The Study of the Interactions Between Heavy Metals with Sulfonylurea Herbicides using ACE

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Abstract

The affinity capillary electrophoresis method was used in the study of interactions between heavy metals (Pb, Mn, La, Cd, and Ni) and the sulfonylurea herbicides (thifensulfuron, nicosulfuron and sulfometuron). A buffer of 50mM acetate (pH 5.85) was employed in the study. The results proved that there are two binding sites in the herbicides when chelating with the heavy metals. Binding constants of the high chelating ability sites between them were in the range of 10^3 to 10^5, and the other were in the range of 10 to 10^3. Thifensulfuron is the most powerful chelator among the three herbicides. Ni is of the weaker chelators, within the herbicides than Pb, Mn, La, and Cd. The binding abilities were proven to be increased with the pH value among the investigated range.

Introduction

The interactions between pollutants are of great importance regarding their environmental behaviors, such as the processes of adsorption/desorption in water-soil interfaces (1,2), degradation (3) in the environment, bioavailability, and bio-toxicity to plants or cultures (4). Sulfonylurea is a family of environmentally compatible herbicides of low cost with a high effectiveness against a large number of weeds by inhibiting the acetolactase synthesize enzyme. Because of their high efficiency (10–100 g of active ingredient per ha), low toxicity towards animals, and short lifetime, they are widely used in the field for crop protection (5). Although they are less poisonous, studying the environmental impact of their residues in soil and water is really important, considering their wide usage.

As shown in the molecular structures (Figure 1), triazin, amide, amino, and benzoate groups of sulfonylurea herbicides are excellent metal complexing ligands. So, the three sulfonylurea herbicides can form complexes with certain kinds of transfer metals, such as Mn(II), Ni(II), Pb(II), etc. Although the interactions between them are considerably important in the environment, there is little information reported about the binding constant between them.

There are several techniques currently available for the study of molecular interactions, including radioimmunoassay (6), fluorescence quenching (7), and slab gel electrophoresis (8). These techniques generally require the separation and quantitation of free or complex molecules in an equilibrium mixture. If the amount of bound and free ligand in solution can be distinguished, these techniques can provide reasonable estimates of binding constants for the interaction.

Capillary electrophoresis (CE) affords a number of advantages over other techniques for the interaction study (8–11). Only a small amount of receptor and ligand are required, and no purified sample is required as long as CE can distinguish the impurities from the analyte of interest. Affinity capillary electrophoresis (ACE) has been widely used in measuring affinity parameters between biological species of receptor–ligand, protein–drug, protein–DNA, peptide–peptide, peptide–carbohydrate, carbohydrate–drug, and antibody–antigen since 1992 (8–13). More than one mode of ACE fit for the interaction study of heavy metals and herbicides in an aqueous system, such as vacancy affinity capillary electrophoresis (14), Hmmel-Dreyer mode (15), Frontal analysis (16,17), the classical ACE mode, which estimates the binding constant by the changes in the moblities of analytes (18), and some other methods developed on those mentioned earlier, such as partial filling technique (19), pro-self-marking partial filling technique (20), and multi-injection partial filling technique (21,22). Krylov and his coworkers developed kinetic capillary electrophoresis (KCE) for the study of the kinetic parameters of the interaction between bio-molecules in the recent years (23,24).

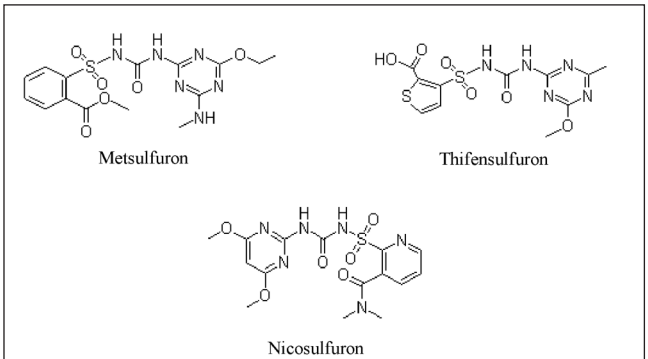

Figure 1. The molecular structures of the sulfonylurea herbicides cited in the research, Metsulfuron (pka = 3.3, MW = 381.4), Thifensulfuron (pka = 4.0, MW = 373.36), Nicosulfuron (pka = 4.3, MW = 410.40).

