

# A thermodynamic study on the binding of mercury and silver ions to urease

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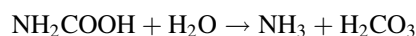
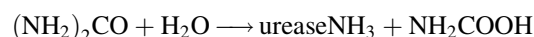
**Abstract** In this article, a thermodynamic study on the interaction of Jack bean urease, JBU, with  $\text{Hg}^{2+}$  and  $\text{Ag}^+$  ions were studied by isothermal titration calorimetry (ITC) at 300 and 310 K in 30 mM Tris buffer solution, pH 7.0. The heats of  $\text{JBU} + \text{Hg}^{2+}$  and  $\text{JBU} + \text{Ag}^+$  interactions are reported and analyzed in terms of the extended solvation model. It was indicated that there are a set of 12 identical and non-cooperative sites for  $\text{Hg}^{2+}$  and  $\text{Ag}^+$  ions. The binding of  $\text{Hg}^{2+}$  and  $\text{Ag}^+$  ions with JBU are exothermic with association equilibrium constants of 5415.65 and 4368.15 for  $\text{Ag}^+$  and 2389 and 2087  $M^{-1}$  for  $\text{Hg}^{2+}$  at 300 and 310 K, respectively.

**Keywords** Jack bean urease · Isothermal titration calorimetry · Inhibitor · Entropy

## Introduction

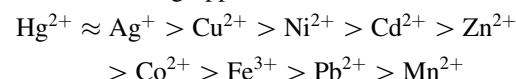
Urease, the first enzyme to be crystallized, is a nickel metallo enzyme that catalyzes the degradation of urea to ammonia and carbamine acid, a reaction of great agricultural and medical importance. The latter compound

decomposes to generate a second molecule of ammonia and carbon dioxide.

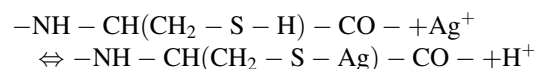


Jack bean urease is important in the nitrogen cycle of nature [1–3]. The inhibition of urease by heavy metal ions has been usually attributed to the reaction of the ions with enzyme thiol groups, resulting in the formation of mercaptides.

Heavy metal ions inhibit both plant and bacteria ureases at the following approximate order of effectiveness [4, 5]:



The mechanism of inhibition of urease by these metals as being due to the blocking of essential thiol groups on the enzyme. The monovalent heavy metals get attached to the sulfur in place of hydrogen in  $-\text{SH}$  groups of cysteine residue on protein chain as follows:



The divalent heavy metals such mercury, cadmium, copper, or lead ions also attach to the sulfur in place of hydrogen:



In this article, we have attempted to find the binding parameters and conformational changes of JBU due to its binding with  $\text{Hg}^{2+}$  and  $\text{Ag}^+$  ions.

## Materials and experiment

Jack bean urease (JBU; MW = 545.34 kDa) and Mercury (II) and silver nitrates were obtained from Merck. The

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**Table 1** The heats of JBU + Hg<sup>2+</sup> interaction, *q*, at 300 (○) and 310 K (□)

