行政院國家科學委員會專題研究計畫　成果報告

利用毛細管電泳測量蛋白質之物理化學性質

計畫類別：個別型計畫
計畫編號：NSC91-2113-M-032-015-
執行期間：91 年 08 月 01 日至 92 年 07 月 31 日
執行單位：淡江大學化學系

計畫主持人：吳俊弘

計畫參與人員：楊承熹、植啟中、王文政、彭士峰

報告類型：精簡報告

處理方式：本計畫可公開查詢

中　華　民　國 93 年 2 月 16 日

行政院國家科學委員會專題研究計畫成果報告

利用毛細管電泳測量蛋白質之物理化學性質
Determination of Physicochemical Properties of Proteins by Capillary Electrophoresis

計畫編號： NSC 91-2113-M-032 -015
執行期限：91 **年** 8 **月** 1 **日 至** 92 **年** 7 **月** 31 **日**
主持人：吳俊弘　　淡江大學化學系

一、中文摘要

我們開發了一個嶄新的分析方法，利用毛細管電泳儀在同一次電泳實驗中同時測得蛋白質構型大小及其電荷。利用此方法我們可快速測得具不同分子量以及等電點的蛋白質，在不同離子環境以及 pH **值的緩衝溶液中所帶電荷以及構型大小的變化。另外，我們也利用此方法探討蛋白質與界面活性劑的結合行為，此結果可應用於測量蛋白質分子量之電泳分析技術。**

關鍵詞：擴散系數，黏度，電泳遷移率，流體動力半徑，有效電荷。

Abstract:

We have developed a novel method of using capillary electrophoresis to simultaneously measure protein size and charge in a single electrophoretic run. By using this method we were able to perform quick measurements of the size and charge changes for proteins with different molecular weights and pI values in aqueous solutions with different pH and ionic strength. In addition, the binding properties between proteins and surfactants were also investigated. The results were useful in molecular weight determination of proteins using electrophoretic analysis technique.

Keywords: diffusion coefficient, viscosity, electrophoretic mobility, hydrodynamic radius, effective charge

二、緣由與目的

The three dimensional structure and electrostatic properties of proteins usually determine their roles in biological functions. The measurements of protein size and effective charges are useful in understanding their biological properties. Protein size can be determined by X-ray diffraction method, light scattering technique, and NMR approach [1-3]. The experimental methods used to estimate protein charges include isoionic point method [4], Donna potential measurement [5,6], time dependent polyacrylamide gradient gel electrophoresis method [7-9], titration method [1, 10] and the combination of capillary electrophoresis and charge ladder [11-14]. These methods have disadvantages of consuming large amount of sample and requiring complicated model fitting [15] and tedious protein-ligand synthesis.

In this project we used our newly developed method to investigate the changes of protein size and charge induced by pH and surfactant denaturations.

三、結果與討論

The designed methods of measuring the physicochemical properties of proteins were described as follows. Protein sample was pressure-injected into capillary column filled with buffer solution. The sample band would pass through the detection window by applying a constant pressure in the injection end of he capillary column with both ends immersed in equal amounts of the buffer solution. The resulting Gaussian profile was used to calculate the protein diffusion coefficient according to Taylor-Aris dispersion method [16]. Moreover, the viscosity of the buffer solution could also be determined from the retention time of the Gaussian peak by using Poiseuille equation. With known solute diffusion coefficient and solvent viscosity and according to the calculation of Stokes-Einstein equation, protein hydrodynamic radius could thus be obtained. After passing the detection window for a period of time, the protein band would stop migration by dropping the applied pressure to zero, and then immediately migrated back toward the detector by applying an electric field. Protein mobility could be calculated from the elution time of the second protein peak. Since protein size and electrophoretic mobility along with the buffer viscosity were all measured, we could use Henry's model [15] of the theory of protein electrophoretic mobility to calculate the protein effective charge.