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The classical ACE method has the advantages of simultaneous determination of more than one receptor in the sample at a time, with an acceptor in the running buffer; therefore, the method was used most often until recently (11,12). The present work introduced the typical ACE method to the study of interactions between heavy metal of Cd, Mn, Ni, Pb, and Ni with the sulfonylurea herbicides.

Materials and Methods

Instrumentation

CE experiments were performed on a Beckman P/ACE MDQ capillary electrophoresis system with a UV detector (Beckman, Fullerton, CA). Separations were carried out on fused-silica capillaries of 57 cm (50 cm effective length) × 75 µm i.d. (Yongnian Optical Fiber factory, Hebei, China). UV detector was set at 254 nm for detection. The pH values were obtained by using an ORION Model-828 pH meter (ORION, USA). UV spectrum was determined with a Shimadzu UV-2400 spectrophotometer (Shimadzu, Japan).

Chemicals

The sulfonylurea herbicides of thifensulfuron, nicosulfuron, and sulfometuron were gifted from The State Key Laboratory of Elemento-Organic Chemistry, Nankai University (Tianjin, China). The acetic acid and ammonium acetate were of analytical grade, purchased from Beijing Chemical Reagents Company (Beijing, Chian). $La(NO_3)_3$, $Pb(NO_3)_2$, $Cu(NO_3)_2$, $Cd(NO_3)_2$, $Ni(NO_3)_2$, and $MnSO_4$ of analytical grade were purchased from Beijing Chemical Reagent Company. Dimethyl phthalate used as electroosmotic flow (EOF) marker was purchased from AccuStandard (New Haven, CT). All other reagents were of analytical grade or higher.

The individual stock solutions of herbicides were prepared at a concentration of 4000 mg/L in methanol, and the heavy metal stoke solutions were at a concentration of 50mM in water. The buffer was prepared with 50mM ammonium acetate and manipulated to designed pH with 50mM acetic acid solution. The heavy metal at a defined concentration was added to the acetate buffer as the running buffer to study the interactions between heavy metals and herbicides. The sample was made up of a mixture of 40 mg/L (about 0.1mM each) sulfonylurea herbicides in the acetate buffer (with no heavy metal appended solution).

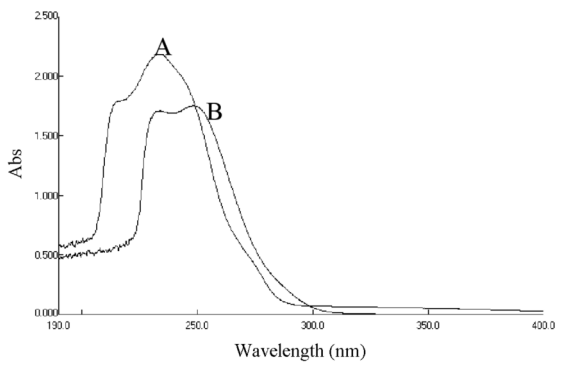

Figure 2. The UV- spectrogram of metsulfuron under the influence of 0.75mM Ni^{2+}. A is the spectrogram of 0.10mM metsulfuron without Ni^{2+} against water, B is the spectrogram of 0.10mM metsulfuron in the buffer of 0.75mM Ni^{2+} against 0.75mM Ni^{2+}.

Analytical conditions

The new capillary was rinsed with water, 1M HNO_3, and water for 5 min, and activated with 1M NaOH and water for 10 min, then rinsed with running buffer for 10 min. Between analysis, the capillary was rinsed with running buffer for 3 min. Injection was performed using a pressure of 0.5 p.s.i. (1 p.s.i. = 6895 Pa) for 5 s. Separation voltage was 25 kV and the capillary was maintained at 20°C in the research.

Calculations

When we assumed that the equilibrium of the interaction between the sulfonylurea herbicides and heavy metals is established very quickly and with the assumption that the complexing reactions happened in a complex (1:1), the apparent binding constant (K) of M with S could be expressed as following: Eq. 1

$$K = [SM] / [S][M] \quad \text{Eq. 1}$$

where [*SM*] is the equilibrium concentration of complex; [S] is the concentration of non-combined herbicides in the complexing equilibrium; [M] is the concentration of heavy metal ions in the equilibrium. And according to Tanaka and Tarabe (8):

$$\Delta\mu / [M] = K(\mu_S - \mu_{MS}) - K\Delta\mu \quad \text{Eq. 2}$$

where µ is the mobility of free analytes, and μ_{MS} is the mobility of the complex of heavy metal with the herbicide. Equation 2 is the x-reciprocal fitting form of Scachard equation for the binding constant calculation, where Δµ is the mobility difference of herbicides in the buffer of heavy metals:

$$\Delta\mu = \mu - \mu_S \quad \text{Eq. 3}$$

The mobility of the herbicides could be calculated according to the migration time of the analytes and the electrophoretic conditions such as separation voltage, capillary total, and effective length.

