Physicochemical Properties of a Combination of 5-Hydroxy-L-tryptophan with β-Aminoethylisothiuronium Bromide Hydrobromide

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A combination of 5-hydroxy-L-tryptophan (HT) and 2-aminoethylisothiuronium bromide hydrobromide (AET) increases radioprotection to a significant extent in biological systems. In the present report some physicochemical nature of the combination has been probed. It has been shown that HT forms a complex with AET in the concentration ratio of 90.82 : 14.23 (μM) by different analytical techniques. Probable structure has also been assigned for the complex. The inference is based on uv spectrophotometry, spectrophotofluorimetry, conductance measurement and infrared spectroscopy. The complex is stable for 6 days at room temperature. With gamma irradiation, there is consistent decrease in pH for HT and AET but for HT + AET the change in pH is biphasic. Possible explanations for these observations have been given.

The functional groups, viz. sidechain NH_2 and SH in case of β-aminoethylisothiuronium bromide hydrobromide (AET)[1] and indole ring and sidechain NH_2 in case of 5-hydroxytryptamine (5 HT)[2] have been ascribed responsible for radioprotection extended by these compounds. A combination of 5-hydroxy-L-tryptophan (HT) and AET has been noted to render very good radioprotection in gamma-irradiated animals[3]. In this study the physicochemical nature of the combination as compared to that of the individual compounds has been probed.

Results and Discussion

The uv absorption maximum of AET in water (concn. 90.82 μM) is obtained at 200 nm. This is due to the formation of NH_3^+ and SH groups, the both being
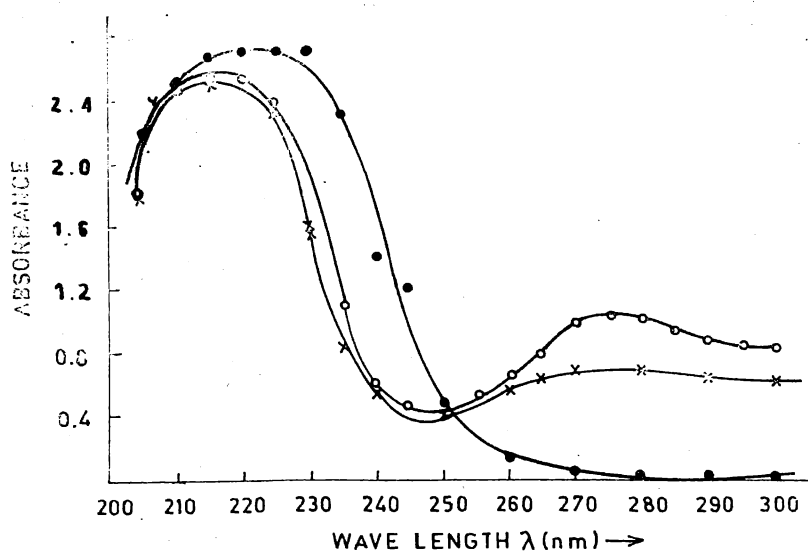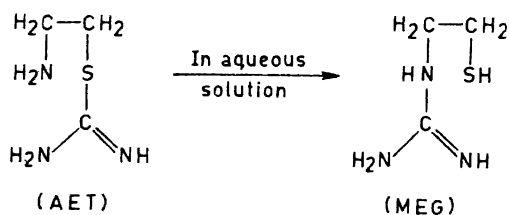

Fig. 1. Uv absorption spectra of HT, AET and HT + AET in 0.9% sodium chloride : (O) HT (concn., 90.82 μM), (x) HT + AET (concn., 90.82 + 14.23) μM + 14.23 μM. and (●) AET (concn., 14.23 μM).

formed as a result of the formation of MEG from AET.