Figure 1 showed the pH effects on protein size. The hydrodynamic radius of protein did not have observable change in pH ranges of 7.00 to 10.88. When pH was less than 5.6, Mb and BSA started to swell mainly due to the denaturation effect and the increase of effective charge. At low pH region, unlike Mb and BCA, Lys and Ov had relatively small changes in their sizes. We found that the existence of disulfide bond in protein would be the main reason to account for this difference. As for BSA, which was also rich in disulfide bond, the rising in size at low pH region was mainly due to its large molecular weight and acidic nature, which would make it possess large positive charge, and thus resulted in larger size. The electrophoretic mobilities of proteins would increase with increasing or decreasing the pH values from the pI of each protein. Figure 2 showed the effects of pH on protein charge. The protein charges measured did approach zero when pH reached the pI of each protein. Although the pIs of Ov and BSA were the same, BSA would bear more charge than Ov due to its larger molecular weight. Within the pH range studied, the effective charge of Lys seemed to be small and change very little.

Similar to the result shown in fiure 3, the sizes of most protein-SDS complexes would increase with increasing SDS concentrations and then reached a plateau value due to the saturated binding of SDS molecule onto protein. At low pH condition, Mb had been denatured by the acidity. The addition of SDS only slightly increased the denaturation of Mb. Therefore the radius of Mb-SDS complex was not much bigger than that of Mb as shown in figure 4. Hb is a tetrapepitide with subunits similar to Mb. In figures 5 and 6, the loss of Hb's quaternary structure by SDS denaturation in high pH region (7~10.88) accounted for the similarity of the plateau radii of Hb-SDS and Mb-SDS. Compared with Mb-SDS complex, further denaturation and thus larger size increase in low pH region for Hb-SDS complex was observed. Owing to the lack of disulfide bond in BCA tertiary structure, the plateau radius of BCA-SDS complex in figure 7 only increased slightly when decreasing pH. The acidity denaturation effect was not obvious because SDS denaturation had almost completely expanded the complex size. The high disulfide bond ratio in Ov tertiary structure made acidity denaturation effect become insignificant. However, the effect of SDS denaturation on the complex plateau radius did show big expansion of SDS-Ov complex as shown in figure 8. Similar to the results of OV, figure 9 showed that the pH dependences of both BSA radius and BSA-SDS plateau radius were almost the same. This was mainly due to the high disulfide bond ratio existing in the BSA tertiary structure. In low pH region, the size increase was probably caused by the high

molecular weight and relatively high hydrophilic nature of BSA.

四、計劃成果自評

The results of this proposal provided us a new method for the measurements of protein size and effective charge. This would make us better understand the conformational and electrostatic properties of proteins as well as their binding behaviors with surfactant molecules. The results were also useful in optimizing CE conditions for the separation and identification of proteins. The newly designed method requires extremely small amounts of protein and is easily automated. This method provides not only quick measurements of protein related physicochemical properties but also the conditions for optimizing protein separations by capillary electrophoresis.

五、參考文獻

[1] Tanford, C. *Physical Chemistry of Macromolecules*, John Wiley & Sons: New York, 1961.

[2] Carcia de la Torre, J.; Maria L. H.; Carrasco, B. *Biophysical Journal*. **2000**, 719-730.

[3] Carbeck, J. D.; Negin, R. S. *J. Am. Chem. Soc*. **2001**, *123*, 1252-1253

[4] Nozaki, Y. and Tanford, C., *Methods Enzymol*, **1967**, 11, 715-734.

[5] Ford, C. L. and Winzor, D. J., *Biochim. Biophys. Acta,* **1982**, 703, 109-112.

[6] Öjteg, G., Lundahl, P. and Wolgast, M., *Biochim. Biophys. Acta,* **1989**, 991, 317-323.

[7] Rothe, G. M. and Weidmann, H., *Electrophoresis*, **1991**, 12, 703-709.

[8] Rothe, G. M., *Electrophoresis of Enzymes*, Springer-Verlag, Berlin **1994**, pp.71-124.

[9] Rothe, G. M., Welschbillig, N. and Reiss, E., *Electrophoresis*, **1998**, 19, 745-751.

[10] Hunter, R. J. *Zeta Potential in Colloid Science: Principles and Applications*, Academic Press: London, 1981.

[11] Gao, J., Gomez, F. A., Härter, R. and Whitesides, G. M., *Proc. Natl. Acad. Sci.USA* **1994**, 91, 12027-12030.

