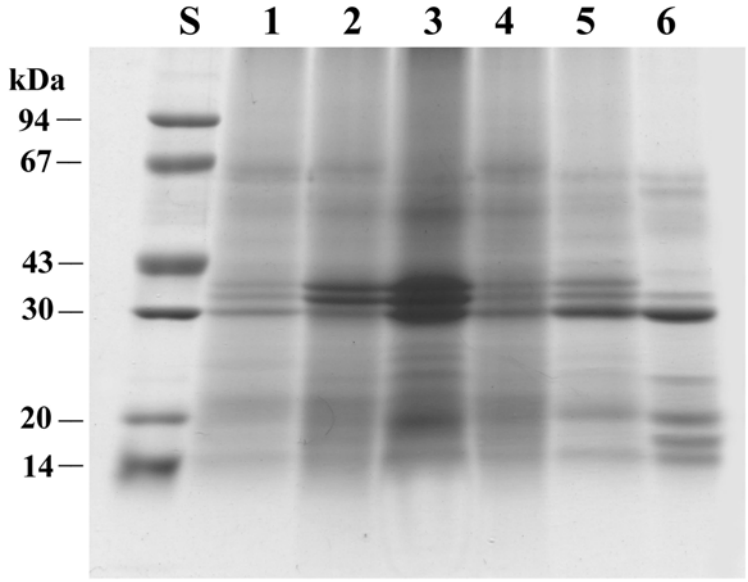

Fig. 43 SDS-PAGE (12% gel) of Triton X-100 extracted proteins from various sludge occurred during the producing of latex concentrate.

S = Molecular weight markers

1 = Proteins extracted from sludge found at the bottom of settling tank

2 = Proteins extracted from sludge found beside the bowl casing, lower portion of bottom fraction of centrifuged sludge

3 = Proteins extracted from sludge found beside the bowl casing, upper portion of bottom fraction of centrifuged sludge

4 = Proteins extracted from sludge found on the upper part of bowl casing, lower portion of bottom fraction of centrifuged sludge

5 = Proteins extracted from sludge found on the upper part of bowl casing, upper portion of bottom fraction of centrifuged sludge

6 = Proteins extract of freshly prepared BFM

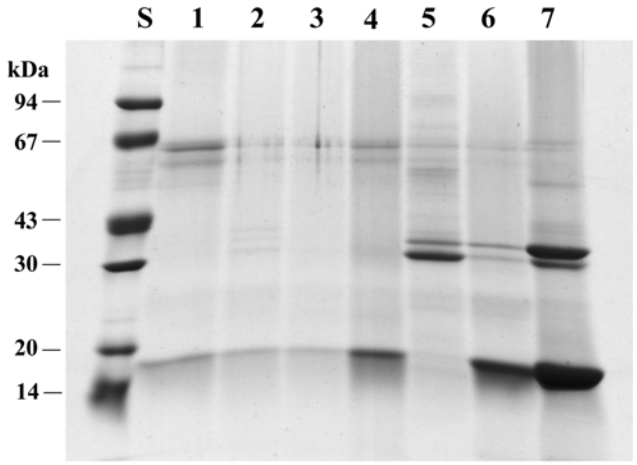

Fig. 44 SDS-PAGE (12% gel) of sludge proteolipids.

S = Molecular weight markers

1 = Proteolipids extracted from washed sludge found at the bottom of settling tank

2 = Proteolipids extract of lower portion of centrifuged washed sludge obtained along the side of bowl casing

3 = Proteolipids extract of upper portion of centrifuged washed sludge found along the side of bowl casing

4 = Proteolipids extract of washed sludge taken from the upper part of bowl casing

5 = Proteolipids extract of unwashed sludge obtained along the side of bowl casing

6 = Proteolipids extract of unwashed sludge taken from the upper part of bowl casing

7 = Proteolipids extract of freshly prepared BFM

Part 4. Latex gloves

Normally latex concentrate is aged under alkaline solution about 2 month to meet demanding physical properties before being further compounded with sulphur vulcanizing ingredients and prevulcanized at 70°C for 2 hours. The compounded latex is then ready to be used as dipping material in producing latex gloves. During the manufacturing process, most of contaminated chemicals including soluble proteins on the surface of latex gloves are leached/washed by water and the latex gloves finally dried in the oven.

