Chapter 7 Kinetics of antigen-antibody reactions at solid-liquid interfaces

7.1 Introduction

When interpreting the data of solid-phase immunoassays, certain assumptions must be made about the kinetics of the antigen-antibody reaction at the solid-liquid interface. Specific knowledge about the kinetics of the antigen-antibody reaction at a solid-liquid interface is of vital importance when optimising a solid-phase assay procedure. Measurements of antibody binding to solid-phase immobilised with antigen have revealed that the kinetics of the reaction differ from the kinetics of the corresponding liquid phase reaction in several aspects. The initial forward reaction often becomes diffusion rate limited at plane surfaces (Stenberg et al., 1982; Nygren and Stenberg, 1985). The dissociation rate of bound antibody is slower at solid-liquid interface than in solution (Nygren et al., 1986) and the binding reaches a concentration-dependent saturation level that is not caused by a dynamic equilibrium (Nygren and Sternberg, 1985). The present study was to further elucidate the mechanisms of the kinetics of antigen-antibody reactions at the solid-liquid interface for both PP and NR surfaces and to find out the main reason for the difference in their sensitivity.

7.2 Materials and experimental methods

7.2.1 Materials - please refer to Section 2.2

7.2.2 Experimental methods

Washed NR coated tubes (Washed five times with 1 ml 0.1 mol dm^{-3} HCl followed by five water washes) and unwashed PP tubes were used as solid phases. The tubes were coated with anti-HBs or HBsAg and blocked with NBCS 50% as described in Section 2.2 . The following kinetic experiments were studied :

a.) Re-equilibration

WNR or PP tube (as a control) was coated with 200 µl of anti-HBs (Horse polyclonal) and HBsAg and was left to equilibrate at 4°C overnight. The solution was decanted and the tubes were washed four times with 1 ml of distilled water. 200 µl of 50% NBCS were added into each tube, and the tubes were left overnight at 4°C. The solution was decanted and tubes were washed four times with 1 ml of the PBS solution. The anti-HBs (HBsAg) coated tube was allowed to react with 200 µl of HBsAg (anti-HBs) positive control serum or negative control serum (or without serum incubation) and incubated for two hours at 45°C. The tubes were washed four times with 1 ml of distilled water. 200 µl of ^{125}I anti-HBs (^{125} I HBsAg) were then added into each tube and incubated for two hours at 45° C. The bound radioactivities were counted with an Abbott Autologic Gamma Counter before and after four washes with 1 ml of distilled water. The above procedures were repeated by re-equilibrating

the same tube with fresh control serum and ^{125}I anti-HBs (or ^{125} I HBsAg) (Ch'ng,1991).

b.) Kinetic studies

1.) Two-step procedure

200 µl ^{125}I anti-HBs (^{125}I HBsAg) were added to the HBsAg (anti-HBs) coated and NBCS blocked tube. The tubes were placed in a water bath, the antigen-antibodies were allowed to react at 45°C for various periods of time. At regular time intervals, the tubes were taken out and washed four times with 1 ml of distilled water. The radioactivities of bound ^{125}I anti-HBs (^{125}I HBsAg) were counted using the Abbott Autologic Gamma Counter (1st count). After counting, 200 µl of ^{125}I HBsAg (^{125}I anti-HBs) were added to the same tubes and incubated in a water bath at the same temperature for various periods of times. The reaction was stopped after various periods of time by washing the tubes four time with 1 ml of distilled water. The radioactivities of bound ^{125}I HBsAg + ^{125}I anti-HBs (or ^{125}I anti-HBs + ^{125}I HBsAg) were counted using the Abbott Gamma Autologic Counter again (2nd count)(Diagram A)

2.)Anti-HBs (HBsAg) positive controlled serum were added to HBsAg coated (anti-HBs coated) and NBCS blocked tube. The tubes were placed in a water bath, the reaction was allowed to continue at 45°C for various periods of time. The tubes were then washed four times with 1 ml of distilled water. ^{125}I HBsAg (^{125}I anti-HBs) were

added to the tubes in a water bath and incubated at the same temperature for various periods of time. The reaction was stopped after various period of incubation by washing the tubes four times with 1 ml of distilled water. The activities of bound ^{125}I HBsAg (^{125}I anti-HBs) were counted using an Abbot Gamma Counter (Diagram B).

3.) One-step procedure

Equal volume of ^{125}I anti-HBs and ^{125}I HBsAg were mixed thoroughly to form complex of antigen-antibody. 200 µl of the ^{125}I complex after incubation at room temperature (26°C) for 10 minutes (A set) and 2 hours (B set) respectively were transferred to anti-HBs and HBsAg coated tubes. The tubes were incubated at 45°C. At different time intervals, the reactions were stopped by washing the tubes four times with 1 ml of distilled water. The radioactivities of the bound complex were counted in an Abbott Autologic Gamma Counter (Diagram C).

c.) Dissociation of bound ^{125}I HBsAg and ^{125}I anti-HBs in assay procedure

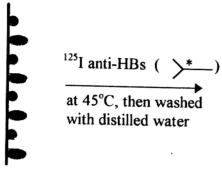
1.) 200 µl of ^{125}I HBsAg (^{125}I anti-HBs) were incubated with the solid-phase (PP & WNR) at 4°C overnight. The tubes were washed four times with 1 ml of distilled water and the radioactivities were counted (Count 1). The tubes were then incubated with different blockers for 24 hours at 4°C. After the blocker was decanted, the tubes were washed four times with 1 of ml distilled water. The radioactivities of immobilised labelled antibody and antigens were counted (Count 2). Finally the tubes were incubated in positive control or negative control serum for two hours at 45°C and the

remaining bound labelled antigen and antibody were counted after four washes with 1 ml of water (Count 3&4).(Series 1 = NR, Series 2 = PP) (Diagram D).

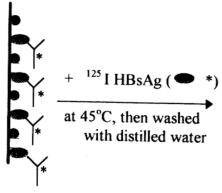
2.) 200 µl ^{125}I anti-HBs (^{125}I HBsAg) were added to the HBsAg (anti-HBs) coated tube. The tubes were incubated in a water bath , the antigen and antibodies were allowed to react at 45°C for various periods of time. At regular intervals, the tubes were removed from the water bath and washed four times with 1 ml of distilled water. The radioactivities of bound ^{125}I anti-HBs (^{125}I HBsAg) were counted using an Abbot Autologic Gamma Counter (1st count). After counting, 200 µl of HBsAg positive control serum (anti-HBs positive control serum) were added to the same tubes and incubated in the water bath at the same temperature for various periods of time. The tubes were then washed four times with 1 ml of distilled water. The final radioactivities of bound ^{125}I anti-HBsAg (^{125}I HBsAg) were counted using an Abbot Autologic Gamma Counter (Diagram E).

Diagram A : Two-step procedure

(ia) HBsAg immobilised tube blocked with 50% NBCS

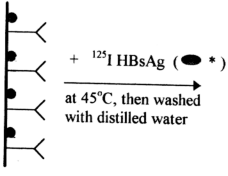


(iia) Radioactivities were counted (1st count)

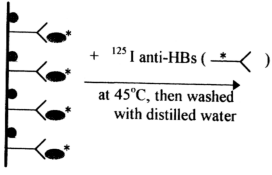


(iiia) Radioactivities were counted (2 nd count)

(ib) anti-HBs immobilised tube blocked with 50% NBCS



(iib) Radioactivities were counted (1st count)



(iiib) Radioactivities were counted (2 nd count)

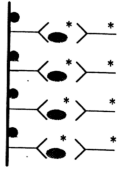
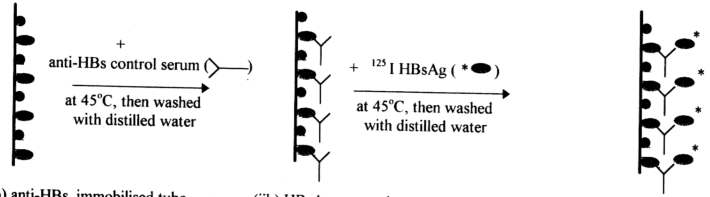


Diagram B :

(ia) HBsAg immobilised tube blocked with 50% NBCS

(iia) Anti-HBs control serum were added

(iiia) Radioactivities were counted



(ib) anti-HBs immobilised tube blocked with 50% NBCS

(iib) HBsAg control serum were added

(iiib) Radioactivities were counted

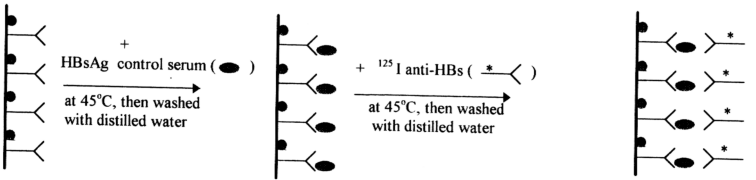
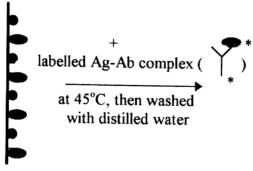


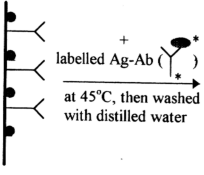
Diagram C : One-step procedure

(ia) HBsAg immobilised tube blocked with 50% NBCS



(iia) Radioactivities were counted

(ib) anti-HBs immobilised tube blocked with 50% NBCS)



(iib) Radioactivities were counted

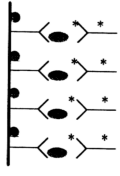
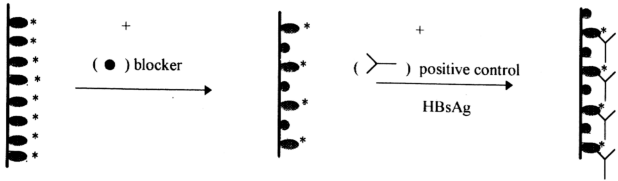


Diagram D : Dissociation of bound ^{125}I HBsAg and ^{125}I anti-HBs in assay

(ia) ^{125}I HBsAg incubated at 4°C with solid phase. The tube was washed and radioactivities were counted (count 1)

(iia) Blocker was added & incubated at 4°C. The tube was washed and radioactivities were counted (count 2)

(iiia) Positive control HBsAg or negative control HBsAg was added and incubated at 45°C The tube was washed and (count 3 & 4)



(ib) ^{125}I anti-HBs incubated at 4°C with solid phase. The tube was washed and radioactivities were counted (count 1)

(iib) Blocker was added & incubated at 4°C. The tube was washed and radioactivities were counted (count 2)

(iiib) Positive control anti-HBs or negative control anti-HBs was added and incubated at 45°C The tube was washed and radioactivities were counted (Count 3 & 4)

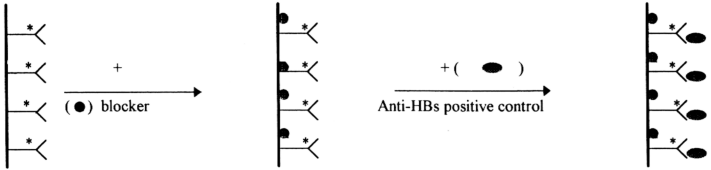
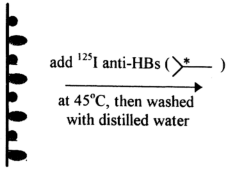
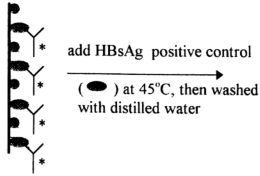


Diagram E : Dissociation

(ia) HBsAg immobilised tube blocked with 50% NBCS

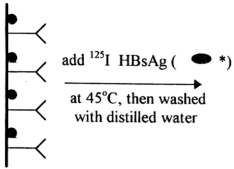


(iia) Radioactivities were counted (1st count)

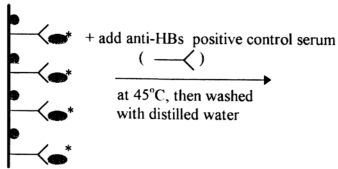


(iiia) Radioactivities were counted (2 nd count)

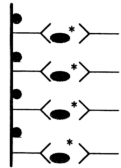
(ib) anti-HBs immobilised tube blocked with 50% NBCS



(iib) Radioactivities were counted (1st count)



(iiib) Radioactivities were counted (2 nd count)



7.3 Results

7.3.1 Re-equilibration

The re-equilibration of both PP and WNR surfaces immobilised with anti-HBs or HBsAg increased the non-specific binding (with /without serum) and specific binding (a-b) in both anti-HBs and HBsAg assays (Table 7.1 a-d). The optimal specific bindings of ^{125}I anti-HBs in the presence of HBsAg positive control sera (in HBsAg assay) on WNR surface immobilised with anti-HBs, were achieved after two re-equilibrations (three equilibrations). However for PP surface, the optimal specific binding was reached after one re-equilibration (two equilibrations) (Table 7.1 a & 7.1 c). In anti-HBs assay, the optimal number of re-equilibration was one for WNR surface. As for PP surface, the specific binding showed a rising trend after two re-equilibrations (Table 7.1b & 7.1d).