[12] Gao, J., Mammen, M. and Whitesides, G. M., *Science*, **1996**, 272, 535-537.

[13] Colton, I. J., Anderson, J. R., Gao, J., Chapman, R. G., Isaacs, L. and Whitesides, G. M., *J. Am. Chem. Soc.*, **1997**, 119, 12701-12709.

[14] Colton, I. J., Carbeck, J. D., Rao, J. and Whitesides, G. M., *Electrophoresis*, **1998**, 19, 367-382

[15] Menon, M. K. and Zydney, A. L., *Anal Chem*, **1998**, 70, 1581-1584.

[16] Bello, M. S.; Rezzonico, R.; Righetti, P. G. *Science* **1994**, *266*, 773-776.

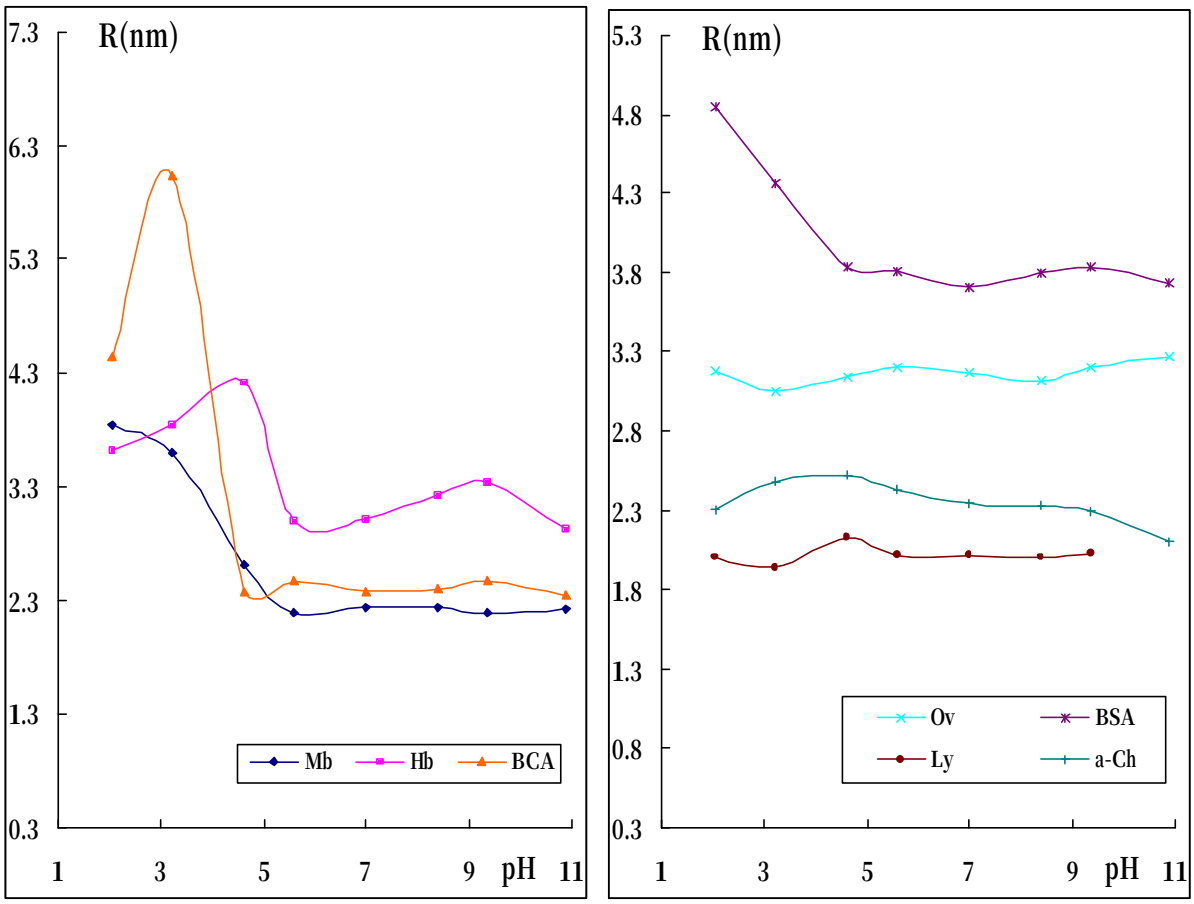

Figure 1: pH dependent hydrodynamic radii of studied proteins

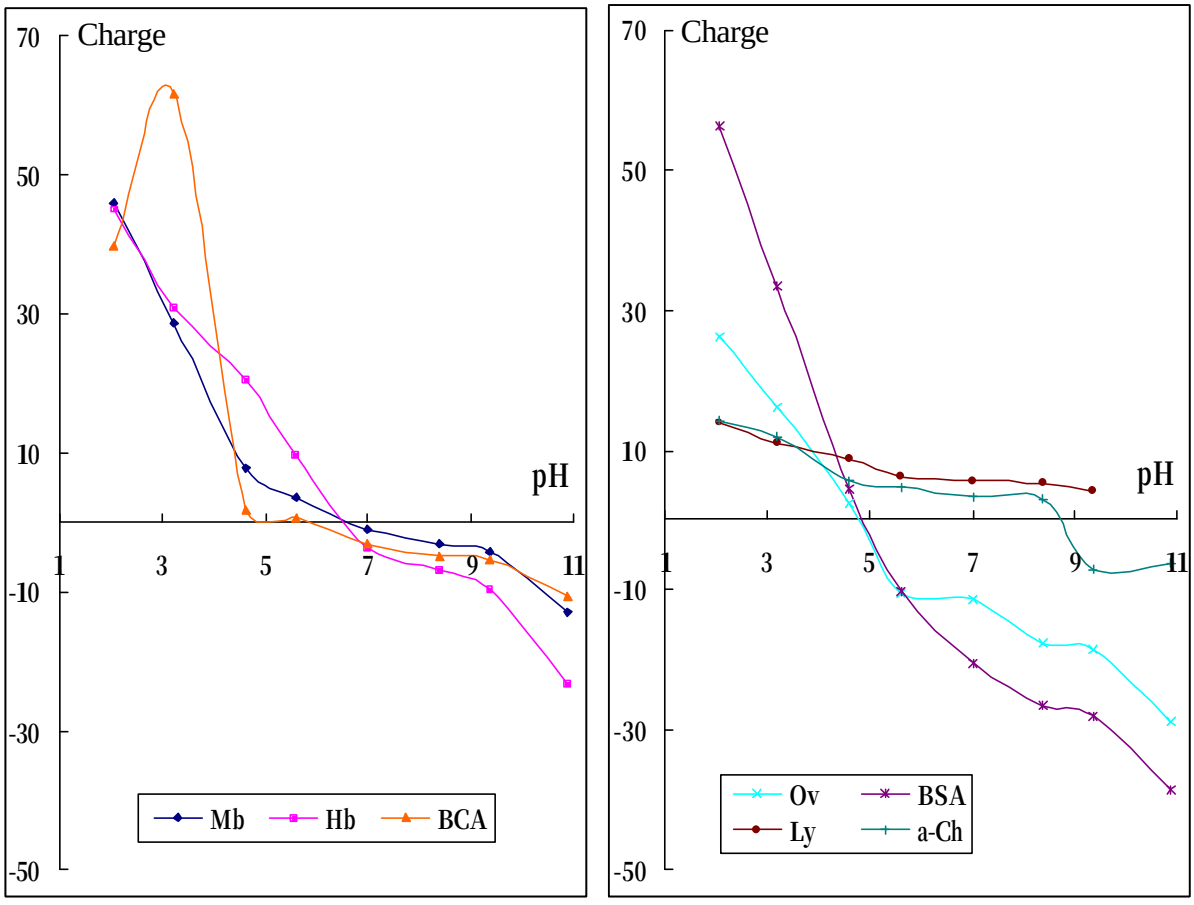

