CHAPTER 4
Elucidating the Protective Effect of Sodium Dodecyl Sulphate on Serum Albumins Against Urea and Temperature at Low pH

4.1. Introduction:

Serum albumins play an important role in transportation and distribution of exogenous and endogenous ligands in the blood such as fatty acids, drugs, steroids, also maintain the physiological pH and osmotic pressure of the blood (Peters, 1985). Human serum albumin (HSA), bovine serum albumin (BSA), porcine serum albumin (PSA), sheep serum albumin (SSA) and rabbit serum albumin (RSA) are globular protein consisting of a single polypeptide chain. HSA and RSA contain one tryptophan while BSA, PSA and SSA contain two tryptophans which are located in hydrophobic cavities of IB and IIA sub domains respectively. All these albumins have high sequence similarity at the gene and protein sequence level, so it become important to know their structural and functional stability. As we have reported earlier that all the albumins, particularly HSA, BSA, PSA and RSA are having a similar aggregation propensity in the presence of acetonitrile (Sen et al., 2009). For understanding the structural and functional relationship of proteins under various stress condition is important for applicative aspects. The stability of serum albumins has been studied in many in-vitro studies (Farruggia and Pico, 1999, Farruggia et al., 2003, Brahma et al., 2005, Galantini et al., 2010). Several methods are used to examine the protein stability such as chemical denaturantion. Urea is a chemical denaturant which is widely exploited for investigating the conformational stability of proteins (Auton et al., 2007, Das and Zhou, 2010). The molecular mechanism of urea induced protein unfolding is still a controversial issue; because it is not completely known what type of forces are responsible in unfolding? There are two types of mechanism proposed on the basis of experimental and theoretical observations. The first is an indirect mechanism, which propose that the urea disrupt the water molecules and help in salvation of hydrophobic groups (Bennion and Daggett, 2003). According to the second mechanism, the urea directly interacted with protein by electrostatic or Van der Waals forces (Lim et al., 2009). The thermal denaturation is another important method to test the conformational stability of proteins. Vast literatures is available on thermal denaturation (Rezaei Tavirani et al., 2006,
Kawamura et al., 2006, Fan et al., 2007). Various strategies have been proposed to achieve stabilization of proteins including chemical modification, protein engineering, use of surfactants and polyhydroxy compounds (Simon et al., 2002). Using these methods, the half-life, water solubility is also increased and self-aggregation property of proteins is reduced (Batton et al., 2001, Marshall et al., 2003). Stabilizers are very extensively used to store the proteins for longer time. In the area of protein expression and purification, inclusion bodies formation is a major problem. Many additives are routinely used for the solubilization of inclusion bodies and surfactant is one of them. The surfactant-protein interaction creates much interest for many physiochemical as well as conformational phenomena. Sodium dodecyl sulphate (SDS) is the most repeatedly studied surfactant. It is well documented that SDS is used for both stabilization and destabilization of proteins (Nielsen et al., 2007). However, it is not clearly known how SDS interacts with proteins. Available literature reveals that SDS interacts with proteins via ionic as well as hydrophobic interactions (Gitlin et al., 2006). SDS also increases the enzymatic activity and conformational stability of many proteins (Kricka and De Luca, 1982, el-Sayed and Roberts, 1985). Protein stabilization property of SDS is not extensively studied so far in details. It is also reported that SDS is having great capacity to unfold the proteins (Otzen et al., 2009, Bhuyan, 2010). In this study we have taken five serum albumins from different source and studied the effect of SDS at pH 3.5 on the conformation of albumin. Further, we have seen the effect of urea and temperature in the presence of 4.0 mM SDS. Other objectives of this work were to investigate the mechanism of urea action.

4.2. Material and Methods:

4.2.1. Materials:

Essentially fatty acid free human serum albumin (068K7538V), bovine serum albumin (110M1661V), porcine serum albumin (094K7636), sheep serum albumin (117K7540) and rabbit serum albumin (117K7565), urea and sodium dodecyl sulphate (SDS), were purchased from Sigma Chemical Co. (St. Louis, Mo, USA). All other reagents used were of analytical grade.
4.2.2. Methods:

4.2.3. Protein concentration determination:

The protein concentrations was determined spectrophotometrically using molar extinction coefficients: $\varepsilon_{280} = 35700 \text{ M}^{-1} \text{ cm}^{-1}$ (HSA), $43827 \text{ M}^{-1} \text{ cm}^{-1}$ (BSA), $43385 \text{ M}^{-1} \text{ cm}^{-1}$ (PSA and RSA) and $42925 \text{ M}^{-1} \text{ cm}^{-1}$ (SSA) at 280 nm on Perkin Elmer (Lambda 25) double beam spectrophotometer. The stock solutions of all proteins were prepared in 20.0 mM Tris-HCl buffer pH 7.4. The stock was diluted to the respective buffer for further uses.