7.3.2 Dissociation of labelled anti-HBs and HBsAg from solid phase

Results in Table 7.2 show that in general less anti-HBs and HBsAg were desorbed from PP surface when compared with WNR surface (compare count 3 & 4 on both PP and WNR surfaces). It was noted that dissociation of the bound labelled protein on either PP or WNR surface occurred in each of the assay procedure. On WNR surface, the dissociation of labelled protein was most marked when NBCS and NEO were used as blockers.

7.3.3 Dissociation of bound labelled anti-HBs and HBsAg from HBsAg and anti-HBs immobilised surface

Bound labelled anti-HBs and HBsAg respectively were found to dissociate from HBsAg and anti-HBs immobilised surfaces respectively (PP or WNR) when unlabelled antigen/antibody was added (Fig.7.1 & 7.2). However the percentage of dissociation was higher on WNR surface when compared to PP surface (Table 7.3 a & b)

7.3.4 Kinetics studies

The percent binding of labelled anti-HBs to HBsAg-immobilised surface in relation to incubation time is shown in Fig.7.3 (Table 7.4a). The percent binding of labelled anti-HBs on both surfaces increased rapidly initially and it levelled off to a constant value at saturation level. However the binding rate of labelled anti-HBs on HBsAg immobilised PP surface was more rapid than that of WNR HBsAg immobilised surface. The percent binding of labelled anti-HBs by HBsAg immobilised PP surface was 3-4 times greater than that of WNR surface at different incubation time and reached a maximum after seven hours. Fig.7.4 (Table 7.6) shows the percent binding of labelled HBsAg to HBsAg immobilised surface which has been preincubated with anti-HBs positive control serum. The percent binding of labelled HBsAg on both of the surfaces increased rapidly initially and than levelled off to a constant value at saturation. The percent binding of labelled HBsAg on to PP surface was about three

times greater than that on WNR surface and reached a maximum after seven hours. The ratio was similar to that observed in Fig 7.3 for labelled anti-HBs.

Fig 7.5 (Table 7.5a) shows the percent binding of labelled HBsAg to anti-HBs immobilised surface. The percent binding of labelled HBsAg onto the anti-HBs immobilised WNR surface increased rapidly initially and reached a constant value after seven hours. On PP surface, the binding increased continuously and approximately linearly with time and saturation was not reached even after 23 hours of incubation. The binding rate of labelled HBsAg onto the anti-HBs immobilised PP surface was greater than that of anti-HBs immobilised WNR surface. However the maximum percent binding of labelled HBsAg reached was 4.49% on PP and 1.21% on WNR anti-HBs immobilised surface respectively after 23 hours (Fig 7.5). This shows that the percent binding of labelled HBsAg to surface immobilised with anti-HBs (Fig 7.5) was lower than that of labelled anti-HBs to surface immobilised HBsAg (Fig 7.3). The percent binding of labelled HBsAg is about four times greater on PP surface than those on WNR surface after 23 hours (Table 7.5a, Fig 7.5). Fig 7.6 (Table 7.6) shows the percent binding of labelled anti-HBs to anti-HBs immobilised WNR surface which had been preincubated in HBsAg positive control serum. On WNR surface, the percent binding of labelled anti-HBs increased rapidly at the beginning and reached a plateau value at 4% over a period of approximately 2.5 hours, then increased linearly with time after seven hours with a rate much higher compared to that of PP. On PP surface which has been immobilised with anti-HBs and preincubated in HBsAg control serum, the amount of labelled anti-HBs bound increased rapidly up to 6 hours of

incubation, beyond which it increased linearly with a slope of 0.30%/hour. WNR surface gave higher binding (specific plus non-specific) of labelled anti-HBs (12.29%) than PP surface (9.15%) after 21 hours (Table 7.6 & Fig 7.3b).

The same behaviour was observed for the binding of ^{125}I complex (mixture of equal volume of labelled anti HBs and HBsAg) to WNR and PP surfaces immobilised with HBsAg (Fig 7.7; Series 2, 3, 5, 6 & Table 7.4c). The binding increased rapidly initially and then levelled off at long incubation time. The percent binding of ^{125}I complex on HBsAg immobilised PP surface at saturation point was about 1.5 times greater than that of WNR surface after 22 hours of incubation (Table 7.4 ci & cii). ^{125}I complex which was allowed to equilibrate for two hours before it was allowed to react with the immobilised HBsAg was seen to bind slightly more effectively on WNR surface as compared with ^{125}I complex which was used immediately (10 minutes) after mixing. However for both type of ^{125}I complex, whether it was mixed and used after 10 minutes or mixed and used after incubation of two hours, no significant difference was observed on PP surface immobilised with HBsAg (Table 7.4 ci & cii).

On WNR surface immobilised with HBsAg (Fig 7.7 Series 1,2,3 & Table 7.4 b & c), the saturation percent binding of ^{125}I complex in the one-step process (Table 7.4(c), Fig 7.7 Series 2&3) was two times bigger compared with the two-step binding procedure (Table 7.4(b) , Fig.7.7 Series 1), i.e. immobilised HBsAg incubated sequentially with ^{125}I anti-HBs followed by ^{125}I HBsAg. However on PP surface, no significant difference was seen.

The binding of labelled ^{125}I complex (in one-step procedure)(Table 7.5(c)) and total ^{125}I HBsAg + ^{125}I anti-HBs (in 2-step procedure) (Table 7.5(b)) on WNR surface immobilised with anti-HBs show similar shape (Fig 7.8 Series 1, 2 &3). The binding increased rapidly initially and levelled off as the incubation times was increased. However, saturation level of labelled protein (total ^{125}I HBsAg + ^{125}I anti-HBs) using the two-step procedure (Table 7.5(b), Fig 7.8 Series 1) was lower than that of the one-step procedure (^{125}I complex) on WNR surface (Table 7.5(c), Fig 7.8 Series 2,3). The binding of ^{125}I complex which was allowed to equilibrate for two hours before incubation shows slightly lower binding on WNR surface immobilised with anti-HBs compared with ^{125}I complex which was used 10 minutes after mixing (Table 7.5 ci & cii. However, the differences was not very significant (Fig 7.8 Series 2 & 3). The percent binding of labelled protein (^{125}I HBsAg + ^{125}I anti-HBs) using the two-step procedure on PP surface immobilised with anti-HBs increased rapidly over a short period of times followed by a linear increase in binding throughout the 23 hours of incubation (Fig. 7.8, series 4). The highest percent binding (at 23 hours incubation) was 2.84 %. The binding of ^{125}I complex (^{125}I complex used after 10 minutes of incubation prior to immobilisation)(Fig 7.8, Series 5,6) showed similar behaviour, except the slope of percent binding against times was lower than that of the two-step procedure (Fig 7.8 Series 4). However in the one-step procedure, the percent binding of ^{125}I complex which was allowed to equilibrate for two hours prior to immobilisation showed different binding characteristics (Fig 7.8, Series 6). The binding increased rapidly and reached the first plateau before five hours of incubation. Beyond five

hours, the binding increased rapidly until it reached the second plateau at 23 hours. On PP surface, the maximum percent binding of ^{125}I complex (^{125}I HBsAg + ^{125}I anti-HBs) using the two-step procedure (Table 7.5(b); Fig 7.8 Series 4) was higher than that of the one-step procedure (Table 7.5(ci & cii), Fig 7.8 Series 5&6) . This phenomenon is in contrast to what was observed on WNR surface (Fig 7.8, Series 1,2,3). ^{125}I complex which was allowed to equilibrate for two hours before incubation showed a higher binding on PP surface immobilised with anti-HBs than ^{125}I complex which was used 10 minutes after mixing (Fig 7.8 Series 5&6).

7.3.5 Calculation

(a) Concentration of ^{125}I anti-HBs and ^{125}I HBsAg used

Concentration of ^{125}I anti-HBs and ^{125}I HBsAg = 20 μCi/μg or 1 μCi/ml

1 μCi = 1/20 μg

1 ml = 1 μCi

200μl = (1/50) x (1/20μg)

= 1/100 μg

Concentration of ^{125}I anti-HBs and ^{125}I HBsAg used

=(1/20) μg/1 ml

=0.05 μg/ml

Area immobilised in sample tube

Area immobilised at bottom of the tube

$= 2\pi (6.0)^2$ mm^2

= 226.19 mm^2

b. Surface concentration of ^{125}I anti-HBs on tube immobilised with HBsAg

1. PP tube

Surface concentration of ^{125}I anti-HBs on tube immobilised with HBsAg at 50.38% (Table 7.4)

=[50.38 x (1/100) x (1/100)]/2.2619 μg cm^{-2}

=[50.38 x (1/100) x (1/100) x 1/ 2.2619] x [10^{-6}/150000] mol cm^{-2} (assuming molecular weight of anti-HBs is 150,000 (Turner, 1977)

=1.4847x10^{-14} mol cm^{-2}

=9.0x10^9 molecules cm^{-2}

2. NR tube

Surface concentration of ^{125}I anti-HBs on tube immobilised with HBsAg at 17.24% (Table 7.4)

=[17.24x (1/100) x (1/100)]/2.2619 μg cm^{-2}

=[17.24x (1/100) x (1/100)x 1/ 2.2619] x [10^{-6}/150000] mol cm^{-2} (assuming molecular weight of anti-HBs is 150,000 (Turner, 1977)

=5.080x10^{-15} mol cm^{-2}

=3.1x10^{9} molecules cm^{-2}

c.)Surface concentration of ^{125}I HBsAg on tube immobilised with anti-HBs

1. PP tube

Surface concentration of ^{125}I HBsAg on tube immobilised with anti-HBs at 4.49% (Table 7.5)

=[4.49 (1/100) x (1/100)]/2.2619 μg cm^{-2}

=[4.49 x (1/100) x (1/100)x 1/ 2.2619] x [10^{-6}/4.0x10^{6}] mol cm^{-2} (assuming molecular weight of HBsAg is 4.0x 10^{6} - Skinhoj et al, 1973)

=5.0 x 10^{-17} mol cm^{-2}

=3.0 x 10^{7} molecules cm^{-2}

2. NR tube

Surface concentration of ^{125}I HBsAg on tube immobilised with anti-HBs at 1.21% (Table 7.5)

=[1.21 (1/100) x (1/100)]/2.2619 μg cm^{-2}

=[1.21 x (1/100) x (1/100)x 1/ 2.2619] x [10^{-6}/4.0x10^{6}]mol cm^{-2} (assuming molecular weight of HBsAg is 4.0x 10^{6} - Skinhoj et al, 1973)

=1.3x10^{-17} mol cm^{-2}

=7.8x10^{6} molecules cm^{-2}

Table 7.1a Re-equilibration of HBsAg positive or negative sera, and ^{125}I anti-HBs with anti-HBs immobilised on WNR coated surface (HBsAg assay)

WNR coated tube immobilised with anti-HBs, blocked with 50% NBCS and pre-incubated with HBsAg positive control serum or HBsAg negative control serum or without serum preincubation before ^{125}I anti-HBs was added. The bound radioactivities were counted after the tubes were washed four times with 1 ml of distilled water. The assay procedures were repeated. (WNR tube = NR coated tube pre washed five times with 1 ml of 0.1M HCl followed by five washes with 1 ml of distilled water)

@The numbers indicated in all the tables are the mean of three determinations together with their standard errors. This applies to all the Tables in the Chapter.