[Hg <sup>2+</sup> ]/μM	[JBU]/μM	<i>q</i> (○)/μJ	<i>q</i> <sub>dilut</sub> (○)/μJ	<i>q</i> (□)/μJ	<i>q</i> <sub>dilut</sub> (□)/μJ
110	9.890	-494.6	-328.1	-424.0	-321.4
217	9.783	-851.8	-610.5	-739.4	-598.1
323	9.677	-1116.5	-841.5	-979.6	-824.5
426	9.574	-1318	-1038	-1166.8	-1017.0
526	9.474	-1475.3	-1206.7	-1315.9	-1182.4
625	9.375	-1600.9	-1343.9	-1436.9	-1316.9
722	9.278	-1703.2	-1463.7	-1536.9	-1434.3
816	9.184	-1788	-1566.9	-1620.7	-1535.4
909	9.091	-1859.3	-1653.6	-1691.8	-1620.3
1000	9.000	-1920	-1723.9	-1752.9	-1689.2
1089	8.911	-1972.3	-1788.8	-1805.9	-1752.7
1176	8.824	-2017.7	-1845.7	-1852.3	-1808.5
1262	8.738	-2057.6	-1897.1	-1893.2	-1859.0
1346	8.654	-2092.8	-1939.1	-1929.6	-1900.2
1429	8.571	-2124.2	-1978	-1962.1	-1938.3
1509	8.491	-2152.3	-2011.4	-1991.3	-1971.0
1589	8.411	-2177.6	-2042.2	-2017.7	-2001.3
1667	8.333	-2200.5	-2070.7	-2041.7	-2029.2
1743	8.257	-2221.3	-2096.8	-2063.6	-2054.8
1818	8.182	-2240.3	-2119.5	-2083.6	-2077.0
1892	8.108	-2257.7	-2139.8	-2102.0	-2096.9
1964	8.036	-2273.7	-2157.7	-2119.0	-2114.5
2035	7.965	-2288.5	-2174.3	-2134.7	-2130.8
2105	7.895	-2302.2	-2189.7	-2149.3	-2145.9
2174	7.826	-2314.9	-2203.9	-2162.9	-2159.8
2241	7.759	-2326.8	-2216.5	-2175.5	-2172.1
2308	7.692	-2337.9	-2227.4	-2187.3	-2182.7
2373	7.627	-2348.2	-2236.5	-2198.4	-2191.7
2437	7.563	-2357.9	-2245.2	-2208.8	-2200.3
2500	7.500	-2367	-2252.7	-2218.6	-2207.7

*q*<sub>dilut</sub> are the heats of dilution of Hg(NO<sub>3</sub>)<sub>2</sub> with water. Precision is ± 0.100 μJ or better

buffer solution used in the experiments was 30 mM Tris, pH 7.0, which was obtained from Merck. Experiments were carried out in 300 and 310 K. The isothermal titration microcalorimetric experiments were performed with the four channel commercial microcalorimetric system, Thermal Activity Monitor 2277, Thermometric, Sweden. The titration vessel was made from stainless steel. Mercury solution (10 mM) was injected by use of a Hamilton syringe into the calorimetric titration vessel, which contained 1.8 mL JBU (10 μM). Thin (0.15 mm inner diameter) stainless steel hypodermic needles, permanently fixed to the syringe, reached directly into the calorimetric vessel. Injection of metal nitrates solution into the perfusion vessel was repeated 30 times, with 20 μL per injection. The calorimetric signal was measured by a digital voltmeter

that was part of a computerized recording system. The heat of each injection was calculated by the “Thermometric Digital 3” software program. The heat of dilution of the metal nitrates solutions were measured as described above except JBU was excluded. The microcalorimeter was frequently calibrated electrically during the course of the study. The measured heats for JBU + Hg<sup>2+</sup> and JBU + Ag<sup>+</sup> interactions were listed in Tables 1 and 2.

## Results and discussion

In the new solvation theory, the heats of the ligand + JBU interactions in the aqueous solvent systems can be calculated via the following Eq. [6–14]:

**Table 2** The heats of JBU + Ag<sup>+</sup> interaction at 300 K (○), 310 K (□) in 30 mM tris buffer solution of pH 7

[Ag <sup>+</sup> ]/mM	[JBU]/μM	q/μJ (○)	q <sub>dilut</sub> /μJ (○)	q/μJ (○)	q <sub>dilut</sub> /μJ (○)
0.110	3.956	-485.8	-318.6	-415.6	-292.0
0.217	3.913	-737.4	-592.8	-649.0	-543.4
0.323	3.871	-885.6	-816.9	-794.4	-748.9
0.425	3.83	-982.0	-1007.7	-892.6	-923.8
0.526	3.789	-1049.3	-1171.5	-963.0	-1074.1
0.625	3.75	-1098.8	-1305.0	-1015.8	-1196.2
0.722	3.711	-1136.6	-1421.4	-1056.8	-1302.9
0.816	3.673	-1166.5	-1521.6	-1089.5	-1394.7
0.909	3.636	-1190.6	-1605.8	-1116.2	-1471.8
1.000	3.6	-1210.5	-1674.0	-1138.4	-1534.5
1.089	3.564	-1227.2	-1737.1	-1157.1	-1592.2
1.1765	3.529	-1241.4	-1792.2	-1173.1	-1642.8
1.262	3.495	-1253.6	-1842.0	-1186.9	-1688.5
1.346	3.461	-1264.2	-1882.8	-1199.0	-1726.0
1.428	3.428	-1273.5	-1920.6	-1209.6	-1760.7
1.509	3.396	-1281.8	-1953.0	-1219.0	-1790.2
1.589	3.364	-1289.2	-1983.0	-1227.4	-1817.7
1.667	3.333	-1295.8	-2010.3	-1235.0	-1843.0
1.743	3.303	-1301.7	-2035.3	-1241.9	-1866.1
1.818	3.273	-1307.1	-2057.4	-1248.1	-1886.4
1.892	3.243	-1312.0	-2077.2	-1253.8	-1904.6
1.964	3.214	-1316.5	-2094.6	-1259.0	-1920.5
2.035	3.186	-1320.6	-2110.8	-1263.8	-1935.4
2.1053	3.158	-1324.4	-2126.2	-1268.2	-1949.1
2.174	3.130	-1327.9	-2139.6	-1272.3	-1961.8
2.241	3.103	-1331.1	-2151.7	-1276.1	-1972.9
2.308	3.077	-1334.1	-2162.4	-1279.6	-1982.8
2.373	3.051	-1336.9	-2171.6	-1282.9	-1991.0
2.437	3.025	-1339.5	-2179.8	-1286.0	-1998.6
2.500	3.000	-1342.0	-2187.0	-1288.9	-2005.3

Precision in q, is ± 0.1 μJ or better

$$q = q_{\max}x'_B - \delta_A^\theta(x'_A L_A + x'_B L_B) - (\delta_B^\theta - \delta_A^\theta)(x'_A L_A + x'_B L_B)x'_B \tag{1}$$

The values of q are the heats of ligand + JBU interactions, and q<sub>max</sub> represents the heat value upon saturation of all JBU. The parameters δ<sub>A</sub><sup>θ</sup> and δ<sub>B</sub><sup>θ</sup> are the indexes of JBU stability in the low and high ligand concentrations, respectively. The positive values for δ<sub>A</sub><sup>θ</sup> or δ<sub>B</sub><sup>θ</sup> indicate that ligand stabilizes the enzyme structure and vice versa. Also, δ<sub>B</sub> < 0 shows electrostatic interactions and δ<sub>B</sub> > 0 shows hydrophobic interactions. δ<sub>A</sub> < 0 shows that non-specific interactions are dominant and δ<sub>A</sub> > 0 shows specific interaction are dominant. If the binding of ligand at one site increases the affinity for ligand at another site, the macromolecule exhibits positive cooperativity. Conversely, if the binding of ligand at one site lowers the

affinity for ligand at another site, the enzyme exhibits negative cooperativity. If the ligand binds at each site independently, the binding is non-cooperative.