(AET) → In aqueous solution → (MEG)

The sidechain NH_2 group of MEG gets protonated and forms NH_3^+ (Ref. 4,5). In EDTA solvent, AET (i.e. MEG in water) shows consistent increase in absorbance value with increasing concentration (concn. $\mu g\ ml^{-1}$ in parenthesis) ; 2.04(20), 2.35(40), 2.48(60), 2.60(80) and 2.70(100); showing thereby that the dissolved compound follows Lambert-Beer's law. However, the $\overset{.}{S}H$ and NH_2 groups being auxochromes, they produce increase in intensity[4].

In Fig. 1, the absorption spectra of HT, AET and HT + AET in 0.9% sodium chloride have been shown. In this solvent also AET gets converted to MEG, but the absorption maxima is obtained at 220 nm. The attachment of Na^+ ions to the $=NH$ and SH groups and chloride ions to the NH_3^+ ions of MEG is possible. This is the likely reason for the shifting of the absorption maxima from 200 to 220 nm.

HT shows absorption maxima at 215 and 275 nm. The absorption at 275 nm seems to be more characteristic of the compound and is not amenable to shift in the maximum wavelength[6]. When HT combines with AET in the micromolar ratio of (90.82 : 14.23), one absorption maximum is obtained close to 215 nm. The absorbance at 270–290 nm for HT + AET though
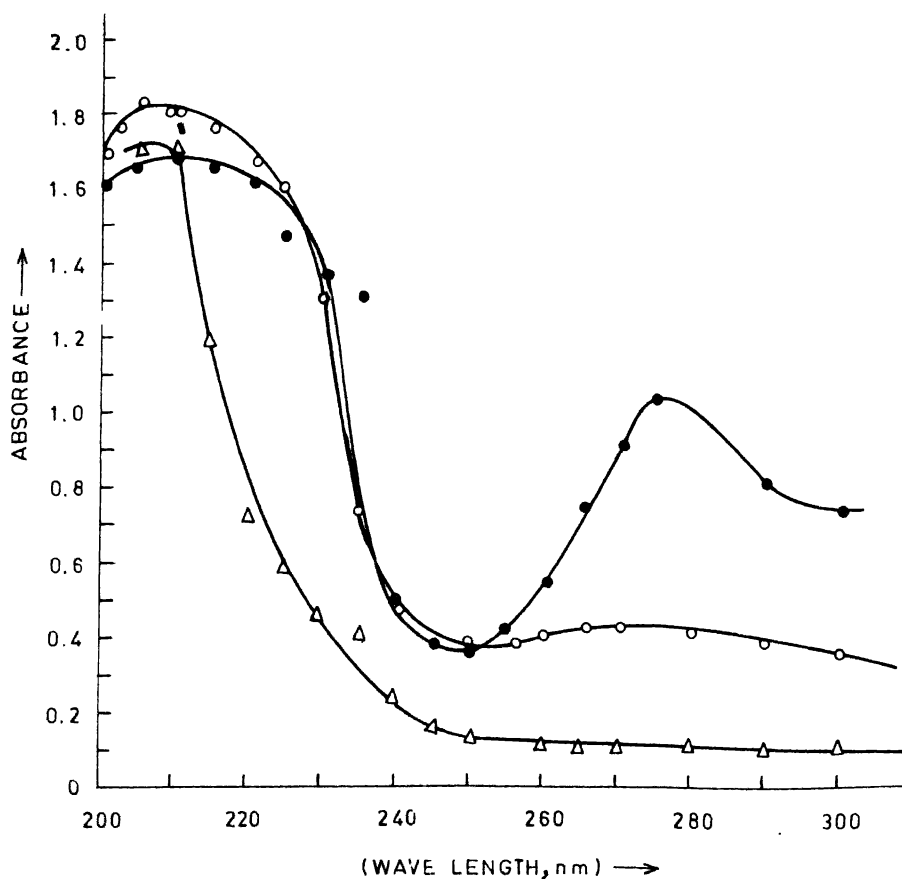

Fig 2   Uv absorption spectra in EDTA (●) HT (concn.. 90.82 µM). (Δ) AET (concn.. 14.23 µM) and (O) HT + AET (concn.. 90.82 µM + 14.23 µM).

slightly high does not form any peak in contrast to HT alone (at 275 nm).