4.2.4. pH measurements:

pH measurements were carried out on Metler Tolado pH meter (seven easy S 20-K) using Exper "Pro3 in 1" type electrode. The least count of the pH meter was 0.01 pH unit. The working protein samples were prepared in 20.0 mM sodium acetate buffer pH 3.5. The buffers were filtered through PVDF 0.45 µm syringe filter (Millipore Milex-HV).

4.2.5. Circular Dichroism

CD measurements were performed with a Jasco spectropolarimeter (J-815), equipped with a Jasco Peltier-type temperature controller (PTC--424S/15). The instrument was calibrated with D-10-camphorsulfonic acid. The measurements were carried out at 25 °C. Far-UV CD spectra were collected with a protein concentration of 5.0 µM with 0.1 cm path length in the range of 200-250 nm. Each spectrum was the average of 2 scans. The results were expressed as mean residual ellipticity (MRE) in deg cm$^2$ dmol$^{-1}$ which is defined as:

$$\text{MRE} = \frac{\theta_{\text{obs}} (\text{mdeg})}{10 \times n \times C_p} \times 1$$

where $\theta_{\text{obs}}$ is the CD in millidegree, $n$ is the number of amino acid residues, $l$ is the path length of the cell in centimeters and $C_p$ is the molar fraction of proteins.

4.2.6. Fluorescence measurements:

Fluorescence measurements were performed on Hitachi spectrofluorometer (F-4500) equipped with a PC. The fluorescence spectra were collected at the 25 °C with a 1 cm path length cell. The intrinsic fluorescence spectra were recorded between 300 to 400 nm with excitation wavelength of 295 nm. The excitation and emission slit width were set at 5 nm. 5.0 µM of protein concentration were taken in all the conditions.
4.2.7. Dynamic light scattering:

DLS measurements were performed with protein concentration of 15 µM using DynaPro—TC-04 dynamic light scattering equipment (Protein Solutions, Wyatt Technology, Santa Barbara, CA) equipped with temperature-controlled microsampler. Before the measurements, all the samples were kept for overnight (24 hrs) incubation. Prior to scanning, all the solutions were spun at 10,000 rpm for 10 mins and filtered through microfilter (Millipore Millex-HV hydrophilic PVDF) having a pore size of 0.45 µm followed by further filtration using 0.22 µm pore sized filter (Whatman International, Maidstone, UK). Measured size was presented as the average value of 50 runs. Data were analyzed by using Dynamics 6.10.0.10 software at optimized resolution. The mean hydrodynamic radius ($R_h$) and polydispersity ($P_d$) were estimated on the basis of an autocorrelation analysis of scattered light intensity based on the translational diffusion coefficient, by Stokes–Einstein equation:

$$R_h = \frac{kT}{6\pi\eta D^{25^\circ}W}$$  \hspace{1cm} (3)

where $R_h$ is the hydrodynamic radius, $k$ is the Boltzman’s constant, $T$ is the absolute temperature, $\eta$ is the viscosity of water and $D^{25^\circ}W$ is the translational diffusion coefficient.

