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Analytical study and Anticancer drug

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Abstract

The methyl red (MR) and the methyl orange (MO) were studied in order to understand the effect of the structure of each azo dye in their analytical and biological properties. The results of the two selected indicators seem to be effected by different solvents and the best solubility of each was in the methanol. The outcomes were indicated, that the changes in the energy of solvation and the dielectric constants were responsible to shape the shifts of the peaks in their spectra. Designated the F(D) and $\phi\ (D)$ functions of MR and MO were also provided a linear relation with the λ_{max} (nm). Furthermore, the toxic effects of dyes were deliberated in vitro due to indicate, that the MR and MO were harmless using diff. concentrations from each. Therefore, the esophagus (SK-GT-4) cancer cells were tested using MR and MO through using the MTT cell viability assay. The dyes, (from low to high concentrations) were revealed high ability to inhibit the growth of cancer cells in contrast with the control (C). The MR was gave high inhibition rates in all selected concentrations, which were equal to around 100% in contrast with the control. But, the MO was giving positive and negative responses in low concentration and the inhibition rate was increased with rises of the concentration and it was reach to 100% in 1000 µg/ml. Our observation, that the MR and MO had actual significant ability to inhibit the esophagus cancer (SK-GT-4) cells. Due to recommend each of them as a new treatment of esophagus cancer, which can apply directly to the disease.

Key words: Methyl red, Methyl orange, Esophagus, Cancer Cells, Rate of Inhibition

1. Introduction

Azo dyes have had high attention in scientific research with an excessive vigorous in chemical analysis.[1,2,3,4] These dyes,[5,6,7,8] correspondingly have gotten a very significant role in the analytical chemistry.[9,10,11] A strongly colored dyes can be yellow, red, orange, blue or even green, depending on the exact structure of the molecule. due to create these compounds as very important pigments for a long time. [12] The presence of C=C, N=O, N=N, aromatic rings, C=O and NO_2 groups in the organic compounds usually responsible to produce a colour. Though, the azo (–N=N–) and nitroso (–N=O) groups are invariably conferred the colour, whereas the other really were fixed consequently under certain conditions.[13] A colored compounds were hold a prominent position in the industrial landscape due to their versatility and applicability in a wide range of manufacturing processes.[14] Alongside these dyes, the chemical arsenal utilized by industries includes surfactants, chelating agents, and pH regulators, each playing a crucial role in shaping the final products. Also the pharmaceutical azo dyes were graded as antidiabetic, antineoplastic, antibacterial,[5] synthesis of protein, DNA and RNA inhibitors and anti-cancer drugs.[15] Human breast cancer cells have been subjected to rigorous analysis in vitro using different synthetic azo dyes.[16,17] The outcomes of these experiments have yielded valuable insights into the cytotoxic effects of these compounds. Through a comprehensive cytotoxicity assay, [18,19] the unique properties of these dyes have been systematically examined and correlated with their potential applications in cancer treatment. In the scope of the current research, we aim to use MR and MO, which are used as an indicator in an acid-base titration with major difference between them, (pH rang 4.4 (red) to 6.2 (yellow) and of 3.1 (red) to 4.4 (yellow) respectively), they will both show a change in pH level at different points. Our study focusing in study of the effect of polar solvents in each dye to explore our understanding about the ability of each in evaluating the individual effect on esophageal cancer (EC) disease though their analytical properties. This study is poised to contribute significantly to the growing body of knowledge surrounding the versatile applications of synthetic azo dyes in industry especially in the field of pharmaceutical azo dyes and medical science.

2. Methodology

Determination of absorption spectra was achieved using a spectrophotometer. Human cell line was intended, (IraqBiotech 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% CO2).

2.1 Influence of solvents in the MR and MO spectra

A set of solutions of each of MR and MO, ($5x10^{-5}$ M) were prepared by using a set of set of solvents, (water (MR1) or (MO1), ethanol (MR2) or (MO2), methanol (MR3) or (MO3), diethylamine (MR4) or (MO4)).

2.2Cellular toxicity

Each of MR and MO solutions, (25, 50, 75, 100, 250, 500 and 1000 $\mu g/ml$) was tested.[20]

2.3 Human esophagus cell viability assay

MTT Assay was intended, (1×10^4 cells/well), the esophagus cells were preserved with MR or MO, followed by eliminating the MTT solution. The DMSO (100 µL) then was added after the cell viability was measured, (37 °C for 15 min). The absorbance was intended at 620 nm.