In this study, extractable protein from different brands of latex gloves were compared and the presence of BFM proteins examined. Furthermore, the native sources of immunogens found in ultra-low proteins glove were also analyzed.

4.1 Proteins in latex gloves

Six brands of latex gloves comprising of three brands of examination glove (Usison, Safe Skin and Mala), two brands of surgical gloves (Doctor Hand and Unknown) and one brand of household gloves (Ansell) were used in this study.

Table 9 showed the amount of extractable soluble proteins from various brands of latex gloves as assayed by the method of ASTM (D 5712-95). The highest extractable soluble proteins (818.2 μg/g glove) was observed on Mala glove. Safe Skin glove has the lowest extractable soluble proteins (86.8 μg/g glove) but are still relatively high when compared to the recommended standard level by the US-FDA (<50 μg/g glove).

Table 9 Protein contents of various brands of latex glove.

Gloves	Type	Protein concentration (μg/g glove)
Unison	Examination glove	128.5
Mala	Examination glove	818.2
Safe Skin	Examination glove	86.8
Doctor Hand	Surgical glove	228.0
Unknown	Surgical glove (reuse)	286.5
Ansell	Household glove	190.5

SDS-PAGE of protein patterns of the extracts obtained among different brands of latex gloves were quite similarity, especially among those with high protein contents (Fig. 45A). The most frequent bands observed in latex glove extracts were in the region of <14, 14, 20, 22, 30-35, 43 kDa.

SDS-PAGE-immunoblotting using rabbit polyclonal antiserum against extractable BFM proteins (Fig. 45B) and 30-35 kDa BFM proteins (Fig. 46) demonstrated the presence of BFM proteins in all brands of latex glove. The immunoblot with antiserum against extractable BFM proteins showed the predominant bands at MW of 43 kDa in all brands of latex glove extracts besides the ones on 35 and 55 kDa observed on Mala glove and Doctor hand, respectively (Fig. 45B). The presence of 30-35 kDa proteins in the Mala was clearly observed in the immunoblot using rabbit antiserum against 30-35 kDa BFM proteins (Fig. 46).

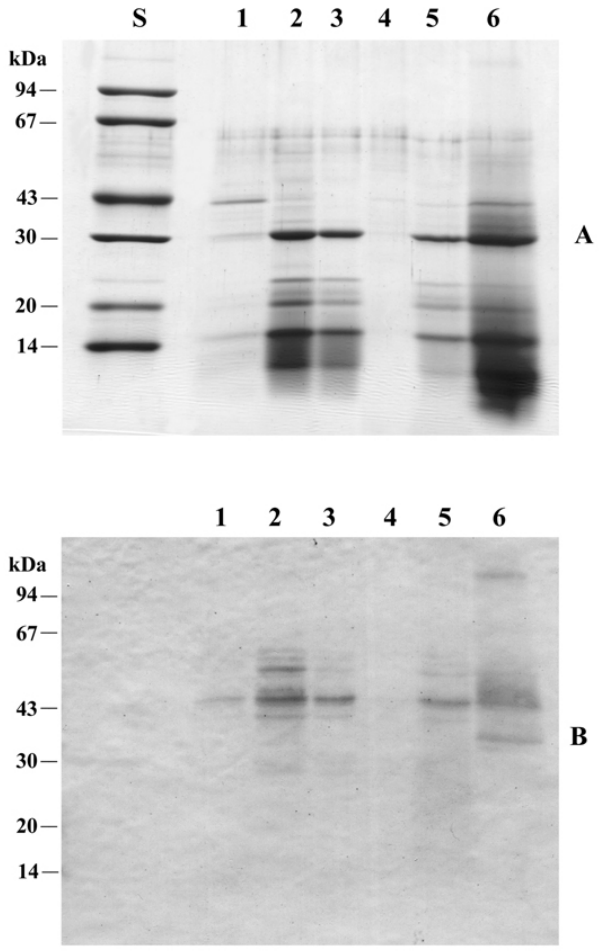

Fig. 45 SDS-PAGE (12% gel) and immunoblotting of extractable proteins from various brands of commercial latex gloves.

