acid ($CH_3NH_2^+SO_3^-$). Using the STO-3G optimized geometry for the N-S and S-O bond lengths of sulphamic acid and standard values[13] for all other parameters, it is found that the eclipsed and staggered conformations of $CH_3NH_2^+SO_3^-$ have almost equal energy (−708.369 hartrees). Further it is found that $CH_3NH_2^+SO_3^-$ is also stable over its neutral analog (CH_3NHSO_3H) by about 30 kcal mol^{-1}.

The stability of the zwitterion form over the neutral form in the isolated molecules of both sulphamic acid and N-methylsulphamic acid suggests that the SO_3^- group is present over a wide pH range and hence is available for calcium (Ca^{2+}) binding in heparin molecule[11].

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ATYPICAL PHYSICOCHEMICAL PROPERTIES OF CYANOGEN BROMIDE FRAGMENTS OF HUMAN SERUM ALBUMIN.

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SEVERAL fragments of serum albumin have been recently used in the study of structural and functional properties of the protein[1-3]. Among others, chemical cleavage with cyanogen bromide (CNBr)* has generally been employed to prepare the fragments. However, very little attention has been paid to the fact that the CNBr cleavage may cause some structural alterations in the resulting fragments. Therefore, the results of such studies are likely to be misinterpreted. In the present report we have shown that CNBr fragments of HSA possess some physicochemical properties which are atypical of other globular proteins. It is probably the first report of its kind and assumes significance as it may affect the inferences made on the basis of the studies involving CNBr fragments of proteins.

All the marker proteins, including albumin and CNBr, were purchased from Sigma Chemical Co., USA. Other chemicals used were of analytical grade. A cystinylated derivative of HSA was prepared[4] and treated with CNBr in 80% (v/v) formic acid for 18 hr in the dark under gentle stirring. Both the reactions *i.e.* cystinylation and CNBr cleavage were completed quantitatively under these experimental conditions. The resulting fragments were first fractionated by a Sephadex G-100 gel chromatography in 0.2 M ammonium formate buffer, (pH 2.8) and subsequently purified by ion exchange chromatography on a DEAE cellulose column equilibrated with 0.005 M sodium phosphate buffer, pH 7.5. The column was eluted using a linear salt-gradient produced by mixing the phosphate buffers containing 0 M and 1 M sodium chloride. The purity of the fragments thus obtained was checked by polyacrylamide gel electrophoresis on 8% gels. The hydrodynamic parameters of CNBr fragments of HSA were determined[5], by calibrating a Sephadex G-200 column having the following characteristics[6]: total volume 316.5 ml; void volume, 126.1 ml and internal volume, 171.7 ml. All light absorption measurements

* Abbreviations: HSA = Human serum albumin
DEAE = Diethylamino ethyl; CNBr = Cyanogen bromide.

were made on a Beckman model 26 spectrophotometer.

Cleavage of HSA with CNBr yielded three fragments. Based on their different properties like molecular weight, spectral characteristics and N- and C-terminal amino acid residues, the three fragments were named as HSA-$CNBr_{1-123}$, HSA-$CNBr_{124-297}$, and HSA-$CNBr_{298-584}$, indicating that they were prepared by CNBr cleavage and consisted of residues 1–123, 124–297, and 298–584 of the primary structure[7] of HSA respectively.

The gel filtration data of HSA fragments were analyzed by the procedures described by Porath[8], Laurent and Killander[9], and Ackers[10], and were found to fit the following equations of straight lines obtained by the method of least squares:

$$V_e/V_0 = 5.79 - 0.94 \log M, \quad (1)$$

$$M^{1/3} = 73.72 - 56.61\ K_d^{1/3}, \quad (2)$$

$$(-\log K_{av})^{1/2} = 0.022r + 0.10, \quad (3)$$

$$erfc^{-1}\ K_d = 0.033\ r - 0.27, \quad (4)$$

where, V_0 is the void volume of the column, V_e is the elution volume of the proteins, K_d and K_{av} are the distribution and available distribution coefficients respectively, and ($erfc^{-1}\ K_d$) is the inverse error function complement of K_d. The average molecular weights (M) and Stokes radii (r) of the three HSA fragments were computed from (1), (2) and (3), (4) respectively and the values thus obtained are given in table 1. The values of M and r were further used in the calculation of frictional ratio, f/f_o, and diffusion coefficient, D, of the fragments using the following relations:

$$f/f_o = r/(3\bar{V}M/4N)^{1/3} \quad (5)$$

$$D = kT/6\pi\eta r, \quad (6)$$

where N is the Avogadro's number (6.002×10^{23} per mole); $\bar{V}$, the partial specific volume of the fragments, was calculated[11] from their amino acid composition[7,12], to be 0.74, 0.71, and 0.74 for the fragments HSA-$CNBr_{1-123}$, HSA-$CNBr_{124-297}$, and HSA-$CNBr_{298-584}$ respectively; r, the Stokes radius in cm; T, the absolute temperature (295°K); k, the Boltzmann constant (1.386×10^{-16} ergs per degree) and η is the coefficient of viscosity (0.01 poise) of the buffer (0.06 M, sodium phosphate, pH 7.0).

A substantial difference between the actual and the expected values of the hydrodynamic parameters listed in the table, suggests the presence of some asymmetry in the three fragments. This can be attributed to partial unfolding of the fragments that may occur during the course of their preparation. Since, CNBr treatment has been known for its side reactions with some of the amino acids[13], it is possible that such side reactions have taken place during CNBr cleavage of HSA that ultimately resulted in fragments with distorted structure. This contention is further supported by the ultraviolet absorption spectra of the three fragments (shown in the figure) which are atypical of other globular proteins. This is especially applicable to spectra B and D of the figure which do not show the usual absorption minima in the 250–270 region. It will be interesting to recall here that chemically modified ovalbumin gave similar absorption spectra[5]. However, the increase in absorbance in 255–270 nm region for the spectra B and D is significantly higher than those seen in the chemically modified ovalbumin. This, at present, is not fully explicable. The presence of the pronounced ultraviolet absorption peaks in the 255–270 region (see figure 1) for the fragments HSA-$CNBr_{1-123}$ and HSA-$CNBr_{298-584}$, indicate that the spectra is dominated by phenylalanine[12]; the two fragments are devoid of tryptophan and they have relatively small number of tyrosine residues[2]. Thus it appears that the fragments prepared by CNBr cleavage of the proteins have some elements of unfolded structures. It is, therefore, advisable to be cautious in interpreting the data based on the studies involving CNBr fragments of proteins.

Table 1 *Physicochemical properties of the fragments of human serum albumin*

Properties	HSA fragments		
	HSA-$CNBr_{1-123}$	HSA-$CNBr_{124-297}$	HSA-$CNBr_{298-584}$
Molecular weight	20,660 (14,030)	27,450 (19,870)	50,730 (32,400)
Stokes radius (nm)	2.08 (1.71)	2.37 (2.03)	3.09 (2.53)
Frictional ratio	1.14 (1.07)	1.18 (1.13)	1.26 (1.20)
Diffusion coefficient (cm^2/sec)	1.04×10^{-7} (1.26×10^{-7})	9.15×10^{-8} (1.07×10^{-7})	7.02×10^{-8} (8.58×10^{-8})

Values outside brackets denote actual values while those in brackets represent expected values. Expected values were calculated from the molecular weight of the fragments based on their amino acid composition.

