ANALYTICAL STUDIES AND THE EFFECT OF TRYPAN BLUE IN HUMAN ESOPHAGUS CANCER CELLS

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Abstract:

Analytical studies of Trypan Blue (TB) diazo dye; showed, highest solubility in water without deviation in the linearity between a dipole moment constants and maximum wavelength. Because of the absorption spectrum of TB affected by using a range of different solvents via solvation and dielectric constant (D). The linear relationship between λ_{max} and each function F(D), $\phi(D)$ and (D-1)/(D+1), Shown that the D is the only operator that controls shift in the peak. Results also showed, that the deviation from the linear relationship of DMSO is explained on the basis of the formation of strong hydrogen bonding between the solvent and the solute molecules. Though, pH effect, also studied in some variety buffer solutions. The protonation (pKb) and ionization (pKa) constants were intended, owing to suggest the ionization scheme in acidic and basic media. TB was non-toxic and it isn't shown any hemolysis effect in the normal human cells. Further, the MTT assay using human esophagus cancer cells (SK-GT-4) was applied using three different concentrations of TB, the inhibition rate of the cell growth was seeming to be higher than (50%) using 250 µg/ml concentration in contrast with the control. But, there is no effect of TB using 500 µg/ml concentration. Furthermore, the results were indicated that the TB was generating a negative response using high concentration (1000 µg/ ml). In this study TB recommended to use as anti- human esophagus cancer cells (SK-GT-4) .

Key Words: Trypan Blue, Analytical studies, Ionization constants, Protonation constant, Esophagus and Cancer Cells.

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Introduction:

In scientific research azo compounds have receiving high attention,[1,2,3,4] because their significance in the analytical chemistry.[5,6,7] A powerfully colored compounds were status as DNA and RNA inhibitors, antidiabetic, antineoplastic, antibacterial,[5] and can use in the synthesis of protein.[8] These compounds also used against human breast MDA-MB231 cancer cells,[9,10] using cytotoxicity assay to receive an appropriate results within a short time in vitro, which is used earlier than in vivo testing. Because of, in vitro cultures can be refined the results under a controlled environment, due to minimize experimental errors.[10] Therefore, in this study, MTT assay applied in esophageal cancer (EC) which is related to high death worldwide, [11] using diazo day TB as model.

1. Materials and Methods

Absorption spectrum was determined on a spectrophotometer. Human cell line was gained from Iraq Biotech Cell Bank/ Basrah, and preserved in RPMI-1640 supplemented with 10% Fetal bovine, 100 units/ ml and 100 µg/ml of penicillin and streptomycin respectively. Cells were passaged using Trypsin-EDTA reseeded at 50% confluence twice a week and incubated at 37 °C and 5% CO_2.

2.1 pH effect

Each buffer solution, ($1x10^{-4}$ M) ranging (2-12).

2.2 The effect of solvents with different polarity

Individually, TB solution in: water (TB1), ethanol (TB2), methanol (TB3), chloroform (TB4), ethylene chloride (TB5), triethylamine (TB6) and dimethylsulphoxide (DMSO) (TB7), was prepared, ($1x10^{-4}$ M).

2.3Cellular poisonousness

Toxity of different concentrations of TB, (50,100, 250, 500 and 1000 $\mu g/ml$) was measured.[12] Then, the results were recorded after the incubation for three hrs.

