# Acarbose Binding to Human Serum Albumin Studied by Affinity Capillary Electrophoresis

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## ABSTRACT

The equilibrium interaction between acarbose and albumin was studied using affinity capillary electrophoresis. The migration time of albumin was shown to increase as a result of increasing concentration of acarbose within the sample. Peak area of albumin was decreasing as a result of acarbose binding. The progressive decrease in peak area associated with increasing acarbose concentration was employed to obtain Scatchard plot and consequently the binding constant. The estimated binding constant was $6.7 \times 10^{-4}$ $M^{-1}$. The rather unexpected increase in migration time of albumin as a result of acarbose binding was explained in terms of potential change in the three dimensional structure of albumin. Such structural changes might facilitate the binding of other compounds such as digoxin. Therefore, these findings might explain the frequently reported decrease in free digoxin concentration when given concomitantly with acarbose.

**Keywords:** Acarbose, Digoxin, Protein binding, Albumin.

## INTRODUCTION

Poor Human serum albumin (HSA) is the most abundant and perhaps important transport protein in blood. Many drugs and other endogenous substances are known to bind reversibly to HSA[(1).] Drug-HSA interactions play a determining role in the overall therapeutic drug action. That is because HSA represents a carrier and a reservoir to replenish free drug concentration after being removed by elimination processes. However, various drugs show different affinities towards HSA which means that the stronger binding drugs may displace the weaker binding compounds resulting in a potentially serious clinical outcomes. Displacement of valproate by aspirin leading to a potential clinical toxicity of valproate is just an example of such interactions[(2)]. Therefore, understanding the interaction of a drug with HSA is important in order to explain and potentially predict its interaction with other drugs.

Acarbose is a glucosidase and/or amylase inhibitor that is used to control postprandial hyperglycemea in diabetic patients[(3)]. Although its site of action is primarily the gut, it is believed that a small percentage could be absorbed ending in the systemic circulation[(4-5)]. Thus, there would be a chance for acarbose to interact with some other drugs; if found to bind to HSA. Indeed, acarbose was reported to significantly decrease the concentration of digoxin when given concomitantly[(6-7)]. The mechanism for such decrease in digoxin concentration has not been conclusively elucidated yet[(6)]. Some reports suggested that acarbose decreases the absorption of digoxin through increasing gastrointestinal motility resulting from accumulation of undigested material [(7-8)]. However, this might not totally explain acarbose-digoxin interaction because the interaction was

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reported only with digoxin but not with other drugs. Moreover, one contradicting report concluded no significant pharmacokinetic interaction between acarbose and digoxin at the usual dosing level of acrbose [9]. Therefore a need to consider other potential mechanisms of interaction was evident in order to further clarify the frequently reported decrease of plasma concentration of digoxin when co-administered with acarbose. To the best of our knowledge no studies have been published pertaining the interaction of acarbose with HSA. In this research the interaction of acarbose with HSA was studied using affinity capillary electrophoresis (ACE).

ACE has been successfully employed for studying the interaction of drugs with HSA[10-12]. In ACE, the shift in the migration time of HAS, which is usually observed as a result of the change in charge to mass ratio, is utilized to derive the binding curve and the classical Scatchard analysis to estimate the binding parameters [10-12].

## Experimental

### *Chemicals and equipment*

Human serum albumin (HSA) and all other chemicals were bought from Sigma Chemical (St. Louis, USA). Agilent CE system (Agilent Technology, Waldbronn, Germany) equipped with a diode array detector was used in this study with the anode on the injection side and the cathode on the detection side. Uncoated fused silica capillaries (Composite Metal, UK) with an internal diameter of 50 mm and an effective length of 54 cm were employed. Electropherograms were monitored at 220 nm. The temperature of the capillary was maintained at 37 $^{o}$C in all experiments.

### *Affinity CE method*

The affinity CE method for studying interaction of acarbose with albumin was based on that described initially by Chu et al.[10]. Samples containing 2.98 μM HSA and increasing concentrations of acarbose (0.2-10 μM) in 50 mM borate buffer at pH 8.2 were prepared. The samples were then incubated for at least 6 hours, and injected onto the CE system by hydrodynamic injection mode of 20 seconds. The electrophoresis was carried out using a running buffer composed of 50 mM borate buffer at pH 8.2 and containing equivalent concentrations of acarbose to that in the sample. A constant voltage of 30 KV was employed during all runs.

The results were analysed using a modified form of Scatchard equation as demonstrated by Chu. et al. and Avila et al.[10-11]. The average of three runs was taken to construct Scatchard plots.