S = Molecular weight markers, 1 = Examination glove (Unison), 2 = Surgical glove (Doctor hand), 3 = Examination glove (Safe skin), 4 = Household glove (Ansell), 5 = Surgical glove (Unknown), 6 = Examination glove (Mala)

A: Coomassie Blue staining

B: Nitrocellulose blot of a replicate gel after incubation with rabbit antiserum against extractable BFM proteins and visual staining with goat anti-rabbit IgG conjugated with peroxidase.

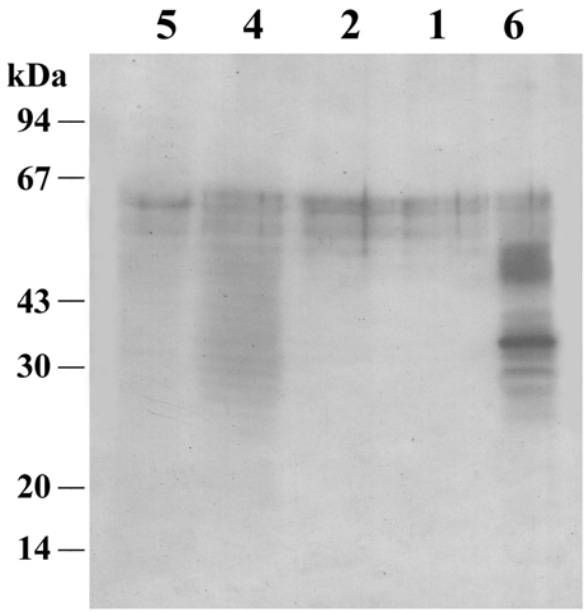

Fig. 46 SDS-PAGE and immunoblotting of extractable proteins from various brands of commercial latex gloves, using rabbit antiserum against 30-35 kDa BFM proteins.

1 = Examination glove (Unison), 2 = Surgical glove (Doctor hand) 4 = Household glove (Ansell), 5 = Surgical glove (Unknown), 6 = Examination glove (Mala)

4.2 Immunogens in ultra-low proteins gloves

Pooled glove protein extracts from nine brands of latex glove, each containing extractable proteins < 50 μg/g glove, were used as the immunogens. Rabbit polyclonal antibodies against glove extractable proteins were used in immunoblot to verify their origins in various isolated fresh latex protein fraction.

The SDS-PAGE of the C-serum, rubber particle membrane, B-serum and BFM extracts and its immunoblot using rabbit antiserum against ultra-low protein glove extracted proteins was demonstrated in Fig. 47A and B, respectively. The antibodies against proteins from ultra-low protein gloves could not recognized proteins on rubber particles which were heavily stained by coomassie blue as 14 and 24 kDa. There were numerous proteins in the C-serum but only the 67 kDa was recognized by the anti-glove antibodies. On the other hand, several bands of B-serum and BFM proteins under a wide range of molecular weights were visualized on the immunoblot. The immunoblot of BFM showed the prominent bands in the region of 17, 20, 30, 33, 35, 43, 45, 55 and 58 kDa (Fig. 47B, lane 3 and 4). However, due to the contamination of BFM proteins in B-serum as previously described, most of these bands were also found in B-serum blot (Figure 47B, lane 2). This study thus provides the evidence that the bottom fraction proteins, especially those released from the membrane, are a major source of antigens found in the ultra-low protein glove.