Figure 2: pH dependent effective charge of studied proteins

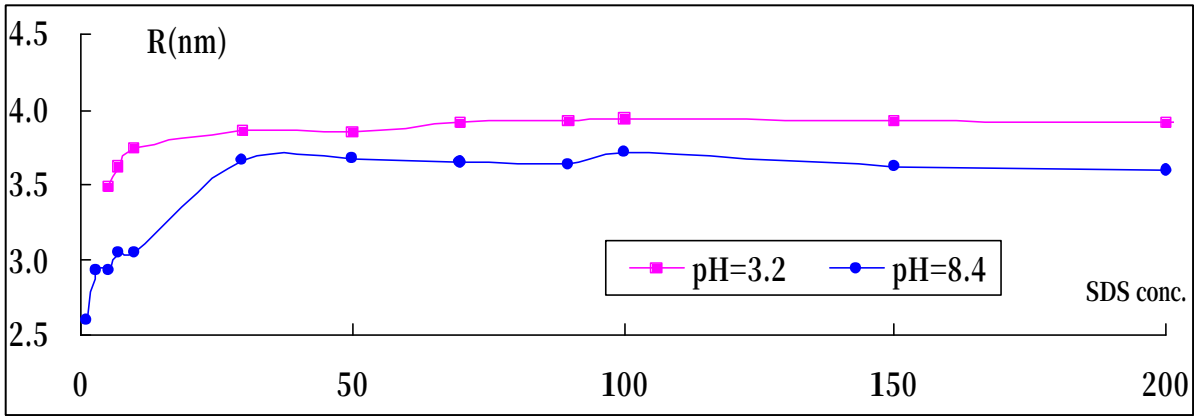

Figure 3: Hydrodynamic radii of Mb-SDS complexes vs. SDS concentration

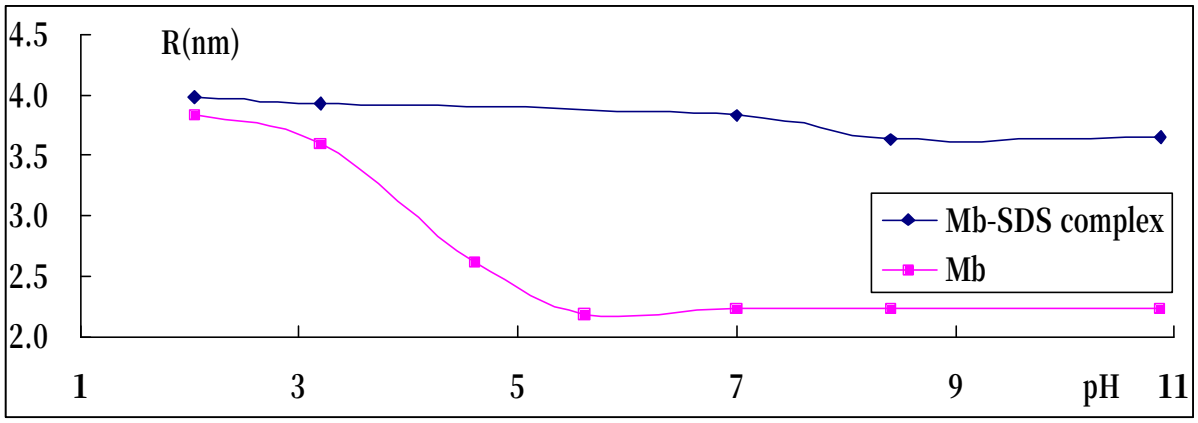

Figure 4: Radius of Mb and plateau radius of Mb-SDS complex at different pH conditions

