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Mohamed Taha
University of Aveiro
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Interactions of TRIS [tris(hydroxymethyl)aminomethane] and related buffers with peptide backbone: Thermodynamic characterization

Mohamed Taha and Ming-Jer Lee*

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In a situation which is far from ideal, many buffers have been found to be quite reactive, besides maintaining their stable pH values. On the basis of apparent transfer free energies ($\Delta G'_{tr}$), through solubility measurements the interactions of zwitterionic glycine peptides: glycine (Gly), diglycine (Gly_2), triglycine (Gly_3), and tetraglycine (Gly_4), with several common neutral pH, amine-based buffers have been studied. The biological buffers studied in this work, including TRIS, TES, TAPS, TAPSO, and TABS are structurally related and all contain TRIS groups. These buffers have pK_a values ranging from 7.5–9.0, which allow them to be used in biological, biochemical or environmental studies. We observed negative values of $\Delta G'_{tr}$ for Gly_3 and Gly_4 from water to buffer, indicating that the interactions are favorable. However, the $\Delta G'_{tr}$ values are positive for Gly and Gly_2, revealing unfavorable interactions, which except for the latter in TRIS buffer are negative. The surprising result in our data is the unexpected extraordinarily high favorable interactions between TRIS buffer and peptides (in comparison with the effect of the most common denaturants, urea and guanidine hydrochloride). The transfer free energies ($\Delta G'_{tr}$) of the peptide backbone unit ($-CH_2C{=}O-NH-$) contributions have been estimated from $\Delta G'_{tr}$ values. We have also investigated the interactions of TRIS buffer with Bovine Serum Albumin (BSA), as a globular protein, using dynamic light scattering (DLS), zeta potential, UV-Visible absorption, fluorescence and Raman spectroscopy measurements. The results indicated that TRIS buffer stabilized the BSA molecules.

1. Introduction

Protein stability is highly dependent on environmental conditions. Major factors include temperature, pH, salt concentration and other buffer conditions. It is well established that protein unfolding can be induced by high or low temperature or extremes of pH. Proteins are flexible molecules and their conformation can change in response to changes in pH. The presence of ligands also alters stability, since anything that binds to the native state more tightly than non-native states will stabilize the protein as a consequence.[1] Significant information on protein structure and interactions can be obtained from solution studies in which the concentration of low molecular mass solutes and cosolvents, causing stabilization, destabilization (denaturation), aggregation, or crystallization effects, is varied over a broad range of protein concentrations, pHs, and temperatures.[2]

In protein crystallization experiments, a buffer is a very standard additive: in the Jancarik and Kim[3] screen, 39 out of the 50 conditions contain a specific buffering chemical. The buffer component is often used at relatively high concentrations, usually 100 mM. Many soluble, cytoplasmatic proteins can be extracted by mechanical and/or osmotic forces, since cell membranes are weak and can be easily disrupted using a homogenizer and a suitable buffer. The buffer can act either by modulating the pH of the protein solution during crystallization or it can act as a chemical in the crystallization cocktail.[4]

Since pH-value control is a central consideration in handling proteins, the selection of different buffers is important. Ideal buffer solutions should be chemically inert, non-toxic, water soluble, no absorption at visible or in the UV region, no complex formation with cations and the pK_a value should not vary with temperature. A commonly assumption that buffers do not interact with DNA and protein was challenged recently.[5–10] Organic amines such as TRIS and several Good's buffers are found to bind to the DNA not only by means of electrostatic interactions but also by hydrogen bonds.[11] In particular, TRIS can form two H-bonds, with purine and pyrimidine, respectively; the third H-bond is shared between two neighbouring TRIS groups.

The extent to which a particular protein may be stabilized or destabilized by a buffer depends on many factors, thereby making the selection of buffer to be a formidable challenge. In conformity with the proposition that "Nature designs the optimum molecules," buffers should mimic the antidenaturant properties of nature exhibited by osmolytes[12–16] that are independent of the evolutionary history of the proteins.[17,18] Such properties may include preferential exclusion from the protein domain[19–22] and stabilization without changing the denaturation Gibbs energy.[23]

Recently, Quan *et al.*[24] have studied the interaction of lysozyme with TRIS buffer and they suggested avoiding using TRIS when the ligands are weak, such as in lysozyme ligand

Department of Chemical Engineering, National Taiwan University of Science and Technology, 43 Keelung Road, Section 4, Taipei 106-07, Taiwan. E-mail: mjlee@mail.ntust.edu.tw

studies. Quan *et al.*[24] have found that TRIS molecule formed H bonds with Asp52, Glu35, and Ala107 in lysozyme.

Formulations of Lys^{B28} Pro^{B29} human insulin analog stabilized against aggregation in which the buffering agent is either TRIS or L-arginine.[25] The heat-induced denaturation of the recombinant human megakaryocyte growth and development factor (rHuMGDF, pI = 10.7) was partially reversible in TRIS buffer.[26] Guanidine hydrochloride (GdnHCl)-induced unfolding of yeast prion protein Ure2P was studied in TRIS buffer.[27] A three-state denaturation profile was observed *via* a dimeric intermediate. The native state was stabilized relative to the dimeric intermediate in TRIS buffer, and the profile switched from the three-state to a two-state with a reduction in the range of GdnHCl concentrations in which the dimeric intermediate state was populated.

Proteins are complex molecules and their behavior in solutions is entirely governed by many specific interactions. Indeed, most interpretations of the thermodynamic properties of such systems are based on data for simpler model compounds. Amino acids and peptides are the building blocks of the proteins, and have obvious limitations in representing portions of protein molecules, but the model compound approach has already provided fundamental key results.

In this study, we present basic data on understanding the principles of stabilizing or destabilizing of glycine model compounds (Gly, Gly_2, Gly_3, and Gly_4) in the presence of some important biological buffers, such as (Tris(hydroxymethyl)-aminomethane (TRIS), *N*-Tris(hydroxymethyl)methyl-2-amino-ethanesulfonic acid (TES), *N*-[Tris(hydroxymethyl)methyl]-3-aminopropanesulfonic acid (TAPS), *N*-[Tris(hydroxymethyl)-methyl]-3-amino-2-hydroxypropanesulfonic acid (TAPSO), and *N*-tris[hydroxymethyl]-4-aminobutanesulfonic acid (TABS)). The transfer free-energies were obtained from solubility measurements, as a function of buffer concentration at 25 °C under atmospheric pressure. Using the principles of functional group additivity, the transfer free energy of the peptide backbone has been determined. The schematic chemical structures of glycine model compounds as well as the buffers are shown in Scheme 1. One more purpose of this work is to examine the interactions of TRIS buffer with Bovine Serum Albumin (BSA) using DLS and a variety of spectrometers. We have analyzed the thermal denaturation process of BSA under increasing the TRIS concentrations (from 0.05–1.8 M) at pH 7.0. BSA, a protein with a molecular weight of 66 300 Da, has 17 disulfide bridges, and the flexibility of the molecule is strongly restricted by these 17 disulfide bridges. BSA is composed of a single polypeptide chain with 583 amino acids. Various molecular spectroscopic investigations for the interactions between BSA and TRIS were also reported.

2. Materials and methods

2.1 Materials

Gly (>99% purity) was obtained from Acros Organics (USA). Gly_2 (>99.5% purity), Gly_3 (>99% purity) and Gly_4 (>99% purity) were purchased from Sigma Chemical Co. (USA). TRIS (>99.9% purity), TES (>99% purity), TAPS (>99.5% purity), TAPSO (>99% purity), and TABS (>99% purity) were supplied by Sigma Chemical Co. (USA). Bovine Serum Albumin (BSA)/Fraction V, pH was obtained from Acros Organics (USA). All the purchased materials were used without further purification. Water used for making the aqueous solutions was obtained from NANO pure-Ultra pure water system that was distilled and deionized with resistance of 18.3 MΩ. All the aqueous solution samples were prepared gravimetrically.

2.2 Methods

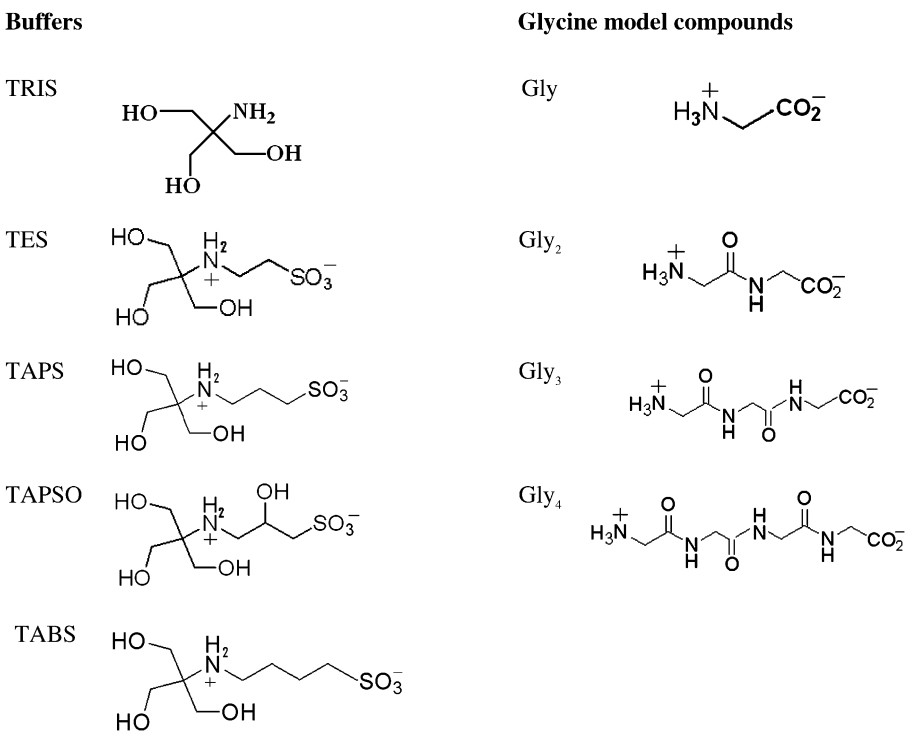
2.2.1 Solubility measurements. The solubility of glycine peptides (GPs) in water and aqueous buffers was obtained from the density (ρ) measurements, as described by Nozaki and Tanford,[28–30] and by Liu and Bolen.[31] The detailed procedure used in this work has been delineated in our earlier articles.[32–35] At least nine sample vials were prepared for each investigated system. To each of the sample vials containing a fixed amount of solvent (water or aqueous buffer solution) was added weighed amounts of GP to provide a series of mixtures with increasing composition of GP mass. The weighed samples were prepared such that approximately five vials would ultimately result in unsaturated solutions and the remaining four vials were saturated. Each vial was sealed with a Teflon coated screw cup. The vials were completely immersed in a thermostatic shaker equipped with water bath (BT-350R, Yih-Der, Taiwan) at T = 25 °C ± 0.03 °C for 36–48 h, and the supernatant of each solution was removed through a syringe and filtered by a 0.22 μm disposal filter (Millipore, Millex-GS) before performing the density measurements. The density of each sample was plotted as a function of its composition and the density *versus* composition data of the unsaturated solutions were fitted to a polynomial, while the data of the saturated solutions were fitted to a straight line. The solubility limit was evaluated at the intersection of the two fitted lines, and the GP concentration and the density of solution at the solubility limit were recorded. Densities were measured with an Anton Paar DMA-4500 vibrating-tube densitometer, Austria, with an uncertainty of $\pm 5 \times 10^{-5}$ g cm^{-3}. The temperature was controlled to within ±0.02 °C. The densitometer was calibrated with air and degassed distilled water. The uncertainty of the solubility limit is within ±0.8%. The concentration of GP in the samples, in units of (grams of GP/100 g of solvent), was calculated from eqn (1).

