[Chem. Pharm. Bull. 24(12)2949—2954(1976)]

UDC 547.962.3.04 : 546.271.04

Interaction between Serum Albumin and Mercaptoundecahydrododecaborate Ion (An Agent for Boron-Neutron Capture Therapy of Brain Tumor). III. Results of Analysis

Toshio Nakagawa[1a] and Tadashi Nagai[1]

Shionogi Research Laboratory, Shionogi & Co., Ltd.[1]

(Received March 17, 1976)

The interaction of mercaptoundecahydrododecaborate with native bovine serum albumin (BSA), human serum albumin (HSA), and BSA denatured with urea has been investigated by the method described in the preceding papers. The results can be reasonably explained with the random-pairing model, rather than with the monogamous-pairing model. It is inferred that the denaturation of BSA increases both the number of active disulfide groups and their ability to react with the borate while native BSA and HSA behave much the same.

In the preceding paper, two reaction models (random-pairing and monogamous-pairing) have been proposed for the interaction between the borate and protein. The objects of the present paper are to find the reaction parameters by which the experimental data are best explained (best-fit parameters) and to decide which of the two models is more adequate.

Table I. Interaction of Bovine Serum Albumin (native[a]) with $B_{12}H_{11}SH^{2-}$

No.	Protein conc. M $[P]\times10^4$	Borate conc. M $[B]\times10^4$	$[B]/[P]_t$	ν_{obs}	No.	Protein conc. M $[P]\times10^4$	Borate conc. M $[B]\times10^4$	$[B]/[P]_t$	ν_{obs}
1	2.83	1.62	0.572	0.054	27	1.68	0.493	0.294	0.026
2	2.83	1.08	0.381	0.043	28	1.40	33.0	23.7	0.265
3	2.83	0.539	0.191	0.031	29	1.40	26.4	18.9	0.283
4	2.81	2.79	0.994	0.057	30	1.40	19.8	14.2	0.271
5	2.81	2.23	0.795	0.052	31	1.38	14.7	10.7	0.287
6	2.68	59.4	22.1	0.244	32	1.35	60.6	44.8	0.291
7	2.68	47.5	17.7	0.261	33	1.35	48.5	35.8	0.300
8	2.68	35.6	13.3	0.203	34	1.35	37.3	27.6	0.209
9	2.68	23.7	8.85	0.201	35	1.35	36.4	26.9	0.261
10	2.68	11.9	4.42	0.140	36	1.35	24.2	17.9	0.219
11	2.67	7.20	2.70	0.139	37	1.35	12.1	8.96	0.166
12	2.23	2.80	1.26	0.068	38	1.34	4.99	3.73	0.125
13	2.23	2.24	1.00	0.050	39	1.11	2.71	2.45	0.126
14	2.23	1.68	0.753	0.062	40	1.11	2.17	1.96	0.067
15	2.23	1.12	0.503	0.049	41	1.11	1.63	1.47	0.065
16	2.23	0.560	0.251	0.030	42	0.676	36.6	54.2	0.363
17	2.10	67.9	32.3	0.271	43	0.676	29.3	43.4	0.289
18	2.10	54.3	25.8	0.208	44	0.676	22.0	32.5	0.271
19	2.10	40.8	19.4	0.259	45	0.676	14.7	21.7	0.214
20	2.10	27.2	12.9	0.230	46	0.676	7.33	10.8	0.199
21	2.10	13.6	6.46	0.155	47	0.558	2.91	5.22	0.165
22	2.03	37.3	18.4	0.200	48	0.558	2.33	4.17	0.140
23	1.68	2.47	1.47	0.085	49	0.558	1.75	3.13	0.093
24	1.68	1.97	1.18	0.056	50	0.558	1.17	2.09	0.113
25	1.68	1.48	0.883	0.050	51	0.558	0.582	1.04	0.075
26	1.68	0.986	0.588	0.044					

a) in 1/15 M phosphate buffer (pH 7.4)

1) Location: *Fukushima-ku, Osaka*; a) Present address: *Furuedai 3-24-4, Suita, Osaka, 565, Japan.*

Experimental Data

Test solutions with various concentrations of $Cs_2B_{12}H_{11}SH$ and native bovine serum albumin (BSA) were made by mixing a protein solution and a borate solution both prepared with a 1/15 M phosphate buffer (pH 7.4) which had been deoxygened and stored under nitrogen atmosphere. A vessel containing the test solution was alternately degased and charged with nitrogen gas several times. After 20 hr incubation at 37°, an aliquot (0.5—4.0 ml) of the solution was poured into an ion-retardation resin (7 or 30 ml) column, and eluted with deionized water. The protein-containing fraction was analysed to measure the protein and borate contents by the methods written in Part I of this series of papers,[2] where the description for the protein, borate, and resin is also given. The data are summarized in Table I.