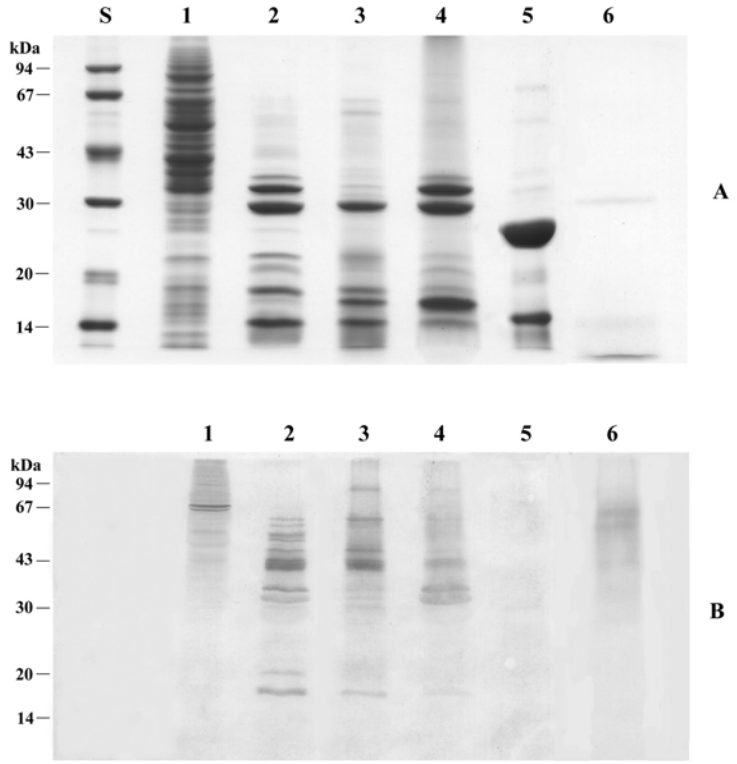

Fig. 47 SDS-PAGE (12% gel) and immunoblotting of proteins from various fraction of fresh latex and ultra-low protein glove extract.

S = Molecular weight markers, 1 = C-serum proteins, 2 = B-serum proteins, 3 = Triton X-100-extracted BFM proteins , 4 = BFM proteolipids, 5 = Rubber particle proteins, 6 = Ultra-low protein glove extract

A: Coomassie Blue staining

B: Nitrocellulose blot of a replicate gel after incubation with rabbit anti-ultra-low protein glove IgG and visual staining with goat anti-rabbit IgG conjugated with peroxidase.

Addition to the analysis of immunoblotting, the source of immunogenic glove proteins was also carried out by the indirect ELISA technique.

Fig. 48 showed a titration of rabbit anti-glove protein where a single dilution of rubber particle, C-serum, B-serum and BFM proteins (50 μg/ml) was coated onto microtitre plates and the rabbit antiserum titrate out in a two fold dilution series. It is clear from the graph shown that the rabbit glove protein titred out to about 1:32,000 against B-serum and BFM extracts whereas the titre against C-serum and rubber particle extract was clearly greater that 1:1,000 and 1:500, respectively.

In a typical dose response experiment using a single dilution of rabbit anti-glove protein (1:16,000) and two fold serial dilutions of rubber particle, C-serum, B-serum and BFM in an indirect ELISA, the rabbit antiserum was shown to be highly reactive towards the proteins from B-serum and BFM extracts in a dose dependent manners. However, only relatively very small reactivity observed with the C-serum and rubber particle extracts (Fig. 49).