In Fig. 2, the absorption spectra of HT (90.82 μM), AET (14.23 μM) and HT + AET (90.82 μM + 14.23 μM) in EDTA have been shown. It is observed that HT + AET shows a distinct peak at 205 nm whereas neither HT nor AET shows any distinct peak. Again the absorption intensity of HT + AET

and HT + AET. The solvent (i.e. 0.9% sodium chloride) has some fluorescence but emission wavelength is different (300 nm) and intensity is small compared to that of HT, AET or HT + AET (Fig. 3). The fluorescence intensity of AET is much less as compared to that of HT in the same wavelength (E_x 287 nm E_m 340 nm) in the given concentrations. Fluorescence intensity of HT + AET is in between HT
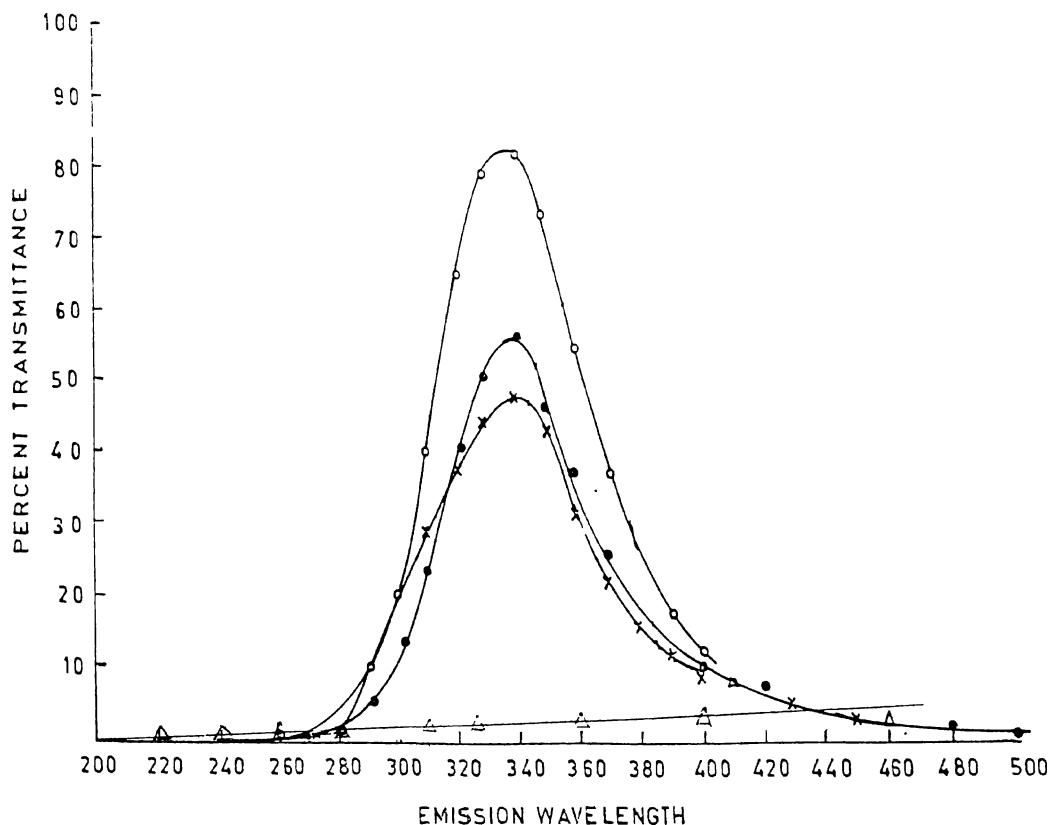

Fig. 3   Relative fluorescence intensity of HT, AET and HT + AET as a function of emission wavelength in 5 ml of 0.9% sodium chloride · (O) HT (5 μg), (●) HT + AET (5 μg + 1 μg), (x) AET (1 μg) and (Δ) 0.9% sodium chloride

does not change to any significant extent from 260 to 290 nm, but the intensity is in between that of HT and AET. Only HT shows an absorption maximum at 275 nm and AET does not show any absorption maximum in this wavelength range. It seems that sidechain of HT is invloved in binding with AET. That is why the absorption maximum of HT is missing. The formation of secondary amide bond is indicated[7].

