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Binding Propensity of Modified Corn Starch and Oat Starch for NR Latex Proteins and Ways to Minimise the Interaction

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The affinity of absorbable dusting powder (ADP) corn starch and oat starch for NR latex proteins was assessed by determining the amount of fresh latex serum proteins and glove proteins bound to the powders. Factors affecting the protein uptake were investigated.

In agreement with earlier reports, ADP corn starch and oat starch bound non-ammoniated latex proteins, with corn starch binding more than oat starch. Corn starch, however, showed preferential binding affinity towards acidic proteins which constituted the major component of extractable glove proteins. The adsorption of glove proteins to corn starch could be markedly reduced by introducing certain anionic constituents into the slurry. Changing the alkaline slurry to neutral pH value, choosing appropriate corn starch powder, lowering slurry temperature, incubation time and powder content and incorporation of post leaching could further reduce the protein uptake to minimal levels.

Oat starch, on the contrary, showed no detectable binding to acidic glove proteins but some interaction with basic serum proteins.

Key words: dusting powder; corn starch; oat starch; NR latex; proteins; serum; glove; binding affinity; powder slurry; buffer

Modified corn starch has long been applied to medical gloves to facilitate glove donning and glove separation as well as to increase comfort and sensitivity for the health care providers. Reports[1,2] on allergic reactions with respiratory symptoms during use of powdered gloves show the possible role of the powder as aeroallergens. Corn starch powder which has been modified to meet the United States Pharmacopoeia (USP) specification for absorbable dusting powder and which has not been in contact with latex does not elicit allergic reactions[3]. The reactions, however, are associated with the latex proteins adsorbed by the powder[3–5]. A study by Tomazic *et al.*[6] demonstrates that corn starch binds ammoniated and non-ammoniated latex proteins and glove proteins, with the binding to non-ammoniated latex proteins predominating. The powder-bound proteins are also tested to be allergenic.

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Oat starch is introduced as an alternative to modified corn starch powder with the claim of having much lower binding propensity for latex allergenic proteins. The claim is substantiated by a comprehensive study[7] comparing the binding affinity of the two powders for latex allergens under both laboratory conditions and in commercial glove production lines. The study also highlights the possibility that corn starch powder may contain more extractable allergens than the finished gloves on a mass basis. The opposite seems to occur with oat starch.

To date, factors affecting the binding affinity of the powders for NR latex proteins remain unclear. It is the objective of this study to not only compare the binding propensity of the two glove powders for NR latex proteins but also attempt to elucidate the mechanism affecting it. For that, the uptake of fresh latex serum proteins and glove proteins by corn and oat starch during powder slurry treatment was studied. Factors such as pH of the slurry, ionic constituents, incubation time, temperature, protein concentration and powder levels were also investigated to assess their influence on the adsorption of proteins by the powders.

MATERIALS AND METHODS

Powders and Proteins

Six types of modified corn starch powders commercially available and an oat starch powder were studied. All the powders contained below detectable levels of total proteins and allergenic proteins except for oat starch and corn starch-5, which showed some quantifiable amount of total proteins (*Table 1*). This indicated no or minimal interference by these powders in the total protein and allergenic protein assays. The modified corn starches also satisfied the USP requirements on pH and sedimentation values (*Table 1*).

The sedimentation value was determined by boiling 100 mL of 10% suspension of the powder in water for 20 min, followed by cooling before transferring the slurry into 100 mL graduated cylinder. The slurry was then diluted with water to 100 mL level and the mixture left to stand for 24 h. The volume occupied by the powder, termed as sedimentation value, was then measured.

The proteins used to test the binding affinity of the powders were from combined B and C-sera of fresh RRIM 600 latex and from water extracts of NR latex examination gloves.

Uptake of Glove Proteins by the Powders

The powder was mixed with protein solution (in water) in a ratio of 100 mg powder to 1 mL protein solution. The mixture was incubated at 55ºC for 3 h and regularly stirred to prevent sedimentation of the powder. The powder mixture was then centrifuged at 6000 g for 15 min and the resulting clear supernatant isolated. The proteins in the supernatant, which were proteins left unbound to the powder, were then assayed.