	@Percent binding		Non-specific binding	Specific binding	
No of equilibration	With HBsAg Positive Serum (*a)	With HBsAg Negative serum (*b)	Without serum	a-b	a/b
1	3.44±0.23	2.25±0.08	2.94±0.03	1.19	1.53
2	4.49±0.21	2.50±0.11	4.50±0.04	1.99	1.80
3	4.23±0.27	2.17±0.13	5.14±0.17	2.06	1.95

Table 7.1b Re-equilibration of anti-HBs positive or negative sera and ^{125}I HBsAg with HBsAg immobilised on WNR coated surface (anti-HBs assay)

WNR coated tube immobilised with HBsAg , blocked with 50% NBCS and pre-incubated with anti-HBs positive or anti-HBs negative control serum or without serum preincubation before ^{125}I HBsAg was added. The bound radioactivities were counted after the tubes were washed four times with 1 ml of distilled water. The assay procedure was repeated. (WNR tube = NR coated tube pre washed five times with 1 ml of 0.1M HCl followed by five washes with 1 ml of distilled water)

	Percent binding		Non-specific binding	Specific binding	
No of equilibration	With anti-HBs Positive Serum (a)	With anti-HBs Negative serum (b)	Without serum	a-b	a/b
1	2.41±0.30	0.34±0.01	0.43±0.01	2.07	7.09
2	3.30±0.32	0.37±0.02	0.62±0.01	2.93	8.93
3	3.09±0.22	0.42±0.01	0.80±0.06	2.67	7.36

Table 7.1c Re-equilibration of HBsAg positive or negative sera and ^{125}I anti-HBs with anti-HBs immobilised on PP surface (HBsAg assay)

PP tube immobilised with anti-HBs , blocked with 50% NBCS and pre-incubated with HBsAg positive or HBsAg negative control serum or without serum preincubation before ^{125}I anti-HBs was added. The bound radioactivities were counted after the tubes were washed four times with 1 ml of distilled water. The assay procedures were repeated.

	Percent binding		Non-specific binding	Specific binding	
No of equilibration	With HBsAg Positive Serum (a)	With HBs Ag Negative serum (b)	Without serum	a-b	a/b
1	2.95±0.20	0.12±0.01	0.11±0.01	2.83	24.50
2	3.92±0.20	0.73±0.04	0.19±0.04	3.19	5.37
3	3.45±0.20	0.64±0.05	0.33±0.04	2.81	5.39

Table 7.1d Re-equilibration of anti-HBs positive or negative sera and ^{125}I HBsAg with HBsAg immobilised on PP surface (anti-HBs assay)

PP tube immobilised with HBsAg , blocked with 50% NBCS and pre-incubated with anti-HBs positive control serum or anti-HBs negative control serum or without serum preincubation before ^{125}I HBsAg was added. The bound radioactivities were counted after the tubes were washed four times with 1 ml of distilled water. The assay procedures was repeated.

	Percent binding		Non-specific binding	Specific binding	
No of equilibration	With anti-HBs Positive Serum (a)	With anti-HBs Negative serum (b)	Without serum	a-b	a/b
1	5.35±0.23	0.12±0.01	0.10±0.01	5.23	44.58
2	7.37±0.20	0.26±0.02	0.16±0.01	7.11	28.35
3	9.09±0.30	0.51±0.04	0.21±0.00	8.58	17.82

Table 7.2 : Percent binding of ^{125}I labelled protein on solid surface and extent of dissociation during the assay (See diagram D)

Blocker	Percent binding of ^{125}I labelled protein retained			
	^{125}I anti-HBs immobilised on WNR tube	^{125}I HBsAg immobilised on WNR tube	^{125}I Anti-HBs immobilised on PP tube	^{125}I HBsAg immobilised on PP tube
gelatin				
Count 1	2.70 (100.00)	0.30(100.00)	0.12(100.00)	0.10(100.00)
Count 2	2.47 (91.48)	0.27(90.00)	0.11(91.67)	0.09(90.00)
Count 3	1.29(47.77)	0.15(50.00)	0.08(66.67)	0.05(50.00)
Count 4	1.40(51.85)	0.14(46.67)	0.06(50.00)	0.05(50.00)
BSA				
Count 1	2.60(100.00)	0.28(100.00)	0.14(100.00)	0.11(100.00)
Count 2	2.28(87.69)	0.23(82.14)	0.08(57.14)	0.08(72.73)
Count 3	1.23(47.30)	0.13(46.43)	0.09(64.28)	0.06(54.54)
Count 4	1.34(51.54)	0.14(50.00)	0.08(57.14)	0.05(45.45)
NEO				
Count 1	2.65(100.00)	0.28(100.00)	0.12(100.00)	0.09(100.00)
Count 2	1.33(50.19)	0.16(57.14)	0.06(50.00)	0.06(66.67)
Count 3	0.77(29.06)	0.10(35.71)	0.08(66.67)	0.04(44.47)
Count 4	0.75(28.30)	0.11(39.29)	0.04(33.33)	0.04(44.36)
NBCS				
Count 1	2.60(100.00)	0.29(100.00)	0.10(100.00)	0.08(100.00)
Count 2	1.77(68.07)	0.21(72.41)	0.12(120)	0.07(87.50)
Count 3	0.99(38.08)	0.13(44.83)	0.04(40.00)	0.04(50.00)
Count 4	1.22(46.92)	0.13(44.83)	0.03(30.00)	0.03(37.50)

Step 1

Solid surface incubated with 200 µl labelled protein overnight at 4°C. After incubation, the tubes were washed four times with 1 ml of distilled water. The radioactivities of the labelled protein were counted using Abbott Autologic Gamma Counter (Count 1).

Step 2

200 µl of blocker (0.5% gelatin, 0.5% BSA, NEO & 50% NBCS) were added into each tube, the tubes were left overnight at 4°C. The solution was decanted and tubes were washed four times with 1 ml of distilled water. The radioactivities of labelled protein were counted using Abbott Autologic Gamma Counter (Count 2).

Step 3

The above labelled protein coated tube was allowed to react for 2 hours with 200 µl of HBsAg (for ^{125}I anti-HBs immobilised tube) or anti-HBs (for ^{125}I HBsAg immobilised tube) positive control serum or negative control serum. The tubes were washed four times with 1 ml of distilled water. The bound radioactivities of labelled protein were counted using Abbott Autologic Gamma Counter. (Count 3 - in positive control serum; count 4- in negative control serum)

* ( ) = Percentage of labelled protein remained bound on solid surface

Table 7.3a Dissociation of bound labelled anti-HBs (See diagram E (ia); (iia); (iiia))

Solid surface was immobilised with HBsAg and blocked with 50% NBCS. 200 µl ^{125}I anti-HBs were added to the HBsAg coated tubes. The tubes were incubated at 45°C for various period of times. At regular intervals, the tubes were taken out and washed four times with 1 ml of distilled water. The radioactivities of bound ^{125}I anti-HBs were counted using Abbott Gamma Counter (1st count). After counting, 200 µl of HBsAg positive control serum were added to the same tubes and incubated at the same temperature for various periods of times. The tubes were then washed four times with 1 ml of distilled water. The final radioactivities of bound ^{125}I anti-HBs were counted using Abbott Gamma Counter (2nd count) (Results were used to plot Fig 7.1)

(WNR tube = NR coated tube pre washed five times with 1 ml of 0.1 M HCl followed by five washes with 1 ml of distilled water)

	WNR coated tube			PP tube		
Time/h	Percent binding (1st count)	Percent binding (2nd count)	*Apparent percent dissociation of anti-HBs (%)	Percent binding (1st count)	Percent binding (2nd count)	*Apparent percent dissociation of anti-HBs (%)
0.17	2.92±0.01	2.17±0.20	25.68	6.22±0.28	4.84±0.21	22.18
0.33	3.32±0.02	2.34±0.20	29.52	11.30±0.26	9.53±0.23	15.66
0.5	3.87±0.03	2.63±0.20	32.04	12.40±0.24	10.19±0.24	17.82
1	7.44±0.04	4.59±0.20	38.30	19.76±0.23	15.92±0.11	19.43
2	6.17±0.01	3.31±0.10	46.35	29.67±0.35	20.93±0.19	29.46
3	8.26±0.10	4.09±0.25	50.48	36.16±0.60	24.40±0.18	32.52
4	13.12±0.09	6.85±0.25	47.79	39.63±0.25	29.39±0.21	25.84
5	11.11±0.20	5.51±0.20	50.40	43.97±0.30	30.43±0.31	30.80
22	17.24±0.20	9.40±0.30	45.48	50.38±0.25	31.02±0.22	38.43

* Apparent percent dissociation of anti-HBs = [(Percent binding of 1st count - percent binding of 2nd count)/(Percent binding of 1st count) x 100%]

Table 7.3b Dissociation of bound labelled HBsAg (See diagram E (ib); (iib); (iiib))
Solid surface immobilised with anti-HBs and blocked with 50% NBCS. 200 µl ^{125}I HBsAg were added to the anti-HBs coated tubes. The tubes were incubated at 45°C for various period of times. At regular intervals, the tubes were taken out and washed four times with 1 ml of distilled water. The radioactivities of bound ^{125}I HBsAg were counted using Abbott Gamma Counter (1st count). After counting, 200 µl of anti-HBs positive control serum were added to the same tubes and incubated at the same temperature for various periods of times. The tubes were then washed four times with 1 ml of distilled water. The final radioactivities of bound ^{125}I HBsAg were counted using Abbott Gamma Counter again (2nd count) (Result were used to plot Fig 7.2)

(WNR tube = NR coated tube pre washed five times with 1 ml of 0.1 M HCl followed by five washes with 1 ml of distilled water)