Results and Discussion

When the herbicides formed complexes with heavy metals, the UV-spectrum would be changed. The UV spectrum of metsulfuron and the mixture of metsulfuron together with Ni(II) was shown, and an obvious red-shift was observed in Figure 2. To well understand the interactions, ACE was used to investigate the binding constants.

The buffering reagents are weak acids or bases, which might form complexes with the heavy metals, or precipitate with heavy metals in the aqueous solution. Therefore, the buffer of phosphate, citrate, oxalate, and succinate was excluded from the candidates. The buffer system of Tris-HNO_3 and acetate buffer was

investigated. The response of thifensulfuron, nicosulfuron, and sulfometuron is relatively low in the Tris system (data not shown). And then the acetate buffer system was selected in the following research.

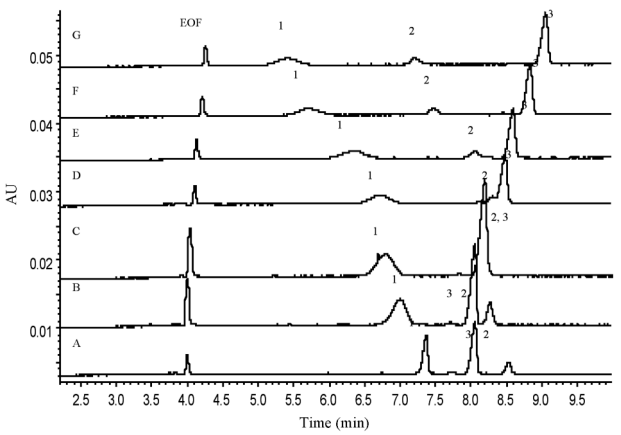

Figure 3. The electrophoregrams of nicosulfuron (1), sulfometuron (2) and thifensulfuron (3), and in the running buffer of 50mM acetate buffer, at pH 5.85, the concentration of $Ni(NO_3)_2$: A, 0; B, 0.010; C, 0.015; D, 0.025; E, 0.050; F, 0.10; G, 0.15mM. Sulfonylureas were in concentration of 40 mg/L as sample. Beckman P/ACE capillary electrophoresis was used, with a capillary of 75 µm i.d., length is 50 cm (40 cm to detector). The temperature was 20°C.

Table I. The Scachard Fitting Equation and Binding Constants of Heavy Metals of Cd, La, Cu, Pb, Mn, and Ni with Nicosulfuron, Thifensulfuron-Methyl, and Metsulfuron-Methyl

Element	Sulfonylurea	Order	Fitting equation	*R*	K(10^3)	log K
Cd	Nicosulfuron	1	$y = -1300x + 220$	0.95	1.30	3.11
		2	$y = -68.4x + 14.7$	0.97	0.68	1.83
	Thifensulfuron	1	$y = -10900x + 1870$	0.98	10.9	4.04
		2	$y = -1004x + 173$	0.85	10.0	3.00
	Metsulfuron	1	$y = -2970x + 548$	0.89	2.97	3.47
		2	$y = -338x + 65.3$	0.95	3.38	2.53
Mn	Nicosulfuron	1	$y = -11400x + 1770$	0.8	11.4	4.06
		2	$y = -493x + 80$	0.96	4.93	2.69
	Thifensulfuron	1	$y = -8930x + 1520$	0.9	8.93	3.95
	Metsulfuron	1	$y = -7660x + 1380$	0.9	7.66	3.88
La	Nicosulfuron	1	$y = -4640x + 4512$	0.86	4.64	3.66
		2	$y = -15.6x + 8.27$	0.85	0.32	1.51
	Thifensulfuron	1	$y = -14760x + 2500$	0.99	14.7	4.17
		2	$y = -45.1x + 10.1$	0.85	0.45	1.65
	Metsulfuron	1	$y = -9350x + 1690$	0.73	9.35	3.97
		2	$y = -39.7x + 10$	0.87	0.40	1.60
Pb	Nicosulfuron	1	$y = -3133x + 500$	0.86	3.13	3.5
		2	$y = -152x + 28$	0.95	1.52	2.18
	Thifensulfuron	1	$y = -12111x + 2075$	0.98	12.1	4.08
		2	$y = -175x + 33.3$	0.94	1.75	2.24
	Metsulfuron	1	$y = -2388x + 448$	0.87	2.39	3.38
		2	$y = -426x + 82.7$	0.96	4.26	2.63
Ni	Nicosulfuron	1	$y = -550x + 104$	0.89	0.55	2.74
		2	$y = -76x + 18.7$	0.97	0.76	1.88
	Thifensulfuron	1	$y = -1999x + 385$	0.97	2.00	3.30
		2	$y = -127x + 28$	0.95	1.27	2.10
	Metsulfuron	1	$y = -663x + 129$	0.97	0.63	2.80