$$\text{Composition } g_{GP}/100\ g_{solvent} = \left(\frac{\text{weight}_{GP}\ (g)}{\text{weight}_{solvent}\ (g)}\right) \times 100 \quad (1)$$

The solubility limits expressed as (grams of GP/100 g of solvent) are converted to more appropriate units of molarity (moles of GP/liter of solution) using eqn (2).

$$\text{molarity} = \frac{W_{GP}\,\rho^*}{M_{GP}\,(W_{GP} + W_{sv})} \times 10^3 \quad (2)$$

where W_{GP} is the weight of GP in grams, W_{sv} = 100 g of solvent, M_{GP} is the GP molecular weight, and ρ^* is the density (g L^{-1}) of the saturated solution at the solubility limit determined experimentally.



Scheme 1 Schematic structures for all investigated biological buffers (TRIS, TES, TAPS, TAPSO, and TABS) and glycine model compounds (Gly, Gly_2, Gly_3, and Gly_4).

2.2.2 Transfer free energy changes. The solubility data were used to calculate the transfer free energy, ΔG_{tr}, for the GPs from water to the aqueous solutions containing TRIS, TES, TAPS, TAPSO, and TABS at 25 °C under atmospheric pressure. At the solubility limit, the solute in the crystalline phase is in equilibrium with the solution phase, and thus the fugacities of solute at the solid state and in the liquid phase should be equal.[36] The detailed description of obtaining ΔG_{tr} has been reported in our earlier work.[32,33] The transfer free energy of the solute from water to aqueous buffer solutions is equal to the difference between the standard state chemical potentials of the solute in each solution, eqn (3). Here, the solute refers to the zwitterionic glycine peptides: Gly, Gly_2, Gly_3, and Gly_4.

$$\Delta G_{tr} = RT \ln\left(\frac{S_{GP,w}}{S_{GP,ws}}\right) + RT \ln\left(\frac{\gamma_{AA,w}}{\gamma_{AA,ws}}\right) \qquad (3)$$

where the subscript w refers to the aqueous and ws to aqueous TRIS, TES, TAPS, TAPSO and TABS. The term ($S_{GP,w}/S_{GP,ws}$) represents the concentration ratios in either mole fraction, molar, or molal units, each quantity being evaluated at the solubility limit of glycine model compounds. It should be noted that the transfer free energies reported by Cohn and Edsall[37] and Tanford[38] are based upon the mole fraction scale, while Robinson and Jencks[39,40] and Bolen and co-workers[31,41] used the molarity scale. Ben-Naim[42] has strongly suggested in the favor of molarity scale for obtaining transfer free energies. The disadvantage of the mole fractions scale is that activity coefficients, which are required to incorporate nonideality in the solvation free energy, have often been ignored because of experimental difficulty.[31] A molarity-based concentration scheme is free from these difficulties and has a clear physical meaning. *i.e.*, the transfer free energy of a solute from a fixed position in a phase to a fixed positioning another phase.[43] Therefore, we are also used molarity scale for calculating the transfer free energies. Eqn (3) can also be expressed in terms of molar concentrations ($C_{GP,w}$ and $C_{GP,ws}$), as given in eqn (4).

$$\Delta G_{tr} = RT \ln\left(\frac{C_{GP,w}}{C_{GP,ws}}\right) + RT \ln\left(\frac{\gamma_{GP,w}}{\gamma_{GP,ws}}\right) \qquad (4)$$

Historically, the term containing the activity coefficients has been ignored[29,30,32,33,40–42,44] because of the difficulty in obtaining these values for multicomponent system. It was found that the contribution due to the activity coefficient ratio considered as small or negligible.[28–30] Because the solubility data of glycine model compounds in water and in aqueous buffer solutions have been reported without activity coefficients, ΔG_{tr} is better denoted as apparent transfer free energies ($\Delta G'_{tr}$), eqn (5).

$$\Delta G'_{tr} = RT \ln\left(\frac{C_{GP,w}}{C_{GP,ws}}\right) \qquad (5)$$

The uncertainty of $\Delta G'_{tr}$ was estimated to be about ±1.6%.

2.2.3 Dynamic light scattering and zeta potential measurements. The hydrodynamic diameter (d_H) has been determined by means of DLS using a Zetasizer Nano ZS90 (Malvern Instruments Ltd., UK). This instrument employs a 4 mW He–Ne laser with a fixed wavelength λ = 633 nm. DLS measurements were performed at affixed scattering angle of 90°; thus, the hydrodynamic diameter obtained was an

apparent z-averaged hydrodynamic diameter. This instrument is equipped with a thermostatic sample chamber for maintaining the desired temperatures within a temperature range of 2–90 °C. Sample used for DLS had a protein (BSA) concentration of 10 mg ml^{-1}. The solutions were prepared by incubating 2 ml screw-capped vials in 0.05, 0.5, 1, and 1.8 M TRIS buffer pH 7.0 solutions at 25 °C for 4 h to attain complete equilibrium. All samples were filtered through Millipore 0.22 μm disposal filters prior to measurements. A bubble-free sample of around 1.5 ml was introduced in a quartz cuvette (QC) sample cell through a syringe. The cell was then sealed airtight with a Teflon-coated screw cap and secured in a sample chamber of DLS. The DLS measures the Brownian motion of particles and correlates this to the particle sizes. The relationship between the size of a particle and its speed due to Brownian motion is defined in the Stokes–Einstein equation:

$$d_H = \frac{kT}{3\pi\eta D} \quad (6)$$

where k is the Boltzmann's constant ($1.3806503 \times 10^{-23}$ m^2 kg s^{-2} K^{-1}), T is the absolute T/K, η is the viscosity (mPa s), and D is the diffusion coefficient (m^2 s^{-1}). All data were obtained from the instrumental software.

The zeta potential of 10 mg ml^{-1} of BSA in 0.05, 0.5, 1.0, and 1.8 M TRIS buffer, pH 7.0, was measured using a Zetasizer Nano ZS90 (Malvern Instruments Ltd., UK).

2.2.4 UV-visible absorption measurements. The difference spectra were recorded for 10 mg ml^{-1} of BSA at 0.05, 0.5, 1.0, and 1.5 M TRIS at room temperature on a V-550 spectrophotometer (JASCO) equipped with 1.0 cm quartz cells.

2.2.5 Fluorescence measurements. Fluorescence spectra were measured for 10 mg ml^{-1} of BSA at 0.05, 0.5, 1.0, and 1.5 M TRIS at room temperature with a RF-5301PC spectrofluorophotometer (SHIMADZU), using 1-cm quartz cell. The excitation wave length was 297 nm, and the emission was read at 280–320 nm.

2.2.5 Raman spectroscopy measurements. Raman spectra of 10 mg ml^{-1} of BSA at 0.05, 0.5, 1.0, and 1.5 M TRIS at room temperature were recorded using a Raman spectrometer employing a diode laser of 10 mV radiating on the sample operating at 785 nm. The laser spot size is *ca.* 1–2 μm. A thermoelectrically cooled charge-coupled device (CCD) with 1024 × 256 pixels was used as the detector with 1 cm^{-1} and each scan takes 15 min. Raman spectra were recorded in the 1560–1750 cm^{-1} range (amide I band region).

3. Results and discussion

Table 1 summarizes the solubility limits (expressed as g of solute per 100 g of solvent) and corresponding densities for Gly, Gly_2, Gly_3, and Gly_4 in aqueous solutions of TRIS, TES, TAPS, TAPSO, and TABS at 25 °C. The solubilities of the model compounds of proteins are significantly affected by the addition of buffers. The solubility values of glycine (Gly) decrease with increasing the concentration all buffer solutions, indicating that salting-out effect is dominant. From Table 1, we can see that the solubilities of Gly_2 in TRIS buffer cause salting-in, while TES, TAPS, TAPSO, and TABS cause salting-out. Both effects increase with increasing buffer concentration. Interestingly, a significant decrease in the solubility of Gly_2 occurred in TAPS solutions (from 21.79–2.98 g/100 g TAPS solution) with increasing the TAPS concentrations (from 0.05–0.5 M). Furthermore, we found that the solubility of Gly_3 and Gly_4 increases with increasing the buffer concentrations. This abrupt change in solubility behavior from higher glycines reveals that salting-in effect is predominant. Surprisingly, substantial increase in the solubilities of Gly_3 and Gly_4 by TRIS buffer with increasing the buffer concentrations from 0–1 M. These values increase (from 6.41–17.11 g/100 g TRIS solution) for Gly_3 and (from 0.395–3.86 g/100 g TRIS solution) for Gly_4, respectively.