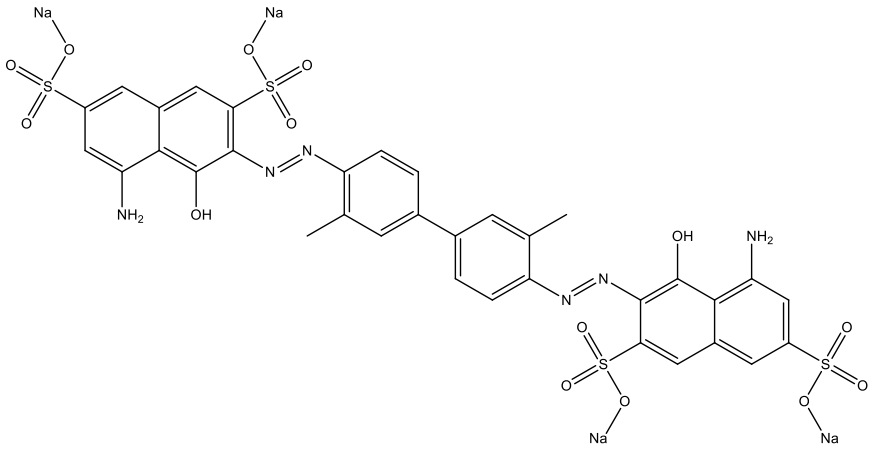
2.4 MTT Assay

Human esophagus cell viability assay was directed on 96-well plates, (1×10^4 cells/well). After day. cells were treated with TB, but the cell viability was measured after three days. After removing the MTT solution, the crystals remaining in the wells were solubilized by the addition of 100 µL of DMSO followed by 37 °C incubation for 15 min with shaking. The absorbance was intended by using microplate reader at 620 nm; the test was repeated thee times. The inhibition rate was calculated as the following

equation: Proliferation rate as (PR)= B/A*100 where A is the mean optical density of untreated wells and B is the optical density of treated wells and IR= 100- PR.

3. Results and Discussion

Analytical studies were focused on Trypan Blue, ,[13] (TB, Figure 1).



sodium 3,3'-((1*E*,1'*E*)-(3,3'-dimethyl-[1,1'-biphenyl]-4,4'-diyl)bis(diazene-2,1-diyl))bis(5-amino-4-hydroxynaphthalene-2,7-disulfonate)

Figure 1. The structure of TB.

The solvents effects in TB was studied using a set of solvents, water (TB1), ethanol (TB2), methanol (TB3), chloroform (TB4), ethylene chloride (TB5), triethylamine (TB6) and DMSO (TB7), (Figure 2).

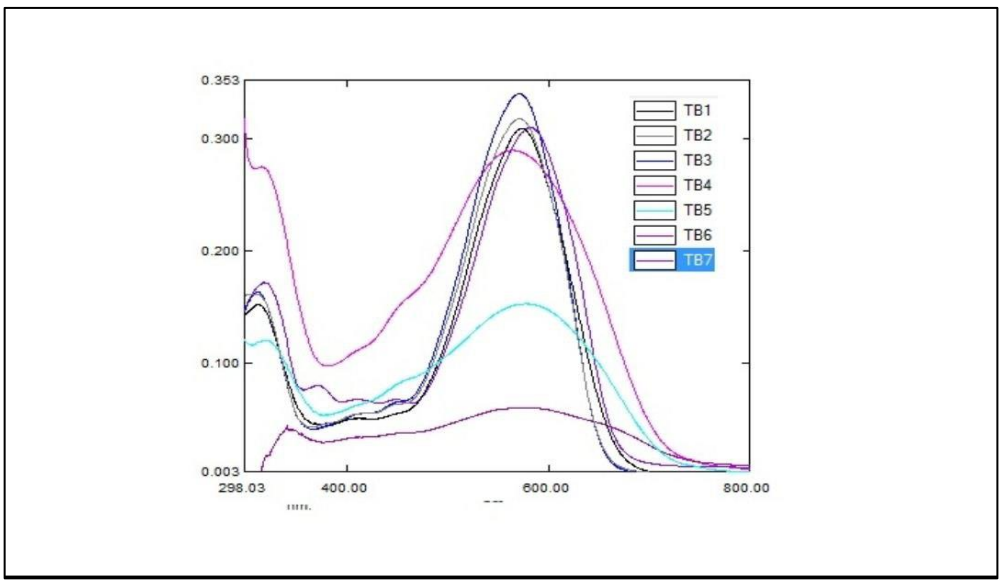


Figure 2. The solvents effects.

Figure (2) above shows different values of λ_{max} and the best solubility of TB was in water, (Table 1).

Table 1. The solvents effects in TB

Solvent	(TB)	
	ƛ max (nm)	ε_{max} ($\times 10^{-4}$)
TB1	315	0.153
TB2	310	0.162
TB3	310	0.164
TB4	315	0.275
TB5	320	0.121
TB6	340	0.044
TB7	370	0.081

The results were revealed that the peaks in the ranges (310-375) were associated with π -π* transitions of N=N due to indicate, that the presence of additional λ_{max}, (550-580) nm is related to the hydrazine form, which is in equilibrium with azo form with respect the symmetry in the structure of TB, (Figure 3).