3. Results and Discussion

The two indicators, named methyl red (MR)[21] and methyl orange (MO) [22], (Figure 1) were studied, in order to understand the effect of their structure in their analytical and biological properties.

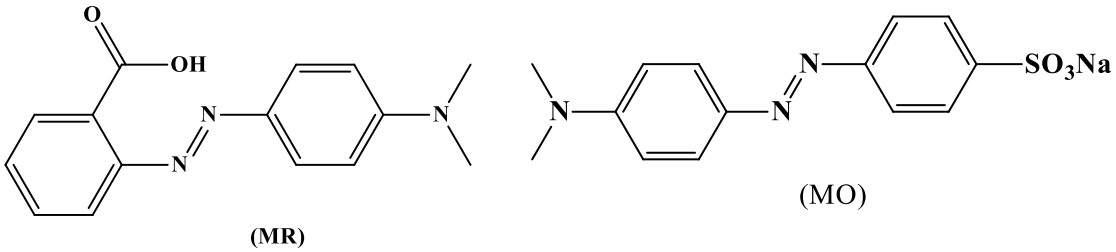


Figure 1. Chemical structure of MR and MO.

The solubility of the two azo dyes MR and MO were affected by selected solvents, (water (MR1, MO1), ethanol (MR2, MO2), methanol (MR3, MO3) and diethylamine (MR4, MO4)) as realized in Figures (2) and (3) below.

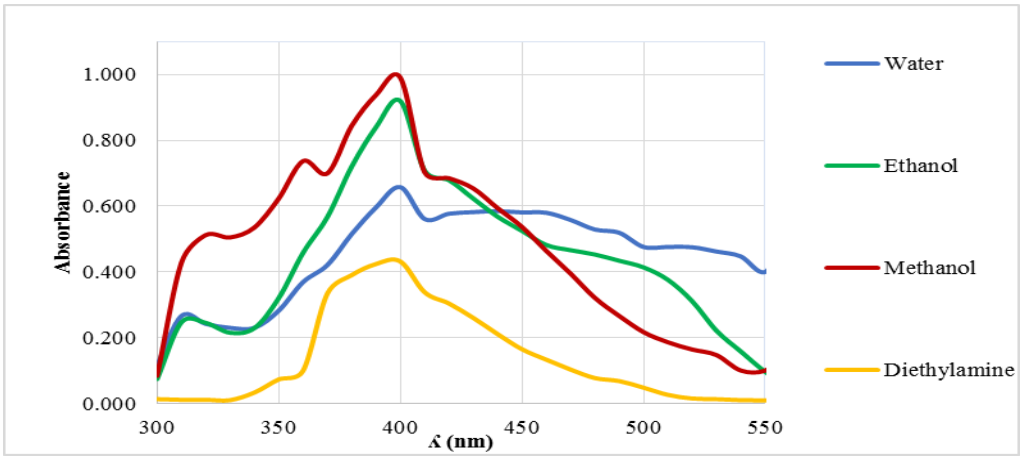


Figure 2. Effectiveness of diff. solvents toward MR.