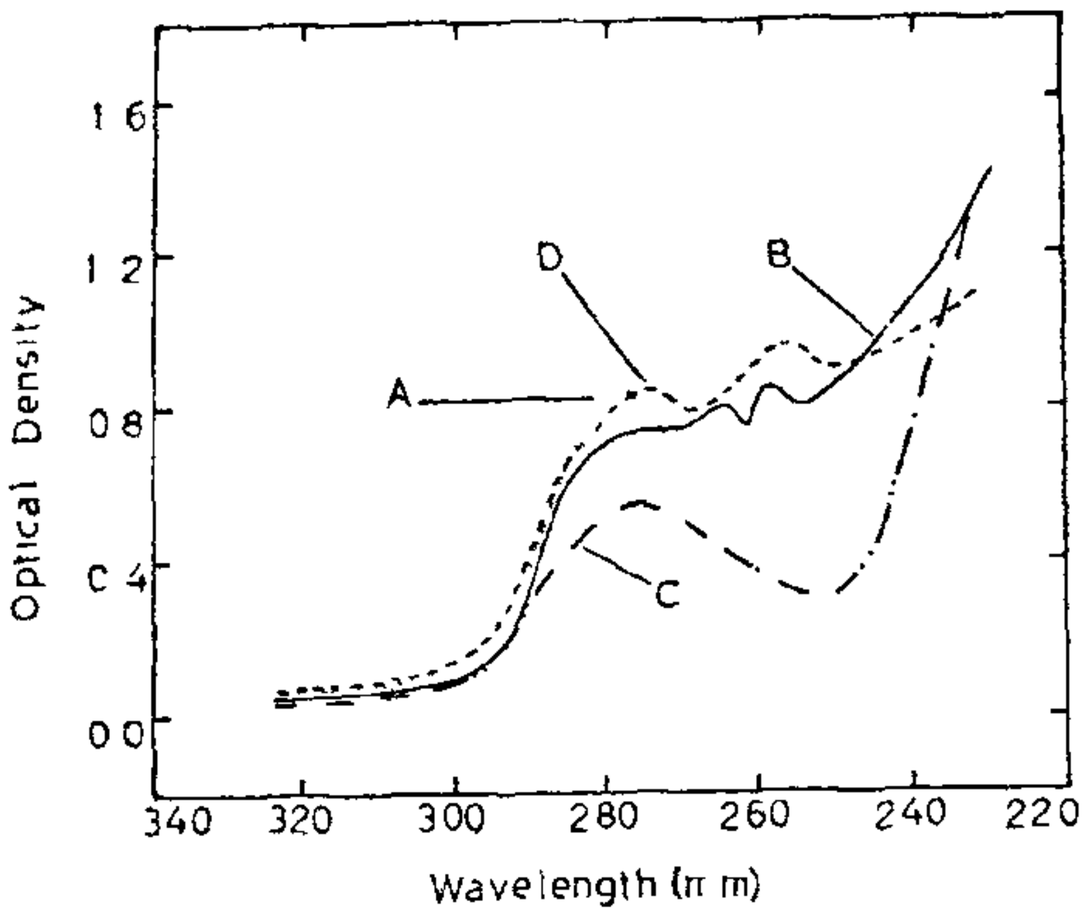

Figure 1. Ultraviolet absorption spectra of human serum albumin, A; and its fragments, HSA-$CNBr_{1-123}$, B; HSA-$CNBr_{124-297}$, C; and HSA-$CNBr_{298-584}$, D. The spectra were recorded at 22°C in 0.06 M sodium phosphate buffer pH 7.0.

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STUDIES ON DIAMAGNETIC SUSCEPTIBILITY OF BIOLOGICALLY IMPORTANT HETEROCYCLES: DIAMAGNETIC SUSCEPTIBILITIES OF PHENOTHIAZINE SULPHONES

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PHENOTHIAZINE sulphones form an interesting class of heterocyclic compounds and find a number of medicinal as well as industrial applications. Phenothiazine sulphones are not only interesting from medicinal and industrial point of view but also from structural stand point. In our previous studies on diamagnetic susceptibilities of structurally interesting series of aliphatic compounds[1-3], some interesting findings regarding molecular diamagnetism have been reported. These diamagnetic studies are extended to medicinally and structurally interesting series of phenothiazine sulphones, since changes in substitution pattern causes changes in electronic structures of these molecules and hence affects their molecular diamagnetism and biological activities.

Phenothiazine sulphones required for the diamagnetic studies have been synthesized[4,5] by the oxidation of corresponding phenothiazines by 30% H_2O_2 in glacial acetic acid. Phenothiazines[4,5] used in the synthesis of phenothiazine sulphones were prepared by the condensation of substituted o-aminobenzenethiols and o-halonitrobenzenes *via* Smiles rearrangement. Magnetic susceptibilities of phenothiazine sulphones have been measured by Gouy method and are summarized in table 1.

It becomes necessary to estimate diamagnetic susceptibilities theoretically in order to analyse the experimental results in the light of structural features. Diamagnetic susceptibilities of phenothiazine sulphones could not be calculated by using incremental system, based on atomic and bond susceptibility concept, because the effects of interactions on diamagnetic susceptibility caused by different substituents cannot be accounted for by this approach. Wave-mechanical approach cannot be applied also for these molecules because of non-availability of susceptibility data required in the wave-mechanical calculations. Semi-empirical approach, which has recently been applied to calculate diamagnetic susceptibilities of aliphatic compounds[1-3,7] and provided excellent results, cannot be applied for phenothiazine sul-