p > 1 or p < 1 indicate positive or negative cooperativity of macromolecule for binding with ligand, respectively; p = 1 indicates that the binding is non-cooperative. x'<sub>B</sub> can be expressed as follows:

$$x'_B = \frac{px_B}{x_A + px_B} \tag{2}$$

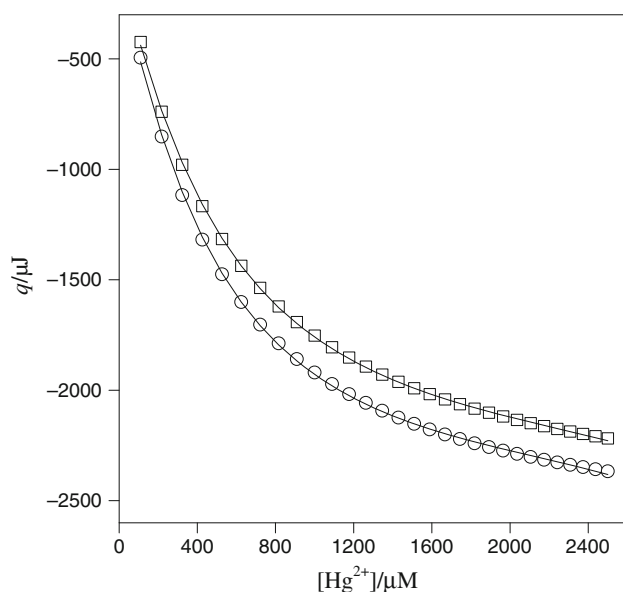
x'<sub>B</sub> is the fraction of the bound Hg<sup>2+</sup> or Ag<sup>+</sup>, and x'<sub>A</sub> = 1 - x'<sub>B</sub> is the fraction of unbound Hg<sup>2+</sup> or Ag<sup>+</sup>. We can express x<sub>B</sub> fractions, as the total Hg<sup>2+</sup> or Ag<sup>+</sup>, L, concentrations divided by the maximum concentration of the metal ions upon saturation of all JBU as follows:

$$x_B = \frac{[L]_f}{[L]_{\max}} \quad (3)$$

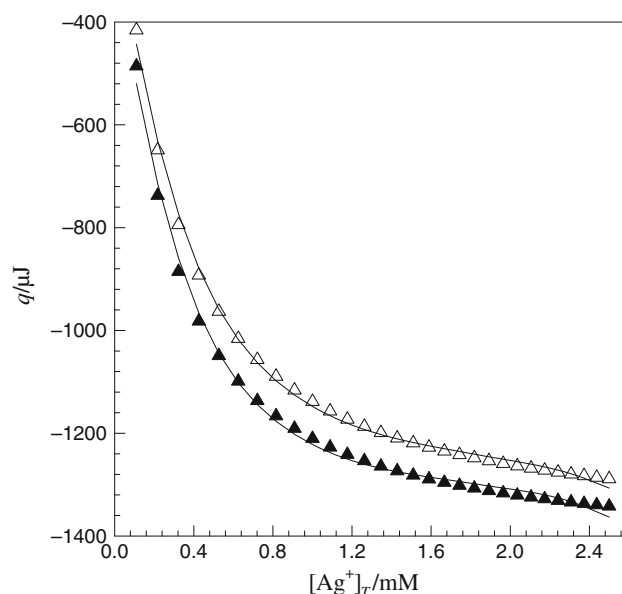
$[L]_f$  is the total concentration of metal ions and  $[L]_{\max}$  is the maximum concentration of  $\text{Hg}^{2+}$  or  $\text{Ag}^+$  upon saturation of all JBU.  $L_A$  and  $L_B$  are the respective contributions of unbound and bound metal ions in the heats of dilution with the exclusion of JBU and can be calculated from the heats of dilution of metal ions in buffer,  $q_{\text{dilut}}$ , as follows:

$$L_A = q_{\text{dilut}} + x_B \left( \frac{\partial q_{\text{dilut}}}{\partial x_B} \right), \quad L_B = q_{\text{dilut}} - x_A \left( \frac{\partial q_{\text{dilut}}}{\partial x_B} \right) \quad (4)$$

