Chapter 8 Concluding remarks

A new solid-phase was constructed from natural rubber (NR) latex for Hepatitis B surface antigen (HBsAg), anti-Hepatitis B surface antigen (anti-HBs), Hepatitis C virus antibody (anti-HCVAg) and Thyroxine (T4) assays with polypropylene (PP) solid phase as control. The study showed that the percent binding of proteins was much higher on NR than on PP surface. NR latex film surface was applicable for both sandwich (HBsAg, anti-HBs, anti-HCVAg assays) and competitive binder ligand immunoassays (T4 assay). Experiment on NR latex film surface stored at 26°C showed that this could be stored for up to at least six months without significant changes in specific binding. In addition, both anti-HBs or HBsAg-immobilised NR/PP tubes showed no significant changes in specific and non-specific bindings for both anti-HBs and HBsAg after 3 months of storage at 4°C. Despite the high percent binding of proteins, the sensitivity of anti-HBs, HBsAg, T4 and anti-HCVAg assays on NR latex film surface was rather low as compared to PP solid phase even after modifications of the NR solid phase by (i) trypsinisation followed by washing with distilled water, (ii) washing with HCl followed by distilled water, (iii) washing with PBS followed by distilled water and (iv) precoating with blockers. Experiments were designed to seek suitable blockers that can reduce the non-specific binding and thus enhanced the sesitivity of the assay. The optimum coating concentration of HBsAg on PP tube was 0.88 μg/ml. However, higher concentration of 1.76 μg/ml was required to saturate the washed

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natural rubber (WNR) latex film surface. In the case of PP surface immobilised with anti-HBs, the optimal coating concentration was 260 μg/ml. In comparison, at 260 μg/ml coating concentration, the surface binding of ^{125}I anti-HBs by NR latex film surface immobilised with anti-HBs was still below its saturated value and there was a concomitant increase in the non-specific binding. In conclusion, NR latex film surface was found to be inferior to PP surface as a solid phase in immunoassay. The NR latex film surface need to be further improved upon, before it can replace the PP surface as solid phase in immunoassay applications.

In the assay analysis, the low sensitivity of NR latex film surface can be explained as follows: -

(i) Occurrence of substantial denaturation of the immobilised polyclonal antibodies and antigens passively adsorbed on NR latex film surface resulting in a decrease in their binding properties.

(ii) Even coating of antibodies/antigens on PP surface provides a bigger surface area for interaction between serum antigens or antibodis with the immobilised macromolecules. On NR latex film surface, the clusters formed were partially embedded in the coating itself resulting in reduced interaction with the antibodies or antigen.

The adsorption of antigen/antibody on both PP and NR surfaces was studied using scanning electron microscopy and atomic force microscopy techniques. Dendrite-like aggregates of protein was observed on PP surface forming a thin evenly spread out layer. However, adsorption of protein on NR latex film surface was in cluster form and submerged into

the NR coating. These could possibly be due to (a) difference in hydrophobicity of the two surfaces: PP surface was more hydrophobic promoting unfolding of proteins molecules and covering the surface more evenly, whereas the adsorbed antigen-antibody molecules did not unfold on the less hydrophobic NR latex film surface. (b) NR latex film surface contained inorganic substances and proteins. These may interact with the immobilised protein resulting in the formation of aggregates.

(iii) Unfolded antigen/antibody proteins on PP surface were tightly adsorbed and could not be displaced with ease by other molecules, whereas on NR latex film surface, added antigen/antibody could penetrate through the NBCS layer (used for blocking the active site of the solid phase after coating with anti-HBs or HBsAg). In addition, desorption of immobilised antigens/antibodies from the NR latex film surface occurred because these were not so tightly bound compared to those on PP surface.

Some suggestions for further work to improve the sensitivity of assay using NR latex film surface are:

(i) To reduce inteference by the inorganic substances and proteins of the NR latex film surface by

- Wet gel leaching: washing the wet gel of latex film formed.
- Dry film-leaching: latex film can be heated up first to effect migration of soluble NR proteins to the surface before the proteins are leached with water.

(ii) To reduce desorption of antigen or antibody from the solid immunosorbents by covalent coupling of antigen or antibody.

(iii) To minimise lose of functional activity as a result of immobilisation of the antigen or antibody at the surface by immobilising them instead via an antiglobulin or streptavidin bridge.