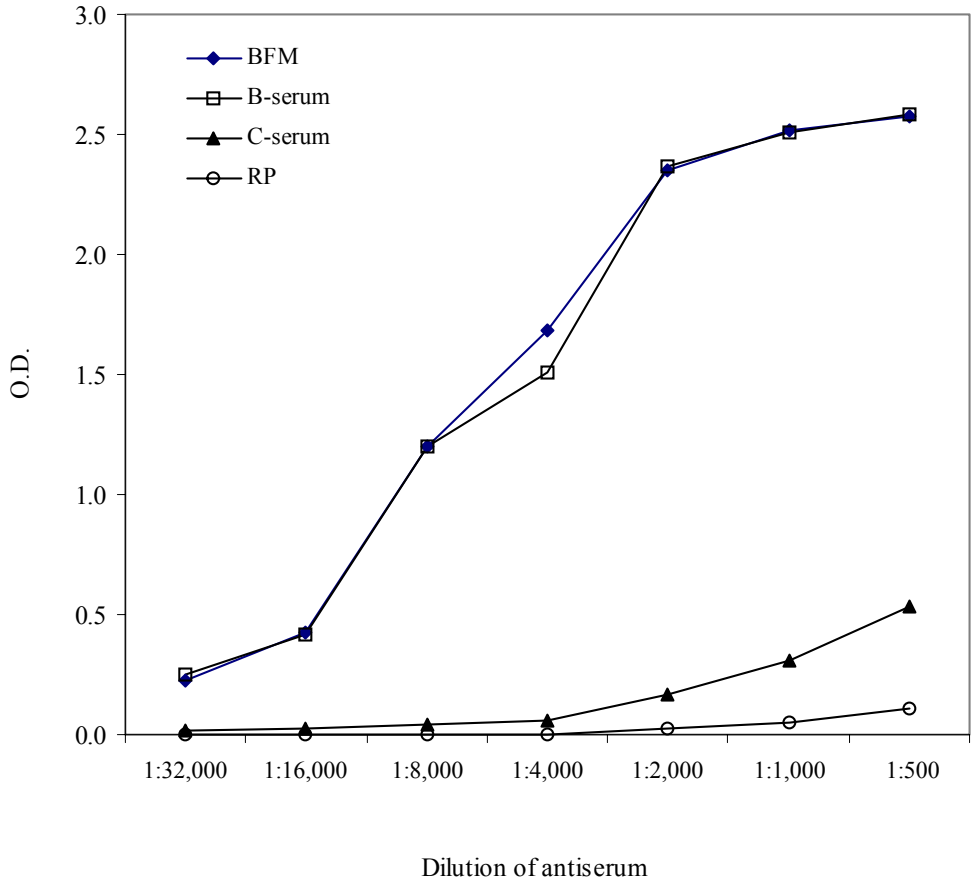

Fig. 48 Tritation of rabbit antiserum against ultra-low protein glove extract in an indirect ELISA using microtitre plates coated with BFM, B-serum, C-serum and rubber particle extracts at respective concentration of 0.5 μg/ml.

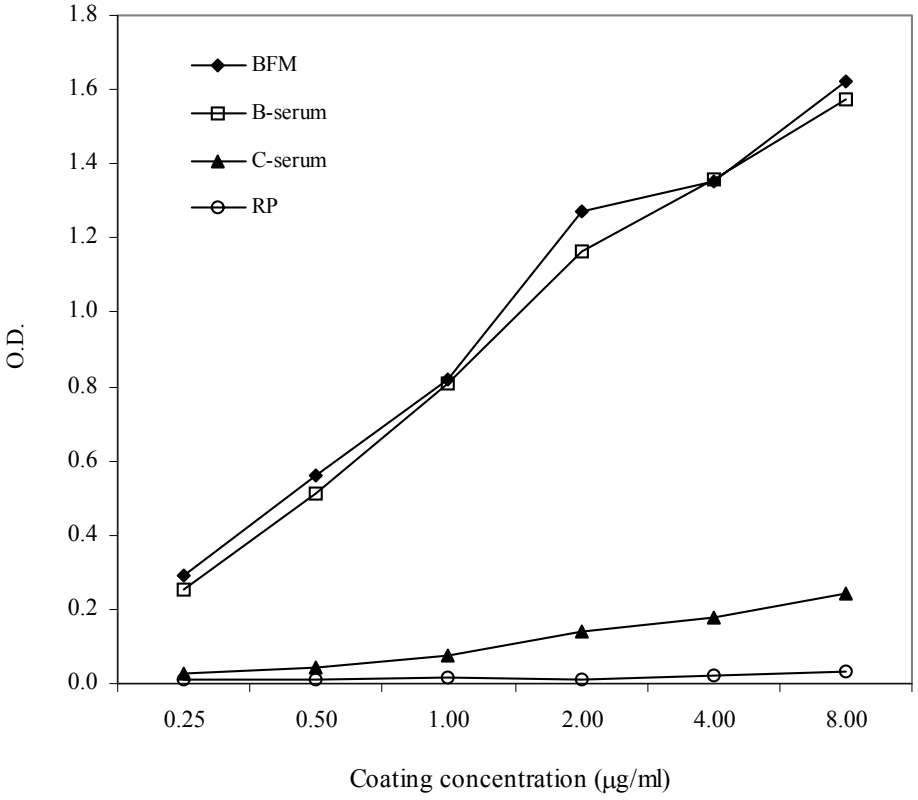

Fig. 49 Dose response relationships of BFM, B-serum, C-serum and rubber particle protein extracts in an indirect ELISA using rabbit antiserum against ultra-low protein glove extract at 1: 16,000 dilution-fold.