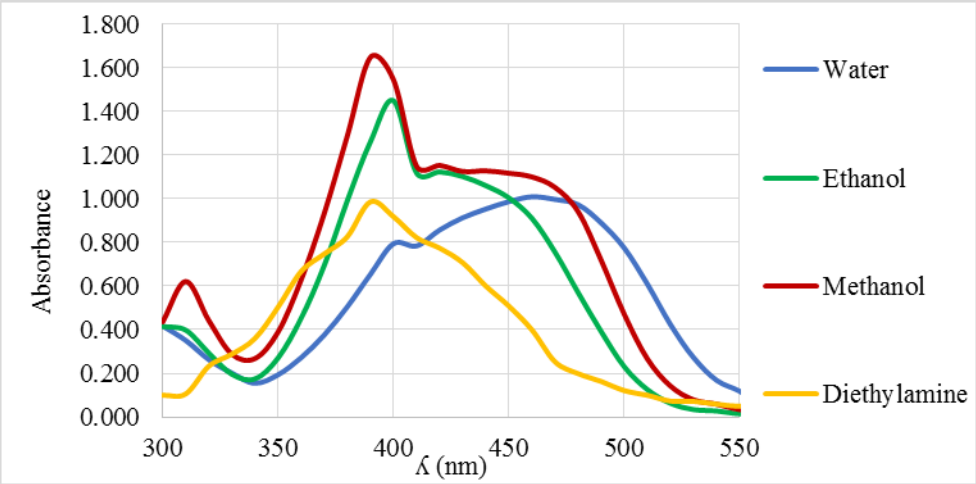
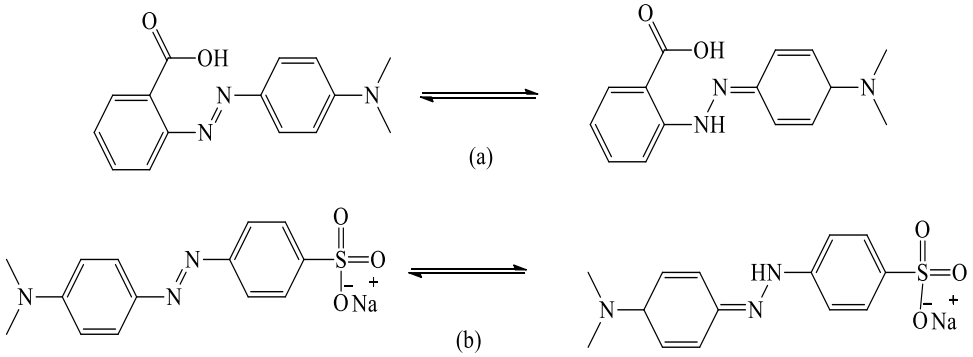


Figure 3. Effectiveness of diff. solvents toward MO.

The obtainable λ_{max} of MR and MO from their spectra were in the range (300-360) nm and (310-345) nm and (490-530) nm and (480-520) nm respectively, which were related to the azo and



hydrazine forms respectively, (Figures 4a and b).

Figure 4. The equilibrium of azo form with hydrazine form of MR (a) and MO (b).

The solvent effect of MR and MO, (Table 1) were displayed, that the best solubility of MR and MO individually were in the methanol.

Table 1. Effecting of selected solvents in the MR and MO

Solvent	(MR)		(MO)	
	ƛ max (nm)	ε_{max} ($\times 10^{-5}$)	ƛ max (nm)	ε_{max} ($\times 10^{-5}$)
Water	400	3.290	400	3.97
Ethanol	400	167.5	400	7.25
Methanol	400	4.955	390	8.24
Diethylamine	390	2.120	390	4.93

The outcomes were indicated the changes in the energy of solvation and the dielectric constants were produced the shifts of the peaks, which occurs in the spectra of each azo dye, (equation1).

$$\Delta\tilde{V} = \left[(a-b)\left(n^2\text{-}1 / 2n^2\text{+}1\right)\right] + b\left(D\text{-}1 / D\text{+}1\right) \ldots\ldots (1)^{23}$$

From equation (1), the F(D) and $\phi\,(D)$ functions were designated for each of MR and MO as below:

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

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

These functions were provided some linear relations with the λ_{max} (nm), that gained from Figures (2) and (3) above as shown in Figures (5) and (6) below.