4.3. Results:

4.3.1. Effect of urea on secondary structure of serum albumins:

Far-UV CD spectroscopy is greatly used to observe the changes in secondary structure, conformation and stability of proteins in solutions (Santra et al., 2005, Moriyama and Takeda, 2005). The far-UV CD is also used to estimate the fraction of secondary structure i.e. $\alpha$-helix, $\beta$-sheet and random coil. Far-UV CD spectra of all the five albumins at pH 3.5 exhibited two minima, one at 208 nm and other at 222 nm which is characteristic of $\alpha$-helical structure. Similar spectral features of serum albumins were also found at pH 7.4 (Mir et al., 2010b). Urea induced secondary structural change has been frequently observed in many proteins including serum albumins also (Tripathi et al., 2009, Galantini et al., 2010). As shown in Figure 4.1, in the presence of 4.0 mM of SDS ellipticity of spectra in all the albumins were same as that of spectra at pH 3.5. From this observation it was concluded that SDS is not
affecting the secondary structures of serum albumins. We further checked the effect of urea on albumins in the absence and presence of 4.0 mM SDS at pH 3.5. For sake of clarity only 4 representative spectra are shown i.e. at 0, 2.0, 8.0 and 9.0 M urea. Up to 2.0 M urea the ellipticity of all albumins did not change but beyond this concentration ellipticity decreased continuously till 9.0 M. However in the presence of 4.0 mM SDS, the ellipticity of spectra was not changed even up to 9.0 M urea. The Figure 4.2, shows urea induced unfolding of serum albumins in the absence and presence of 4.0 mM SDS as monitored the change in CD (mdeg) at 222 nm. Urea unfolds the serum albumins without SDS at pH 3.5 with $C_m$ values 3.44 M, 2.28 M, 2.49 M, 2.58 M, 3.41 M in an HSA, BSA, PSA, RSA and SSA respectively. However in the presence of 4.0 mM SDS urea was unable to unfold albumins even at higher concentration. From CD measurements it can be concluded that in the presence of 4.0 mM SDS the secondary structures of serum albumins was stable against urea denaturation even up to 9.0 M.
Figure 4.1. Secondary Structural change of albumins was monitored by Far-UV CD. Effect of increasing concentration of urea in the absence and presence of 4.0 mM SDS on (A) HSA (B) BSA (C) PSA (D) SSA and (E) RSA at pH 3.5. In all the measurements albumin concentration was fixed 5.0 µM and sample were incubated 12 hrs before measurements.
Figure 4.2. Change in α-helicity of albumins were demonstrated by urea in the absence and presence of 4.0 mM SDS at pH 3.5. The overall helicity of (A) human, (B) bovine, (C) porcine, (D) sheep and (E) rabbit serum albumins at 222 nm in the absence and presence of 4.0 mM SDS as a function of varying urea concentration. The albumins concentration was taken 5.0 μM.

4.3.2. Intrinsic fluorescence measurements:

Intrinsic fluorescence measurement gives information about the polarity of envimino acids and residues. Proteins contain three type of aromatic amino acid residues (Trp, Tyr and Phe) which may contribute to their intrinsic fluorescence but only tyrosine and tryptophan is used experimentally because their quantum yields is high enough to give a good fluorescence signal. The intrinsic fluorescence of proteins reveals the environment-dependent solvent exposure of the Trp indole ring and the tyrosine aromatic side chains. HSA RSA contains one Trp, while BSA, PSA and SSA contain two Trp which are buried in the hydrophobic core of the albumins at physiological pH. In Figure 4.3, shows the fluorescence emission spectra The maximum emission of fluorescence intensity was observed at 333 nm (HSA), 332 nm (BSA), 336 nm (PSA), 338 nm (SSA) and 336 nm (RSA). It is reported that if wavelength maximum is in the
range of 330-340 nm, the protein is well folded and tryptophan is buried in a hydrophobic core (Lakowicz, 1983). After addition of 4.0 mM SDS in all albumins incubated at pH 3.5, a drastic change in the fluorescence emission spectrum was noticed and wavelength maximum showed a blue shift on an average of 8 nm. We studied the effect of increasing concentration of urea on albumins in the absence and presence of 4.0 mM SDS. As shown in Figure 4.3, in the absence of SDS the fluorescence emission spectrum exhibited a slight red shift upon incubation with 2.0 M urea while maximum red shift was noticed (8.0 nm). The fluorescence emission spectrum was slightly red shifting in the presence of 2.0 M urea and maximum red shift was noticed at 9.0 M urea with low fluorescence intensity. However in the presence of 4.0 mM SDS the emission maxima was very minutely shifted even in the presence of 9.0 M urea with slight increase in fluorescence intensity. The shift in wavelength maximum of five albumins in the absence and presence 4.0 mM SDS are plotted at varying concentration of urea as shown in Figure 4.4. Albumins incubated at pH 3.5 revealed a sigmoidal change in emission maxima with increasing concentration of urea (Figure 4.4A) but in the presence of 4.0 mM SDS (Figure 4.4B) no sigmoidal change was noticed even at 9.0 M urea. The change observed in emission maxima values are given in Table 4.1.
Figure 4.3. Effect of urea on 4.0 mM SDS-incubated serum albumins. Fluorescence emission spectra of (1) human, (2) bovine, (3) porcine, (4) sheep and (5) rabbit serum albumins at increasing concentration of urea in the (A) absence and (B) presence of 4.0 mM SDS at pH 3.5. The samples containing protein, SDS and urea were incubated overnight and protein concentration were 5.0 μM.
Figure 4.4. Effect of Urea on albumins in the absence and presence of 4.0 mM SDS at pH 3.5. Change in wavelength maximum of HSA (■), BSA (○), PSA (△), RSA (▼) and SSA (▲) in the (A) absence and (B) presence of 4.0 mM SDS with respect to increasing concentration of urea. Before performing the experiments samples were incubated overnight with 5.0 µM of serum albumin concentration throughout the study.