The heats of JBU +  $\text{Hg}^{2+}$  and JBU +  $\text{Ag}^+$  interactions were fitted to Eq. 1 over the whole range of metal ions concentrations (Figs. 1, 2). In the procedure, the only adjustable parameter ( $p$ ) was changed until the best agreement between the experimental and calculated data was approached. The negative values of  $\delta_A^\theta$  (Table 3) prove that JBU +  $\text{Hg}^{2+}$  complexes are not stable, indicating that the non-specific interactions (i.e., N- and O-containing groups) have no contribution in JBU inhibition in this region. The positive values of  $\delta_B^\theta$  indicate that the inhibition of JBU was governed by the reaction with the enzyme thiols (specific interactions), and the complete loss of enzyme activity involved all thiols available in the enzyme under non-denaturing condition. The positive  $\delta_B^\theta$



**Fig. 1** Comparison of the agreement between the experimental (symbols) and calculated (line) results obtained from Eq. 1, with  $p = 1$ , at 300 (open circle) and 310 K (open square). The small standard errors and the high  $r^2$  values (0.9999) support the method. These results imply to non-cooperative interaction between JBU and  $\text{Hg}^{2+}$  ions



**Fig. 2** Comparison between the experimental heats at  $T = 300$  K (filled triangle), at  $T = 310$  K (open triangle) for JBU +  $\text{Ag}^+$  interactions and the calculated data (lines) via Eq. 1.  $[\text{Ag}^+]_T$  are the total concentrations of  $\text{AgNO}_3$  solution in mM

**Table 3** Binding parameters for JBU +  $\text{Hg}^{2+}$  interaction

	$T = 300$ K	$T = 310$ K
$K_a/M^{-1}$	$2457.97 \pm 0.12$	$2086.90 \pm 0.14$
$p$	1	1
$\delta_A^\theta$	$-0.52 \pm 0.03$	$-0.59 \pm 0.04$
$\delta_B^\theta$	$1.81 \pm 0.05$	$1.09 \pm 0.03$
$\Delta H/\text{kJ mol}^{-1}$	$-12.80 \pm 0.02$	$-12.30 \pm 0.03$
$\Delta G/\text{kJ mol}^{-1}$	$-19.47 \pm 0.05$	$-19.70 \pm 0.05$
$\Delta S/\text{kJ mol}^{-1}\text{K}^{-1}$	$0.02 \pm 0.001$	$0.024 \pm 0.002$

The negative  $\delta_A^\theta$  values for JBU +  $\text{Hg}^{2+}$  interaction indicate that, non-specific interactions have no contributions in the inhibition of JBU. The positive  $\delta_B^\theta$  values show that the JBU +  $\text{Hg}^{2+}$  complexes are stable, indicating that  $\text{Hg}^{2+}$  ions inhibit the ureolytic activity by blocking sulfhydryl groups via specific interactions and aggregating JBU molecules

values (Table 4) prove that  $\text{RSAgH}_2\text{O}$  complexes are stable, indicating that  $\text{Ag}^+$  ions most likely promote JBU aggregation or inhibit the ureolytic activity by inducing protein polymerization along with the blockage of thiol groups. The positive  $\delta_A^\theta$  values at low  $\text{Ag}^+$  concentration indicates that  $\text{Ag}^+$  ions binding to functional groups in urease other than thiols, i.e., N- and O-containing groups (non-specific interactions), cannot be excluded.