Urea and guanidine hydrochloride (GdnHCl) have been used to dissolve coagulated systems and to unfold proteins. Of the two most commonly used protein denaturants, GdnHCl[45] is generally found to be 2–2.5 times more effective as a denaturant than urea.[45,46] Recent studies have tended to support the idea that these denaturants promote protein unfolding by favorable interaction with groups exposed upon unfolding.[47–50] By comparing the solubility values for Gly_3 and Gly_4 in TRIS buffer with their solubilities in urea[51] and GdnHCl,[30,52] it is surprising that the solubilities of Gly_3 and Gly_4 in TRIS buffer solution are much higher than those in urea and guanidine hydrochloride, as illustrated in Fig. 1. The peptide is being transferred to a good solvent (*i.e.*, a solvent that supports the peptide's presence well) and the solubility of the peptide will necessarily be increased in the presence of the TRIS buffer; one has to ask, does TRIS buffer stabilize or destabilize protein structure?

Amino acid transfer free energy measurements have played a key role in understanding the effect of various solvent media on protein stability.[28] If a protein moiety is more soluble in water than in the presence of cosolvent, the free energy of transfer will be positive (unfavorable interactions). In contrast, if the protein moiety is more soluble in cosolvent than it is in water, ΔG_{tr} will be negative (favorable interactions).

Table 2 lists the values of $\Delta G'_{tr}$ at 25 °C calculated from solubilities, and graphically illustrated in Fig. 2. Unfavorable interactions of glycine (Gly) were found with all five investigated biological buffers, according to their positive values of $\Delta G'_{tr}$, and these positive values increase with increasing the concentrations of buffer. Since Gly has no peptide groups the buffer cannot interact with Gly. The interactions of Gly_2 with TES, TAPS, TAPSO, and TABS are also unfavorable. While TRIS interacts favorably with Gly_2, that is with negative values of $\Delta G'_{tr}$, TRIS possibly forms a complex or direct hydrogen bond with the peptide group of Gly_2. A study involving molecular modeling showed that Ser-His dipeptide interacts with the TRIS molecule through intermolecular H-bonds, which results in formation of a Ser-His···TRIS complex.[53] The results in Fig. 2 show that $\Delta G'_{tr}$ values are negative for Gly_3 and Gly_4 in aqueous buffer solutions. Since these buffers can form hydrogen bond with proton donors and acceptors, the negative contributions reveal that buffers interact preferentially binding with higher glycines. As shown in Fig. 2, the negative $\Delta G'_{tr}$ values for Gly_3 or Gly_4 increase significantly with increasing buffer concentrations. It is worth

Table 1 Solubilities of glycine, diglycine, triglycine, and tertraglycine in water or aqueous buffer solutions and densities limits (ρ^*_{AA}) at 25 °C

	Solubility (g/100g solvent)					ρ^*_{AA}/g cm^{-3}				
Solvent	0 M	0.05 M	0.2 M	0.5 M	1.0 M	0 M	0.05 M	0.2 M	0.5 M	1.0 M
Gly (MW = 75.06 g mol^{-1})										
Water	25.09[a]					1.08299[a]				
TRIS		24.80	24.31	23.49	21.94		1.08463	1.08729	1.09354	1.10306
TES		23.89	23.13	22.48			1.08608	1.09580	1.11694	
TAPSO		24.54	23.30	21.61			1.08751	1.09834	1.12140	
TAPS		24.71	23.48	22.54			1.08582	1.09506	1.11467	
TABS		23.63	22.77	20.99			1.08423	1.09184	1.11146	
Gly_2 (MW = 132.12 g mol^{-1})										
Water	22.75[a]					1.07731[a]				
TRIS		23.29	24.98	26.49	28.54		1.08131	1.09019	1.10371	1.12194
TES		22.28	22.02	20.97			1.08192	1.09275	1.11367	
TAPSO		22.15	21.99	20.75			1.08241	1.09567	1.12097	
TAPS		21.79	11.18	2.98			1.08096	1.05797	1.05338	
TABS		22.02	21.23	19.72			1.08007	1.08978	1.10845	
Gly_3 (MW = 189.20 g mol^{-1})										
Water	6.41[a]					1.02205[a]				
TRIS		8.08	10.28	13.96	17.11		1.02971	1.04184	1.06262	1.08670
TES		7.54	7.75	8.18			1.03156	1.04478	1.07136	
TAPSO		7.38	7.66	8.01			1.03083	1.04699	1.07789	
TAPS		7.22	7.54	7.92			1.02956	1.04385	1.07113	
TABS		6.88	6.93	7.06			1.02834	1.04164	1.06746	
Gly_4 (MW = 246.23 g mol^{-1})										
Water	0.395[a]					0.99859[a]				
TRIS		1.02	1.94	2.95	3.86		1.00270	1.01070	1.02373	1.04150
TES		0.482	0.533	0.569			1.00343	1.01694	1.04347	
TAPSO		0.399	0.413	0.437			1.00396	1.01984	1.05043	
TAPS		0.411	0.470	0.495			1.00352	1.01722	1.04367	
TABS		0.505	0.536	0.612			1.00356	1.01677	1.04337	

[a] Ref. 34.

mentioning that TRIS buffer interacts more favorably than urea or GdnHCl with Gly_3 and Gly_4 (for example, the absolute value of $\Delta G'_{tr} = -5671.01$ J mol^{-1} for Gly_4 in 1 M TRIS is greater than that in 8 M urea, -1936.10 J mol^{-1}).[51] Thus our results reveal that the contribution of the buffers is thermodynamically the same contribution as that of urea[51] or GdnHCl[30,52] in the systems of higher glycines.

Protein surface contains a wide range of functional groups. It would be of interest to know the preference of each functional group on the protein for the two solvent components, water and buffer. The contribution of a backbone peptide group to $\Delta G'_{tr}$ was calculated as the effect of inserting a glycyl residue (–CH_2C═O–NH–) between an H_3N^+ and a CH_2COO^- group. This contribution, denoted as $\Delta G'_{tr}$, is calculated by assuming additivity of constituent atomic groups. From the apparent transfer free energies of Gly, Gly_2, Gly_3, and Gly_4, the transfer free energy of the peptide backbone is calculated. Because these series have been used by many researchers[28–30,37,51,54] in their studies, as presented in eqn (7), where m and n are the number of the peptide units in the glycine peptides such that $m \geq n + 1$.

$$\Delta g'_{tr} = (\Delta G'_{tr}[H_3\overset{+}{N}(CH_2C{=}ONH)_mCH_2COO^-] - \Delta G'_{tr}[H_3\overset{+}{N}(CH_2C{=}ONH)_nCH_2COO^-]/(m-n) \quad (7)$$

This subtraction provides a mathematical means to remove the contribution of the end groups. Two types of mathematical constructs arise from that equation, simple subtractional constructs for which $m = n + 1$ and composite constructs for which $m > n + 1$. The simple subtractional constructs consist of subtracting the $\Delta G'_{tr}$ of two consecutive glycines, such as Gly_2 and Gly; Gly_3 and Gly_2; or Gly_4 and Gly_3. On the other hand, the composite constructs consist of subtracting the $\Delta G'_{tr}$ of two glycines that differ in chain length by more one peptide unit, such as Gly_4 and Gly, then dividing the difference by three, the number of remaining peptide units, [(Gly_4 – Gly)/3]. It is similar for other two different chain-length peptide units, such as [(Gly_4 – Gly_2)/2] and [(Gly_3 – Gly)/2].

The results in Table 3 reveal that the transfer free energies of the peptide backbone from water to the buffer solutions ($\Delta g'_{tr}$) are negative and also their absolute values increase with increasing the buffer concentration, except for the $\Delta g'_{tr}$ of (Gly_2 – Gly) with TAPS system and 0.05 M TAPSO, (Gly_4 – Gly_3) with TAPSO system and 0.05 M TAPS. The negative contribution indicates that the interactions between the solvent and the peptide backbone are favorable. Besides, different values were observed for the peptide backbone contribution, depending on the molecule into which the glycyl reside is inserted. These results show that the value of $\Delta g'_{tr}$ for peptide group is very sensitive to the nature of neighboring groups. Such a feature of transfer free energies has been observed by other researchers.[28–30,37,54] From Table 3, it can be seen that the interactions of the zwitterionic buffers (TES, TAPS, TAPSO, and TABS) with the peptide backbone are smaller than those of TRIS buffer.