Table 4.1. Shift in wavelength maximum of albumins at different conditions and a transition mid point by CD (mdeg) at 222 nm.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Protein name</th>
<th>Wavelength maximum (nm)</th>
<th>Transition midpoint (Cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pH 3.5</td>
<td>pH 3.5+SDS</td>
<td>Urea 10 M</td>
</tr>
<tr>
<td>1</td>
<td>HSA</td>
<td>332.4</td>
<td>325.4</td>
</tr>
<tr>
<td>2</td>
<td>BSA</td>
<td>331.6</td>
<td>325.6</td>
</tr>
<tr>
<td>3</td>
<td>PSA</td>
<td>336</td>
<td>326.6</td>
</tr>
<tr>
<td>4</td>
<td>SSA</td>
<td>338</td>
<td>326.8</td>
</tr>
<tr>
<td>5</td>
<td>RSA</td>
<td>334</td>
<td>327.4</td>
</tr>
</tbody>
</table>

4.3.3. Hydrodynamic Radii measurements:

DLS is used to characterize the hydrodynamic radii ($R_h$) of particles as well as to detect the conformational change of proteins. The change in hydrodynamic radii of five albumins at pH 7.4 alone and at pH 3.5 in the absence and presence of 4.0 mM SDS are shown in Figure 4.5. The effect of urea was also seen under both the conditions (pH 3.5 and pH 3.5 + 4.0 mM SDS). The $R_h$ of albumins at pH 7.4 was found to be 3.4 nm (HSA), 3.7 nm (BSA), 3.9 (PSA) and 3.4 (RSA, SSA). At pH 3.5,
a slight increase in \( R_h \) was observed i.e. 4.0 nm (HSA), 4.5 nm (BSA), 4.4 nm (PSA), 4.0 nm (RSA) and 4.4 nm (SSA) probably due to the partial unfolding of protein under acidic condition. The addition of 4.0 mM SDS at pH 3.5 caused decrease in \( R_h \) of partially unfolded albumins to 3.2 nm (HSA), 3.1 nm (BSA), 3.6 nm (RSA) and 3.9 nm (PSA, SSA) respectively owing to compaction of structure induced by SDS. The effect of urea was also seen on albumins at pH 3.5. The urea unfolds the albumins in the absence of SDS and hydrodynamic radii increases accordingly is shown in Table 4.2. However in the presence of 4.0 mM SDS, urea was incapable of unfolding protein as the hydrodynamic radii did not increase and is more or less like native albumins. The hydrodynamic radii at different conditions are summarized in Table 4.2.

Figure 4.5. Change in hydrodynamic radii monitored by DLS. Change in hydrodynamic radii of HSA were monitored (A) at pH 7.4, (B) pH 3.5, (C) pH 3.5 + 4.0 mM SDS, (D) pH 3.5 + 3M urea, (E) pH 3.5 + 4.0 mM SDS + 5 M urea, (F) pH 3.5 + 9.0 M urea and (G) pH 3.5 + 4.0 mM SDS + 9.0 M urea in HSA.
Table 4.2. Hydrodynamic radii of serum albumins at different condition.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Proteins</th>
<th>pH 7.4</th>
<th>pH 3.5</th>
<th>pH 3.5+SDS</th>
<th>pH3.5+5MU</th>
<th>pH3.5+9MU+4.0mMSDS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>HSA</td>
<td>3.4</td>
<td>4.0</td>
<td>3.2</td>
<td>5.1</td>
<td>9.3</td>
</tr>
<tr>
<td>2</td>
<td>BSA</td>
<td>3.7</td>
<td>4.5</td>
<td>3.1</td>
<td>6.2</td>
<td>11.3</td>
</tr>
<tr>
<td>3</td>
<td>BSA</td>
<td>3.9</td>
<td>4.4</td>
<td>3.9</td>
<td>5.2</td>
<td>14.3</td>
</tr>
<tr>
<td>4</td>
<td>PSA</td>
<td>3.4</td>
<td>4.4</td>
<td>3.9</td>
<td>5.8</td>
<td>12.4</td>
</tr>
<tr>
<td>5</td>
<td>RSA</td>
<td>3.4</td>
<td>4.0</td>
<td>3.6</td>
<td>6.0</td>
<td>10.8</td>
</tr>
</tbody>
</table>