Consider a solution containing ligand  $L$ , and a biomacromolecule ( $M_g$ ) that contains “ $g$ ” sites capable of binding the ligand. If the multiple binding sites on a

**Table 4** Binding parameters for JBU + Ag<sup>+</sup> interactions

	T = 300 K	T = 310 K
$K_a/M^{-1}$	5415.79 ± 0.12	479.18 ± 0.14
$p$	1	1
$\delta_A^\theta$	0.055 ± 0.03	-0.02 ± 0.04
$\delta_B^\theta$	2.85 ± 0.05	2.65 ± 0.03
$\Delta H/kJ mol^{-1}$	-16.70 ± 0.11	-16.30 ± 0.03
$\Delta G/kJ mol^{-1}$	-21.44 ± 0.05	-21.60 ± 0.05
$\Delta S/kJ mol^{-1} K^{-1}$	0.016 ± 0.002	0.017 ± 0.002

$p = 1$  indicates that the binding is non-cooperative. The positive  $\delta_A^\theta$  and  $\delta_B^\theta$  values prove that the JBU + Ag<sup>+</sup> complexes are stable, indicating that Ag<sup>+</sup> most likely promote JBU aggregation or inhibit the ureolytic activity by inducing protein polymerization along with the blockage of thiol groups. Positive or little values of  $\delta_A^\theta$  are indicative of non-specific interactions (i.e., N- and O-containing groups) in the inhibition of JBU in the low concentrations of Ag<sup>+</sup> ions

biomacromolecule are identical and independent, the ligand binding sites can be reproduced by a model system of monovalent molecules ( $M_g \rightarrow gM$ ) with the same set of dissociation equilibrium constant ( $K_d$ ) values. It has been shown previously that the binding parameters can be determined using the following equation [6–14]:

$$\frac{\Delta q}{q_{max}} M_0 = \left(\frac{\Delta q}{q}\right) L_0 \frac{1}{g} - \frac{K_d}{g} \tag{5}$$

where  $\Delta q = q_{max} - q$  and  $q$  represents the heat value at a certain ligand ( $L_0$ ) biomacromolecule ( $M_0$ ) concentrations and  $q_{max}$  represents the heat value upon saturation of all biomacromolecule. If  $q$  and  $q_{max}$  are calculated per mole of macromolecule then the molar enthalpy of binding for each binding site ( $\Delta H$ ) will be  $\Delta H = \frac{q_{max}}{g}$ .

Therefore, the plot of  $\left(\frac{\Delta q}{q_{max}}\right)M_0$  vs.  $\left(\frac{\Delta q}{q}\right)L_0$  should be a linear plot slope of “ $\frac{1}{g}$ ” and the vertical-intercept of  $\frac{K_d}{g}$ , from which  $g$  and  $K_d$  can be obtained. The linearity of the plot has been examined by different estimated values for  $q_{max}$  (Tables 3, 4) to find the best value for the correlation coefficient (near to one).

The molar enthalpy of each binding site and its dissociation constant in a set of JBU binding sites can be obtained via simple following equation:

$$[L]_t = [L] + [ML] \tag{6}$$

$$[M]_t = g[M] + [ML] = \frac{K_d[ML]}{[L]} + [ML] \tag{7}$$

Equation 6 can be solved for  $[L]$  and then substituted into the Eq. 7, and rearranged to give a quadratic equation with the real root.

$$[ML] = \frac{1}{2} \left\{ (B + K_d) - \left[ (B + K_d)^2 - C \right]^{\frac{1}{2}} \right\} \tag{8}$$

where

$$B = g[M]_t + [L]_t \quad C = 4g[M]_t \cdot [L]_t \tag{9}$$

The sum of heat evolutions following the  $i$ th titration step,  $q_i$ , can be expressed as:

$$q_i = \Delta H V_i [ML]_i \tag{10}$$

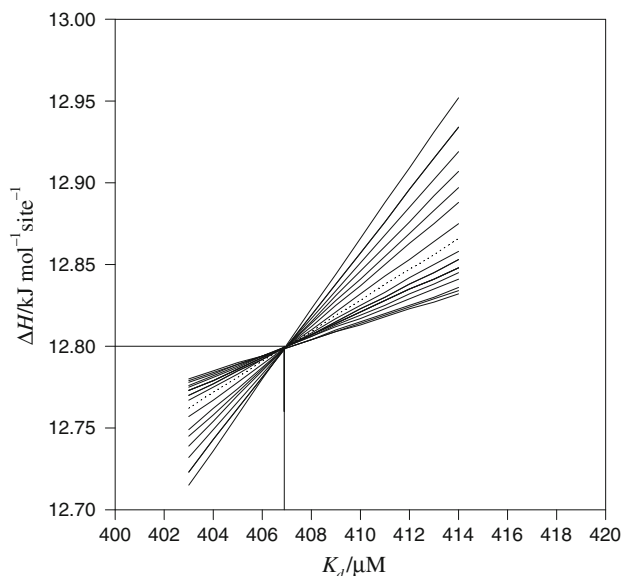
where  $V_i$  is the volume of the reaction solution in the calorimetric sample cell in each injection step and  $\Delta H$  is the enthalpy of binding. Combination of Eqs. 8 and 10 will lead to:

$$\Delta H = \frac{1}{\{(B_i + K_d) - [(B_i + K_d)^2 - C]^{1/2}\} \times A_i} \tag{11}$$

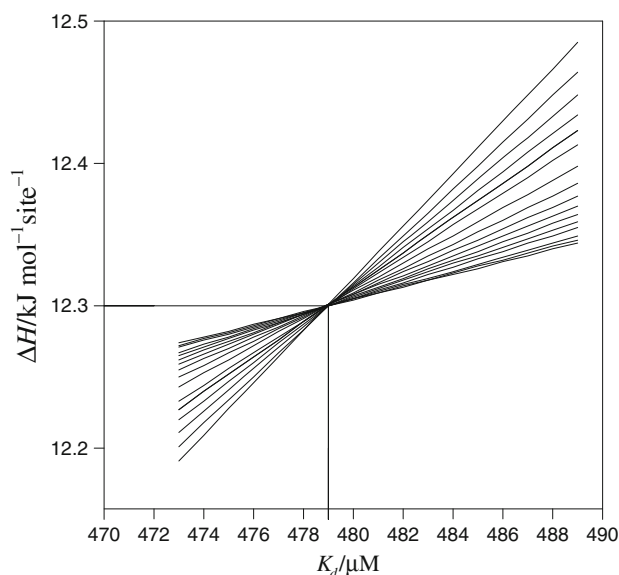
where

$$A_i = \frac{V_i}{2q_i} \tag{12}$$

Equation 11 contains two unknowns,  $K_d$  and  $\Delta H$ . A series of reasonable value for  $K_d$  is inserted into Eq. 11 and corresponding values for  $\Delta H$  are calculated, and the graph  $\Delta H$  vs.  $K_d$  is constructed. Curves of all titration steps will intersect in one point, which represents the precise value for  $\Delta H$  and  $K_d$ . The plots of  $\Delta H$  vs.  $K_d$ , according to Eq. 11, for 16 injections of Hg<sup>2+</sup> at 300 and 310 K are shown in Figs. 3 and 4, respectively.



**Fig. 3**  $\Delta H$  vs.  $K_d$  by using Eq. 11, for 16 injections at 300 K. The intersection of curves gives  $K_d = 406.9 \mu M$  and  $\Delta H = -12.8 kJ mol^{-1} site^{-1}$



**Fig. 4**  $\Delta H$  vs.  $K_d$  by using Eq. 11, for 16 injections at 310 K. The intersection of curves gives  $K_d = 479 \mu\text{M}$  and  $\Delta H = -12.3 \text{ kJ mol}^{-1} \text{ site}^{-1}$

The change in the standard Gibbs free energy and change in the standard entropy of binding could be calculated by using  $K_a$  and  $\Delta H$  values in Eqs. 13 and 14, respectively.

$$\Delta G^0 = -RT \ln K_a \quad (13)$$

$$T\Delta S^0 = \Delta H^0 - \Delta G^0 \Rightarrow \Delta S^0 = \frac{\Delta H^0 - \Delta G^0}{T} \quad (14)$$

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