	WNR coated tube			PP tube		
Time/h	Percent binding (1st count)	Percent binding (2nd count)	* Apparent percent dissociation of HBsAg (%)	Percent binding (1st count)	Percent binding (2nd count)	* Apparent percent dissociation of HBsAg (%)
0.17	0.27±0.15	0.22±0.11	18.52	0.28±0.10	0.22±0.03	21.43
0.33	0.35±0.02	0.25±0.12	28.57	0.42±0.10	0.35±0.01	16.67
0.5	0.39±0.00	0.31±0.13	20.51	0.43±0.01	0.39±0.05	9.30
1	0.50±0.10	0.41±0.11	18.00	0.83±0.08	0.72±0.04	13.25
2	0.67±0.10	0.45±0.10	32.84	0.73±0.09	0.66±0.03	9.59
3	0.70±0.09	0.47±0.11	32.86	0.89±0.07	0.77±0.02	13.48
4	0.99±0.08	0.65±0.08	34.34	1.19±0.01	1.04±0.06	12.60
6	0.92±0.07	0.66±0.09	28.26	1.30±0.06	1.16±0.05	10.76
23	1.21±0.08	0.90±0.10	25.62	4.49±0.06	3.84±0.01	14.48

* Apparent percent dissociation of HBsAg = [(Percent binding of 1st count - percent binding of 2nd count)/(Percent binding of 1st count) x 100%]

Table 7.4 Percent of specific and non-specific binding of (a) labelled anti-HBs (b) labelled anti-HBs and labelled HBsAg (c) ^{125}I complex on solid surface immobilised with HBsAg as a function of reaction time

Two-step procedure (See diagram A)

(ia)

200 µl ^{125}I anti-HBs were added to the HBsAg coated and NBCS blocked tubes. The tubes were incubated at 45°C for various period of times. At regular intervals, the tubes were taken out and washed four times with 1 ml distilled water. The radioactivities of bound ^{125}I anti-HBs were counted using the Abbott Autologic Gamma Counter (1st count). (Results were used to plot Fig 7.3)

(iia) & (iiia)

After counting, the same tubes were used, 200 µl of ^{125}I HBsAg were added and incubated in a water bath at the same temperature for various periods of times. The tubes were then washed four time with 1 ml of distilled water. The radioactivities of bound protein were counted using Abbott Autologic Gamma Counter (2nd count) (Results were used to plot Fig 7.7)

One-step procedure (See diagram C(ia) & (iia))

Equal volume of ^{125}I anti-HBs and ^{125}I HBsAg were mixed thoroughly to form complex of antigen-antibody . 200 µl of ^{125}I complex that has been mixed and incubated for 10 minutes (ci) and 2 hours (cii) at 26°C respectively were added to the HBsAg coated tubes(WNR). The tubes were immersed in a water bath at 45°C. At different intervals, tubes were taken out and the radioactivities of the complex bound were counted by using Abbott Gamma Counter (Results were used to plot Fig 7.7)

(WNR tube = NR coated tube pre washed five times with 1 ml of 0.1M HCl followed by five washes with 1 ml of distilled water)

	Two-step procedure				One-step procedure			
	(a) Percent binding of ^{125}I anti-HBs (First count) **		(b)Percent binding of total ^{125}I anti-HBs + ^{125}I HBsAg (Second count) ***		(ci) Percent binding of ^{125}I complex * (10 minutes incubation)***		(cii) Percent binding of ^{125}I complex* (2 hours incubation)***	
Time/ h	WNR tube	PP tube	WNR tube	PP tube	WNR tube	PP tube	WNR tube	PP tube
0.17	2.92± 0.32	6.22± 0.39	0.98± 0.09	1.81± 0.10	1.17± 0.06	3.32± 0.14	1.30±0.05	3.04± 0.70
0.34	3.32± 0.40	11.30± 0.27	1.12± 0.21	3.27± 0.20	1.17± 0.45	4.16± 0.38	1.95±0.31	3.85± 0.10
0.50	3.87± 0.37	12.40± 0.75	1.33± 0.14	3.60± 0.15	2.26± 0.21	5.51± 0.15	2.70±0.06	5.08± 0.10
1.00	7.44± 0.78	19.76± 1.19	2.20± 0.32	5.66± 0.20	2.42± 0.59	6.65± 0.22	3.47±0.12	6.10± 0.25
2.00	6.17± 0.49	29.67± 0.06	1.88± 0.28	8.56± 0.20	4.38± 0.53	10.87 ±0.58	5.58±0.48	10.68± 0.54
3.00	8.26± 0.49	36.16± 2.73	2.46± 0.23	10.33± 0.20	5.67± 0.21	11.86 ±0.69	6.56±0.62	11.70± 0.43
4.00	13.12± 0.87	39.63± 0.09	3.12± 0.29	11.89± 0.30	6.05± 0.07	12.40 ±1.49	7.00±1.25	11.72± 0.31
5.00	11.11± 0.78	43.97± 1.79	2.94± 0.35	13.25± 0.25	6.88± 0.21	14.29 ±0.40	7.81±0.36	13.48± 0.30
6.00	13.54± 2.05	44.97± 0.34	3.89± 0.20	14.77±0 0.30	6.43± 0.98	14.39 ±0.20	7.83±0.41	14.23± 0.57
7.00	14.53± 1.86	45.56± 0.51	3.80± 0.20	13.86± 0.30	7.60± 0.05	14.73 ±0.29	9.19±1.10	14.26± 0.31
8.00	13.13± 2.71	48.57± 0.80	3.83± 0.13	15.23±0. 25		15.23 ±0.35		15.07± 0.34
8.50	11.74± 0.62	48.13± 0.70	3.44±	14.60± 0.23		14.28 ±0.83		15.24± 0.47
21.00					10.28± 0.20		11.52± 0.30	
22.00	17.24± 0.23	50.38± 0.52	5.02± 0.14	15.78± 0.21	11.28± 0.43	15.71 ±0.16	11.82± 0.16	15.75± 0.31

* ^{125}I complex = ^{125}I HBsAg + ^{125}I anti-HBs

** Used to plot fig 7.3

*** Used to plot fig 7.7

Table 7.5 Percent of specific and non-specific binding of (a) labelled HBsAg (b) total labelled anti-HBs and labelled HBsAg (c) ^{125}I complex on solid surface immobilised with anti-HBs as a function of reaction time

Two-step procedure (See diagram A)

(ib)

200 µl ^{125}I HBsAg were added to the anti-HBs coated & NBCS blocked tubes. The tubes were incubated at 45°C for various period of times. At regular intervals, the tubes were taken out and washed four times with 1 ml distilled water. The radioactivities of bound ^{125}I HBsAg were counted using the Abbott Autologic Gamma Counter (1st count) (Results were used to plot Fig 7.5)

(iib) & (iiib)

After counting, the same tubes were used, 200 µl of ^{125}I anti-HBsAg were added and incubated in a water bath at the same temperature for various periods of times. The tubes were then washed four times with 1 ml of distilled water. The bound radioactivities were counted using the Abbott Autologic Gamma Counter (2nd count) (Results were used to plot Fig 7.8)

One-step procedure (See Diagram C (ib) &(iib))

Equal volume of ^{125}I anti-HBs and ^{125}I HBsAg were mixed thoroughly to form complex of antigen-antibody. 200 µl of ^{125}I complex that has been mixed and incubated for 10 minutes (ci) and 2 hours (cii) at 26° C respectively were added into the anti-HBsAg coated tubes (WNR). The tubes were immersed in a water bath at 45°C. At different intervals, tubes were taken out and the activities of the complex bound were counted using Abbott Gamma Counter (Results were used to plot Fig 7.8)

(WNR tube = NR coated tube pre washed five times with 1 ml of 0.1 M HCl followed by five washes with 1 ml of distilled water)

	Two-step procedure				One-step procedure			
	(a) Percent binding of ^{125}I HBsAg (First count)**		(b)Percent binding of total ^{125}I anti-HBs + ^{125}I HBsAg (Second count) ***		(ci) Percent binding of ^{125}I complex* (10 minutes incubation) ***		(cii) Percent binding of ^{125}I complex* (2 hours incubation) ***	
Time/h	WNR tube	PP tube	WNR tube	PP tube	WNR tube	PP tube	WNR tube	PP tube
0.17	0.27± 0.02	0.28± 0.01	0.63± 0.02	0.16± 0.05	0.66± 0.05	0.17± 0.03	0.66± 0.03	0.16± 0.03
0.34	0.35± 0.01	0.42± 0.07	0.74± 0.01	0.26± 0.01	0.77± 0.04	0.24± 0.02	0.79± 0.06	0.18± 0.05
0.50	0.39± 0.01	0.43± 0.07	0.82± 0.01	0.26± 0.01	0.89± 0.04	0.25± 0.04	0.83± 0.02	0.28± 0.10
1.00	0.50± 0.05	0.83± 0.16	1.07± 0.02	0.47± 0.02	1.20± 0.04	0.25± 0.04	1.23± 0.02	0.38± 0.07
2.00	0.67± 0.08	0.73± 0.08	1.48± 0.01	0.48± 0.03	1.79± 0.01	0.33± 0.09	1.88± 0.08	0.39± 0.06
3.00	0.70± 0.06	0.89± 0.23	1.50± 0.03	0.62± 0.01	2.23± 0.10	0.46± 0.09	2.16± 0.25	0.40± 0.05
4.00	0.99± 0.11	1.19± 0.22	1.76± 0.01	0.78± 0.01	2.42± 0.20	0.45± 0.04	2.60± 0.14	0.35± 0.08
5.00		1.45± 0.15	1.76± 0.01	0.84± 0.02	2.78± 0.10	0.49± 0.04	2.58± 0.02	0.53± 0.09
6.00	0.92± 0.07	1.30± 0.17	1.73± 0.01	1.53± 0.02	2.72± 0.23	0.56± 0.04	2.70± 0.13	0.55± 0.08
7.00	0.91± 0.14	1.59± 0.23	1.88± 0.05		2.77± 0.11	0.65± 0.07	2.56± 0.47	0.64± 0.03
8.00	1.01± 0.03	1.81± 0.09	1.85± 0.01		2.96± 0.24	0.60± 0.02	2.79± 0.17	1.40± 0.09
9.00		1.97± 0.20			2.96± 0.05	0.71± 0.11	2.88± 0.12	1.66± 0.00
10.00	0.98± 0.03							
21.00	1.20± 0.06		2.09± 0.05		3.10± 0.03		2.84± 0.40	
23.00	1.21± 0.12	4.49± 0.63	1.91± 0.01	2.84± 0.01	3.06± 0.17	1.06± 0.11		2.23± 0.00

* ^{125}I complex = ^{125}I HBsAg + ^{125}I anti-HBs

** Used to plot fig 7.5

*** Used to plot fig 7.8

Table 7.6 Percent binding (specific and non-specific) of ^{125}I anti-HBs (^{125}I HBsAg) on anti-HBs (HBsAg) immobilised tube (See diagram B)

Anti-HBs (HBsAg) positive controlled serum were added to HBsAg (anti-HBs) coated and NBCS blocked tubes. The tubes were incubated at 45°C for various period of times. The tubes were then washed four times with 1 ml of distilled water. The same tubes were used to incubate with ^{125}I anti-HBs (^{125}I HBsAg) in a water bath at the same temperature for various periods of time. After incubation the tubes were washed four times with 1 ml of distilled water. The bound radioctivities of ^{125}I anti-HBs (^{125}I HBsAg) were counted using Abbott Autologic Gamma Counter. (Results were used to plot fig 7.4 & 7.6)

(WNR tube = NR coated tube pre washed five times with 1 ml of 0.1M HCl followed by five washes with 1 ml of distilled water)