4.3.4. Thermal Denaturation:

We also checked stabilizing effect of SDS on albumins against temperature changes. We incubated albumins at pH 7.4 and 3.5 and in the absence and presence of 4.0 mM SDS. Thermal unfolding of albumins in all above conditions were determined by observing the change in θ222 nm with respect to temperature. Figure 4.6, summarizes the changes in CD ellipticity at 222 nm of native albumins and in the absence/presence of 4.0 mM SDS at pH 3.5. The native albumins show broad sigmoidal change with respect to temperature and the transition temperature (Tm) were 76 °C (HSA), 79 °C (BSA), 70 °C (PSA), 75 °C (SSA) and 70 °C (RSA) while at pH 3.5 the Tm was decreased significantly to 54 °C (HSA), 57 °C (BSA, PSA), 59 °C (SSA) and 63 °C (RSA) which suggest that secondary structure of albumins at pH 3.5 is very labile due to partial unfolding. We further incubated the albumins with 4.0 mM SDS at pH 3.5 and monitored the changes in secondary structure as a function of temperature. A sigmoidal change was found and Tm increased significantly to 74 °C (HSA), 75 °C (BSA), 70 °C (PSA, SSA) and 75 °C (RSA) which is close to native albumins. The Tm values of albumins under different conditions are shown in Table 4.3. From the thermal denaturation profile it can be concluded that SDS is protecting the secondary structure of all albumins at pH 3.5.
Table 4.3. Fm were measured in all albumins at different condition.

Change in ellipticity at 222 nm. Albumin concentration was 3 mg/mL.

(-) Fm 3.5 (-) in the presence of 4 mM SDS at pH 3.5 (measured by)
human (A) bovine (B) porcine (C) sheep and (E) rabbit serum albumins at pH 7.4. (-)

Figure 4.6. Thermal denaturation of albumins. Thermal denaturation of (A)

Chapter 4
4.5. Discussion:

Protein denaturation and stabilization is very emerging topic in molecular biology due to its large application in the industry. Various methods and additives are used for protein stabilization and denaturation (Dobson, 2003, Paschek and Garcia, 2004). The surfactants, particularly SDS is frequently used in the stabilization and destabilization of proteins. SDS mimics the lipid of biological membrane so its interaction with protein gives important clues to understand the effect of the lipid on protein in vivo. In this study we have taken five serum albumins from different sources and observed the effect of SDS, urea and temperature at pH 3.5. First we investigated the effect of pH on all albumins. It is reported that at neutral pH all the albumins exist in a compact form native or 'N' form while at low pH (pH 3.5) it is less compact i.e. 'F' form and this form comes due to conformational isomerization (Nakamura et al., 1997). 'F' form is very fast moving and is less stable than the 'N' form because at lower pH the positively charge residues become protonated, due to this protein turn out to be partially unfolded state. Further we observed the effect of SDS on the fast moving ('F') state of serum albumins. The secondary structures of fast moving serum albumins remained unchanged in the presence of 4.0 mM SDS while the tertiary structure shows some alteration with shift in wavelength maximum by 8.0 nm. The blue shifting behavior of proteins signifies that aromatic chromophores are shifting into the more non-polar environment. Similar results were also found in case of HSA where around 16 nm blue shift was noticed in the presence of 7.0 mM SDS at pH 7.0 (Anand et al., 2010). From these results it appears that SDS is stabilizing the proteins at acidic pH because the negative head of SDS interact with positive part of albumins while the hydrophobic tail interacts with non-polar part of proteins. Lysozyme also showed similar patterns in the presence of SDS (Yonath et al., 1977).