The amount of proteins bound to the powder was calculated by subtracting the amount of unbound proteins from the original amount of protein added to the powder. When the level of proteins bound to the powder was below the detection limit of the protein assay, the powder was considered to exhibit no detectable binding to the proteins

To verify that the loss of proteins was mainly due to their binding to the powder, a

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separate experiment was carried out to test the effects of powder slurry water on the glove and serum proteins. For this, the powder was first mixed with water at the ratio of 1 g powder to 10 mL water. The mixture was then centrifuged at 6000 g and the clear supernatant (the powder slurry water) isolated. One mL of the latter was incubated with glove proteins and serum proteins according to the amount specified in *Table 2*. No traces of precipitated proteins were observed in the resulting mixture after incubation at 55ºC for 3 h. The solution was then assayed for protein content. Results in *Table 2* showed that there was no marked loss (fraction of proteins recovered was >1) in the glove and serum proteins on incubation with alkaline slurry water of all modified corn starches or with acidic slurry water of oat starch. This indicated that any latex protein loss on incubation with modified corn starch or oat starch powder was attributed to the binding of the protein to the powder.

TABLE 1. EXTRACTABLE PROTEINS, pH AND SEDIMENTATION VALUES OF OAT AND CORN STARCH

Powder	Total proteins (μg/100 mg powder)	Allergenic proteins (AU/100 mg powder)	pH	Sedimentation (mL)
Oat starch	11	<0.5	4.5	–
Corn starch -1	<4.7	<0.5	10.4	52
Corn starch -2	<4.7	<0.5	10.8	62
Corn starch -3	<4.7	<0.5	10.8	57
Corn starch -4	<4.7	<0.5	10.9	50
Corn starch -5	6	<0.5	10.8	57
Corn starch -6	<4.7	<0.5	10.5	52

TABLE 2. FRACTION OF PROTEINS RECOVERED AFTER INCUBATION WITH POWDER SLURRY WATER

Powder	Serum proteins		Glove proteins	
	Total protein (170 μg/mL)	Allergenic protein (265 AU/mL)	Total protein (65 μg/mL)	Allergenic protein (20 AU/mL)
Oat starch	1.2	1.1	1.3	1.5
Corn starch -1	1.0	1.2	1.1	1.2
Corn starch -2	1.1	1.4	1.2	1.7
Corn starch -3	1.2	1.9	1.1	1.3
Corn starch -4	1.2	0.8	1.3	1.3
Corn starch -5	1.6	1.4	1.3	1.2
Corn starch -6	1.2	1.3	1.3	1.2

Note: Fraction of proteins recovered was calculated from the amount of proteins obtained after incubation at 55ºC for 3 h over the original amount of proteins added.

Extraction of Proteins Bound to the Powder

The proteins bound to modified corn starch powder were extracted with 25 mM phosphate buffered saline (PBS) at 25ºC for 2 h with constant shaking (on shaker). The extraction ratio was maintained at 100 mg powder to 1 mL PBS.

Protein Determinations

The total protein content was determined by *ASTM D 5712-99* while the allergenic protein content was determined by IgE-ELISA inhibition method adapted from the Finnish test method[8]. The IgE antibodies were pooled human sera, which had been tested to contain specific antibodies to latex proteins. Fresh latex serum proteins were used as the standard allergens and a total protein concentration of 10 mg/mL was assigned to contain 100 000 allergen units (AU) per mL[8].

The detection limits of *ASTM D5712-99* and IgE-ELISA inhibition are 4.7 µg/mL and 0.5 AU/mL, respectively. This is equivalent to 4.7 µg/100 mg powder and 0.5 AU/100 mg powder, respectively.

Hev b 5 was assessed following the protocols outlined in the FITkits from FIT Biotech.

RESULTS AND DISCUSSION

Binding Affinity of Corn and Oat Starch for NR Latex Proteins

Table 3 shows that oat starch and modified corn starches 1–3 adsorbed fresh latex serum allergenic proteins, with corn starch adsorbing 62% to 76% more than oat starch. Corn starches 4–6, however, exhibited no binding affinity for the fresh latex serum allergens. On incubating the powders in glove allergenic proteins, all the corn starch powders attracted 65%–83% of the proteins except corn starch-6, which attracted the lowest amount of 16%. Oat starch, on the other hand, showed no affinity for glove allergens.