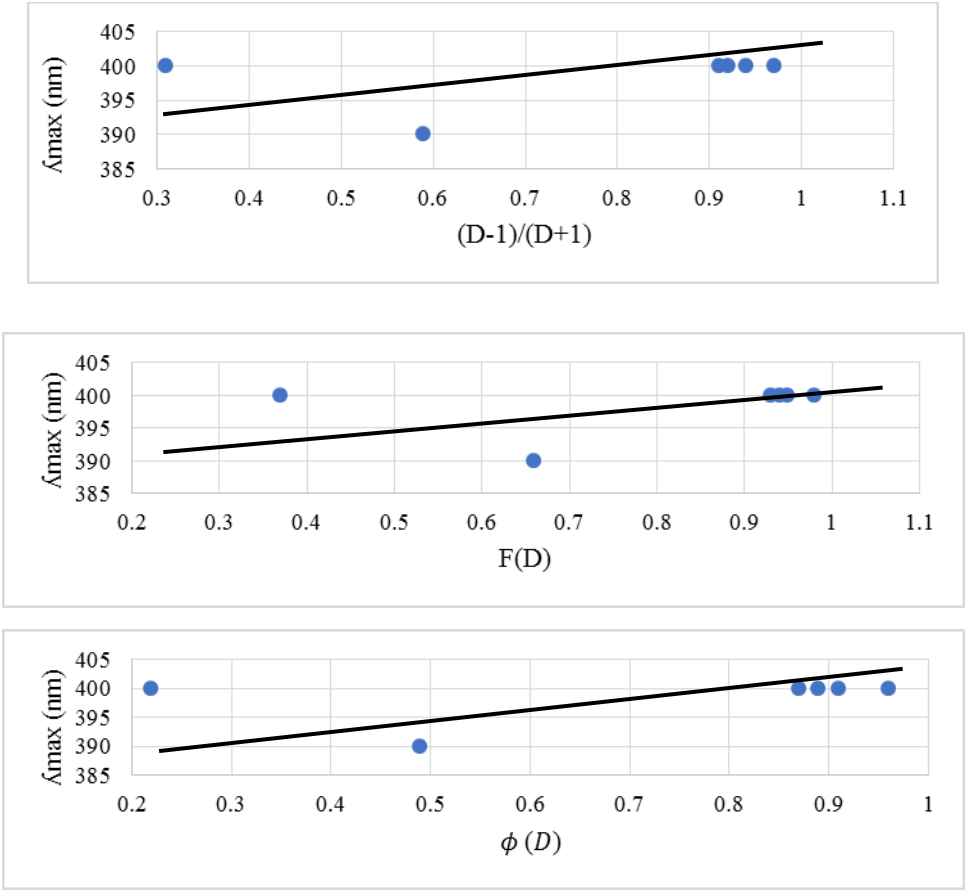


Figure 5. Designated functions of MR.