We further explored the effect of urea and temperature on albumins conformation in the absence and presence of 4.0 mM SDS at pH 3.5. Urea unfolds the albumins in the absence of SDS at a little bit lower concentration (2.0 M) while in the presence of 4.0 mM SDS the urea is unable to unfold both secondary as well as tertiary structures of albumins even up to 9.0 M concentration. Urea unfold albumin at pH 7.4 and helicity of proteins is almost lost but at low concentration with the added SDS the helicity of proteins is again recovered which is very close to the native state of albumins. These results indicate that small amount of SDS refolded the unfolded
protein (Takeda K, 1987). Hydrodynamic radii of albumins were monitored by DLS. The albumins at pH 7.4 have compact structure and hydrodynamic radii (Table 2) which is inconsistent with other reports (Gull et al., 2011). But at acidic pH, the hydrodynamic radii was found to increases due to partial unfolding. In the presence of 4.0 mM of SDS the hydrodynamic radii was decreased. The hydrodynamic radii of partially unfolded albumins were markedly increases in the presence of 9.0 M urea but in the presence of 4.0 mM SDS the urea is not capable of unfold and changing the hydrodynamic radii as also supported our CD data. The protective behavior of SDS was also observed in earlier reports and it is reported that SDS is wrapped around proteins with the help of hydrophilic head and a hydrophobic tail. This wrapping provides enough strength to proteins. At above CMC, SDS-protein interaction is based on two type of models one is a necklace and other is bead model. According to these two models the SDS is stabilizing the protein (Guo et al., 1990, Turro, 1995). From above results it is concluded that urea is uable to unfold the albumins in the presence of SDS. It is reported that urea has two types of denaturing property either directly or indirectly. From the above observation we can say that initially urea is favorably interacting with albumins electrostaticaly and through hydrogen bonding because albumins are positively charged at pH 3.5. But in the presence of 4.0 mM SDS at same pH the urea is unable to interact directly, the reason is hydrophobicity of environments is high and protein charge is masked with the head of SDS monomer. The similar protective behavior of SDS was also reported in cytochrome C. At pH 4.0 urea were unable to unfold the cytochrome C when it was incubated with 2.0 mM SDS and its secondary as well as tertiary structures was not altered (Chattopadhyay and Mazumdar, 2003). CD, fluorescence and DLS data reveled that if proteins are having charge on the surface, then urea interacts with protein directly and unfolding will occur on the another hand if the protein is in hydrophobic environment the urea is unable to interact directly with protein, but it will interact with environmental hydrophobicity. This lead us to conclude that urea interact with proteins directly if protein is having a charge on the surface while it interacts indirectly if the protein is in hydrophobic environments. Apart from urea denaturation we also performed thermal denaturation for better understanding of the protective effect of SDS. Anionic surfactants are capable of protecting the proteins also by the thermal denaturation (Moriyama et al., 2008, Chodankar et al., 2008). The thermal denaturations of albumins was studied in the presence and absence of 4.0 mM SDS at pH 3.5 and also
at neutral pH. The albumins, whether at neutral or acidic pH, in the presence of 4.0 mM SDS showed sigmoidal transition with respect to increasing temperature. One thing is important to note that the albumins at pH 3.5 have very less thermal stability than at pH 7.4. While in case of SDS stabilized albumins, stability is similar to that at pH 7.4 as evident from \( T_m \) values. Similar results were also found when BSA; was incubated with SDS the secondary structure of BSA was not disrupted even at 130 °C (Moriyama et al., 2008). Earlier we have reported that RSA is very heat tolerable in the presence of 20.0 mM CTAB and RSA is not unfolded in the presence of CTAB (Ali et al., 2010). From these observation we can concluded that the SDS is having strong protective effects against urea and thermal denaturation. Overall results are summarized in (Figure 4.7) as graphical presentation.

**Figure 4.7.** Schematic presentation of SDS-stabilized albumins and their unfolding by urea and temperature.
4.6. Conclusion:

Effect of SDS, urea and temperature in 'F' form of serum albumins was investigated. We found that 4.0 mM SDS concentration has great potential to protect the albumins from unfolding by urea (0-9.0 M). SDS is also showing a protective effect against temperature changes (20-90 °C). In the presence of 4.0 mM SDS, the urea was unable to unfold the serum albumins because SDS interact with albumins via electrostatic as well hydrophobic interactions. From this study we have also proposed the mechanism of interaction of urea. Urea will interact with protein directly if the protein is in charged state otherwise indirect interaction will take place. Thermal stability is also provide by SDS due to electrostatic as well as hydrophobic interaction.

Stability of protein is a major concern in industries because some proteins are very unstable at physiological pH. This study will satisfy the stability problem.