## RESULTS AND DISCUSSION

Typical electropherograms for a series of albumin solutions incubated with increasing concentrations of acarbose are shown in Fig. 1. Electropherograms in Fig. 1 show that a progressive increase in the migration time of the albumin peak is observed as a result of increasing the concentration of acarbose in the incubation mixture. In addition to the successive increase in migration time, the peak area of HSA was successively decreasing and getting broader. Thus the change in migration time, which presumably results from the change in charge-to-mass ratio of the protein, as well as the decrease in peak area were both taken as evidence of acarbose binding to HSA [10-11]. Typical binding curve could be obtained by plotting the peak area of HSA (P) against the concentration of acarbose in the electrophoresis buffer (Fig. 2). With the assumption that the binding stoichiometry was 1:1, then Scatchard analysis could be performed according to the formula developed by Chue et al.[10] The obtained Scatchard plot is shown in Fig. 3. The plot in Fig. 3 shows a typical Scatchard plot with an estimated binding constant of $6.7 \times 10^{-4}$ $M^{-1}$. Accordingly, acarbose can be said to reasonably bind HSA with the potential of displacing less tightly bound drugs. Consequently, some drug-drug interaction between acarbose and other drugs might result from displacement process. However, the only drug-drug interaction reported for acarbose was that with digoxin[6-7]. Previous reports have demonstrated that concomitant adminstration of acarbose with digoxin resulted in decreasing the free concentration of the latter in plasma[6-7]. Some studies have attributed these observations to the effect of acarbose on the digestive enzymes in the gut with the

consequent increase in bowl movement and decrease in the amount of digoxin absorbed [7-8]. Nevertheless, this explanation might not be absolutely correct due to the fact that this effect was observed specifically with digoxin but not with other drugs. Even digitoxin, the closely related compound, was not shown to be affected by the concomitant use of acarbose.

Therefore, it is proposed based on the results obtained from this work that the well-documented interaction between acarbose and digoxin might be a result of interaction with albumin. Digoxin was reported to have only little affinity (if any) towards albumin and this is unlike digitoxin which binds strongly to albumin [13]. It is proposed in this study that the binding of acarbose results in allosteric changes in the albumin structure that facilitates the binding of digoxin and consequently lowers its free concentration in plasma.

The evidence that acarbose binding results in allosteric changes in albumin could be seen from the electrophoretic behaviour of albumin in the presence of acarbose. The observed shift in the migration time of albumin as a result of interaction with acarbose (Fig.1) was not in the expected directions i.e. if the shift in migration time was due only to simple change in charge-to-mass ratio, as a consequence of binding, then the bound albumin molecule should migrate at shorter time due to higher positive charge acquired by binding of the positively charged acarbose [10-11]. However, an increase in migration time was progressively associated with the increase of acarbose (0.2-10 μM). Therefore, the only plausible explanation for the observed increase in migration time is a change in the three dimensional structure (shape and size) of the albumin molecule which might occur as a result of binding to acarbose. Such allosteric modification of albumin has been reported to occure with diazepam which facilitates the binding of tenoxicam to HSA [14].

Thus, it could be concluded that the allosteric changes that have been observed as a result of acarbose binding to albumin, potentiates the binding of digoxin to albumin. Leading to a net decrease in the concentration of free digoxin in plasma as reported in previous studies.

## CONCLUSION

Acarbose binds to albumin with a binding constant = $6.7 \times 10^{-4}$ $M^{-1}$ for at least one binding site. The binding of acarbose to albumin appears to alter the three dimensional structure of albumin (allosteric binding) with a concomitant facilitation for the binding of digoxin (cooperative binding). The net effect would be a decrease in the free concentration of digoxin in plasma. These observations might evidently explain the frequently appearing reports that administration of acarbose concomitantly with digoxin leads to a decrease in free plasma concentration of the latter. However, further studies are required to firmly show that the allosteric changes caused by acarbose result in increasing the binding of digoxin and hence decreasing its free fraction.