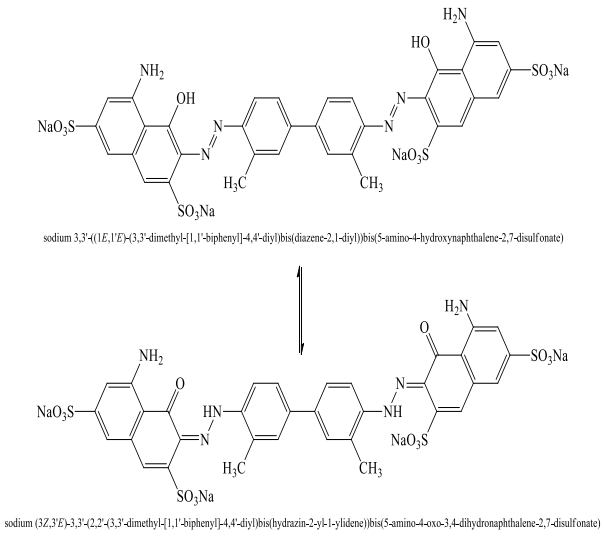


Figure 3. The equilibrium of di azo form with di hydrazine form.

Designated absorption spectrum of TB affected by using a range of different solvents by meaning of solvation and D, which can be stated,[14] (equation1).

$$\Delta\tilde{V} = \left[(a-b)\left(n^2\text{-}1 \;/\; 2n^2\text{+}1\right)\right] + b\left(D\text{-}1 \;/\; D\text{+}1\right) \ldots\ldots (1)$$

The results from equation (1) are giving an indication that the shift in the peak can occur in response to the change in the solvation energy or in related to the exact dielectric constant. In more precisely the functions F(D) and $\phi(D)$ lead to linear relation, when the D is the only operator that controls the shift.

$$\mathbf{F(D)} = \frac{\mathbf{2(D-1)}}{\mathbf{2D+1}}$$

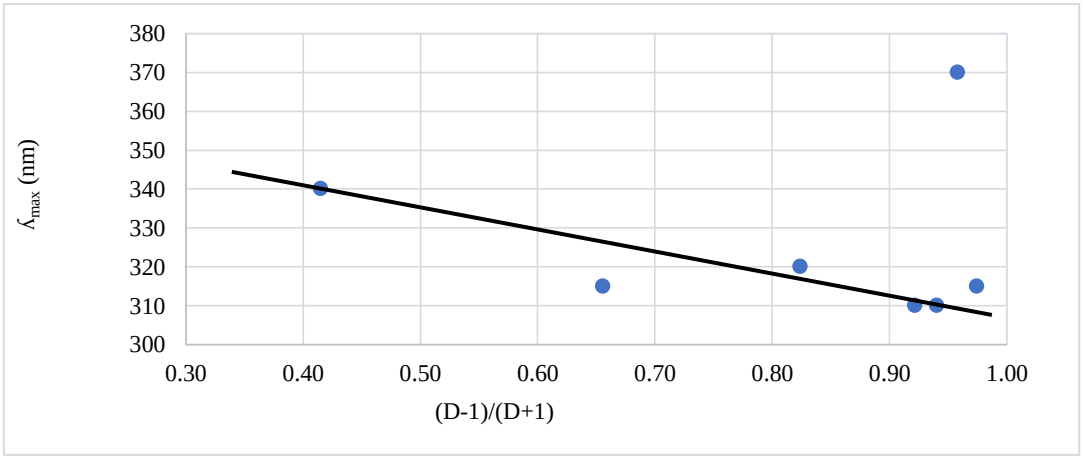
$$\boldsymbol{\phi}\mathbf{(D)} = \frac{\mathbf{D-1}}{\mathbf{D+2}}$$