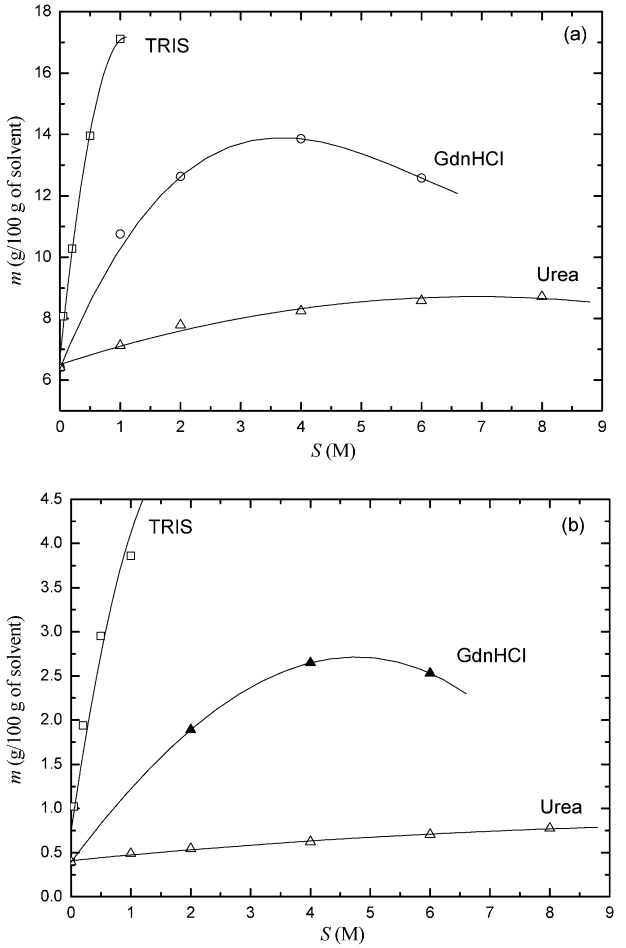


Fig. 1 Solubility limits for (a) Gly_3; (b) Gly_4 in water and aqueous solutions of TRIS, GdnHCl, and urea: (□) this work, (○) ref. 30, (△) ref. 51 and (▲) ref. 52. Solid lines show only smoothness of the solubility data points. The symbol *S* is the concentration of solvents or buffers and *m* is the solubility of glycine peptides.

DLS measurements were performed at various temperatures for Bovine Serum Albumin (BSA) in 0.05, 0.5, 1.0, and 1.8 M TRIS buffer at pH = 7.0 to obtain the absolute value of hydrodynamic diameter (d_H). Fig. 3 shows the intensity distribution graph of BSA in 0.05 M TRIS buffer solution (pH 7.0) at various temperatures. For the sake of clarity we do not present all the distribution graphs. The size distribution curve obtained by intensity (Fig. 3) exhibited two peaks at lower temperatures (from 25–55 °C). One of these peaks was obtained from a major population (*i.e.*, native state) of small particles with d_H values from 11.4–13.5 nm at temperatures from 25–55 °C. The other population corresponded to particles with $d_H > 100$ nm, with a lower percentage of intensity. These d_H values give information about the different particles present in the sample. From Rayleigh approximation, the intensity is proportional to d^6 (*d* is the particle diameter). Thus, the contribution of mean size is minimal compared with that of large particles, and, thereby, the larger particles in the second population are negligibly small.[55,56] The values of the polydispersion indexes (PDIs) don't exceed 0.5. In Fig. 3, it can be seen that the native state peaks decrease and the denatured state peaks increase with increasing temperature. Moreover,

Table 2 Apparent transfer free energies of glycine, diglycine, triglycine, and tertraglycine from water to aqueous buffer solutions at 25 °C and at atmospheric pressure

	$\Delta G'_{tr}$/J mol^{-1}			
Solvent	0.05 M	0.2 M	0.5 M	1 M
Gly				
TRIS	19.3	53.0	107	224
TES	90.5	133	144	
TAPS	23.8	105	143	
TAPSO	33.7	113	214	
TABS	117	174	295	
Gly_2				
TRIS	−56.5	−217	−363	−548
TES	31.7	30.8	83.5	
TAPS	79.0	1560	4660	
TAPSO	42.4	26.9	88.9	
TABS	59.7	112	222	
Gly_3				
TRIS	−554	−1130	−1860	−2350
TES	−399	−494	−680	
TAPS	−294	−429	−606	
TAPSO	−348	−472	−647	
TABS	−180	−228	−332	
Gly_4				
TRIS	−2350	−3940	−4980	−5670
TES	−503	−785	−1010	
TAPS	−110	−475	−666	
TAPSO	−38.2	−162	−375	
TABS	−619	−798	−1190	

only a single peak appears at higher temperatures (56–70 °C), and the native population has vanished absolutely.

The thermal denaturation of proteins often involves unfolding of the protein molecules and is accompanied by irreversible aggregation of the unfolded molecules, which changes the protein size that can be monitored by DLS. At the melting point of the protein, a marked increase in hydrodynamic size and scattering intensity is observed. This melting temperature is indicative of the thermal stability of a protein. Significantly unfolding leads to exposure of the hydrophobic residues to solvent, when the protein is heated above its characteristic thermal stability point (protein melting point). This entropically unfavorable state is soon replaced, however, with one wherein the hydrophobic residues on one protein chain associate with those on another protein chain.[57–59] Light scattering is ideal for studying this non-specific aggregation of denatured proteins, as the technique is extremely sensitive for studying the protein melting point phenomenon.[60]

An automatic temperature scan of the sample chamber allows observation of both the size and the scattering intensity as a function of temperature. The marked point where both the size and the intensity start to increase significantly is called the melting point or T_{den}, the critical denaturation temperature (beginning of the denaturation process). Fig. 4 shows the melting curves reporting the values of d_H as a function of temperature for BSA in 0.05, 0.5, 1.0, and 1.8 M TRIS buffer (pH 7.0) and the protein size (d_H) values are collected in Table 4. From Fig. 4, it can be seen that the native protein in 0.05 M TRIS buffer solution has a d_H = 11.4 nm at 25 °C. The diameter remains constant up to 55 °C, followed by a significant increase of d_H beyond T_{den} = 55 °C. This indicates that BSA is in folded form up to 55 °C and BSA starts to

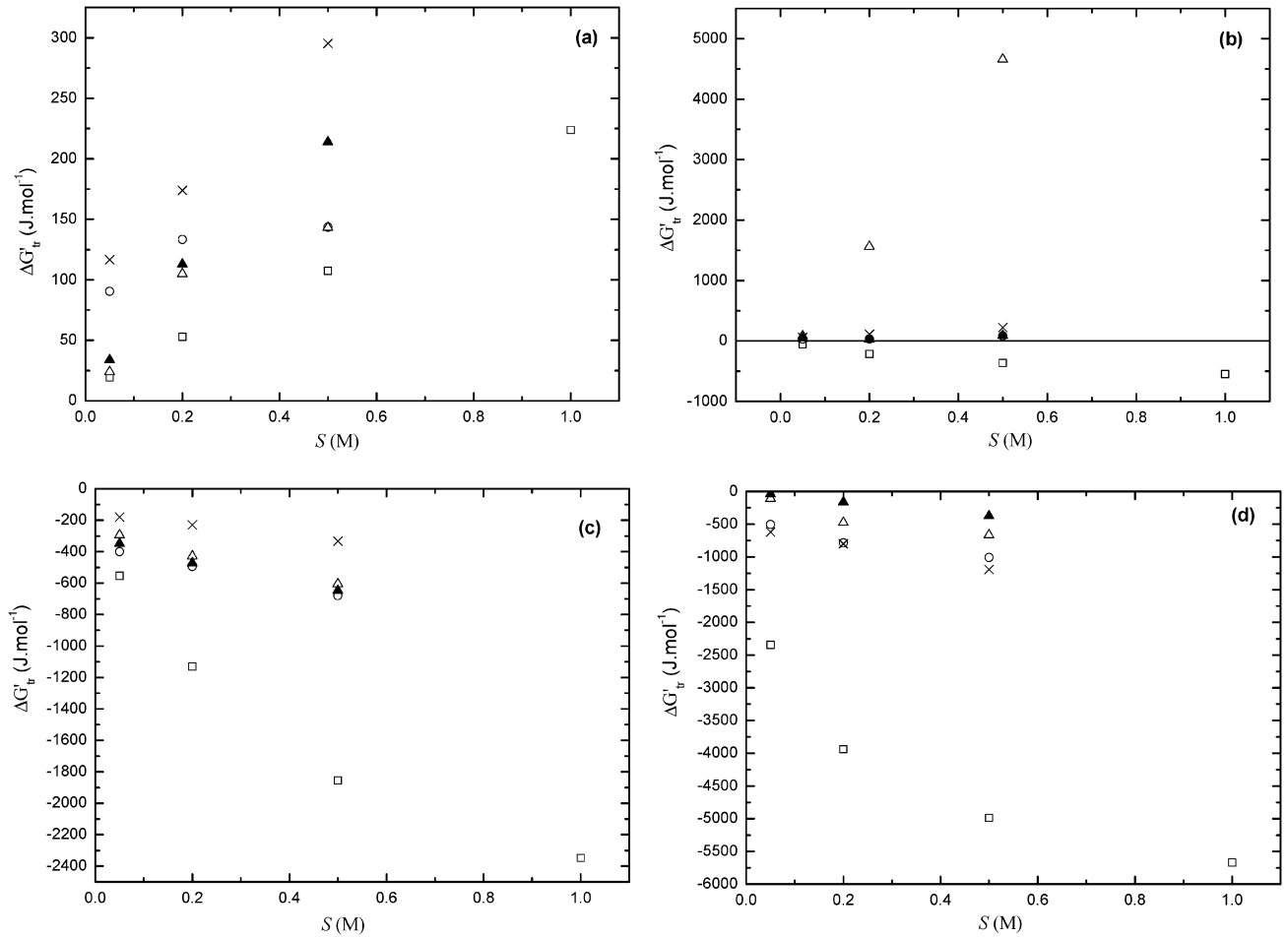


Fig. 2 The apparent transfer free energies ($\Delta G'_{tr}$) of glycine peptides of (a) Gly; (b) Gly_2, (c) Gly_3, (d) Gly_4 in aqueous solutions of TRIS (□), TES (○), TAPS (△), TAPSO (▲) and TABS (×). The symbol *S* is the concentration of buffers.

denature with increasing temperature (for example, d_H = 104 nm at 70 °C). The observed process was essentially irreversible, as can be seen from the unchanged value (d_H ~ 104 nm) of the hydrodynamic diameter as the sample cools back to 25 °C after the denaturation (from the evidence of our DLS measurements). Interestingly, from Fig. 4 the values of T_{den} at 0.05, 0.5, 1.0, and 1.8 M TRIS buffer (pH 7.0), are 55, 60, 62, and 65 °C, respectively. The results presented here demonstrate that the thermal stability of BSA increases with increasing TRIS buffer concentration. It is worth noting that in the sample photographs in Fig. 5, when the sample chamber was heated to 85 °C, the BSA aggregation decreased with increasing TRIS concentration. The sample with 0.05 M TRIS became a white cream. With increasing TRIS concentration, the white cream precipitates became more transparent. When the concentration of TRIS reached 1.8 M, the sample became quite transparent. Taken together, these results indicate that increasing TRIS buffer concentration suppresses the aggregation of a thermal denatured protein (BSA).