The percent fluorescence transmittance against emission wavelength has been plotted for HT, AET

and AET and the value is close to that of AET. This indicates that a complex has been formed whose fluorescence intensity is much lower than that of HT alone.

In EDTA solvent, fluorimetric response (E_x 287 nm and E_m 340 nm) for HT, AET and HT + AET have been shown in Fig. 4. It is observed that HT shows maximum, AET shows minimum and HT + AET shows intermediate fluorescence intensity indicating a possible complex formation. EDTA does not have a
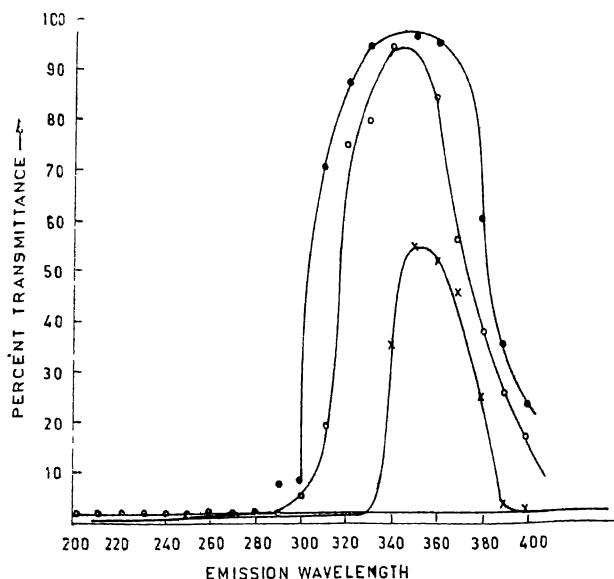
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Fig 4 Relative fluorescence intensity of HT, AET and HT + AET as a function of emission wavelength in 5 ml of EDTA (●) HT (5 μg), (○) HT + AET (5 μg + 1 μg), (×) ALT (1 μg) and (○) EDTA

fluorescence at this wavelength. Fluorescence quenching in both the solvents in case of HT + AET is an indication of binding of HT with AET.

Conductance measurement shows that at a concentration ratio of HT with AET of 90.00 : 14.23 μM, the conductance becomes minimum both in water and in 0.9% sodium chloride (Tables 1 and 2) indicating again a possible complex formation. The relative values in water are much less compared to that in 0.9% sodium chloride. In water, the COOH groups of HT get neutralised with the NH_3^+ groups of MEG. The electronic excess charge of SH groups also gets neutralised at the above concentration ratio. In 0.9% sodium chloride also the same phenomenon takes place. In addition, Na^+ and Cl^- ions that are present in excess get clustered around any excess negative and positive charge centres.

Table 1—Conductance of HT + AET in Different Proportions of Solvent-Water

Solution no	HT μM	AET μM	Conductance μ Ω⁻¹
1	30	14 23	344
2	40	14 23	350
3	60	14 23	354
4	80	14 23	358
5	90	14 23	339
6	100	14 23	363

Table 2—Conductance of HT + AET in Different Proportions of Solvent 0 9% Sodium Chloride