The different binding propensities of oat and corn starch for NR latex serum and glove proteins could be due to the differences in the physico-chemical properties of the powders and in the composition of the proteins. All corn starch powder slurries showed alkaline pH of above 10 while the oat starch slurry had an acidic pH of 4.5 (*Table 1*). The different pH values could affect the net protein charge as an earlier study[9] showed that fresh latex serum proteins contained acidic and basic proteins while glove proteins contained mainly acidic proteins.

In the alkaline medium of corn starch slurry, the acidic glove proteins would have net negative charge and the fact that they were attracted to corn starch could imply that the powder had positively charged groups on its matrix. In the acidic pH of the oat starch slurry the negative charge of the glove proteins would be neutralised to zero net charge and these seemed not to be attracted to oat starch. As oat starch did not bind acidic glove proteins, it could be assumed that the powder would not bind acidic serum proteins either. Its uptake of fresh latex proteins could be to the basic proteins, which in acidic pH medium would exert a net positive charge. This implied that the oat starch could possibly have negatively charged groups on its matrix, which explained its uptake of the basic serum proteins but not the acidic glove proteins. The low uptake of serum proteins could be related to the low level of basic proteins in fresh latex serum compared to the acidic proteins[9].

TABLE 3. PERCENTAGE UPTAKE OF ALLERGENIC PROTEINS BY MODIFIED CORN STARCH AND OAT STARCH

Powder	Allergens of fresh latex serum (1013 AU/mL)[a]	Allergens extractable from gloves (453 AU/mL)[a]	Hev b 5 of fresh latex serum (6 µg/mL)[a]
Oat starch	14	Nb	Nb
Corn starch-1	90	83	99
Corn starch-2	76	80	99
Corn starch-3	77	80	98
Corn starch-4	Nb	74	Nb
Corn starch-5	Nb	65	Nb
Corn starch-6	Nb	16	Nb

[a]Initial allergen concentration of 10% powder slurry
Nb: No detectable binding of proteins by the powder.

The preferential binding of corn starch to acidic proteins was further proven by their affinity for an acidic protein, Hev b 5, derived from fresh latex serum (*Table 3*). Like fresh latex serum proteins, Hev b 5 too was observed to bind mainly to corn starch powders 1–3 but not to corn starch powders 4–6. As expected Hev b 5 did not bind to oat starch.

The ability of corn starches 4–6 to bind to acidic proteins from gloves but not to acidic proteins from fresh latex serum could be attributed to the differences in the molecular weight of the proteins. SDS-PAGE[9] showed that fresh latex serum proteins consist mainly of high molecular weight (>14 kD) components while glove proteins consist mainly of low molecular weight (<14 kD) components. The varying affinity of corn starches 1–3 to bind to both low and high molecular weight acidic proteins and corn starches 4–6 to only low molecular weight acidic proteins reflected differences in certain properties of the different grades of modified cornstarch powder used in the glove industry.

Factors Affecting the Binding Propensity of Corn and Oat Starch for Glove Proteins

Apart from differences in the types of powder, other factors that might affect the binding propensity of glove powders for latex proteins were investigated.

pH and ionic constituents of the powder slurry. *Table 4* shows that incubating oat starch in buffers of different pH and ionic constituents did not change the negative binding affinity of the powder for glove proteins. Corn starch, on the other hand, was markedly influenced by these factors. *Table 3* shows that all corn starch powders suspended in water (pH 10) adsorbed glove proteins. Similar interactions of the powders with proteins occurred when corn starches-1 and 6 were suspended in 0.5 M Tris-HCl buffer of pH of 10 (*Table 4*). However, when the pH was reduced to neutral level as in 1.5 M Tris-HCl and 0.025 M PBS the binding of modified corn starch to glove proteins was drastically reduced to non-detectable level. Similar effects were observed

TABLE 4. PERCENTAGE UPTAKE OF NR GLOVE PROTEINS BY CORN STARCH AND OAT STARCH IN BUFFERS OF DIFFERENT pH AND IONIC CONSTITUENTS

Powder and protein	PBS buffer (0.025 M) pH 7.4	Tris-HCl buffer (1.5 and 0.5 M)[a] pH 7.3	Tris-HCl buffer (1.5 and 0.5 M)[a] pH 10.0	CO_3-HCO_3 buffer (0.05M) pH 9.8
Oat starch	Nb	Nb	Nb	–
Corn starch-1	Nb	Nb	84	8
Corn starch-6	Nb	Nb	17	1
Proteins added (µg/100 mg powder)	141	248	244	247

[a]Molarity of Tris-HCl buffer of pH 7.3 and 10.0, respectively.