	Percent binding of ^{125} I anti-HBs		Percent binding of ^{125} I HBsAg	
Time/h	WNR tube immobilised with anti-HBs	PP tube immobilised with anti-HBs	WNR tube immobilised with HBsAg	PP tube immobilised with HBsAg
0.17	1.38±0.04	0.46±0.14	0.38±0.05	0.37±0.03
0.34	1.64±0.11	0.79±0.19	0.59±0.00	0.81±0.13
0.50	1.91±0.13	0.93±0.22	0.80±0.15	0.88±0.14
1.00	1.96±0.29	1.69±0.20	1.35±0.18	1.81±0.24
2.00	3.07±0.20	2.37±0.23	2.40±0.33	4.04±0.18
3.00	3.27±0.44	2.96±0.28	2.73±0.23	5.39±0.22
4.00	3.80±0.29	3.95±0.17	2.57±0.26	6.57±0.95
5.00		4.29±0.36	2.98±0.20	7.71±0.32
6.00	4.22±0.14	4.36±0.15	3.50±0.12	8.77±0.59
7.00	4.10±0.59	4.87±0.57	3.72±0.46	8.24±0.37
8.00	4.70±0.40			
10.00	6.26±0.17			
21.00	12.29±0.24	9.15±0.70		
22.00			3.60±0.27	10.13±0.35

Table 7.7 The calculated surface concentration of labelled anti-HBs bound to surface immobilised with HBsAg as compared to theoretical surface concentration in relation to time. 200 µl ^{125}I anti-HBs were added to the HBsAg coated and NBCS blocked tubes. The tubes were incubated at 45°C for various period of times. At regular intervals, the tubes were removed and washed four times with 1 ml distilled water. The radioactivities of bound ^{125}I anti-HBs were counted using the Abbott Autologic Gamma Counter (1st count). The surface concentration of labelled anti-HBs were calculated as shown in section 7.3.5. (Results were used to plot Fig 7.9a & b)

Time/h	*Theoretical surface concentration x 10^{-3} (µg/cm^2)	Percent binding on WNR	Surface concentration on WNR x 10^{-4} (µg/cm^2)	Percent binding on PP	Surface concentration on PP x 10^{-3} (µg/cm^2)
0.17	0.87	2.92	1.29	6.22	0.27
0.34	1.24	3.32	1.47	11.30	0.50
0.5	1.51	3.87	1.71	12.40	0.55
1	2.14	7.44	3.29	19.76	0.87
2	3.03	6.17	2.73	29.67	1.31
3	3.71	8.26	3.65	36.16	1.60
4	4.28	13.12	5.80	39.63	1.75
5	4.79	11.11	4.91	43.97	1.94
6	5.24	13.54	5.99	44.97	1.98
7	5.66	14.53	6.42	45.56	2.01
8	6.06	13.13	5.80	48.57	2.15
22	10.04	17.27	7.62	50.38	2.22

*The theoretical surface concentration were counted using equation 1. Diffusion constant used = 4.00×10^{-7} cm^2/s

Table 7.8 The calculated surface concentration of labelled HBsAg bound to surface immobilised with anti-HBs as compared to theoretical surface concentration in relation to time.
200 µl ^{125}I HBsAg were added to the anti-HBs coated and NBCS blocked tubes. The tubes were incubated at 45°C for various period of times. At regular intervals, the tubes were removed and washed four times with 1 ml distilled water. The radioactivities of bound ^{125}I HBsAg were counted using the Abbott Autologic Gamma Counter (1st count). The surface concentration of labelled HBsAg were calculated as shown in section 7.3.5. (Results were used to plot Fig 7.10a & 7.10b)

Time/h	*Theoretical surface concentration x 10^{-3} (µg/cm^2)	Percent binding on WNR	Surface concentration on WNR x10^{-5} (µg/cm^2)	Percent binding on PP	Surface concentration on PP x 10^{-5} (µg/cm^2)
0.17	0.66	0.27	1.19	0.28	1.24
0.34	0.93	0.35	1.55	0.42	1.86
0.5	1.14	0.39	1.72	0.43	1.90
1	1.61	0.50	2.21	0.83	3.67
2	2.28	0.67	2.96	0.73	3.23
3	2.80	0.70	3.09	0.89	3.93
4	3.23	0.99	4.38	1.19	5.26
5	3.61		4.07	1.45	6.41
6	3.95	0.92	4.02	1.30	5.75
7	4.27	0.91	4.47	1.59	7.03
8	4.56	1.01	4.33	1.81	8.00
9	4.84			1.97	8.71
10	5.10	0.98			
21	7.40	1.20	5.31		
23	7.75	1.21	5.35	4.49	19.85

*The theoretical surface concentration were counted using equation 1. Diffusion constant used = 2.278×10^{-7} cm^2/s

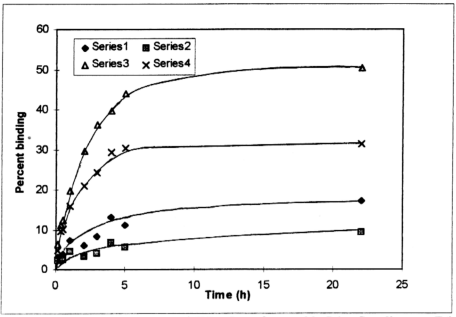


Fig 7.1 Dissociation of bound labelled anti-HBs (Data from table 7.3a; See diagram E(ia) & (iia) & (iiia)

Solid surface immobilised with HBsAg and blocked with 50% NBCS. 200µl ^{125}I anti-HBs were added to the HBsAg coated tubes. The tubes were incubated at 45°C for various period of times. At regular intervals, the tubes were taken out and washed four times with 1 ml of distilled water. The radioctivities of bound ^{125}I anti-HBsAg were counted using Abbott Autologic Gamma Counter (1st count). After counting, 200 µl of HBsAg positive control serum were added to the same tubes and incubated in a water bath at the same temperature for various period of times. The tubes were then washed four times with 1 ml of distilled water. The final radioactivities of bound ^{125}I anti-HBs were counted again using Abbott Gamma Counter (2nd count)

Series 1 - WNR tube immobilised with HBsAg (1st count)
Series 2 - WNR tube immobilised with HBsAg (2nd count)
Series 3 - PP tube immobilised with HBsAg (1st count)
Series 4 - PP tube immobilised with HBsAg (2nd count)

(WNR tube = NR coated tube pre washed five times with 1 ml of 0.1M HCl followed by five washes with 1 ml of distilled water)

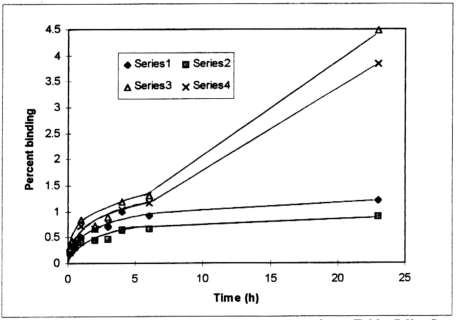


Fig 7.2 Dissociation of bound labelled HBsAg (Data taken from Table 7.3b; See diagram E(ib)&(iib)&(iiib))

Solid surface immobilised with anti-HBs and blocked with 50% NBCS. 200 µl ^{125}I HBsAg were added into the anti-HBs coated tubes. The tubes were incubated at 45°C for various period of times. At regular intervals, the tubes were taken out and washed four times with 1 ml distilled water. The radioactivities of bound ^{125}I HBsAg were counted using Abbott Autologic Gamma Counter (1st count). After counting, 200 µl of anti-HBs positive control serum were added to the same tubes and incubated in a water bath at the same temperature for various period of times. The tubes were then washed four times with 1 ml of distilled water. The radioactivity of bound ^{125}I HBsAg were counted using Abbott Autologic Gamma Counter again (2nd count)

(WNR tube = NR coated tube pre washed five times with 1 ml of 0.1M HCl followed by five washes with 1 ml of distilled water)

Series 1 - WNR tube immobilised with anti-HBs (1st count)
Series 2 - WNR tube immobilised with anti-HBs (2nd count)
Series 3 - PP tube immobilised with anti-HBs (1st count)
Series 4 - PP tube immobilised with anti-HBs (2nd count)

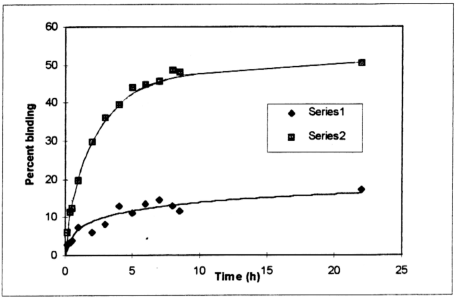


Fig 7.3 Percent binding of labelled anti-HBs on solid surface immobilised with HBsAg as a function of reaction time (Data from Table 7. 4a; See diagram A(ia))

200 µl ^{125}I anti-HBs were added to the HBsAg coated & NBCS blocked tubes. The tubes were incubated at 45°C for various period of times. At regular intervals, the tubes were removed and washed four times with 1 ml of distilled water. The radioactivities of bound ^{125}I anti-HBs were counted using an Abbott Autologic Gamma Counter

Series 1 -WNR as solid phase
Series 2- PP as solid phase

(WNR tube = NR coated tube pre washed five times with 1 ml of 0.1M HCl followed by five washes with 1 ml of distilled water)

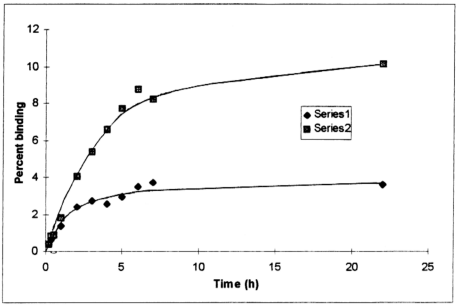


Fig 7.4 Percent binding of labelled HBsAg on surface immobilised with HBsAg after preincubation with anti-HBsAg positive control serum as a function of time (Data from Table 7.6; See diagram B(ia)&(iia)&(iiia))

Anti-HBs positive controlled serum were added to HBsAg coated tubes (blocked with NBCS). The tubes were incubated at 45°C for various period of times. The tubes were then washed four time with 1 ml of distilled water. The same tubes were then allow to react with ^{125}I HBsAg at the same temperature for various periods of times. After incubation the tubes were washed four times with 1 ml of distilled water. The radioactivities of ^{125}I HBsAg bound were counted using Abbott Gamma Counter .