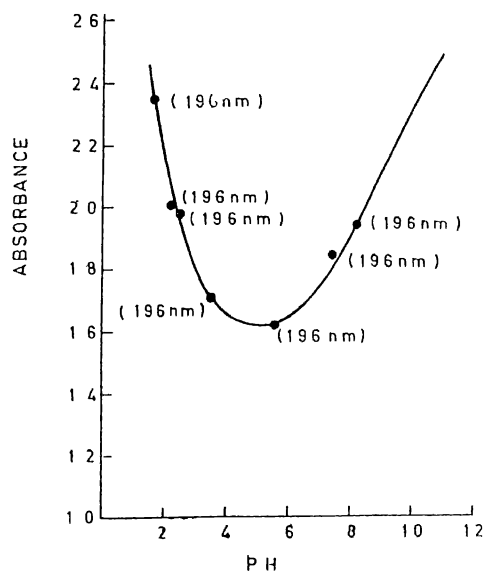| Solution no	HT μM	AET μM	Conductance μ Ω⁻¹
1	10	14 23	11 800
2	20	14 23	11 600
3	30	14 23	11 300
4	40	14 23	11 200
5	50	14 23	10 500
6	60	14 23	10 000
7	80	14 23	9 000
8	90	14 23	8 500
9	100	14 23	10 100

Fig 5 Optical absorbance of AET as a function of pH in water (concn , 10 μg)

The isoelectric point of AET is observed at pH 5 as the absorbance is minimum at this pH. Charge arising due to the chromophoric groups like NH_3^+, =NH, SH and H_3N^+-C(=NH)-NH- get mutually neutralised at this pH (Fig. 5).

The absorbance versus pH curve of HT (solvent water) passes through a stable minimum in the pH range 4.0–9.0 at 207, 222 and 275 nm. It can therefore be concluded that the chromophoricity of sidechain -CH-CH(NH_2)-COOH (absorbance at 275 nm) does not alter to any significant extent in the pH range 4.0– 9.0 (Fig. 6). The chromophoricity of pyrrole ring (absorbance at 207 nm) and indole ring (absorbance at 222 nm) also remains unaltered in the same pH range[7].

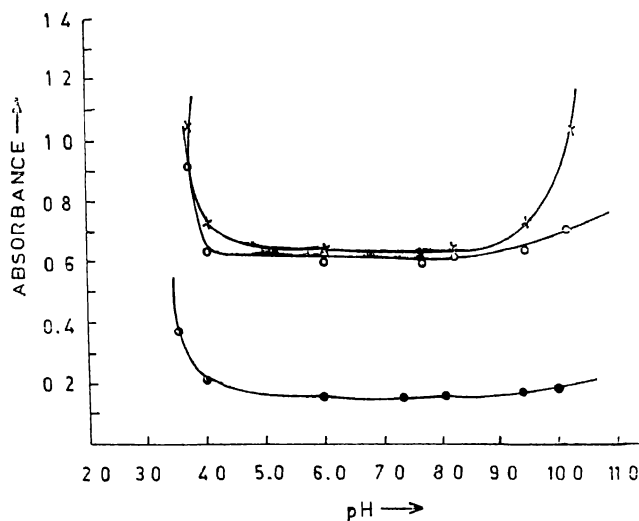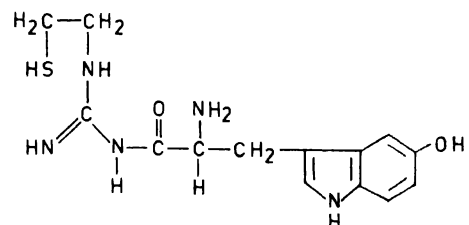For HT + AET, the absorbance values at 207 and

Fig 6   Absorbance versus pH curve of HT in water concn 90 82 μM absorbance at (x) 207 nm (○) 222 nm and (●) 275 nm

275 nm pass through a minimum in the pH range 6–9. The chromophoricity decreases sharply at acidic pH and increases slowly at alkaline pH at 275 nm (Fig. 7). On scrutiny of the nature of the absorbance versus pH curve of HT + AET compared to that of AET or HT alone (Figs. 5, 6 and 7) it is clear that there is distinct difference. This is again an indication that a complex has been formed.