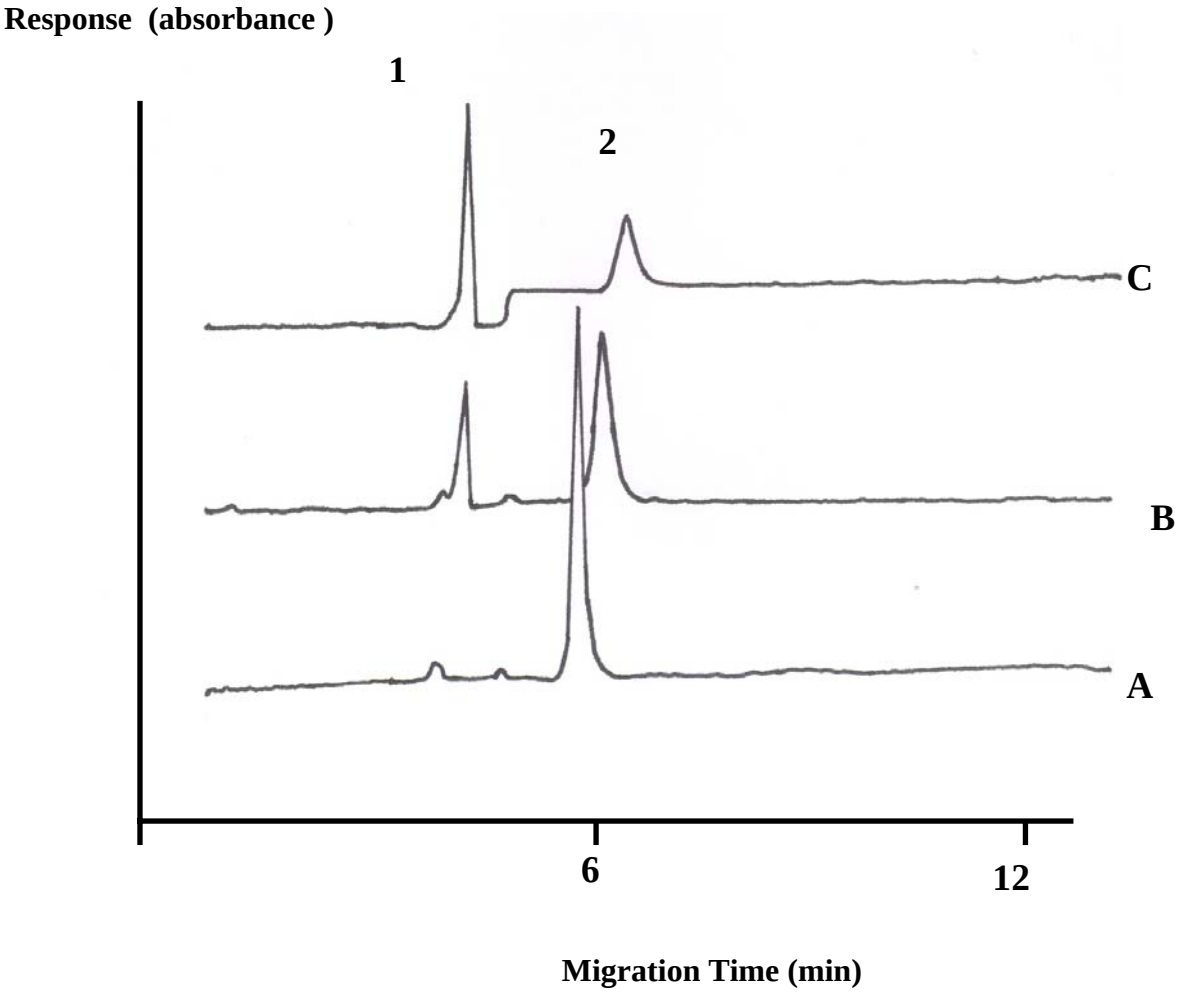


**Fig. (1): Selected electropherograms for samples of albumin incubated with 0, 5 and 8 μM of acarbose in A, B and C, respectively. The running electrolyte also contained amounts of acarbose equivalent to that added to each sample. Other electrophoretic conditions are detailed in the experimental section. Peak identification: 1 = acarbose and 2 = albumin. Note the change in migration time and peak area of albumin.**

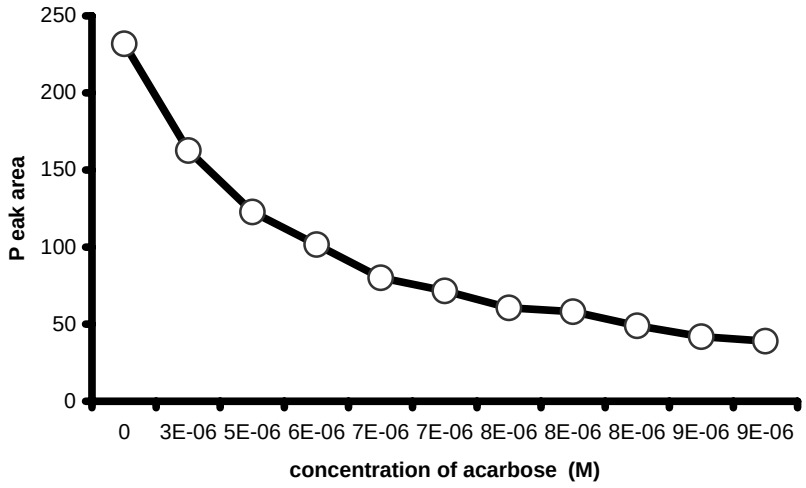


**Fig. (2): binding curve for acarbose and albumin. Peak area (P) was plotted against molar concentration of acarbose. The percentage of error at all points was less than 4 %.**