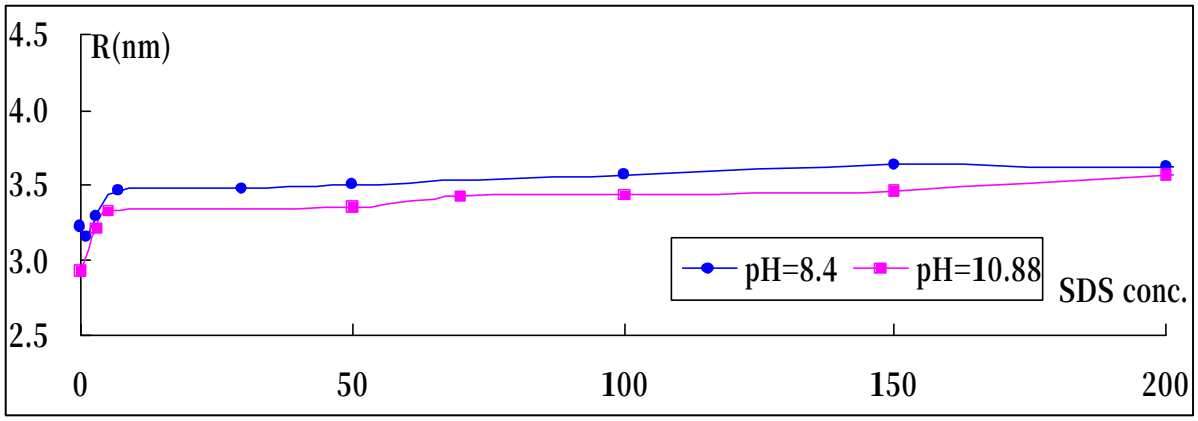

Figure 5: Hydrodynamic radii of Hb-SDS complexes vs. SDS concentration

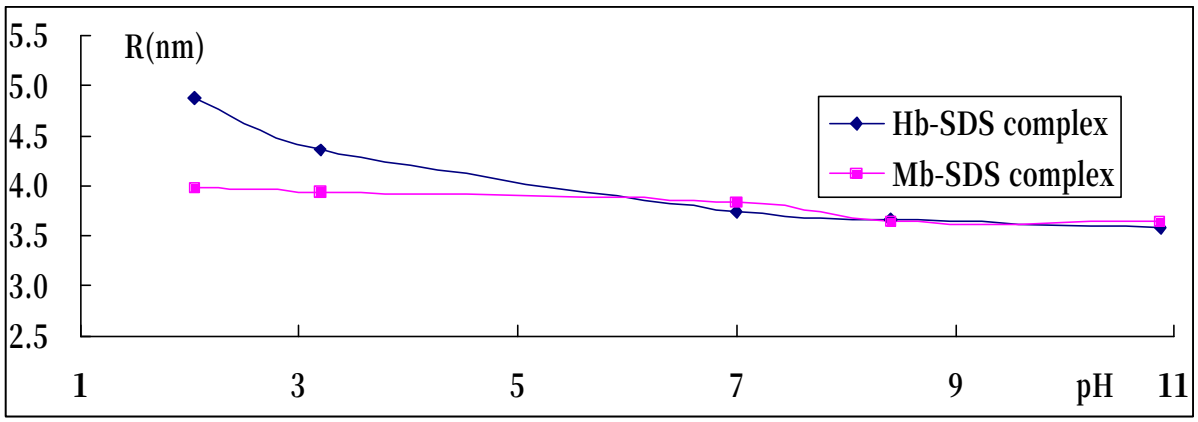

Figure 6: pH dependent plateau radii of Hb-SDS and Mb-SDS

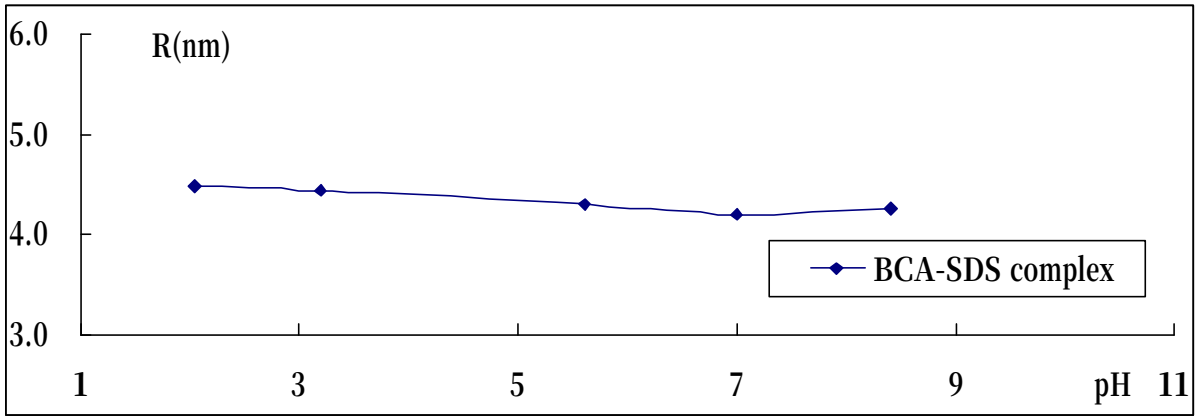

Figure 7: pH dependent plateau radius of BCA-SDS complex