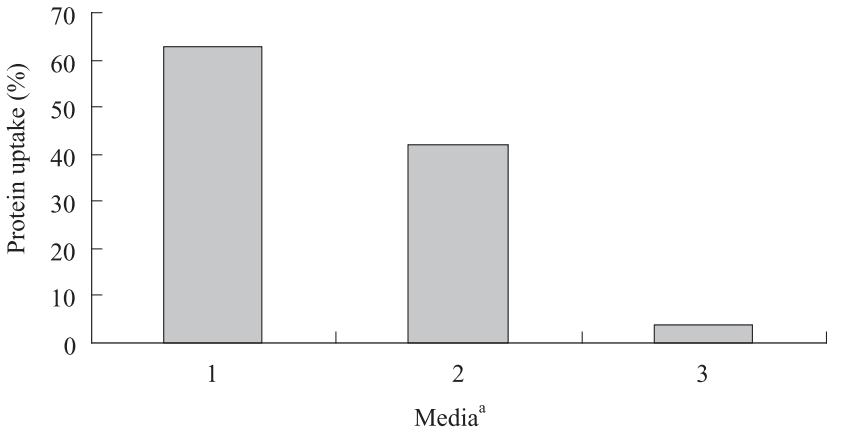
when the powders were incubated in 0.05 M carbonate-bicarbonate buffer of pH 9.8

The above observation further supported the postulation on the electrostatic interaction between the positively charged groups on corn starch and negatively charged acidic proteins. As the pH values decreased, the net negative charge on the proteins decreased. This reduced the amount of proteins adsorbed to corn starch. In carbonate-bicarbonate buffer, the negatively charged proteins competed with the carbonate/bicarbonate anions for the binding sites on the powder. Possibly due to higher concentration, more carbonate/bicarbonate anions adsorbed on the powder, leaving less binding sites for the proteins and thus less protein uptake. This mechanism resembled that of an anion exchanger, which has a matrix of polysaccharide and positively charged groups covalently bound to it[10].

A supplementary study (*Figure 1*) showed that reducing the pH of the powder slurry alone could not give the desired result of low protein binding to the powder. It was the incorporation of anionic constituents into the powder slurry that effectively reduced the protein uptake by the powder.

Protein level and its source. It could be expected that with continuous dipping of gloves into the slurry tank there will be a protein build-up in the slurry with time. Such conditions could induce a higher protein uptake by the corn starch powder. This is evidenced in *Table 5* where the level of proteins bound to 100 mg of corn starch-1 increased from 9 µg to 151 µg when incubated in a glove extract of increasing concentration of 14 µg/mL to 215 µg/mL. The level of protein uptake, however, depended on the source of proteins. *Table 5* shows that although the protein concentrations from 3 brands of gloves were not markedly different (195, 197 and 215 µg/mL) the amount of proteins adsorbed to corn starch-1 varied from 61 to 151 µg/100 mg powder. This could mean that differently processed gloves generated different types of extractable protein, which exhibited different binding affinity to the corn starch powder or the extracts may contain certain ionic constituents which affect the binding as observed earlier.

Incubation time, temperature and powder level. *Figure 2* shows that increasing the incubation time increased the protein uptake by corn starch-1. The level seemed to reach an optimum value after 3 h incubation. At this



[a]Media 1: water, pH 10.0; medium 2: water, acidified to pH 7.0 and medium 3: 0.01 M PBS containing 0.0027 M KCI and 0.137 M NaCI, pH 7.4.

Figure 1. Percentage protein uptake by corn starch-1 incubated at 55ºC for 3 h in different media, each containing a protein concentration of 108 µg/mL.