UV-Visible absorption is a simple method that is applicable to explore the structural changes and complex formation.[61,62] Difference spectrophotometry, a technique introduced to protein chemistry by Laskowshi *et al.*,[63] has been applied by several investigators to problems in which changes in protein conformation are involved. Peaks in the difference spectra of proteins in the 292–294 nm region are assigned to tryptohan,[64] in the 285–288 nm region to tyrosine[63,65] (plus a small contribution by tryptohan) and below 270 nm to phenylalanine.[64] Difference spectrophotometry also offered an opportunity to obtain information about the chromophoric groups of BSA in its interactions with different ligands; such information might be correlated with data obtained by using other techniques to yield specific information about the involvement of tyrosyl and tryptophyl residues in the conformational changes. The blue shift at 285–295 nm has been considered to be a criterion for disorganization of the native structure of the protein.[66] Similar blue shifts have been observed when aromatic chromophores are transferred from organic environments to aqueous solutions,[67] and have been ascribed to a change of the $\pi \rightarrow \pi^*$ transition brought about by changes in the polarizability of the solvent. Therefore, exposure of "hidden" aromatic chromophores to the aqueous solution would cause blue shifts. The opposite process would cause red shifts, as would shielding the chromophores involved from the aqueous environments.[68] In the present work, the difference spectra observed for BSA at 0.05, 0.5, 1.0, and 1.5 M TRIS are shown in Fig. 6. These difference spectra have a maximum at ~297 nm from 0.05–1.0 M TRIS. The ΔA values at ~297 nm increase from 0.05 M

Table 3 Contribution of peptide backbone unit transfer free energy ($\Delta g'_{tr}$) from water to aqueous buffer solutions at 25 °C

		$\Delta g'_{tr}$/J mol^{-1}			
Scheme	Solvent	0.05 M	0.1 M	0.5 M	1 M
$Gly_2 - Gly$					
	TRIS	−75.8	−270	−470	−772
	TES	−58.9	−103	−60.0	
	TAPS	55.2	1460	4520	
	TAPSO	8.7	−85.9	−125	
	TABS	−57.0	−61.9	−73.6	
$Gly_3 - Gly_2$					
	TRIS	−497	−913	−1490	−1800
	TES	−431	−525	−764	
	TAPS	−373	−1990	−5260	
	TAPSO	−390	−499	−736	
	TABS	−239	−340	−554	
$Gly_4 - Gly_3$					
	TRIS	−1790	−2810	−3130	−3320
	TES	−104	−290	−329	
	TAPS	184	−46.3	−60.7	
	TAPSO	310	310	272	
	TABS	−439	−570	−857	
$(Gly_3 - Gly)/2$					
	TRIS	−287	−591	−982	−1290
	TES	−245	−314	−412	
	TAPS	−159	−267	−374	
	TAPSO	−191	−293	−431	
	TABS	−148	−201	−314	
$(Gly_4 - Gly_2)/2$					
	TRIS	−1150	−1860	−2310	−2560
	TES	−267	−408	−546	
	TAPS	−94.6	−1020	−2660	
	TAPSO	−40.3	−94.6	−232	
	TABS	−339	−455	−705	
$(Gly_4 - Gly)/3$					
	TRIS	−789	−1330	−1700	−1960
	TES	−198	−306	−384	
	TAPS	−44.7	−193	−270	
	TAPSO	−24.0	−91.7	−196	
	TABS	−245	−324	−495	

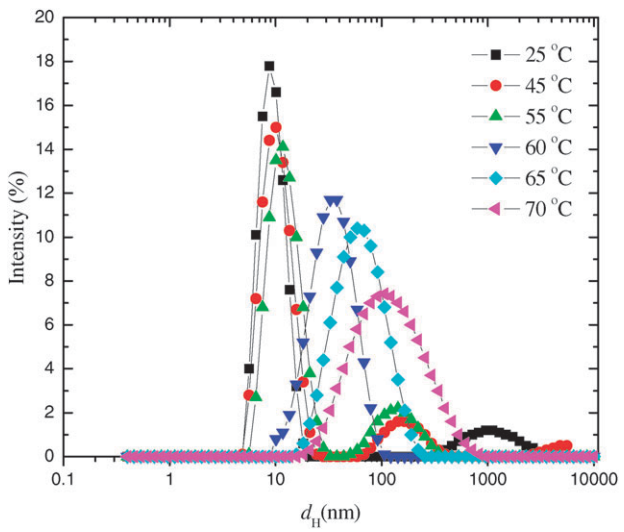


Fig. 3 DLS spectra of intensity distribution graph as a typical size distribution in nanometres of BSA in 0.05 M TRIS buffer at various temperatures.

to reach a maximum at 0.5 M TRIS and then decrease at 1.0 M TRIS, but at 1.8 M TRIS there is a red shift with a

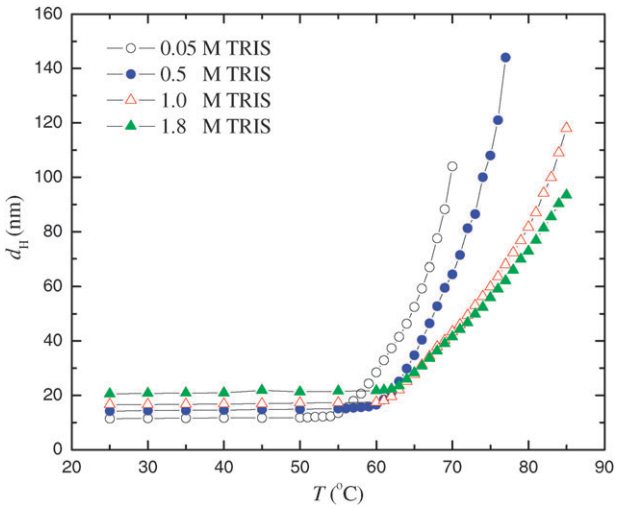


Fig. 4 Hydrodynamic diameter (d_H) obtained from the intensity distribution graph for BSA in 0.05, 0.5, 1.0, and 1.8 M TRIS at pH 7.0, as a function of temperature.

Table 4 DLS data of hydrodynamic diameter (d_H) for bovine serum albumin (BSA) in 0.05, 0.5, 1, and 1.8 M TRIS solutions at different temperatures

T/°C	d_H	T/°C	d_H	T/°C	d_H	T/°C	d_H
0.05 M TRIS		0.5 M TRIS		1 M TRIS		1.8 M TRIS	
25	11.4	25	14.2	25	16.6	25	20.5
30	11.5	30	14.4	30	16.6	30	20.7
35	11.6	35	14.5	35	16.7	35	20.8
40	11.7	40	14.6	40	16.8	40	20.9
45	11.7	45	14.8	45	16.9	45	21.8
50	11.8	50	14.9	50	17.2	50	21.3
51	11.9	55	15.1	55	17.3	55	21.5
52	12.1	56	15.2	60	17.5	60	21.6
53	12.2	57	15.4	61	18.0	61	21.9
54	12.3	58	15.6	62	19.6	62	22.3
55	13.5	59	15.9	63	22.0	63	23.5
56	15.1	60	16.5	64	25.1	64	25.9
57	17.9	61	18.5	65	27.7	65	28.3
58	20.6	62	21.0	66	31.1	66	30.7
59	24.3	63	25.1	67	34.3	67	33.6
60	28.4	64	29.8	68	37.8	68	36.3
61	32.9	65	34.7	69	40.2	69	39.0
62	37.3	66	40.4	70	43.4	70	41.5
63	41.5	67	46.4	71	46.0	71	44.2
64	46.3	68	52.7	72	49.4	72	46.7
65	52.5	69	59.5	73	53.0	73	49.9
66	59.2	70	64.4	74	56.4	74	52.4
67	67.0	71	71.4	75	59.8	75	55.9
68	77.5	72	81.3	76	63.6	76	59.1
69	88.2	73	86.5	77	68.0	77	62.1
70	104	74	100	78	72.2	78	66.0
		75	108	79	76.7	79	69.8
		76	121	80	81.7	80	72.8
		77		81	87.0	81	76.8
				82	94.2	82	81.3
				83	99.9	83	85.4
				84	109	84	90.3
				85	118	85	93.4

maximum at ~311 nm. This change in the absorbance spectrum of the protein is due to the effect of the TRIS buffer, because in the spectrum range between 270 and 370 nm TRIS does not absorb light. The fact that there is a peak at ~297 nm indicates that tryptophyl residues are implicated.[69]

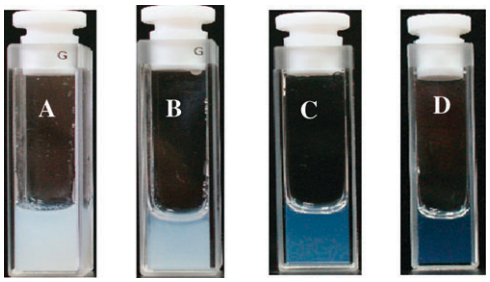


Fig. 5 Sample photographs of 10 mg ml^{-1} of BSA in 0.05 M TRIS (A), 0.5 M TRIS (B), 1.0 M TRIS (C), and 1.8 M TRIS (D), after heating the sample to 85 °C.