EOF marker

In ACE analysis, the EOF marker is of great importance to calculate the mobilities of analytes. Methanol or acetone was commonly employed as the EOF marker. However, while the EOF marker of methanol or acetone was employed, a fluctuation of current commonly happened, induced by the conductivity wave in the capillary for the influence of methanol or acetone, which might influence the mobility calculation according to the migration time. Therefore, dimethyl phthalate was selected as EOF marker instead of organic solvents.

Binding constant investigation

Figure 3 shows a series of electropherograms of thifensulfuron, nicosulfuron, and sulfometuron in different concentrations of Ni^{2+}. The migration time of the herbicides changed as a result of the complex formation between the herbicides and heavy metals in the buffer. In the same time, the resolution and separation of the three herbicides were influenced by the heavy metals. As a result of different interaction intensity, the peak order could be changed by the heavy metals. Thifensulfuron had a shorter migration time than sulfometuron did, while the concentration of Ni was higher than 0.15mM, migration time of sulfometuron was shorter than thifensulfuron as shown in Figure 3.

The binding consant by the ACE method was based on the assumption that the dissociation and formation of complex was a thermodynamic reaction, which needs infinitesimal time for the equilibrium. However, because the reaction is not a perfect thermodynamic reaction, which needs a period of time to reach equilibrium; this leads to broadening of the peaks when the concentration of heavy metals in buffers were higher as shown in Figure 3.

The non-linear Scachard plot is observed when multiple binding sites have different binding constants (6). Figure 4 shows the Scachard plots of Pb with the three herbicides. As shown in Figure 4, the fitting curves were nonlinear plots of most of the heavy metals and herbicides. The plots were made up of two joint lines, according to which, two binding constants could be obtained from the slope of fitting equation (6) as shown in Table I. It is proved that the herbicides had two binding sites in facing the heavy metals employed in the work. The binding sites of higher binding constants were defined as a specific interaction, which is a result of the interaction between metals with the specific binding sites of the herbicides molecules, while the weak interaction was defined as the other, which is the result of the interaction of aspecfic binding sites with heavy metals. The binding constants of specific interactions were in the range of 10^3 to 10^5, while the binding constants of the weak interactions are in the range of 10 to 10^3. The specific interaction is

Table II. The Scachard Fitting Parameters and Binding Constants of Ni with Nicosulfuron, Thifensulfuron-methyl, and Metsulfuron-methyl

			Fitting equations	*R*	K(10^3)	Log K
pH 5.00	Nicosulfuron	1	$y = -287x + 290$	0.97	0.29	2.46
		2	$y = -48x + 15$	0.75	0.048	1.68
	Thifensulfuron	1	$y = -1643x + 88$	0.98	1.65	3.22
		2	$y = -74x + 21$	0.94	0.074	1.87
	Metsulfuron	1	$y = -602x + 39$	0.89	0.62	2.79
pH 5.85	Nicosulfuron	1	$y = -550x + 104$	0.89	0.55	2.74
		2	$y = -76x + 18.7$	0.97	0.76	1.88
	Thifensulfuron	1	$y = -1999x + 385$	0.97	2.00	3.30
		2	$y = -127x + 28$	0.95	1.27	2.10
	Metsulfuron	1	$y = -663x + 129$	0.97	0.63	2.80
pH 7.70	Nicosulfuron	1	$y = -762x + 055$	0.92	0.762	2.88
		2	$y = -17x + 8$	0.74	0.017	1.23
	Thifensulfuron	1	$y = -3460x + 0.05$	0.87	3.46	3.54
		2	$y = -157x + 109$	0.85	0.157	2.20
	Metsulfuron	1	$y = -881x + 0.74$	0.93	0.771	2.89