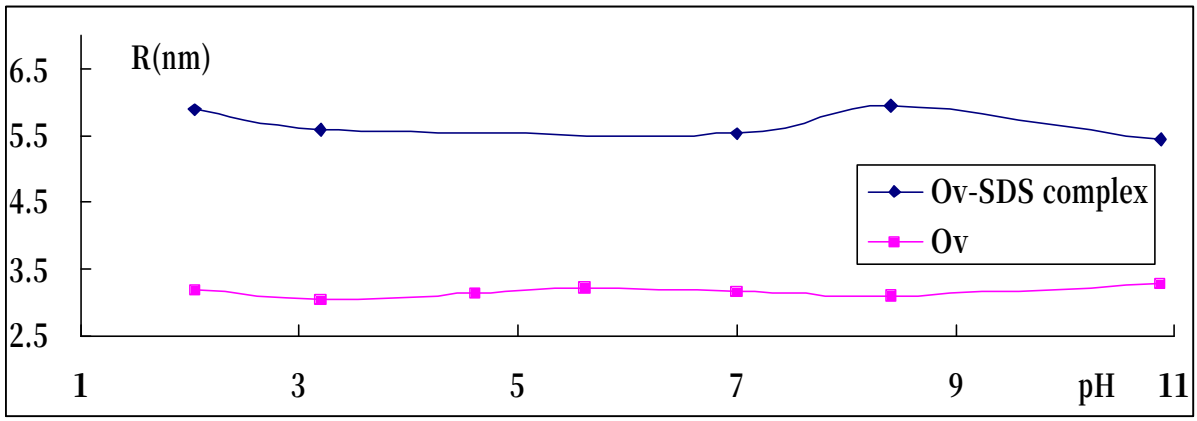

Figure 8: Radius of Ov and plateau radius of Ov-SDS complex at different pH conditions

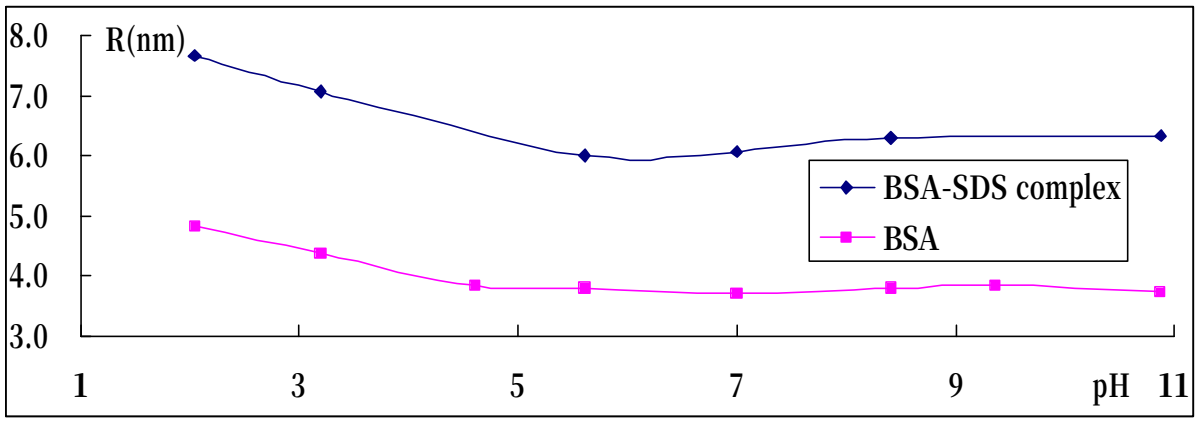

Figure 9: Radius of BSA and plateau radius of BSA-SDS complex at different pH conditions