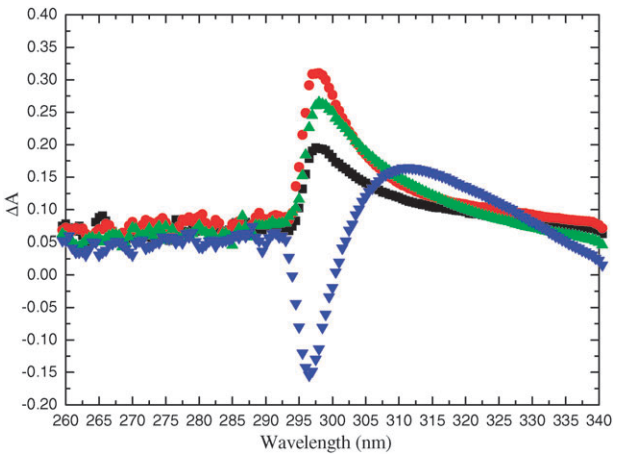


Fig. 6 Difference spectra of 10 mg ml^{-1} of BSA in 0.05 M TRIS (■), 0.5 M TRIS (●), 1.0 M TRIS (▲), and 1.8 M TRIS (▼).

Tryptophan is highly sensitive to the local environment and also displays a substantial spectral shift. As a result, the position of the spectra maximum (λ_{max}) depends on the properties of the environment of the tryptophyl residues.[70] It can be noted that the solvent effects of the TRIS buffer at 1.8 M on exposed tryptophyl residues result in a significantly large red shift (297–311) nm. The spectral shifts due to binding are obviously of a complex nature. From these spectral changes, it is concluded that tryptophyl residues are at or very near the highest energy binding sites.

To investigate whether TRIS buffer binds to BSA, fluorescence measurements are carried out. Fluorescence measurements give information about the molecular environment in a vicinity of the chromophore molecules. Fig. 7 shows the typical fluorescence spectra of 10 mg ml^{-1} BSA in the presence of 0.05, 0.5, 1.0, and 1.8 M TRIS at pH 7.0. The fluorescence measurements were conducted in the range of 280–320 nm upon excitation at 297 nm. The intrinsic fluorescence of BSA excited at 297 nm is mainly due to the presence of the two tryptophyl residues: Trp-134 and Trp-212. Trp-212 is located within a hydrophobic binding pocket in the IIA sub-domain of the protein, while Trp-134 is located on the surface of the albumin molecule and more exposed to the environment.[71] Although the tyrosine residue can also contribute to fluorescence, it presents a very weak emission when was excited at 297 nm. TRIS buffer causes a remarkable decrease in the fluorescence intensity of BSA with increasing concentration of

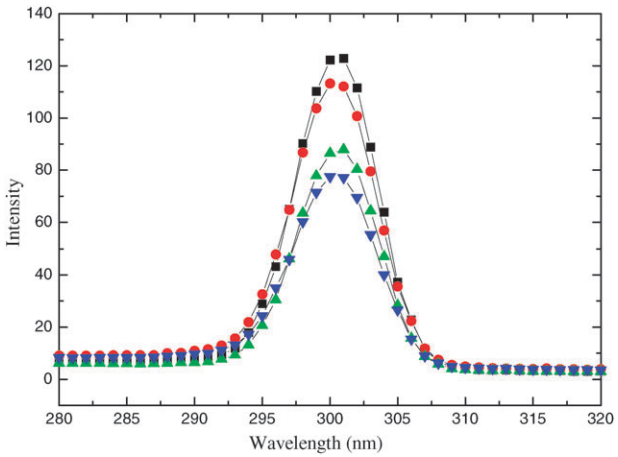


Fig. 7 Fluorescence spectra of 10 mg ml^{-1} of BSA in 0.05 M TRIS (■), 0.5 M TRIS (●), 1.0 M TRIS (▲), and 1.8 M TRIS (▼).

TRIS (Fig. 7), while the emission maximum and shape of the peaks remained unchanged. This indicated that TRIS could bind to BSA without altering the local dielectric environment.

The interactions of BSA with TRIS could alter their charge and size. We measure the zeta potential (net surface charge) of the BSA/TRIS complexes at 25 °C. The particle size of BSA at 25 °C of BSA in 0.05, 0.5, 1.0, and 1.8 M TRIS is, respectively, 11.4, 14.2, 16.6, and 20.5 nm, while the zeta potential was −5.22, −1.34, 1.05, and 3.25 mV, respectively. As the concentration of TRIS increased, there was an increase in the particle size of BSA, whereas there was a slight increase of the zeta potential from negative to positive. It is suggested that interactions between BSA and TRIS are not based on electrostatic interactions. Taken together, it can be noted that TRIS buffer, by virtue of its –OH and amine groups, interacts with BSA mainly through hydrogen-bonding.

The Raman spectra of BSA in 0.05, 0.5, 1.0, and 1.8 M TRIS in the range of 1500–1750 cm^{-1} at 25 °C are shown in Fig. 8. This region is dominated by a broad Raman band near 1659 cm^{-1} corresponding to C═O stretching vibration of amide groups coupled to the in-phase bending at the N–H bond and the stretching of the C–N bond.[72] Fig. 8 shows a

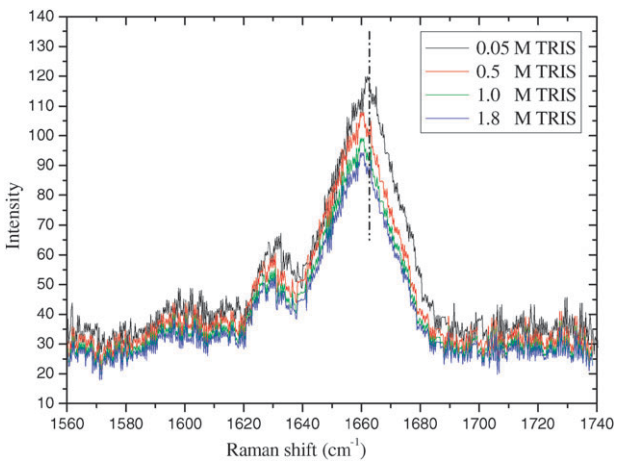


Fig. 8 Raman spectra in the amide I band region of 10 mg ml^{-1} of BSA in 0.05–1.8 M TRIS.

downshift of the amide I band frequency in 0.5, 1.0, and 1.8 M TRIS solutions with respect to that in 0.05 M TRIS solution. This frequency shift indicates that amide group interacts with TRIS buffer at high concentrations. In addition, the intensity of the amide I band decreases with the increasing of TRIS concentration.

4. Conclusions

In the present work, the interactions between glycine peptides: Gly, Gly_2, Gly_3 or Gly_4 and some important biological buffers: TRIS, TES, TAPS, TAPSO, and TABS, were explored based on the solubility data. We have determined the apparent transfer free energies ($\Delta G'_{tr}$) of glycine peptides from water to buffer solutions. We have calculated transfer free energies ($\Delta g'_{tr}$) of the peptide backbone unit (–CH_2C═O–NH–) contributions from $\Delta G'_{tr}$values. The results suggested that TRIS buffer strongly interacts with the peptide backbone. However, TAPS and TAPSO have smaller interactions with the backbone, in comparison with the other zwitterionic buffers. The combined results suggested that most amine-based buffers interact with peptides. This study reinforces the importance of buffer selection under study in biochemical studies. We found that the buffer concentration had a significant effect on the interactions with the peptides, and those influences increased with increasing concentration. Therefore, it is necessary to have a preferable concentration in order to have low buffer–protein interactions without significantly reducing buffer capacity. The interactions of TRIS with BSA, as a globular protein, were investigated through DLS, zeta potential, UV-Visible absorption, fluorescence, and Raman spectroscopy measurements. The experimental evidence shows that TRIS buffer stabilized the BSA molecules.

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MohaMed Taha ahMed aMIN*

Statement of Research and Teaching Interests

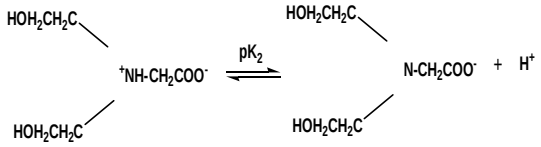
MD Analytical Chemistry, 2005, Cairo University, EGYPT
PhD Applied Chemistry (has equivalent to an Egyptian PhD Physical Chemistry), 2010,
National Taiwan University of Science and Technology, Taiwan.

Research Experience

My primary research interests are in the areas of physical chemistry, specifically: (1) Co-solvents effect on protein folding/unfolding. (2) Solubility of biological compounds in aqueous organic solvents. (3) Phase separation. (4) Phase equilibria: Particularly, solid–liquid equilibria, liquid–liquid equilibria, solid–liquid–liquid equilibria, and vapour-liquid equilibria. (5) The study of nanometric or submicro particles formation with supercritical fluid technology. (6) Determination of dissociation constants of biologically important ligands. (7) Complexation equilibria for binary and mixed ligand complexes and determination of stability constants using potentiometric and spectrophotometeric techniques.

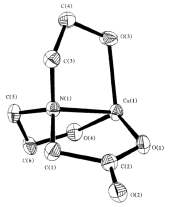
BUFFER INTERACTIONS.

Maintaining a stable pH value in many fields, such as chemistry, biology, pharmacology, medical science, industry, and our daily life as well, by adding a suitable buffer to the medium is major prerequisite. In a situation which is far from ideal, many buffers have been found to be quite reactive, besides maintaining their stable pH values. At the beginning of my research career, and during my Master studies, I got an opportunity to work with Prof. Dr. Mohamed M. Khalil, my Master supervisor (Cairo University). We studied the complexation equilibria between biologically important metal ions, biological buffers, amino acids, carboxylic acids, and hydroxamic acids, by determining their protonation constants as well as their metal complexes. Since, these systems mimic many biological reactions [1-16].