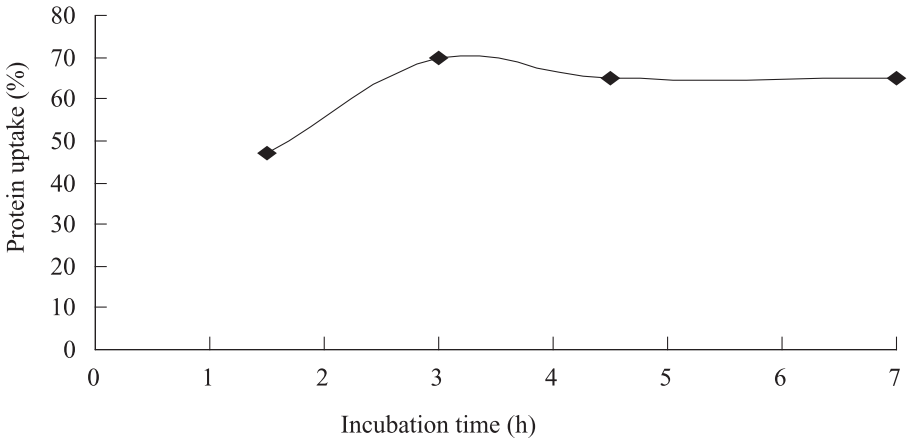


Figure 2. Effect of incubation time on protein uptake by corn starch-1.

time corn starch-1 adsorbed 140 µg (70%) of the 200 µg of proteins initially present in the slurry. The corn starch powder seemed to have a saturation point of adsorbing certain types of proteins. Similar observation was reported by Tomazic *et al*.[11]

Commercial glove powder slurries are normally run at elevated temperature of about 55ºC. Studies presented in *Table 6* show that lowering the slurry temperature from 55ºC to 4ºC lowered the level of proteins adsorbed to corn starch powder from 55% to 21%. Similar decrease in protein uptake was achieved by lowering the powder level from 10% to 5% (*Table 6*). This was as expected, as the availability of binding sites decreased with decreasing powder content.

The results of the present study clearly showed that the level of proteins adsorbed to modified corn starch used in the manufacture of NR gloves could be drastically reduced by the incorporation of certain anionic constituents into the slurry, rather than by adjusting the pH of the slurry. Further improvement could be achieved by using corn starch powder (corn starch-6) with minimal binding affinity to glove proteins or reducing the slurry temperature and powder level. Ensuring low protein concentration in the slurry through long post-leaching process could further reduce the level of protein uptake.

Protein Concentration in Some Commercial Glove Powder Slurries

A survey of corn starch slurries from 3 commercial glove lines (*Table 7*) showed a low protein concentration in the slurry supernatant. The amount varied from 5 µg/mL to 45 µg/mL total protein (0.5 AU/mL to 120 AU/mL allergenic proteins) in slurries of 22 to 57 days old. The low protein concentration could result from long post-leaching of gloves prior to slurry dip or to a substantial amount of proteins being adsorbed by the powder. The latter reasoning is disputable as further tests showed an equally low amount of proteins extractable from the powder. The amount extracted from 100 mg powder by 25 mM PBS varied from 8 µg to 24 µg total protein (11 AU to 89 AU allergenic proteins).

NR powdered gloves normally contained extractable allergen content of higher than 500 AU/g (50 µg/g) glove. Thus, on a gram to a gram basis, the amount of allergens extracted from the powder would be 0.2 to 1.8 times more than the amount extractable from gloves. The ratio proved to be much lower than that postulated in the Mealey's Ligitation Report[12], which quoted a value of 1000 times higher.

CONCLUSION

The study confirmed the established data that modified corn starch binds NR latex serum proteins and glove proteins. The present study, however, showed its specific binding to acidic proteins, which constituted a major component of glove proteins. The interaction between the two resembled an electrostatic interaction whereby the negatively charged proteins were adsorbed to the positively charged groups apparently present on the powder.

This ionic type of interaction was found to be more sensitive to changes in ionic constituents, than in pH. Other factors involved in the slurry treatment such as type of corn starch powder, powder level, protein concentrations, incubation time and temperature of the slurry could also affect the protein uptake by the corn

TABLE 5. EFFECTS OF PROTEIN LEVELS AND SOURCE ON THE AMOUNT OF PROTEINS BOUND TO CORN STARCH-1

Protein source	Level of protein added (µg/100 mg powder)	Protein bound to the powder (µg/100 mg powder)
Glove A	14	9
Glove A	53	35
Glove A	138	84
Glove A	215	151
Glove B	195	135
Glove C	197	61