Series 1 HBsAg immobilised on WNR surface
Series 2 HBsAg immobilised on PP surface

(WNR tube = NR coated tube pre washed five times with 1 ml of 0.1M HCl followed by five washes with 1 ml of distilled water)

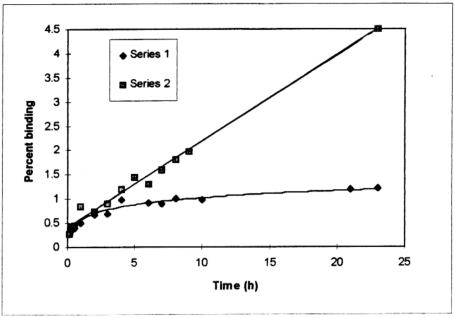


Fig 7.5 Percent binding of labelled HBsAg on solid surface immobilised with anti-HBs as a function of time (Data taken from Table 7.5a ; See diagram A(ib))
200 µl ^{125}I HBsAg were added to the anti-HBs coated & NBCS blocked tubes. The tubes were incubated at 45°C for various period of times. At regular intervals, the tubes were taken out and washed four times with 1 ml of distilled water. The radioactivities of bound ^{125}I HBsAg were counted using the Abbott Autologic Gamma Counter

Series 1 - WNR as solid phase
Series 2 - PP as solid phase

(WNR tube = NR coated tube pre washed five times with 1 ml of 0.1M HCl followed by five washes with 1 ml of distilled water)

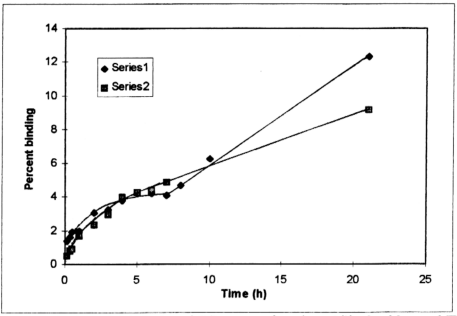


Fig 7.6 Percent binding of labelled anti-HBs on surface immobilised with anti-HBs after preincubation with HBsAg positive control serum as a function of time (Data from Table 7.6; (See diagram B(ib)&(iib)&(iiib))

HBsAg positive control serum were added to anti-HBs coated tubes(and blocked with NBCS). The tubes were incubated at 45°C for various period of times. The tubes were then washed fours times with 1 ml of distilled water. The same tubes were allowed to react with ^{125}I HBsAg at the same temperature for various periods. After incubation the tube were washed four times with 1 ml of distilled water. The radioactivities of bound ^{125}I anti-HBs were counted using Abbott Autologic Gamma Counter.

Series 1- anti-HBs immobilised on WNR surface
Series 2 - anti-HBs immobilised on PP surface

(WNR tube = NR coated tube pre washed five times with 1 ml of 0.1M HCl followed by five washes with 1 ml of distilled water)

Fig 7.7 Percent binding of (a) total ^{125}I labelled anti-HBs and ^{125}I labelled HBsAg (b) ^{125}I complex on surface immobilised with HBsAg as a function of reaction time. (Data taken from Table 7.4)

(a)Two-step procedure (See diagram A)

(ia)

200 µl ^{125}I anti-HBs were added to the HBsAg coated & NBCS blocked tubes. The tubes were incubated at 45°C for various period of times. At regular intervals, the tubes were taken out and washed four times with 1 ml of distilled water. The bound radioactivities were counted (See Table 7.4(a)).

(iia & (iiia)

The same tubes were used. 200 µl of ^{125}I HBsAg were added and incubated in a water bath at the same temperature for various periods of times. The tubes were then washed four times with 1 ml of distilled water. The radioactivities of bound protein were counted using Abbott Autologic Gamma Counter (Two-step incubation - Series 1&4) (See Table 7.4(b))

(b)One-step procedure (See diagram C(ia))

Equal volume of ^{125}I anti-HBs and ^{125}I HBsAg were mixed thoroughly to form a complex of antigen-antibody . 200 µl of ^{125}I complex that had been mixed and incubated for 10 minutes (ci) and 2 hours (cii) at 26° C respectively were transferred to the HBsAg coated tubes. The tubes were incubated at 45°C. At different intervals, tubes were removed , washed and the radioactivities of the bound complex were counted using Abbott Autologic Gamma Counter (See Table 7.4(c)) (1-step incubation - Series 2,3,5,6)

Series 1 - WNR surface immobilised with HBsAg (2-step procedure)
Series 2 - WNR surface immobilised with HBsAg (1-step procedure, mixture was used after 10 minutes of mixing)
Series 3 - WNR surface immobilised with HBsAg (1-step procedure, mixture was used after two hours of mixing)
Series 4 - PP surface immobilised with HBsAg (2-step procedure)
Series 5 - PP surface immobilised with HBsAg (1-step procedure, mixture was used after 10 minutes of mixing)
Series 6 - PP surface immobilised with HBsAg (1-step procedure, mixture was used after two hours of mixing)

(WNR tube = NR coated tube pre washed five times with 1 ml of 0.1 M HCl followed by five washes with 1 ml of distilled water)

Fig 7.7 Percent binding of (a) total labelled anti-HBs and labelled HBsAg (b) Labelled complex on solid surface immobilised on HBsAg as a function of reaction time

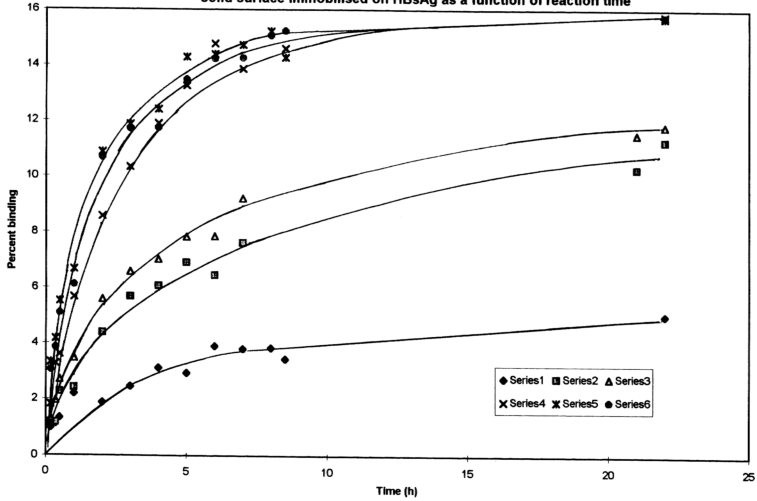


Fig 7.8 Percent binding of (a) total ^{125}I labelled anti-HBs and ^{125}I HBsAg (b) ^{125}I complex on solid phase immobilised with anti-HBs as a function of reaction time (Data taken from Table 7.5)

(a)Two-step procedure (See diagram A)
(ib)
200 µl ^{125}I HBsAg were added to the anti-HBs coated & NBCS blocked tubes. The tubes were incubated at 45°C for various period of times. At regular intervals, the tubes were taken out and washed four times with 1 ml of distilled water. The bound radioactivities of ^{125}I HBsAg were counted (see table 7.5a)

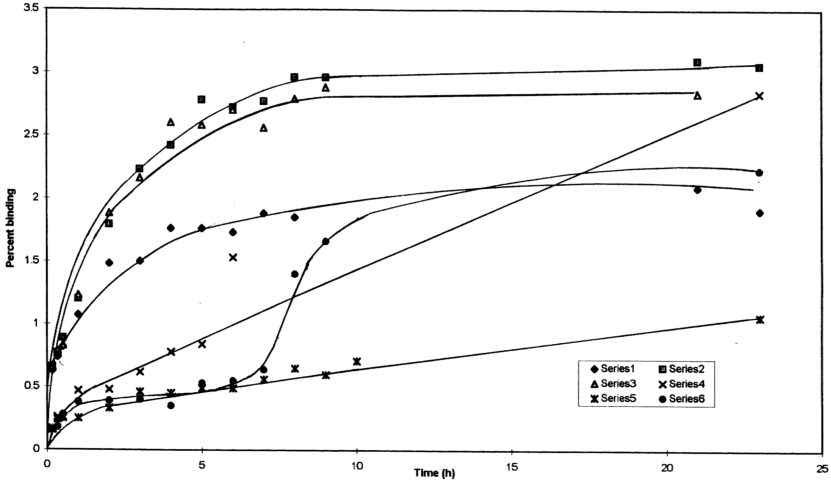
(iib)& (iiib)
200 µl of ^{125}I anti-HBs were added to the same tube and incubated in a water bath at the same temperature for various periods of times. The tubes were then washed four time with 1 ml of distilled water. The radioactivities of bound protein were counted using the Abbott Gamma Autologic Counter (2-step incubation procedure) (Series 1,4)(vide infra)(See Table 7.5b)

(b)One-step procedure (See diagram C(ib))

Equal volume of ^{125}I anti-HBs and ^{125}I HBsAg were mixed thoroughly to form complex of antigen-antibody. 200 µl of ^{125}I complex that has been mixed and left for 10 minutes (ci) and 2 hours (cii) at 26°C respectively were transferred to the anti-HBs coated tubes. The tubes were immersed in a water bath at 45°C. At different intervals, tubes were taken out and the radioactivities activity of the complex bound were counted using Abbott Autologic Gamma Counter (1-step procedure - Series, 2,3,5,6)(See Table 7.5c)

Series 1 - WNR surface immobilised with anti-HBs (2-step procedure)
Series 2 - WNR surface immobilised with anti-HBs (1-step procedure, used after 10 minutes of mixing)
Series 3 - WNR surface immobilised with anti-HBs (1-step procedure, used after two hours of mixing)
Series 4 - PP surface immobilised with anti-HBs (2-step procedure)
Series 5 - PP surface immobilised with anti-HBs (1-step procedure, used immediately after mixed)
Series 6 - PP surface immobilised with anti-HBs (1-step procedure, used after two hours of mixing)
(WNR tube = NR coated tube pre washed five times with 1 ml of 0.1M HCl followed by five washes with 1 ml of distilled water)

Fig 7.8 Percent binding of (a) total labelled HBsAg and labelled anti-HBs (b) Labelled complex on solid surface immobilised with anti-HBs as a fuction of reaction time



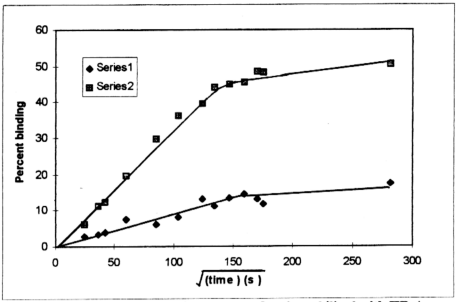


Fig 7.9(a) Percent binding of labelled anti-HBs on surface immobilised with HBsAg as a function of $t^{1/2}$ of incubation (Data from table 7.7)

200 µl ^{125}I anti-HBs were added to the HBsAg coated & NBCS blocked tubes. The tubes were placed in a water bath, the binding of antigen to antibodies was allowed to continue at 45°C for various periods of times. At regular intervals, the tubes were removed and washed four times with 1 ml of distilled water. The radioactivities of bound ^{125}I anti-HBs were counted using the Abbott Autologic Gamma Counter .Series 1 - WNR as solid phase. Series 2- PP as solid phase. (WNR tube = NR coated tube pre washed five times with 1 ml of 0.1M HCl followed by five washes with 1 ml of distilled water)

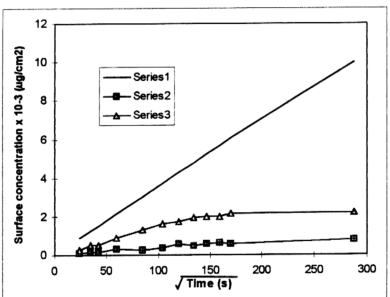


Fig 7.9(b) The surface concentration of labelled anti-HBs on HBsAg immobilised on WNR and PP tube in relation to the square root of time (Amount of antibody that could reach the surface by diffusion) (Data taken from Table 7.7) Series 1 = theoretical line; Series 2 = WNR; Series 3 = PP (Diffusion constant used = 4.00×10^{-7} cm^2 / s)