Protonation Equilibria of Bicine

Cu^{2+} + (bicine)$^-$ ⇌ [Cu (bicine)]$^+$

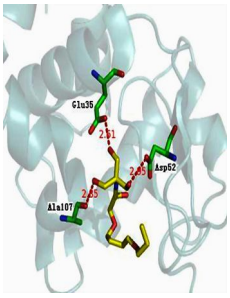


Cupper Complexation of Bicine

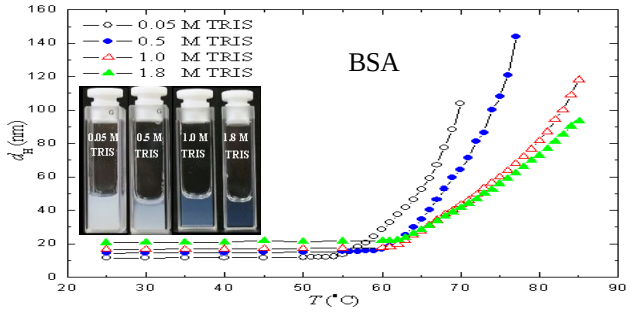
I have published 10 articles in international journals at the beginning of my research career (Master Degree, 2 years), as corresponding author. More excitement came-when I was suffering thru trying to find answers for reviewers' criticisms on my work. This was the most excitement I have ever seen!

To shed more light on the buffer-solvent interactions, I made up my mind to pursue my doctoral study. I have been fortunate to have the opportunity to work in Professor Ming-Jer Lee's lab, Department of Chemical Engineering, National Taiwan University of Science and Technology (NTUST), Taiwan. My advisor Professor Ming-Jer Lee is an exceptional scientist who is extremely creative. Among the commercial available pH buffers, we have selected some important buffers (TRIS, TAPS, TAPSO, TABS, MES, MOPS, MOPSO, and MOBS), as example to study the interactions of these buffers with a series of ionic salts, aqueous solutions of some water-miscible organic solvents [17-22] in terms of solubility and transfer free energies. These buffers have pKa values ranging from 6.0 to 9.0, which allow them to be used in biological, biochemical or environmental studies. Carefully performed measurements on these buffers would not only provide important data on these biological buffers, but could also be used to estimate the effect of the contribution of some functional groups on the interaction of these substances with solvent, such knowledge is an important to design new biological buffers.

TRIS-Protein Interactions.



The interactions of Gly oligomers with various biological buffers was examined in terms of solubility and transfer free energies. These include TRIS, TES, TAPS, TAPSO, and TABS. More and more, we find that buffers can interact preferentially with proteins, providing thermodynamic stabilization. Here we find that TRIS differs from similar buffers in its ability to interact with the peptide backbone, leading to net stabilization of bovine serum albumin (BSA), based on a variety of biophysical measurements [23].

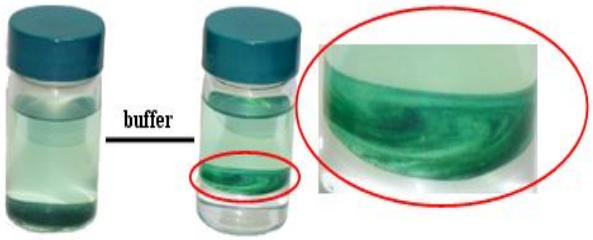


Once again, within two years (2008-2010), by using time effectively, not just efficiently, I have finished 18 credit hour courses, 7 articles published in SCI journals, and another two papers still in preparations. Hence, I spent only two years to earn my PhD. At NTUST, it is difficult for local students or international students to earn a Ph.D. in a short time. An average student has to spend at least 3-4 years.

E-mail: mtaha978@yahoo.com

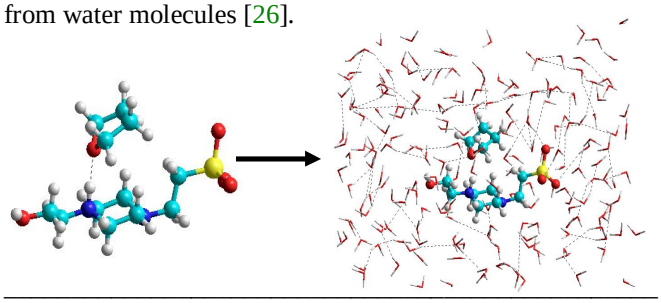
PHASE SEPARATION

Currently, I am working as a postdoctoral fellow in Prof. Ming-Jer Lee's lab. I have been given again an opportunity to learn more and more. My work is started with investigating **Buffering-out**, a new phase separation systems. Buffering-out may be helpful in the recovery of the organic solvents from their aqueous solutions, and may find potential applications in the separation of chemicals having substantially different solubilities in water and in the organic solvent [18, 24, 25].



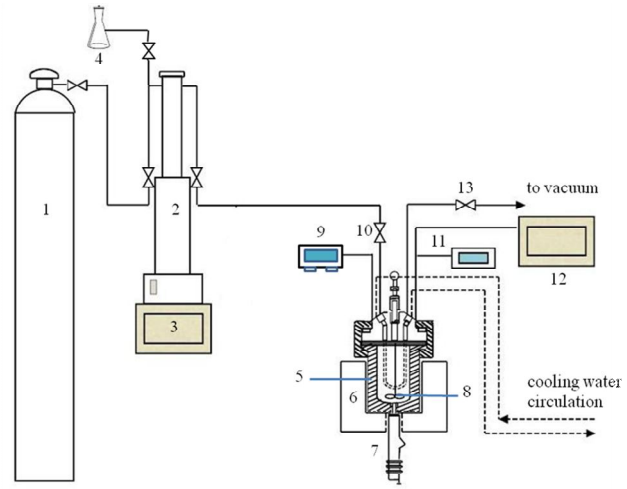
MOLECULAR MODELLING

In order to investigate a possible mechanism of buffer-induced phase separation, we thoroughly examined the intermolecular interactions between buffer, water, and the organic solvents using quantum chemical modeling. The simulations showed that buffer is dominantly hydrogen-bonded with water. On the other hand, the organic solvents are rarely hydrogen-bonded with water molecules. Therefore, it is likely that the competitive interaction of water molecules with buffer through hydrogen bonding, in comparison with the interaction of water molecules with the organic solvent, forces the organic molecules to separate from water molecules [26].



I have been fortunate to have the opportunity to work in Prof Lee's lab again. I have learned a lot from him. **Working on several projects** from the National Science Council of Taiwan, and some of them were from domestic chemical companies and research institutions (Taiwan), using various apparatuses as given below enriched my experience and refreshed my spirit.

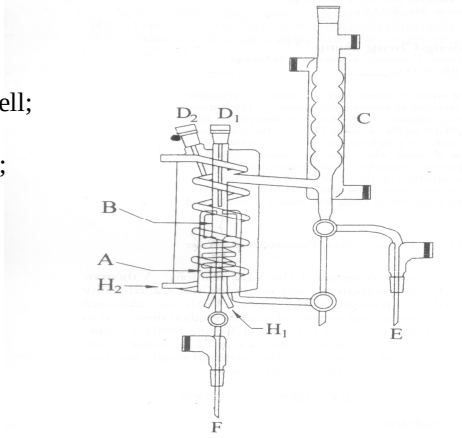
Isothermal Vapor-Liquid Equilibrium Apparatus. This apparatus was installed to measure isothermal vapor-liquid equilibrium (VLE) data over wide ranges of temperature and pressure.



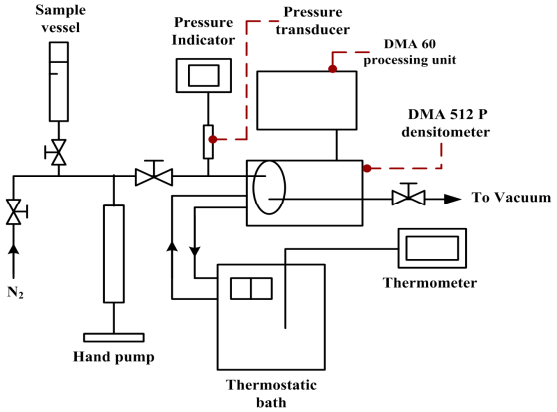
(1) N_2 cylinder;(2)Syringe pump; (3)Syringe pump controller; (4) Degassed solution; (5)Equilibrium cell; (6) heater; (7) Bottom drain valve; (8)Magnetic stirrer; (9)Pressure transducer; (10) Gas inlet/sample valve; (11) Digital thermocouple; (12) Equilibrium cell controller; (13) Gas release valve.

Isobaric Vapor-Liquid Equilibrium Apparatus

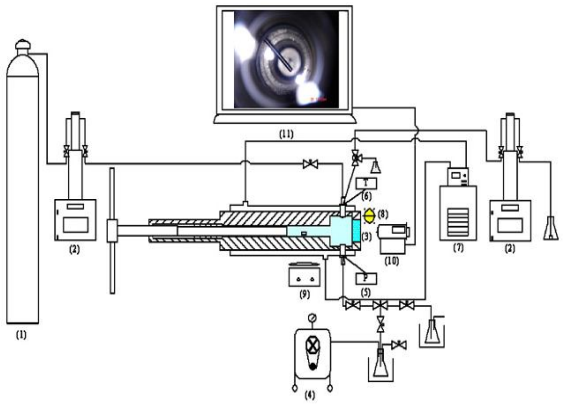
(A) Liquid-phase chamber;
(B) Vapor-phase cell;
(C) Condenser;
(D) Thermometers;
(E) Liquid-phase sampling port;
(F) Vapor-phase sampling port;
(G) Coil heaters.



Pressure-Volume-Temperature apparatus. This was installed to measure density data over wide ranges of temperature and pressure.