TABLE 6. EFFECT OF TEMPERATURE AND POWDER LEVELS ON PROTEIN (%) UPTAKE BY CORN STARCH-1

Protein values	Slurry Temperature				Powder level	
	4ºC	27ºC	40ºC	55ºC	5%	10%
Protein (%) uptake	21	29	39	55	48	60
Proteins added (µg/100 mg powder)	232	240	230	222	86	88

TABLE 7. PROTEIN CONCENTRATION IN THE CORN STARCH SLURRIES OF THREE COMMERCIAL GLOVE LINES

Glove factories	Age of the slurry (days)	Slurry supernatant		Slurry powder	
		Total proteins (µg/mL)	Allergens (AU/mL)	Total proteins (µg/100 mg)	Allergens (AU/100 mg)
I- Line 1	31	21	120	18	89
I-Line 2	45	8	10	13	58
I-Line 3	45	21	44	17	74
I-Line 4	57	12	92	15	83
II	22	5	0.5	8	13
III	30	45	4	24	11

Corn starch-1 and corn starch-6 were used to prepare slurries of factories II and I, respectively. The powder used by factory III was not disclosed.

starch powder. Adjusting the powder slurry in commercial glove lines to the optimum conditions could result in powdered gloves with minimal levels of proteins bound to the powders.

The present study also confirmed earlier reports that oat starch attracted a lower level of latex serum proteins compared to certain brands of corn starch, which attracted a higher level of the proteins. Furthermore, oat starch was shown to have no affinity towards acidic proteins. The binding of the powder to NR latex proteins could be targeted to non-acidic proteins.

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REFERENCES

1. SEATON, A. (1990) Latex as Aeroallergen. *Lancet*, **336**, 808–809.

2. SEGGEV, J.S., MAWHINNEY, T.P., YUNGINGER, J.W. AND BRAUN, S.R. (1990) Anaphylaxis due to Cornstarch Surgical Glove Powder. *Ann. Allergy*, **65**, 152–155.

3. TURJANMAA, K., REUNALA, T., ALENIUS, H., BRUMMER-KORVENKONTIO, H. AND PALOSUO, T. (1990) Allergens in Latex Surgical Gloves and Glove Powder. *Lancet*, **336**, 1588.

4. BAUR, X. AND JAGER, D. (1990) Airborne Antigens from Latex Gloves. *Lancet*, **335**, 912.

5. JAEGER, D., KLEINHANS, D., CZUPPON, A.B. AND BAUR, X. (1992) Latex-specific Proteins Causing Immediate-type Cutaneous, Nasal, Bronchial and Systemic Reactions. *J. Allergy Clin. Immunol.*, **89**, 759–768.

6. TOMAZIC, V.T., SHAMPAINE, E.L., LAMANNA, A., WILTHROW, T.J., ADKINSON, N.F. AND HAMILTON, R.G. (1994) Cornstarch Powder on Latex Products is an Allergen Carrier. *J. Allergy Clin. Immunol.*, **93**, 751–758.

7. SWANSON, M. AND OLSON, D.W. (2000) Latex Allergen Affinity for Starch Powders Applied to Natural Rubber Gloves and Released as an Aerosol: From Dust to Don. *Canadian J. Allergy Clin. Immunol*., **5**, 328–334.

8. PALOSUO, T., MAKINEN-KILJUNEN, S., ALENIUS, H., REUNALA, T., YIP, E. AND TURJANMAA, K. (1998) Measurement of Natural Rubber Latex Allergen Levels in Medical Gloves by Allergen-specific IgE-ELISA Inhibition, RAST Inhibition, and Skin Prick Test. *Allergy*, **53**, 59–67.

9. HASMA, H. AND AMIR-HASHIM, M.Y. (1997) Changes to NR Latex Proteins on Processing the Latex to its Products. *J. nat. Rubb. Res*., **12(1)**, 21–32.

10. *Ion Exchange Chromatography: Principles and Methods* (1999) Sweden: Pharmacia, Biotech.

11. TOMAZIC, V.T., LUCAS, A.D. AND SANCHEZ, B.A. (2003) Factors Affecting Binding of Natural Rubber Latex Proteins to Glove Powder. http:/ www.cfsan.fda.gov/~frf/forum03/abs03ct.html.

12. OLSON, L. AND WOODWARD, W.S. (2002) Powdering Process Causes Material Defect in Latex Gloves. *Mealey's Litigation Report*, **5(11)**.