On assuming that the reaction equilibrium has been established in 20 hr incubation and that no reaction has taken place during the elution process of about 5 minutes, x_{obs} in mole/mole unit is obtained as the borate concentration divided by the protein concentration (*cf.* Part II for the definitions of x_{obs} and x_{theo}). The adequacy of these assumptions was supported by the finding that the prolongation of incubation time and/or elution time within a reasonable range brought about no significant differences in the results.

Determination of Best-Fit Parameters by Simplex Method

A set of parameters (K_1, K_2, α, β) that makes $S=\sum_{i=1}^{51}\left(\frac{(x_{obs})_i-(x_{theo})_i}{(x_{theo})_i}\right)^2$ minimum has been found out by the modified simplex method detailed in Part II.[3] To increase the confidence that the "global" optimum (not a "local" optimum) has been attained, the calculation was started from two widely differing regions in the four-dimensional hyperspace, according to the suggestion given by Deming and Morgan.[4]

Table II. Simplex Calculation for Native BSA-$B_{12}H_{11}SH^{2-}$ (Random-Pairing Model)

	No. of cycles	K_1	K_2	α	β	S
a)	0	2.555×10^{-2}	4.028×10^{-3}	7.305×10^{-2}	5.559×10^{-1}	1.610×10^{0}
	218	1.241×10^{-2}	1.581×10^{-3}	2.062×10^{-2}	6.005×10^{-1}	1.189×10^{0}
	276	1.243×10^{-2}	1.583×10^{-3}	2.072×10^{-2}	6.003×10^{-1}	1.189×10^{0}
	325	1.243×10^{-2}	1.578×10^{-3}	2.072×10^{-2}	5.999×10^{-1}	1.189×10^{0}
	376	1.243×10^{-2}	1.583×10^{-3}	2.072×10^{-2}	6.000×10^{-1}	1.189×10^{0}
	422	1.243×10^{-2}	1.585×10^{-3}	2.075×10^{-2}	6.002×10^{-1}	1.189×10^{0}
	478	1.245×10^{-2}	1.564×10^{-3}	2.068×10^{-2}	5.989×10^{-1}	1.189×10^{0}
	583	1.247×10^{-2}	1.566×10^{-3}	2.075×10^{-2}	5.986×10^{-1}	1.189×10^{0}
	655	1.246×10^{-2}	1.562×10^{-3}	2.068×10^{-2}	5.986×10^{-1}	1.189×10^{0}
	713	1.246×10^{-2}	1.565×10^{-3}	2.073×10^{-2}	5.986×10^{-1}	1.189×10^{0}
b)	0	1.753×10^{-1}	4.435×10^{-1}	5.782×10^{-1}	3.406×10^{0}	27.19×10^{0}
	147	5.865×10^{-3}	1.012×10^{-2}	2.507×10^{-2}	1.397×10^{0}	1.213×10^{0}
	353	1.250×10^{-2}	1.536×10^{-3}	2.061×10^{-2}	5.961×10^{-1}	1.189×10^{0}
	416	1.252×10^{-2}	1.541×10^{-3}	2.074×10^{-2}	5.963×10^{-1}	1.189×10^{0}
	467	1.251×10^{-2}	1.549×10^{-3}	2.075×10^{-2}	5.968×10^{-1}	1.189×10^{0}
	545	1.250×10^{-2}	1.551×10^{-3}	2.071×10^{-2}	5.969×10^{-1}	1.189×10^{0}
	592	1.250×10^{-2}	1.546×10^{-3}	2.072×10^{-2}	5.968×10^{-1}	1.189×10^{0}
	658	1.250×10^{-2}	1.551×10^{-3}	2.072×10^{-2}	5.970×10^{-1}	1.189×10^{0}
	724	1.249×10^{-2}	1.553×10^{-3}	2.071×10^{-2}	5.974×10^{-1}	1.189×10^{0}
c)[a]	7	1.247×10^{-2}	1.554×10^{-3}	2.071×10^{-2}	5.979×10^{-1}	1.189×10^{0}

a) the best-fit parameters

Table II shows how the four parameters and S-value of the best apex of a new-born simplex changed with the number of calculation cyclings (*cf.* Fig. 4 in Part II) in the case of random-pairing model. After about 700 cyclings, these five values nearly coincide between