Table 3–Ir Spectral Characteristics of HT AET and HT+AET

Wave number	HT % Absorb	AET % Absorb	HT+AET % Absorb	Assignment
1 000	65	—	40	
1 100	68	—	45	C–O stretch
1 200	70	6	87	
1 500	70	73	64	R–CONHR amide
1 600	69	60 (1 650)	98 (1 650)	Variation of β-cardon
				Atom of HT due to complex formation
2 800	73 (2 850)	90	—	
2 900	74	95	—	OH stretch for
3 400	75 5	—	—	carboxylic acids

Pertinent wavenumbers for ir spectral studies on HT, AET and HT + AET have been shown in Table 3. Differences in absorbances for the individual components versus the combination in these wavenumbers

are clearly noted. It is concluded from the ir spectral study that (i) the carboxylic acid group is involved in complex formation and (ii) –CONH group is formed during complex formation[8]. Based on ir studies a probable structure of the complex is assigned as

Stability of the complex in aqueous solution has been studied following the absorbance at 275 nm on different days (number of days in parenthesis) : unirradiated 0.440(1), 0.450(4), 0.450(6), 0.380(11), 0.380 (15) and 0.380(19). In unirradiated state, the complex is stable up to 6 days, beyond that the absorbance is lowered.

Variations in pH with gamma radiation dose of AET, HT and HT + AET have been shown in Table 4. For both HT and AET, there is a consistent decrease of pH with radiation dose up to 1500 Gy. But HT + AET behaves in different manner where the variation of pH with radiation dose is biphasic. Gamma radiation-induced reactions of HT are reported is as
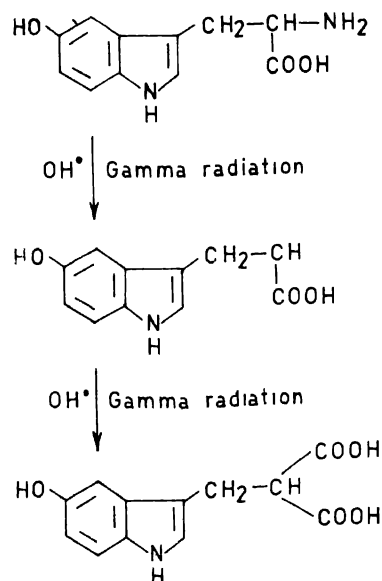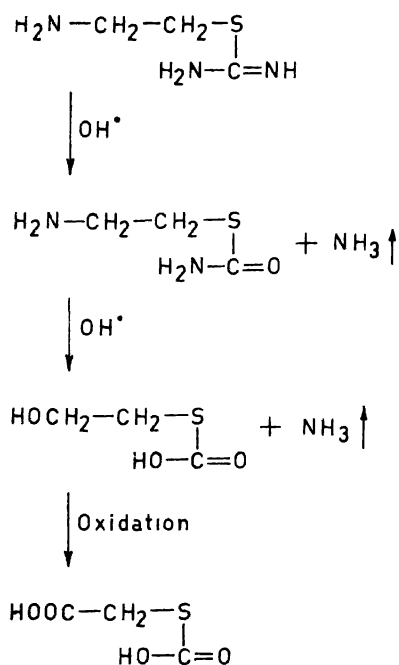
TABLE 4—pH OF RADIOPROTECTORS AND THEIR MIXTURES AS A
FUNCTION OF GAMMA IRRADIATION DOSE

Radioprotectors	Unirradiated	Gamma Irradiated (Gy)			
		100	300	500	1500
AET					
(concn 14 23 μM)	3 85	3 65	3 38	3 11	3 05
HT					
(concn 90 82 μM)	5 98	5 85	5 25	5 21	5 06
HT+AET					
(90 82 μM + 14 23 μM)					
Concn ratio	5 20	5 45	5 50	5 37	4 91

It is therefore inferred that ionisable carboxyl groups are responsible for decrease in pH with increasing radiation dose.