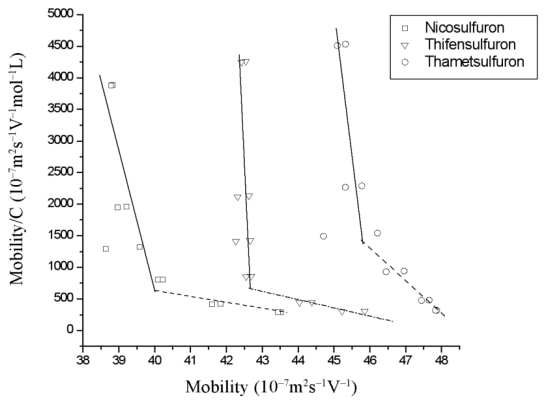

Figure 4. Scatchard plot of Pb^{2+} with sulfonylureas interaction with a two binding constants. The solid lines are the sepcital binding sites and the dash lines are the wake one. The conditions are the same as that of Figure 3.

much higher than the weak interaction (the binding constants are 10 to 10^3 times different).

All the following comparisons were based on the specific interactions. The heavy metals are of different binding abilities to the herbicides from one to each other. In Table I, the Ni had the poorest binding ability among the five heavy metals. Other heavy metals having a binding constant are different from one to each other. Mn has the highest binding constant with nicosulfuron (Mn > La > Pb > Cd > Ni) and the highest binding constants with metsulfuron as well (Mn > La > Pb, Cd > Ni). Thifensulfuron is the most powerful chelating ligands among the three herbicides, which has binding constants over 10^4 with the heavy metals (Ni except). The thiophene group of thifensulfuron might play an important role in the interactions.

Effect of pH on complex formation

The pH value is an important parameter for the resolution and separation in CE, which decides the degree of ionization of the sulfonylurea herbicides and the solubility of heavy metals as well. The pH values of 5.04, 5.85, and 7.00 were selected in the research. The binding constants of niccolum with thifensulfuron, nicosulfuron, and sulfometuron were investigated. A slight increase in binding constants with pH was observed (shown in Table II). This may be induced by the structures of the herbicides and Ni species changes caused by pH.

Conclusion

The binding constants between sulfonylurea herbicides of metsulfuron, thifensulfuron, and nicosulfuron with heavy metals of Ni, Cd, Pb, La, and Mn were studied with ACE by estimating the relative migration time ratio. The work proved the complex formation in the aqueous system between heavy metals and sulfonylurea herbicides. The three herbicides of metsulfuron, thifensulfuron, and nicosulfuron have two binding sites when they are chelating with the heavy metals.

Acknowledgments

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References

1. Y.G. Mishael, T. Undabeytia, O. Rabinovitz, B. Rubin, and S. Nir. Sulfosulfuron incorporated in micelles adsorbed on montmorillonite for slow release formulations. *J. Agric. Food Chem.* **51:** 2253–59 (2003).
2. C. Ashburn Poppell, R.M. Hayes, and T.C. Mueller. Dissipation of nicosulfuron and rimsulfuron in surface soil. *J. Agric. Food Chem.* **50:** 4581–85 (2002).
3. I. Braschi, L. Calamai, M.A. Cremonini, P. Fusi, C. Gessa, O. Pantani, and A. Pusino. Kinetics and hydrolysis mechanism of triasulfuron. *J. Agric. Food Chem.* **45:** 4495–99 (1997).
4. G.M. Fahl, L. Kreft, R. Altenburger, M. Faust, W. Boedeker, and L.H. Grimme. pH-Dependent sorption, bioconcentration and algal toxicity of sulfonylurea herbicides. *Aquatic. Toxicol.* **31:** 175–81 (1995).
5. G. Boschin, A.D. Agostina, C. Antonioni, D. Locati, and A. Arnoldi. Hydrolytic degradation of azimsulfuron, a sulfonylurea herbicide. *Chemosphere* **68:** 1312–17 (2007).
6. M. Malmqvist and R. Karlsson. Biomolecular interaction analysis: affinity biosensor technologies for functional analysis of proteins. *Curr. Opin. Chem. Biol.* **1:** 378–83 (1997).
7. T.D. Gauthier, E.C. Sham, W.F. Guerln, W.R. Seltz, and C.L. Grant. Fluorescence quenching method for determining equilibrium constants for polycyclic aromatic hydrocarbons binding to dissolved humic materials. *Environ. Sci. Technol.* **20:** 1162–66 (1986).
8. Y. Tanaka and S. Terabe. Estimation of binding constants by capillary electrophoresis. *J. Chromatogr. B* **768:** 81–92 (2002).