2) T. Nakagawa and T. Nagai, *Chem. Pharm. Bull.* (Tokyo), **24**, 2934 (1976).
3) T. Nakagawa and T. Nagai, *Chem. Pharm. Bull.* (Tokyo), **24**, 2942 (1976).
4) S.N. Deming and S.L. Morgan, *Anal. Chem.*, **45**, 278A (1973).

(a) and (b) series. Restarting from a new simplex whose two apexes are the best apexes in the last simplexes of (a) and (b) series, the best-fit parameters have been determined after 7 cyclings, when any of the five values changed no more in the range of four digits. It should be noticed that the S-value is rather insensitive to small changes in the four parameters. After 218 cyclings in (a) series and 353 cyclings in (b) series, the S-value varies within a range of 0.001 although the four parameters still change considerably. The best-fit parameters can be determined precisely on a purely mathematical standpoint. Too much practical significance, however, should not be placed on small variance in their values. Similar calculations for the monogamous-pairing model are shown in Table III.

TABLE III. Simplex Calculation for Native BSA-$B_{12}H_{11}SH^{2-}$ (Monogamous-Pairing Model)

No. of cycles		K_1	K_2	α	β	S
a)	0	2.555×10^{-2}	4.028×10^{-3}	7.305×10^{-2}	5.559×10^{-1}	4.093×10^{6}
	277	2.206×10^{1}	2.847×10^{-6}	1.257×10^{1}	4.001×10^{-1}	3.997×10^{0}
	425	6.866×10^{1}	2.066×10^{-6}	4.072×10^{0}	3.819×10^{-1}	3.978×10^{0}
	625	2.721×10^{2}	5.626×10^{-10}	9.577×10^{-1}	3.473×10^{-1}	3.912×10^{0}
	674	5.420×10^{2}	1.034×10^{-9}	3.952×10^{-1}	3.204×10^{-1}	3.872×10^{0}
	750	6.429×10^{2}	1.158×10^{-9}	3.283×10^{-1}	3.111×10^{-1}	3.865×10^{0}
	813	6.433×10^{2}	1.155×10^{-9}	3.278×10^{-1}	3.111×10^{-1}	3.865×10^{0}
	883	6.442×10^{2}	1.150×10^{-9}	3.274×10^{-1}	3.110×10^{-1}	3.865×10^{0}
	943	6.423×10^{2}	1.146×10^{-9}	3.286×10^{-1}	3.112×10^{-1}	3.865×10^{0}
b)	0	9.753×10^{2}	4.435×10^{-3}	3.005×10^{-1}	1.114×10^{0}	2.646×10^{1}
	75	2.491×10^{2}	3.511×10^{-4}	1.005×10^{0}	3.531×10^{-1}	3.936×10^{0}
	200	5.600×10^{2}	4.121×10^{-7}	3.944×10^{-1}	3.182×10^{-1}	3.868×10^{0}
	262	6.420×10^{2}	4.385×10^{-7}	3.287×10^{-1}	3.113×10^{-1}	3.865×10^{0}
	328	6.429×10^{2}	4.409×10^{-7}	3.280×10^{-1}	3.113×10^{-1}	3.865×10^{0}
	394	6.426×10^{2}	4.441×10^{-7}	3.285×10^{-1}	3.112×10^{-1}	3.865×10^{0}
c)[a]	43	6.423×10^{2}	2.902×10^{-8}	3.285×10^{-1}	3.113×10^{-1}	3.865×10^{0}

a) the best-fit parameters

Superiority between the Two Models

The superiority between the random- and monogamous-pairing models can be judged on three criteria.