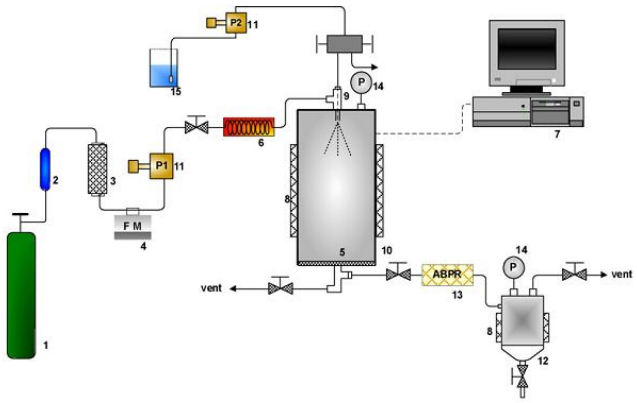


Visual and Volume-variable High-Pressure Phase Equilibrium Analyzer (PEA). This apparatus was installed for determining the vapor-liquid phase boundaries over a wide pressure rang, including near critical region, of mixtures containing supercritical carbon dioxide near.



(1) CO_2 cylinder, (2) Syringe pump, (3) Sapphire window, (4) Vacuum pump, (5) Pressure transducer, (6) Thermocouple, (7) Thermostatic bath, (8) LED light, (9) Magnetic stirrer, (10) Digital camera and (11) TV monitor.

Supercritical Anti-Solvent(SAS) Precipitation Apparatus. This method possesses great potential to produce solvent-free, ultra-fine particulates and micro-capsules of pharmaceutical materials in polymer matrix for controlled relase systems. Moreover, the selection of organic solvent is more flexible for the particle formation process and the use of supercritical carbon dioxide as anti-solvent to precipitate proteins causes les damage to their biological activity due to the mild precipitation conditions.



(1) CO_2 cylinders; (2) Filter; (3) Cooler; (4) Mass flow meter; (5) Metal frit; (6) Pre-heater; (7) Computer; (8) Heating jackets; (9) Coaxial injector; (10) Precipitator; (11). High pressure pumps; (12) Separator; (13) Automatic back pressure regulator; (14) Pressure gauges; (15) Sample solution.

I realize how important it is, as a researcher, to interact with other people working in the same field, and at the same time, am able to work individually.

Teaching

Before pursuing my Ph.D. degree, I have had the opportunity for two major teaching experiences. The first as analytical chemistry instructor at Industrial Education College, EGYPT. In this course, I taught and graded all lab assignments. ***The second is the most excitement with particular challenge,*** when I was working at Arabian Fire Safety Academy, Kingdom of Saudi Arabia, as a Director of Planning & Evaluation. I was responsible to prepare some CERTIFICATE ASSEMBLY (7 levels) in fire science which trained internationally according to the National Fire Protection Association Professional Qualifications (NFPA) (http://www.nfpa.org) and to be accredit by the International Fire Service Accreditation Congress Criteria of Certification (IFSAC) (http://www.ifsac.org), Oklahoma University. The Seven Training Courses are the following:

1. Firefighter I, *NFPA 1001, 2002 Edition.*
2. Firefighter II, *NFPA 1001, 2002 Edition.*
3. Hazardous Material Awareness level, *NFPA 472, 2002 Edition.*
4. Hazardous Material Operations level**,** *NFPA 472, 2002 Edition.*
5. Fire Service Instructor I, *NFPA 1041, 2002 Edition.*
6. Fire Officer I**,** *NFPA 1021, 2003 Edition.*
7. Fire Officer II**,** *NFPA 1021, 2003 Edition.*

I prepared all its documents (Lesson plan, test bank, skill sheets, correlation sheets, policy manual, self study, and IFSAC worksheets).

27-31 January 2007, the site visit had visited us.

ACCREDITATION TEAM MEMBERS

1. James K Heim, Executive Director, Minnesota Fire Service Certification Board
2. Paul Covington, Board member, Indiana Board of Fighters Personnel Standards & Education

The recommendation from the Site Team to the Board of Directors and members of IFSAC is to ***recommend full accreditation of the Seven (7) levels.***
May, 2007: I sent another two levels for administration review:

8. Fire Inspector I, *NFPA 1031, 2003 Edition.*
9. Fire Inspector II, *NFPA 1031, 2003 Edition.*

August 2007, the above two levels are accredited

As for me, this is the greatest achievements I've had in my career life. To contribute to make these important vocational certificates for the first time given in a country I love.

Also, I taught several courses at Arabian Fire Safety Academy, such as Fire Chemistry, Hazardous Material, and Fire Behaviour.

I would like to do a mix of teaching and independent research. There is no better way to learn something than to learn to teach it.

I've the ability to teach the following courses: Physical Chemistry, Chemical Thermodynamics, General Chemistry, Inorganic Chemistry, Organic Chemistry, Green Chemistry, and Environmental Science.

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22. **Mohamed Taha,** Ming-Jer Lee,* Buffer Interactions: Solubilities and transfer free energies of TRIS, TAPS, TAPSO, and TABS from water to aqueous ethanol solutions. *Fluid Phase Equilibria* **2010**, *289*, 122-128.
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24. **Mohamed Taha,** Ming-Jer Lee,* Solubility and phase separation of 4-morpholinepropanesulfonic acid (MOPS), and 3-morpholino-2-hydroxypropanesulfonic acid (MOPSO) in aqueous 1,4-dioxane and ethanol solutions. *J. Chem. Thermodynamics* **2011** (DOI:10.1016/j.jct.2011.05.036).

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Note: * Corresponding author

MohaMed Taha ahMed aMin

Current Address: Department of Chemical Engineering,
National Taiwan University of Science & Technology,
43 Keelung Road, Section 4, Taipei 106-07, Taiwan.

Cell Phone: +**886-987814211**
E-mail: mtaha978@yahoo.com

Personal Information

Nationality: Egyptian
Marital Status: Single
Date of Birth: 29/08/1978
Place of Birth: Beni-Suef, EGYPT

Education

3/2010-Now	Post Doctor Fellow, Chemical Engineering department, National Taiwan University of Science and Technology, Taiwan
[2/2008-1/2010]	PhD in Chemical Engineering (has equivalent to Physical Chemistry, Egypt), National Taiwan University of Science & Technology, Taiwan.
[2002-2005]	Master Degree in Analytical Chemistry, Cairo University, Beni-Suef Branch, EGYPT.
[1995-1999]	Bachelor of Science, Chemistry, Cairo University, Beni-Suef Branch, EGYPT.

Areas of Interest

Co-Solvents Effect on Protein Folding/Unfolding.

Solubility of Biological Compounds in Aqueous Organic Solvents.

Phase Separation.

Phase Equilibria: Solid –Liquid Equilibria, Liquid-Liquid Equilibria, Solid-liquid-liquid equilibria, and Vapor-Liquid Equilibria.

Determination of Dissociation Constants of Biologically Important Ligands.

Complexation Equilibria for Binary and Mixed Ligand Complexes and Determination of Stability Constants using Potentiometric Technique.

Publications List

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Note: * Corresponding author

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Memberships &Activities

1. Active Member in American Chemical Society (ACS), since 2005.
2. Active Member in American Association for Advancement of Science (AAAS), since 2006.
3. My Biographical Data published in Who's Who in Science and Engineering, 9th Edition, 2006 (USA).
4. Reviewer in Journal of Chemical Engineering Data, Asian science Journal, and International Journal of Biological and Chemical Sciences.

Additional Interests	I am interested to study in the field of Fire protection, includes procedures for prevention, detecting, and extinguishing fires. The procedures in these three areas of fire prevention aim to save lives, reduce injuries, and protect property. Also, I am interested to the Occupational Safety & Health as well.
Vocational Certificates	1. **Portable Fire Extinguishers**, Arabian Fire Safety Academy, Kingdom of Saudi Arabia, from 10/12/2005 to 15/12/2005. 2. **Automatic extinguishing system**, Arabian Fire Safety Academy, Kingdom of Saudi Arabia, from 17/12/2005 to 22/12/2005. 3. **Detection and Alarm System**, Arabian Fire Safety Academy, Kingdom of Saudi Arabia, from 24/12/2005 to 05/01/2006. 4. **Preplanning for Fire prevention & Fire Fighting**, Arabian Fire Safety Academy, Kingdom of Saudi Arabia, from 22/10/2005 to 24/11/2005. 5. Advanced **Fire Fighting, Arabian Fire Safety Academy**, Kingdom of Saudi Arabia, from 26/11/2005 to 08/12/2005. 6. **Safe Handling of Hazardous Materials**, Arabian Fire Safety Academy, Kingdom of Saudi Arabia, from 14/01/2006 to 26/01/2006. 7. **Building Safety Requirements**, Arabian Fire Safety Academy, Kingdom of Saudi Arabia, from 07/01/2006 to 12/01/2006. 8. **Occupational Safety and Health**, Arabian Fire Safety Academy, Kingdom of Saudi Arabia, from 03/09/2005 to 05/10/2005. 9. **Safety and loss Prevention**, Arabian Fire Safety Academy, Kingdom of Saudi Arabia, from 28/01/2006 to 09/02/2006. 10. **Modern Safety Management**, Arabian Fire Safety Academy, Kingdom of Saudi Arabia, from 08/10/2005 to 19/10/2005.
Professional Experiences	3/2010-Now — Post Doctor Fellow, Chemical Engineering department, National Taiwan University of Science and Technology, Taiwan [2002-Now] — Occupational Safety & Health Inspector, Manpower & Migration Authority, EGYPT. 4/2008-2/2010 — PhD NTUST Scholarship, Chemical Engineering department, National Taiwan University of Science and Technology, Taiwan [08/2006-8/2007] — Director of Planning & Evaluation, Arabian Fire Safety Academy, Kingdom of Saudi Arabia. [02/2005-8/2006] — Fire Safety Instructor, Arabian Fire Safety Academy, Kingdom of Saudi Arabia. [08/2005-2/2006] — Fire Safety Assistance's Instructor, Arabian Fire Safety Academy, Kingdom of Saudi Arabia [2004-2005] — Chemistry Instructor, Industrial Education College, EGYPT, Part Time. [2000-2001] — Chemist, Dying Factory, EGYPT [1999-2000] — Science teacher, Private School, EGYPT