We suggest that for AET the possible gamma radiation-induced reactions that lead to decrease of pH are as follows :

The initial increase of pH for HT + AET up to 300

$H_2N-CH_2-CH_2-S$
$\quad\quad\quad H_2N-C=NH$

↓ OH•

$H_2N-CH_2-CH_2-S$ + NH_3↑
$\quad\quad\quad H_2N-C=O$

↓ OH•

$HOCH_2-CH_2-S$ + NH_3↑
$\quad\quad HO-C=O$

↓ Oxidation

$HOOC-CH_2-S$
$\quad\quad HO-C=O$

Gy is likely to be due to the formation of NH4OH from ammonia, liberated from sidechain of HT and =NH group of AET of the complex. Afterwords with further increase in radiation dose carboxyl groups both from HT and AET cause the pH to decrease.
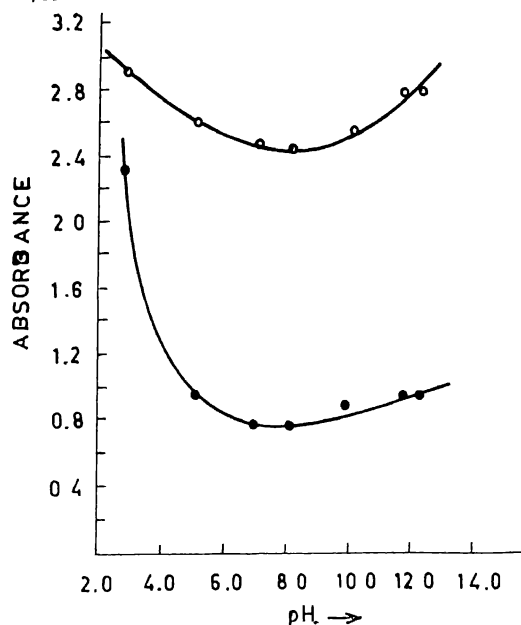
Experimental

Fig 7 Absorbance versus pH curve of HT + AET (concn, 90 82 μM + 14 23 μM) in water absorbance at (O) 205 nm and (●) 275 nm

Anhydrous HT (Lot no. 33, F0402, H3753, mol. wt. 220.2) and AET (Lot no. 122, F0369, mol. wt. 281.02) were dissolved in water on warming to 37^0. Other than water, EDTA $(2.5 \times 10^{-4} M)$ and 0.9% sodium chloride were also used as solvents. Triple-distilled water was used for preparing the solutions.

The nature of interaction was studied by spectrophotometry.

A Cambridge spectrophotometer was used; special care was taken for measuring the absorbance (190–220 nm) by keeping the cuvette assembly in nitrogen atmosphere. Other equipments used were on Aminco-Bowman spectrophotoflourimeter (accuracy 0.001 unit), a Perkin-Elmer infrared spectrophotometer (accuracy 1%), a CM 82T conductivity bridge (accuracy ± 0.01 μ $\Omega^{-1)}$ and a Toshniwal pH meter (accuracy ± 0.01).

HT (concn., 10 μg/ml^{-1}) and AET (concn., 10 μg/ml^{-1}) were dissolved separately in water. For preparation of HT + AET, 10 μg/ml^{-1} HT was mixed with 2 μg/ml^{-1} AET. The mixture was stirred and kept for 3 h at 37^0. HT, AET and HT + AET solutions were

then lyophilised at -30^0 and a pressure of 6 mbar for 6 h. These were then pelletted with KBr, made a thin film and ir spectra obtained.

The melting points of AET and HT were 188^0 and 270^0 and that for HT + AET 190^0 and 230^0 (first and second melting). The data indicate binding between the two compounds. The dose rate for the gamma irradiated solutions was noted to be 0.077 Gy s^{-1} (Ref. 10).

Conclusion : Present studies show that HT forms a complex with AET in the concentration (μM) ratio of 90.82 : 14.23.

Acknowledgement

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