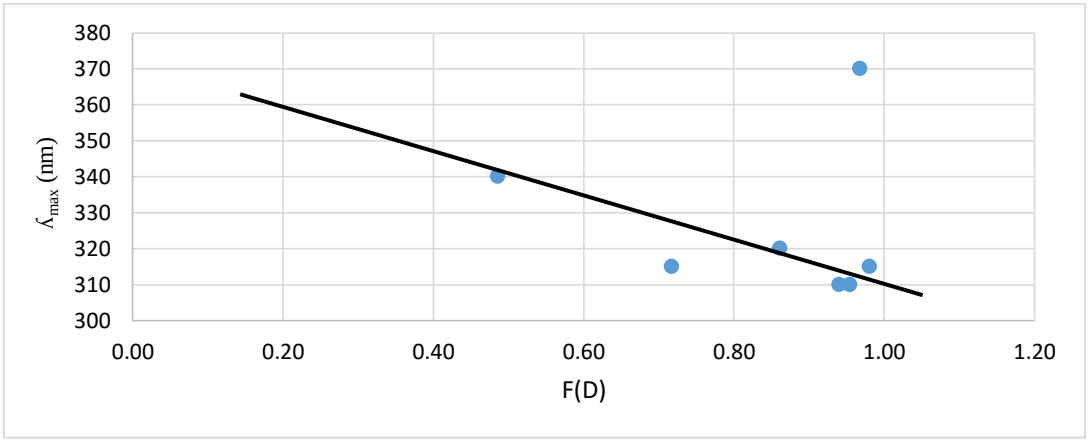
Therefore, F(D), $\phi(D)$ and (D-1)/(D+1) were presented in Table (2) below.

Table 2. Dielectric constants of selected solvents and some functions

Solvent		(TB)			
		D	(D-1)/(D+1)	F(D)	$\phi(D)$
TB1	Water	78.30	0.97	0.98	0.96
TB2	Ethanol	24.55	0.92	0.94	0.89
TB3	Methanol	32.70	0.94	0.95	0.91
TB4	Chloroform	4.810	0.66	0.72	0.56
TB5	Ethylene chloride	10.36	0.82	0.86	0.76
TB6	Triethylamine	2.420	0.42	0.49	0.32
TB7	DMSO	46.68	0.96	0.97	0.94

Therefore, the linear relationship or close it of selected solvents were designated in Figure (4) below.





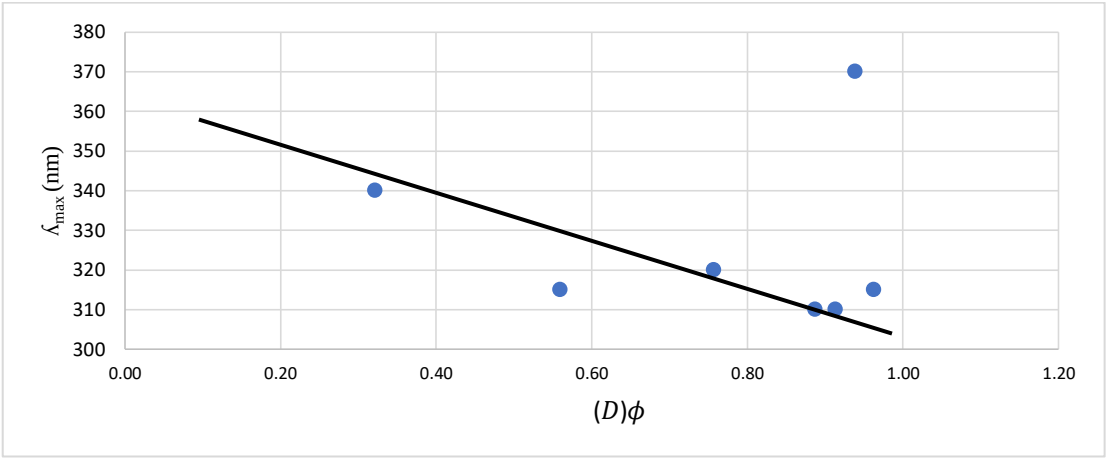


Figure 4. The linear relationship between λ_{max} and some functions of different solvents.

The results were showed that the deviation from the linear relationship of DMSO is explained on the basis of the formation of strong hydrogen bonding between the solvent and the solute molecules. Absorption of each solution of TB, (pH 2-12) was also attended at λ (200-800) nm as seen in Figure (5) below.