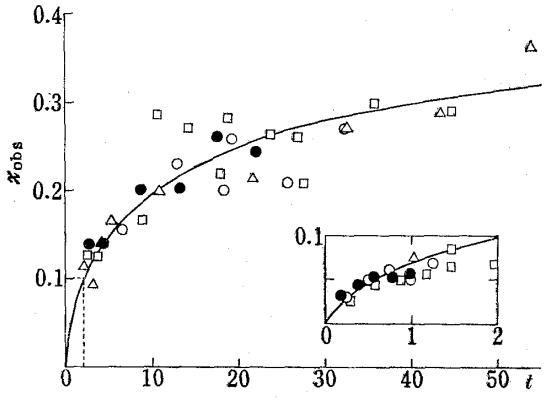

Fig. 1. x_{obs} plotted against t=[B]/[P] for the Native BSA-$B_{12}H_{11}SH^{2-}$ System

[P]: ● 3.0—2.5, ○ 2.5—2.0, □ 2.0—1.0, △ 1.0—0.5×10^{-4}M

The referential $x_{theo}\sim t$ curve has been calculated on assuming the random-pairing model with $K_1=1.247\times10^{-2}$, $K_2=1.554\times10^{-3}$, $\alpha=2.071\times10^{-2}$, and $\beta=5.979\times10^{-1}$.

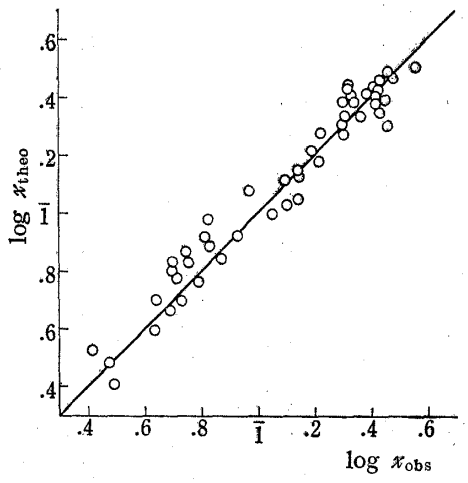

Fig. 2. log x_{theo} (Random-Pairing Model) plotted against log x_{obs}

a) As seen in Table II and III, the sum of squared relative errors, S, for the best-fit parameters of the random-pairing model is considerably smaller than that of the monogamous-pairing model. The average relative error calculated by $|(x_{obs}-x_{theo})/x_{theo}|_{av}=(S/n)^{1/2}$ with $n=51$ is 15.3% for the former model and 27.5% for the latter model. The former value is comparable with the error of boron analysis,[2] while the latter value is far larger. On this criterion, the random-pairing model is better than the monogamous-pairing model.

b) The plot of x_{obs} against t shown in Fig. 1 also supports the superiority of the random-pairing model. In spite of a wide distribution of total protein concentrations, all data can be delineated by a single curve whose slope at the origin is smaller than unity (*cf.* the Appendix of Part II, Fig. 3).

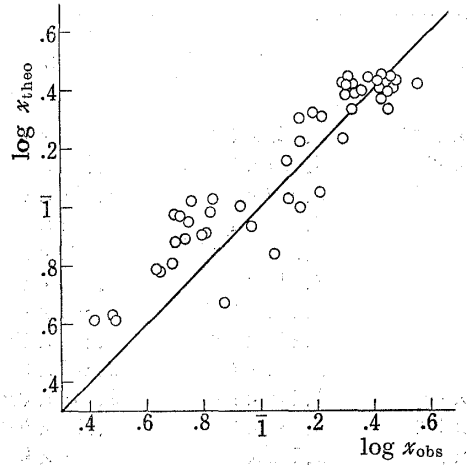

Fig. 3. log x_{theo} (Monogamous-Pairing Model) plotted against log x_{obs}

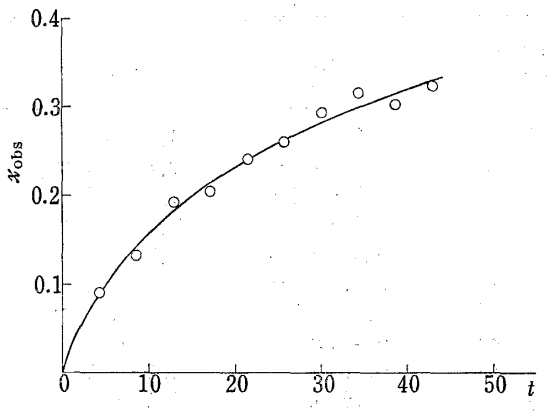

Fig. 4. x_{obs} plotted against t for the Native HSA-$B_{12}H_{11}SH^{2-}$ System

The referential $x_{theo}\sim t$ curve has been calculated on assuming the random-pairing model with $K_1=8.447\times10^{-3}$, $K_2=1.157\times10^{-3}$, $\alpha=2.454\times10^{-1}$, and $\beta=9.355\times10^{-1}$.