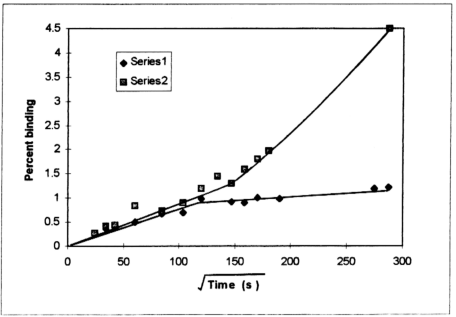
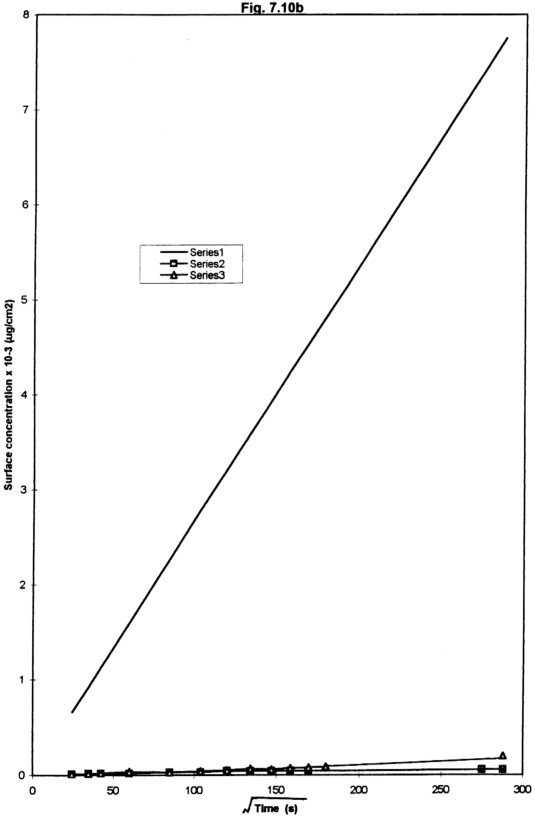


Fig 7.10a Percent binding of labelled HBsAg on surface with immobilised anti-HBs as a function of $t^{1/2}$ of incubation (Data from table 7.8)

200 µl ^{125}I HBsAg were added to the anti-HBs coated & NBCS blocked tubes. The tubes were incubated at 45°C for various period of times. At regular intervals, the tubes were removed and washed four times with 1 ml distilled water. The radioactivities of bound ^{125}I anti-HBs were counted using the Abbott Autologic Gamma Counter . Series 1 - WNR as solid phase. Series 2- PP as solid phase .(WNR tube = NR coated tube pre washed five times with 1 ml of 0.1M HCl followed by five washes with 1 ml of distilled water)

Fig 7.10b The surface concentration of labelled HBsAg bound to anti-HBs immobilised on WNR and PP tube in relation to the square root of time (Amount of HBsAg that could reach the surface by diffusion) (Data taken from Table 7.8) Series 1 = theoretical line; Series 2 = WNR; Series 3 = PP (Diffusion constant 2.278×10^{-7} cm^2/s)

Fig. 7.10b


Series1
Series2
Series3
Surface concentration x 10-3 (ug/cm2)
√Time (s)
0
1
2
3
4
5
6
7
8
0
50
100
150
200
250
300

7.4 Discussion

7.4.1 Percent binding of labelled anti-HBs (HBsAg) on PP/NR surfaces immobilised with HBsAg (anti-HBs)

The kinetics of the binding of labelled anti-HBs and HBsAg to immobilised HBsAg and anti-HBs on both NR and PP surfaces are shown in Fig 7.3 & 7.5. From the Fick's law of diffusion it can be deduced that for an unstirred solution and flat surface geometry, the diffusion-limited amount of bound antibody S is given by

$$S = 2Co(Dt)^{1/2} / (\pi)^{1/2} \text{ '(Rodbard \& Drazier 1975)} \quad (1)$$

where Co is the concentration of protein in solution introduced at time zero and D is the diffusion constant of protein molecules. To check this relation, the percent binding of labelled anti-HBs and HBsAg onto HBsAg and anti-HBs immobilised surface were plotted as a function of the square root time ($t^{1/2}$) as shown in Fig 7.9 a & &.7.10a. The amount of bound anti-HBs or HBsAg (in percent binding) increased linearly with $(t)^{1/2}$ at short incubation time. However, at longer incubation time the rate of increase in percent binding became slower on both WNR and PP surfaces (except for binding of labelled HBsAg on to PP surface immobilised with anti-HBs). The surface concentrations of bound labelled anti-HBs on HBsAg immobilised tube and labelled HBsAg on anti-HBs immobilised tube plotted as a function of the square root of time were shown in Fig 7.9(b) & Fig 7.10(b). The theoretical amount of bound labelled anti-HBs (on HBsAg immobilised surface) and labelled HBsAg (on anti-HBs immobilised surface) was calculated according to equation (1) given above based on a diffusion constant of 4.00×10^{-7} cm^2/s (Nygren

et al., 1987) for anti-HBs and 2.28 x 10^{-7} cm^2/s (Kim & Tilles, 1973) for HBsAg. On the surface immobilised with HBsAg (Fig 7.9 b), the surface concentration of bound labelled antibody increased slowly during the initial stage. The rate of antibody-binding was slower at saturation level. As it can be seen, the experimentally measured initial binding was well below that predicted by theory. The disparity between experimental values and theoretical prediction became wider at longer incubation times. Between WNR and PP surfaces, the disparity was the worse for WNR surface than for PP surface. The agreement between theoretical and experimental amount bound of anti-HBs onto WNR immobilised with HBsAg surface was poor right from very short incubation time (Table 7.7; Fig 7.9b). The experimentally determined concentrations on WNR and PP surfaces were very much lower than the theoretical value on surface immobilised with anti-HBs (Fig 7.10b). The amount of labelled HBsAg bound onto both WNR and PP surface was two orders of magnitude smaller than the theoretical value even at very early incubation time. The discrepancy between the theoretical and experimental values could be due to some factors as discussed in section 7.4.2.

The percent bindings of the labelled anti-HBs by HBsAg immobilised on PP and WNR surfaces at 50.38% and 17.24% (From Table 7.4(a)) percent binding (highest percent binding) are equivalent to a corresponding surface concentration of $1.48x10^{-14}$ and $5.08x10^{-15}$ mol/cm^2 respectively . These amounts of antibody bound

by the immobilised HBsAg are equivalent to a corresponding surface densities of $9.0x10^9$ and $3.1x10^9$ IgG molecules/cm^2 respectively (See Section 7.3.5b).

Binding of labelled anti-HBs to PP surface with immobilised HBsAg reached saturation after 22 hours to give a percent binding of 50.38 % (Fig 7.3). For labelled HBsAg bound to PP surface immobilised with anti-HBs surface only 4.49% was bound after 23 hour. Moreover the saturation level was not achieved at this time (Fig 7.5). The surface coverage of ^{125}I HBsAg on PP surface immobilised with anti-HBs was $5.0x10^{-17}$ mol/ cm^2 which is equal to a surface density of $3.0x10^7$ molecules /cm^2 (refer 7.3.5c). The percent binding of labelled HBsAg immobilised on WNR surface reached a saturation level of 1.21% (Table 7.5) equivalent to a labelled HBsAg surface concentration of $1.3x10^{-17}$ mol/cm^2. This amount of bound antigen was equal to a surface density of $7.8x10^6$ IgG molecules/cm^2. The high percent binding of labelled anti-HBs to surface (PP & WNR) immobilised with HBsAg as compared to the binding of labelled HBsAg on surface (PP & WNR) immobilised with anti-HBs could possibly be due to

(i)Surface concentration of HBsAg immobilised on PP surface was higher than that of anti-HBs, thus higher percent of labelled anti-HBs is bound by the surface immobilised with the antigen.

(ii) Higher molecular weight of HBsAg would result in its slower diffusion to the solid interface, consequently the rate of antigen binding by the antibody was slower. For WNR surface, high percent binding of anti-HBs on HBsAg immobilised surface could be additionally caused by high non-specific binding

of anti-HBs, because anti-HBs had higher affinity towards WNR surface. The decrease in the rate of binding may be due to depletion of the available immobilised surface protein.

The initial binding rate of anti-HBs or HBsAg to solid phase immobilised with HBsAg or anti-HBs was diffusion controlled at both WNR and PP surfaces. The rate of diffusion of the initial reaction had previously been used to compute the coupling between the intrinsic reaction rate (Stenberg et al., 1982) and mass transport in the field of heterogeneous catalyst (Wagner, 1943; Weisz & Hiches', 1962). Diffusion-rate limiting of biospecific reactions at solid surfaces have been shown experimentally previously for enzyme-substrate reactions (Trurnit, 1954), binding of cholera toxin to ganglioside GM1 (Stenberg et al., 1982), protein adsorption (De Feijter, 1978), binding of polyclonal antibodies to protein antigen (Stenberg et al., 1982; Nygren and Stenberg, 1985), and monoclonal antibodies to surface immobilised with antigen (Nygren et al, 1987). There was no correlation between the antibody affinity for the surface immobilised antigen and the diffusion rate limitation of the association reaction (Nygren et al., 1987).The mechanisms behind the diffusion rate limitation have been described theoretically by Stenberg et al., 1986. Measurements of antibody binding to solid-phase immobilised with antigen have revealed that the kinetics of the reaction differ from the kinetics of the corresponding liquid phase reaction in several respects (Sternberg et al; 1986). Theories of possible diffusion-rate limitation in forward reaction rates are compared with experimental work (Stenberg & Nygren, 1988). It is found that the intrinsic

forward reaction rate in the biomolecular antigen-antibody reaction is normally not limited by diffusion either in solution or at the solid-liquid interface. However, reactions at the solid-liquid interface can be diffusion limited due to depletion of reactants close to the surface. The effect depends on the geometry, intrinsic reaction rate and surface concentration of binder molecules. Normally cell surface reactions are not diffusion-rate limited whereas reactions at artificial surfaces often are limited by diffusion. For reactions not limited by diffusion, it is found that the intrinsic forward and reverse reaction rates are lower for surface reactions compared to reactions in solution.

7.4.2 Low binding activity of immobilised-HBsAg and anti-HBs on WNR surface as compared to PP immobilised surface

As discussed in Chapter 2, there are three possible factors which could affect the low percent binding of HBsAg or anti-HBs by WNR surfaces immobilised with anti-HBs or HBsAg respectively. These are as follows

(i) Occurrence of substantial denaturation when antigen and polyclonal antibodies are adsorbed onto the WNR surface (resulting in low binding capacity)

(ii) Clusters formation of immobilised antigen and antibodies after immobilisation and their partial submersion into the natural rubber layer. This could cause steric hindrance and reduction in binding capacity of the immobilised antibodies or antigens.

(iii) Exchange of immobilised protein on WNR surface with added protein during the assay. PP surface caused less denaturation of protein upon immobilisation on

the solid substrate and it also resulted in even spreading of the immobilised protein layer on the solid surface, thus reducing the dissociation of protein from the solid surface. Further investigations by kinetics studies were carried out to verify these :

(iiia) Experiments were carried out to determine the desorption of labelled anti-HBs or HBsAg from both solid surfaces. Williams (1988) had reported that more immobilised labelled HSA was desorbed from the solid phase in the presence of unlabelled HSA than in buffer solution, indicating occurrence of exchange of protein (antibody/antigen) molecules between the solid phase and the reactant. Therefore the desorption of labelled protein from solid surface in the presence of BSA, gelatin, NEO & NBCS was investigated. The percentage of labelled anti-HBs and HBsAg desorbed from the PP surface was less than that from WNR surface for each step of the assay procedure (Table 7.2 Count 1 to 4). Proteins immobilised on PP surface can unfold to form a tigthly bound thin layer on the solid phase and is difficult to displace, whereas protein (antibody/antigen) immobilised on WNR surface formed clusters or aggregates and probably can be easily displaced. Though the amount desorbed from WNR surface was relatively high, the percent binding of labelled protein remained on WNR surface after each step was still higher than those on PP surface. This could be due to a high initial concentration of immobilised antigen or antibody per unit surface area (Table 7.2). Thus desorption of bound labelled proteins from WNR surface cannot account totally for its low binding capacity.