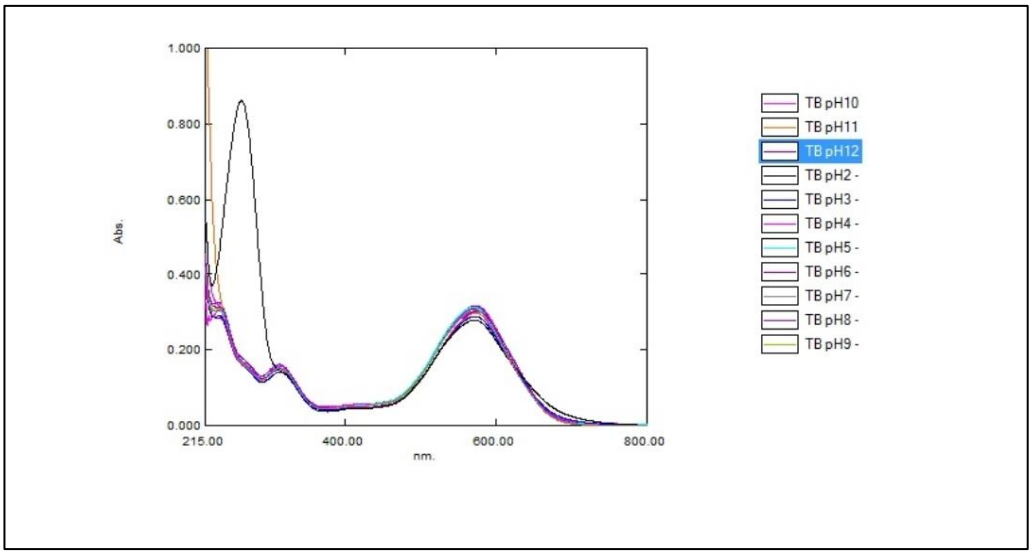


Figure 5. Absorption of TB solutions, (pH=2-12).

Figure (5) above shows two isopiestic points in the spectrum at (540 and 630 nm) and the suitable pH value was in the pH10. Add to which, the half- height method was used to calculate the pKb and pKa of nitrogen atom and hydroxyl group respectively via equations (2) and (3) underneath, (Table 3).

pK_a= pH (at $A_{1/2}$) ..... (2)

$A_{1/2}= \frac{A_l + A_{min}}{2}$ ..... (3)

Table 3. The values of pK

Id	λ_{max}	$A_{1/2p1}$	pK_{P1}	$A_{1/2p2}$	pK_{p2}	$A_{1/2a1}$	pK_{a1}	$A_{1/2a2}$	pK_{a2}
TB	573 nm	0.295	3.5	0.313	7. 6	0.315	9.5	0.300	11.5

The pK at ($A_{1/2}$) of (TB) in acidic and basic media was envisioned since the pH - absorbance curve identified, (Figure 6).