TABLE IV. Simplex Calculation for Native HSA-$B_{12}H_{11}SH^{2-}$ and for Denatured BSA-$B_{12}H_{11}SH^{2-}$

No. of cycles	K_1	K_2	α	β	S
Native HSA					
a) 0	1.753×10^{-1}	4.435×10^{-1}	5.782×10^{-1}	3.406×10^{0}	4.827×10^{0}
106	4.175×10^{-3}	1.079×10^{-1}	9.094×10^{-1}	5.523×10^{0}	2.018×10^{-2}
1493	7.547×10^{-3}	1.552×10^{-3}	2.400×10^{-1}	1.024×10^{0}	1.770×10^{-2}
1951	7.570×10^{-3}	1.510×10^{-3}	2.393×10^{-1}	1.020×10^{0}	1.770×10^{-2}
b) 0	4.809×10^{-3}	2.374×10^{-4}	3.562×10^{-1}	8.571×10^{-1}	5.240×10^{0}
257	1.114×10^{-2}	1.647×10^{-4}	2.596×10^{-1}	7.534×10^{-1}	1.775×10^{-2}
616	8.605×10^{-3}	1.068×10^{-3}	2.455×10^{-1}	9.197×10^{-1}	1.770×10^{-2}
c)[a] 73	8.447×10^{-3}	1.157×10^{-3}	2.454×10^{-1}	9.355×10^{-1}	1.769×10^{-2}
Denatured BSA					
a) 0	2.555×12^{-2}	4.028×10^{-3}	7.305×10^{-2}	5.559×10^{-1}	7.220×10^{3}
263	1.825×10^{0}	4.010×10^{-6}	4.766×10^{-2}	1.101×10^{1}	2.137×10^{-1}
510	1.818×10^{0}	3.993×10^{-7}	4.947×10^{-5}	1.100×10^{1}	2.131×10^{-1}
b) 0	1.753×10^{-1}	4.435×10^{-1}	5.782×10^{-1}	3.406×10^{0}	1.036×10^{3}
448	1.383×10^{0}	3.962×10^{-2}	2.262×10^{-9}	1.344×10^{1}	2.191×10^{-1}
609	1.818×10^{0}	2.545×10^{-6}	2.214×10^{-9}	1.100×10^{1}	2.131×10^{-1}
c) 0	9.380×10^{-3}	7.998×10^{-4}	2.511×10^{-1}	8.618×10^{-1}	1.118×10^{4}
142	1.972×10^{-1}	5.638×10^{-3}	8.373×10^{-6}	3.513×10^{1}	5.674×10^{-1}
329	1.814×10^{0}	4.681×10^{-7}	5.903×10^{-5}	1.101×10^{1}	2.131×10^{-1}
d)[a] 5	1.817×10^{0}	1.791×10^{-6}	1.623×10^{-5}	1.100×10^{1}	2.131×10^{-1}

a) the best-fit parameters

c) A more commonsense judgement may be made by the comparison between Figs. 2 and 3. Data points for the random-pairing model scatter more closely along the 45°-line than those for the monogamous-pairing model do, thus indicating again that the former model is preferable.

Additional Data and Interpretation of Them

By using human serum albumin (HSA) in place of BSA, similar experiments have been carried out with ten solutions. These solutions contained various amounts ($6.1—61\times10^{-4}$M) of $Cs_2B_{12}H_{11}SH$ and a definite amount (1.42×10^{-4}M) of HSA in a 1/15M phosphate buffer of pH 7.4. The results have been treated with the simplex method on assuming the random-pairing model to derive the best-fit parameters through the calculation course shown in Table IV. The data points for x_{obs} in Fig. 4 fall on or near the $x_{theo}\sim t$ curve obtained from these parameters.

The effect of denaturation of protein on the protein-borate interaction has been investigated with ten solutions containing 5.97×10^{-5}M BSA, 8M urea, and $1.59—43.4\times10^{-4}$M $Cs_2B_{12}H_{11}SH$ in a 1/15M phosphate buffer (pH 7.8). The results are summarized in Table IV and Fig. 5.