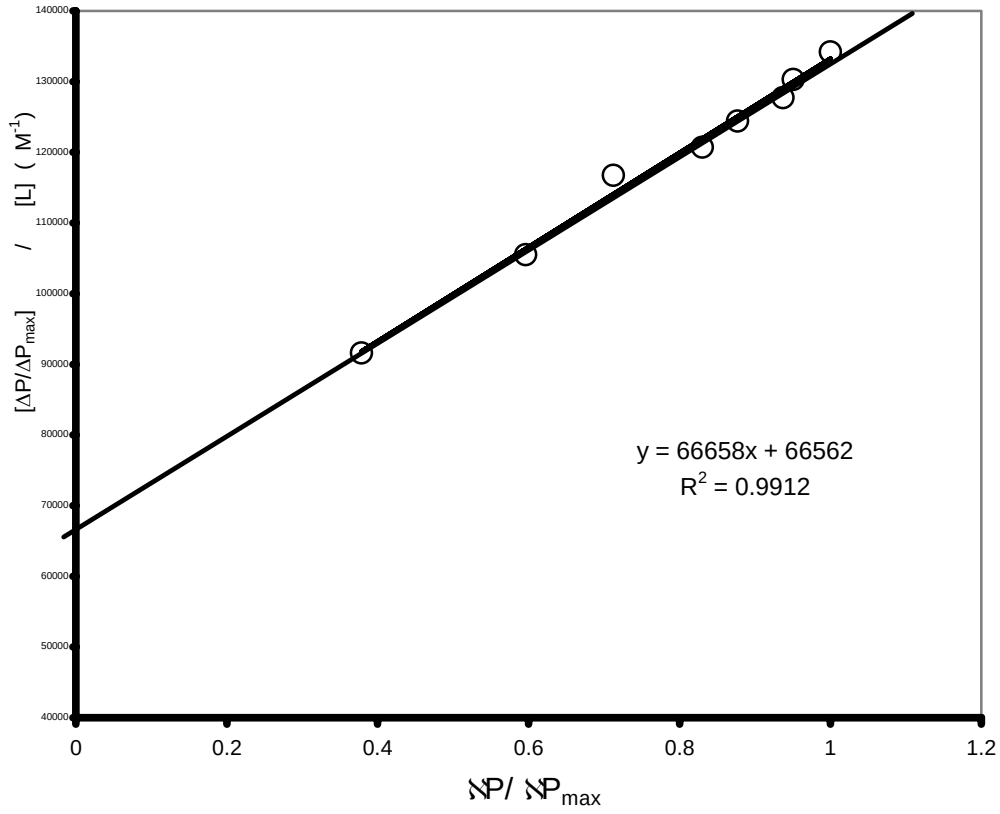


**Fig. (3): Scatchard plot for the binding of acarbose to albumin. The slope and intercept indicate a binding constant of 6.7 x $10^{-4}$ $M^{-1}$. The percentage of error at all points was less than 4 %.**

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# ارتباط الأكاربوز بنيتروجين الألبومين:
# دراسة تمت باستخدام تقنية الارتحال الكهربائي بالشعيرات الدقيقة

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## ملخـص

لقد تمت دراسة ارتباط الأكاربوز ببروتين الألبيومين باستعمال تقنية الارتحال الكهربائي بالشعيرات الدقيقة. تبين أن زمن ارتحال الألبيومين يزداد بزيادة تركيز الأكاربوز بداخل العينة. أما المساحة الخاصة بالألبيومين فكانت تتناقص عل رسم الارتحال الكهربائي كنتيجة لتزايد الأكاربوز. تم توظيف التناقص المتتابع في مساحة الألبيومين للحصول على منحنى سكاتشارد ومنه تم احتساب ثابت الربط بين الأبيومين والأكاربوز على أنه = (6.7 x 10-4 M-1). لقد كانت زيادة زمن ارتحال الألبيومين بعكس ما هو متوقع وبالتالي فإن ذلك يعكس تغير في الشكل ثلاثي الأبعاد للبروتين نتيجة ارتباط الأكاربوز. لذا فمن الممكن أن مثل هذا التغير في شكل البروتين قد يؤدي إلى تسهيل ارتباط مواد أخرى مثل الديجوكسين. وبالتالي فإن هذا قد يفسر التقارير المنشورة سابقا حول تناقص مستوى الديجوكسين الطليق في البلازما إذا ما تم تناوله مع الأكاربوز.

**الكلمـات الدالـة:** أكاربوز، ديجوكسين، ربط بروتيني، البيومين.

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