9. C. Schou and N.H.H. Heegaard. Recent applications of affinity interactions in capillary electrophoresis. *Electrophoresis* **27:** 44–59 (2006).
10. K. Uselova-Vcelakova and I. Zuskova. Gas, stability constants of amino acids, peptides, proteins, and other biomolecules determined by CE and related methods: Recapitulation of published data. *Electrophoresis* **28:** 2145–52 (2007).
11. M. Gayton-Ely, T. Pappas, and L. Holland. Probing affinity via capillary electrophoresis: advances in 2003–2004. *Anal. Bioanal. Chem.* **382:** 570–80 (2005).
12 M. Castagnola, D.V. Rossetti, R. Inzitari, A. Vitali, A. Lupi, C. Zuppi, T. Cabras, M.B. Fadda, I. Podda, R. Petruzzelli, B. Giardina, and I. Messana. Affinity capillary electrophoresis study of the linkage existing between proton and zinc ion binding to bacitracin A1. *Electrophoresis* **24:** 801–807 (2003).
13. M. Castagnola, D.V. Rossetti, R. Inzitari, A. Vitali, A. Lupi, C. Zuppi, T. Cabras, M.B. Fadda, I. Podda, R. Petruzzelli, B. Giardina, and I. Messana. Capillary electrophoretic study of the binding of zinc(II) ion to bacitracin A1 in water-2,2,2-trifluoroethanol. *Electrophoresis* **24:** 1612–19 (2003).
14. F.B. Erim and J.C. Kraak. Vacancy affinity capillary electrophoresis to study competitive protein–drug binding. *J. Chromatogr. B* **710:** 205–10 (1998).
15. J. Heintz, M. Hernandez, and F.A. Gomez. Use of a partial-filling technique in affinity capillary electrophoresis for determining binding constants of ligands to receptors. *J. Chromatogr. A* **840:** 261–68 (1999).
16. W. Yan and C.L. Colyer. Investigating noncovalent squarylium dye–protein interactions by capillary electrophoresis–frontal analysis. *J. Chromatogr. A* **1135:** 115–21 (2006).
17. T. L. Saux, A. Varenne, F. Perreau, L. Siret, S. Duteil, L. Duhau, and P. Gareil. Determination of the binding parameters for antithrombin–heparin fragment systems by affinity and frontal analysis continuous capillary electrophoresis. *J. Chromatogr. A* **1132:** 289–96 (2006).
18. H. Kajiwara. Motif 2 in adenosine kinase homologous ginseng polypeptide showed affinity to ribose by capillary zone electrophoresis and surface plasmon resonance. *J. Chromatogr. A* **817:** 173–79 (1998).
19. A. Brown, R. Desharnais, B.C. Roy, S. Malik, and F.A. Gomez. Optimization of conditions for flow-through partial-filling affinity capillary electrophoresis to estimate binding constants of ligands to receptors. *Anal. Chim. Acta* **540:** 403–10 (2005).
20. B.Y. Guo, Z.N. Xia, G.H. Chen, Y.G. Yin, and H.M. Xu. Study on the interaction of ephedrine, caffeine, and acetaminophen with bovine serum albumin by capillary electrophoresis with partial filling technique. *Chin. J. Chormatogr.* **21:** 367–70 (2003).
21. Y. Zhang and F.A. Gomez. Multiple-step ligand injection affinity capillary electrophoresis for determining binding constants of ligands to receptors. *J. Chromatogr. A* **897:** 339–47 (2000).
22. J. Zavaleta, D.B. Chinchilla, A. Pao Ramirez, A.K. Martinez, S. Nilapwa, J.E. Ladbury, S. Mallik, and F.A. Gomez. Partial filling multiple injection affinity capillary electrophoresis (PFMIACE) to estimate binding constants of receptors to ligands. *Talanta* **71:** 192–201 (2007).
23. A. Petrov, V. Okhonin, M. Berezovski, and S.N. Krylov. Kinetic capillary electrophoresis (KCE): a conceptual platform for kinetic homogeneous affinity methods. *J. Am. Chem. Soc.* **127:** 17104–17110 (2005).
24. Z. Liu, A.P. Drabovich, S.N. Krylov, and J. Pawliszyn. Dynamic kinetic capillary isoelectric focusing: A powerful tool for studying protein-DNA interactions. *Anal. Chem.* **79:** 1097–1100 (2007).

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