Table V compares the best-fit parameters among native BSA, native HSA, and denatured BSA. Denaturation of BSA causes prominent increases in β and K_1, indicating that both the number of active disulfide groups and their ability to react with the borate are augmented by the denaturation. Simultaneous decreases in α and K_2 may not be significant because S

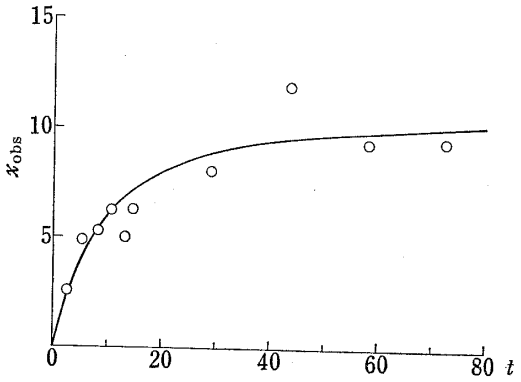

Fig. 5. x_{obs} plotted against t for the Urea-Denatured BSA-$B_{12}H_{11}SH^{2-}$ System

The referential $x_{theo}\sim t$ curve has been calculated on assuming the random-pairing model with $K_1=1.817\times10^0$, $K_2=1.791\times10^{-6}$, $\alpha=1.623\times10^{-5}$, and $\beta=1.100\times10^1$.

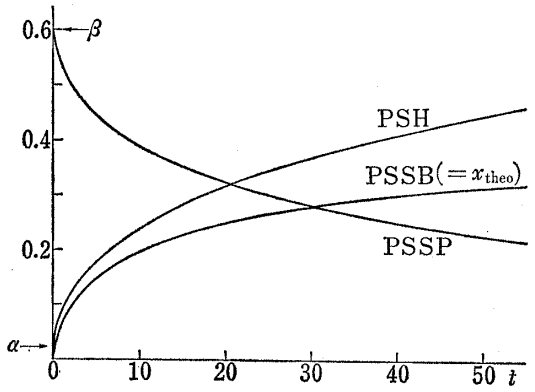

Fig. 6. [PS-SB]/[P]$\sim t$, [PS-SP]/[P]$\sim t$, and [PSH]/[P]$\sim t$ Curves expected for the Native BSA-$B_{12}H_{11}SH^{2-}$ System (Random-Pairing Model)

TABLE V. Best-Fit Parameters for Albumin-$B_{12}H_{11}SH^{2-}$ Interaction (Random-Pairing Model)

	Native BSA[a]	Native HSA[a]	Denatured BSA[b]
K_1	1.247×10^{-2}	8.447×10^{-3}	1.817×10^0
K_2	1.554×10^{-3}	1.157×10^{-3}	1.791×10^{-6}
α	2.071×10^{-2}	2.454×10^{-1}	1.623×10^{-5}
β	5.979×10^{-1}	9.355×10^{-1}	1.100×10^1
S	1.189×10^0	1.769×10^{-2}	2.131×10^{-1}
Number of data	51	10	10
$\left\lvert\frac{x_{obs}-x_{theo}}{x_{theo}}\right\rvert_{av}\times100$	15.3%	4.2%	14.6%

a) incubated for 20 hr at 37° in 1/15 M phosphate buffer (pH 7.4)
b) incubated for 20 hr at 37° in 1/15 M phosphate buffer with 8 M urea (pH 7.8)

is rather insensitive to the variation of these parameters as seen, for instance, in the underlined data of Table IV. No significant differences are found between the parameters for native BSA and HSA, suggesting that the two proteins have similar properties. Because of limited number and variety of available data, especially because of only one protein concentration examined, these interpretations should be considered as tentative ones, although they are compatible with our current knowledge of these proteins.

Once the best-fit parameters are determined, the theoretical concentrations of PS-SB, PS-SP, PSH, BS-SB, and BSH can be calculated as a function of total protein concentration [P] and total borate concentration [B], or as a function of [P] and t=[B]/[P]. In the case of random-pairing model, each of these five concentrations divided by [P] is a function of t only, as pointed out in Appendix of Part II.[3] Figure 6 illustrates [PS-SB]/[P]-t, [PS-SP]/[P]—t, and [PSH]/[P]-t curves for the native BSA-borate system in the range of t=0—54.2. In this range, both [BS-SB]/[P] and [BSH]/[P] increased almost linearly from 0 to 0.0584, and from 0 to 53.7, respectively. The [PS-SB]/[P]-t curve rises monotonically in Fig. 6, but it reaches a maximum of 0.350 at about t=140 and then falls gradually. Similar figures have been depicted for the native HSA-borate and denatured BSA-borate systems but are not reproduced here.