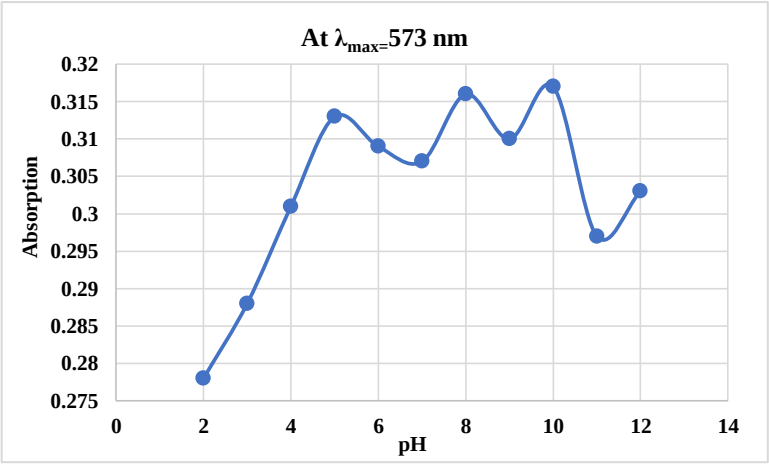
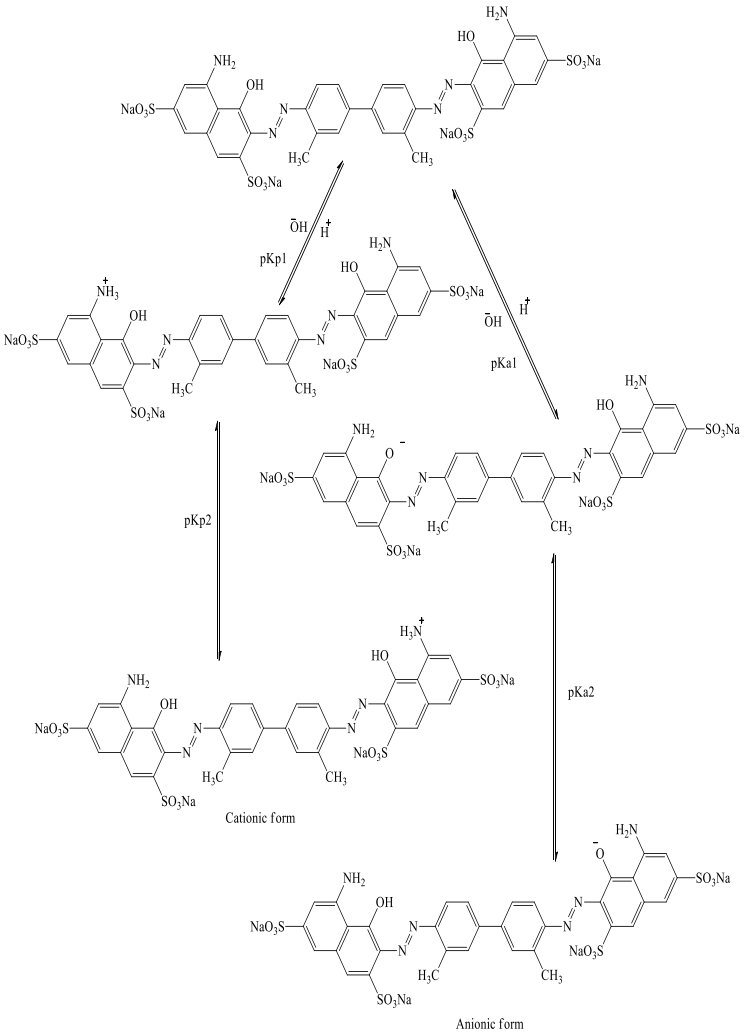


Figure 6. Curve of pH versus absorbance of TB.

The results of TB in acidic and basic media were displayed the suggesting ionization scheme, (1).



Scheme 1. Suggested ionization scheme of TB.

TB toxity using hemolytic red blood cells in vitro was also studied and the results were indicated the non-toxic effects of different concentrations of TB, that did not display hemolysis effect. MTT cell viability assay was used, (Figure 7). TB was diminishing the growth of esophagus (SK-GT-4) cancer cells.

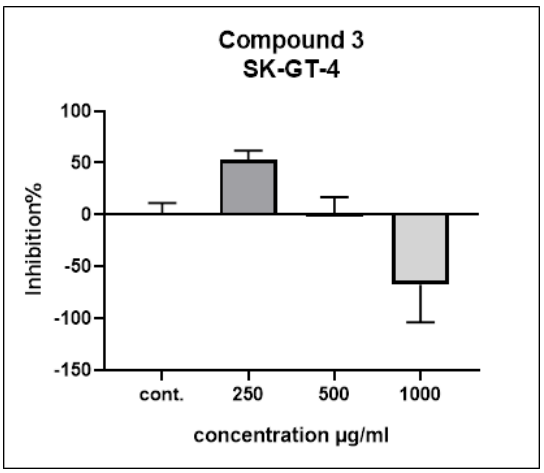


Figure 7. Ability of TB in destroying esophagus cancer cells.

Cytotoxic effect of TB achieved using three different concentrations, inhibition rate of the cell growth seeming to be higher than (50%) using 250 µg/ml in contrast with the control. But, there is no effect of TB using 500 µg/mL concentration on the cancer cells. Further, the results were giving negative response using 1000 µg/ ml concentration in contrast with the control.

4. Conclusion

TB was gained good color, high soluble in water, have non-toxic effects and non-hemolysis effect in the human cells. TB also had variable results using different buffer solution, besides their ability to bind esophagus cancer (SK-GT-4) cells and affect their viability. The inhibition rate of the cell growth was more than 50% and start a negative response at concentration higher than 500 µg/ml. Owing to indorse the diazo dye as anti-human esophagus cancer cells (SK-GT-4).

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