(iiib) The binding rate of labelled HBsAg and anti-HBs onto the PP surface immobilised with anti-HBs or HBsAg was faster than that of WNR surface (Fig 7.3 & 7.5). On PP surface, the percent binding was three to four times higher than those on WNR surface at the plateau level. As shown in Chapter 2 both anti-HBs and HBsAg were bound abundantly more on WNR than on PP surface. The results suggest that low sensitivity of HBsAg or anti-HBs assay using WNR as a solid phase is due to reduced interaction between the second (labelled anti-HBs or HBsAg) and the first layer immobilised protein (HBsAg & anti-HBs). The severe deactivation and steric effect on the immobilised protein could be a result of cluster formation which reduced the effective binding activity of the immobilised anti-HBs and HBsAg on the NR surface (See Chapter 2).

(iiic) Dissociation of bound labelled anti-HBs and HBsAg from HBsAg and anti-HBs immobilised surface was observed when antigen/antibody was added (Fig 7.1 & 7.2). However percentage of dissociation was higher on WNR surface when compared with PP surface (Table 7.3a & 7.3b). In antigen-antibody interactions, specific interaction occurs between the epitope (of the antigen) and the paratope (of antibody). In general, all immunodominant epitopic sites are hydrophilic and they may possess a net electrical charge in some cases, and are electrically neutral in others. When a net epitopal charge exists, the corresponding paratopes will have an electrical charge of the opposite sign. However, when there are alternating positively and negatively

charged amino acids on one site and a complementary array on the other site (e.g. +-+- on the epitope and -+-+ on the paratope), then a very low overall charge and no long range overall net electrostatic (EL) attraction on either complementarity-determining regions (CDR) would result. However, such CDR are very hydrophobic (Oss & Good, 1988) and thus will instead engage in a long range hydrophobic AB (Lewis acid-base) attraction (Oss, 1995). In the subsequent short range interaction, however, an EL attraction between, for instance, +-+- and -+-+ sites, as used in the example given above, can play a role in strengthening the bond. In addition, paratopes tend to be hydrophobic, which necessitate them to be situated on the inside of a concave 'cleft' of the antibody molecule. Complementary epitopes and paratopes need to be fitted together sterically as precisely as possible, so as to be able to interact with the largest possible surface area, over the shortest possible distance. The surface areas of epitopes and paratopes are rather small, i.e. of the order of 0.4-10 n^{m2}. After initial contact is made between epitope and paratope (the primary interactions), secondary interactions set in. First, interstitial water is expelled, which can change the hydrophobic interaction into a direct contact interaction. Then, peptides on antigen and antibody molecules in the vicinity of the epitope and paratope are brought closer together through the neighbouring primary epitope-paratope reaction, and these then often engage in a secondary hydrophobic attraction, which further strengthens the overall antigen-antibody bond (Oss, 1995). All antigen- antibody bonds are weak physical bonds. The

main bonds involved are : (I) Coulombic bonds; (II) Ca^{2+}- bridges; (III) hydrogen bonds (IV) Lifshitz-van der Waals bonds. Combinations of III and IV are normally known as hydrophobic interactions. The degree of involvement of the above interactions varies considerably among different antigen-antibody combinations (Oss, 1986). Since antigen-antibody are bonded by weak forces, therefore dissociation of antigen-antibody can occur. Various factors like surface tension of the aqueous medium, pH , ionic strength affect the degree of dissociation (Oss, 1986).

Previous studies of kinetics of antibody binding to surface-immobilised with antigen, using polyclonal hyperimmune sera, have shown that the dissociation rate of antibody from antigen-antibody complex is a slow process, with a half-life of more than 240 h (Nygren and Stenberg,1985), and is much slower at an interface than in solution. This slow dissociation has been attributed to the bivalence of antibodies in combination with the high local concentration of antigen on the surface (De Lisi, 1980; Berzowsky and Berkower,1984). Nygren (Nygren et al., 1987) showed that both the bivalence of antibody and surface concentration at the solid-phase contributed to the stability of surface-bound antigen-antibody complexes. It was found by ellipsometry that the bound antibodies did not dissociate when rinsed with saline for up to 20 hours, but dissociation occurred in the presence of antigen (Nygren, 1987). The dissociation did not follow any identifiable rate constant and as such it is not possible to interpret the data from the dissociation experiments based on local equilibrium at the interface. Nygren (1987) showed that

stirring the rinsing buffer increased the dissociation rate of Fab fragments in PBS, suggesting that the diffusion of the dissociated ligand at the interface may limit the dissociation rate. The findings that antibodies dissociated only in the presence of antigen could be interpreted in two ways, either as blocking of a reassociation or as an induced dissociation of bound antibodies. The dissociation of bound antibodies from the immobilised antigen in this experiment is similar to the dissociation of adsorbed protein through an exchange reactions with other protein (Vroman et al., 1980). Dissociation of anti-HBs or HBsAg bound to HBsAg or anti-HBs surface was further demonstrated by Ch'ng et al. (1985). He showed that dissociation of HBsAg bound to anti-HBs immobilised on solid phase occurred in the presence of low as well as high doses of antigen. Dissociation was not due to lack of immobilised antibody or HBsAg non-specifically bound to the immobilised antibody. As shown in Table 7.1a-d, the results obtained agree closely with those of Ch'ng et al. who showed that there still available immobilised antibody for formation of complex with antigen after the first re-equilibration as indicated by re-equilibration of positive serum with WNR tube or PP tube immobilised with anti-HBs (Table 7.1a-d). The dissociated antigen sequestered the ^{125}I labelled antibody resulting in a decrease in percent binding of the assay (Fig 7.2). This proposed mechanism can also be applied to anti-HBs assay system. High percentage of dissociation of labelled anti-HBs, labelled anti-HBs - HBsAg complex or labelled HBsAg - anti-HBsAg from solid surface immobilised with anti-HBs or HBsAg could be another

factor that contributes to a lower binding in assay when WNR is used as a solid surface instead of PP surface.

7.5 Conclusion

The binding of anti-HBs or HBsAg to surface immobilised with HBsAg or anti-HBs could be regarded as a reversible reaction involving an equilibrium state. The amount of antibody or antigen bound to the surface increased during the entire experiment on both surfaces in contrast to a reversible reaction. The saturation level of binding of labelled anti-HBs or labelled HBsAg to surface immobilised with HBsAg or anti-HBs could be explained by several reasons. (a) Saturation of binding sites according to a simple Langmuir isotherm (binding of labelled anti-HBs on HBsAg immobilised surface). (Langmuir,1918) (b) Steric blocking of available antigen/antibody by bound antibody/antigen (Nygren et al., 1986) (c) Equilibrium according to the law of mass action. The low sensitivity of WNR rubber was affected by (i) Desorption of immobilised proteins from solid surface (ii) High deactivation and steric effect due to cluster formation which could reduce the binding activity of anti-HBs and HBsAg immobilised on WNR surface (iii) Dissociation of bound anti-HBs and HBsAg from HBsAg and anti-HBs immobilised surface .

References

1. Berzowsky, I.A. and Berkower, I. J. (1984) in : " *Fundamental Immunology"* Paul, W.E (Raver Press, New York) p.595
2. Ch'ng S.L (1991) Developments in Radioimmunoassay and Related Procedures. *"Proceedings of International Symposium on Radioimmunoassay and related procedures "* : Perspectives in developing countries organised by International Atomic Energy Agency in co-operation with the World Health Organisation, Vienna, 26-30 August 1991
3. Ch'ng, S.L., How V.J.L., Soon, K.F., Tan, H.T. (1985) *J. Immunoassay* **8(2&3),** 237-245
4. De Feijtler,J.A., Benjamins, I. and Veer, F.A.(1978) Ellipsometry as a tool to study the adsorption behaviour of synthetic and biopolymers at the air-water interface. *Biopolymers* **17**, 1975
5. De Lisi, C. (1980) *Quantum . Review. Biophys.* **13**, 201
6. Kim,C.Y and Tilles, J.G. (1973) *J. Clin. Invest.* **52**, 1173-1180
7. Langmuir, I.(1918) *J. Am. Chem. Soc.* **40**, 1361
8. Li, C.K.N. (1985) ELISA-based determination of immunological binding constant. *Mol. Immunol.* **22**, 321
9. Nygren, H. and Stenberg, M., (1985) *J. Coll. Interface Sci.* **107,** 560
10. Nygren, H., Werthen, M. and Sternberg, M.(1987) Kinetics of antibody binding to solid-phase-immobilised antigen *J. Immunol. Methods* **101,** 63-71

11. Nygren, H., Kaartinen, M., Sternberg, M. (1986) Determination by ellipsometry of the affinity of monoclonal antibodies. *J. Immunol. Methods* **92,** 219

12. Oss, C.J. van and Good, R.J. (1988) *Protein Chem.* **7,** 179

13. Oss, C.J. Van., Good, R.J., Chaudhury, M.K. (1986) *J. Chromatography* **376,** 111-119

14. Oss, C.J.van. (1995) *Mol. Immunol.* **32,** 199

15. Rodbard, D. and Drazier, G.R. (1975) in ' *Methods in Enzymology* ' **37B**, 1

16. Skinhoj, P. and Hansen, J.F.(1973) *Scand. J. Lab. Invest.* **32**, 199-203

17. Stenberg, M. and Nygren, H.(1983) The use of the isoscope ellipsometer in the study of adsorbed proteins and biospecific reactions. *J. Phys. (Paris) Colloq.* **10**, 12.

18. Sternberg, M., Nygren, H. (1988) Kinetics of antigen-antibody reactions at solid-liquid interfaces. *J. Immunol. Methods* **113,** 3-15

19. Sternberg, M., Elwing, H. and Nygren, H. (1982) Kinetics of reaction zone formation with radial diffusion in solid -phase immunassays. *J. Theor. Biol.* **98**, 307

20. Sternberg, M., Stiblert, L. and Nygren, H.(1986) External diffusion in solid phase immunoassays. *J. Theor. Biol.* **120**, 129

21. Trurnit, H. J.(1954) Studies on enzyme systems at a solid-liquid interface.II. The kinetics of adsorption and reaction. *Arch. Biochem.* **51**, 176.

22. Turner, M.W. (1977) Structure and function of Immuglobulins. In (Glynn, L.E & Steward, M.E eds) : *"Structure and function of antibodies"* , John Wiley, London, p3-4

23. Vroman, L; Adams, A.L; Fischer, G.C and Munoz, P.C. (1980) Interaction of high molecular weight kininogen, Factor XII & fibrinogen in plasma at interfaces. *Blood* **55**, 156

24. Wagner, C.Z. (1943) *Physiol. Chem.* **A 193**, 1

25. Weisz, P.B. and Hichs, I.S. (1962) *Chem. Eng. Sci.* **17**, 265

26. Williams, R.L. and Williams, D.F. (1988) *Biomaterial* **9**, 206