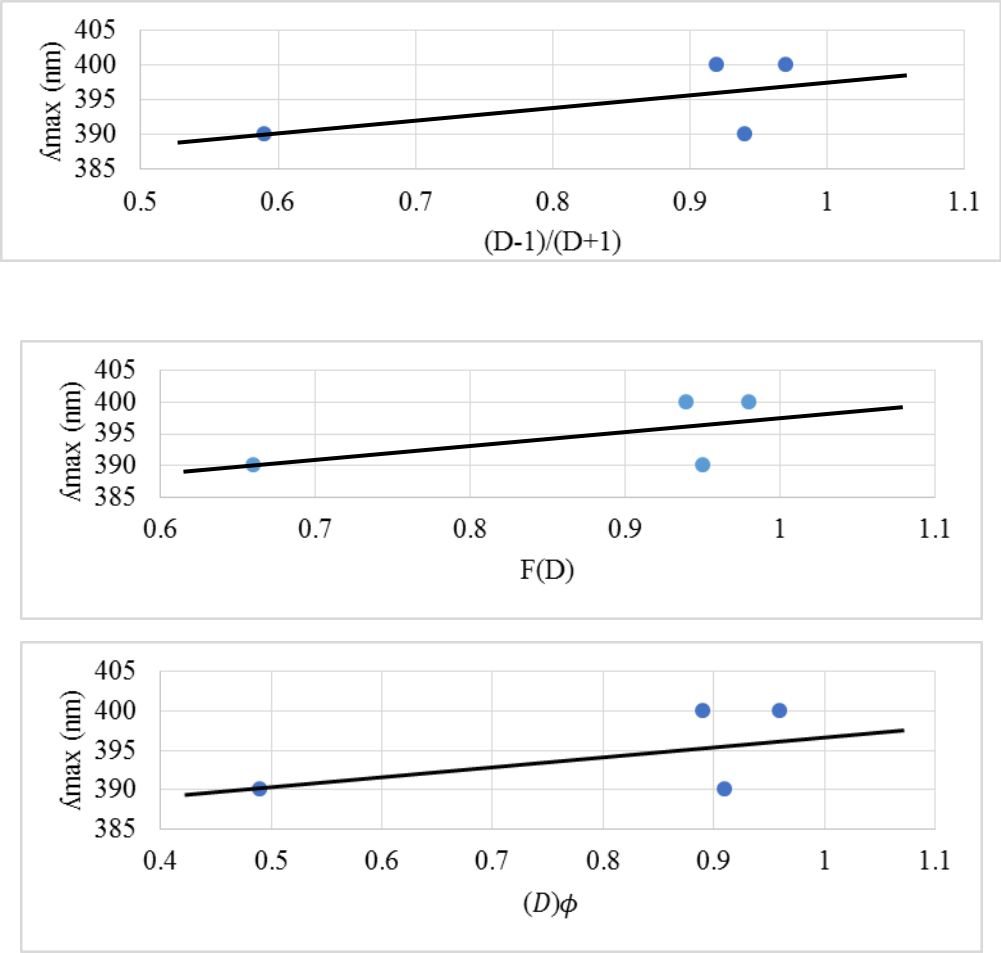
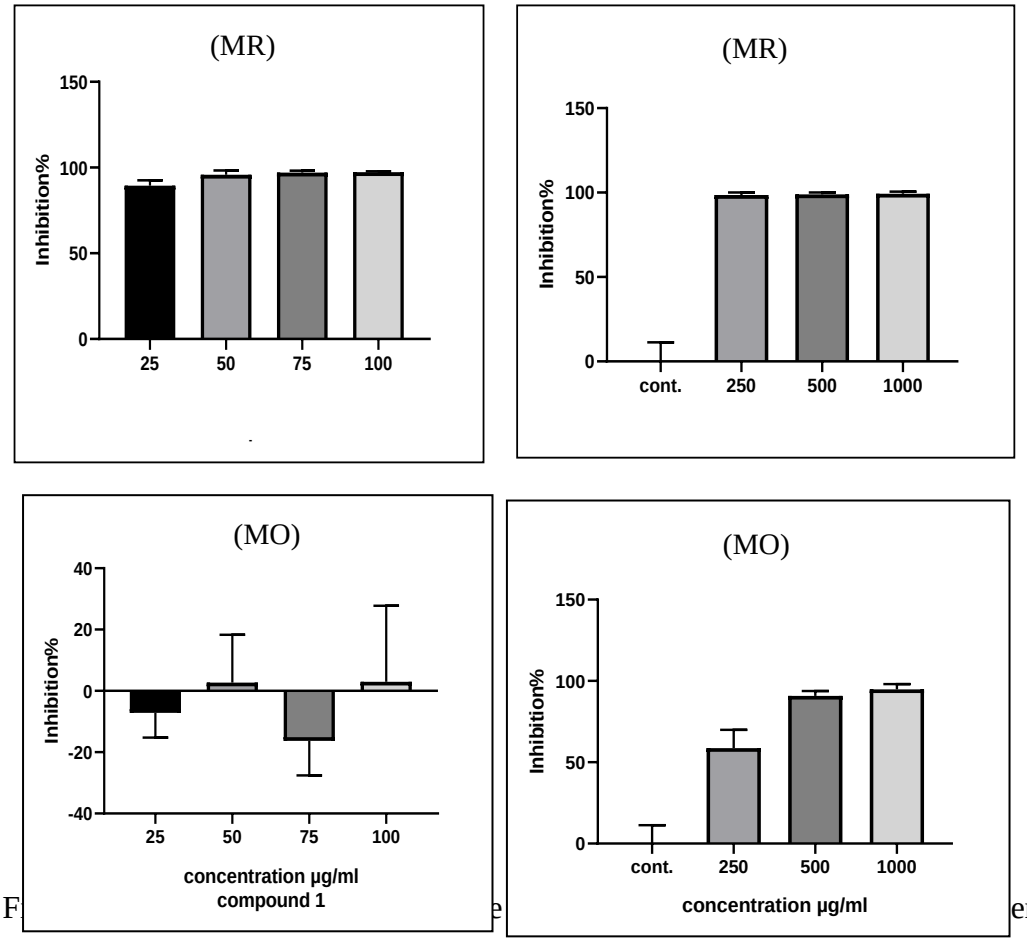


Figure 6. Designated functions of MO.

Figures (5) and (6) above are reveal, that the D was influence the shift in the spectra of MR and MO and these results were indicated the earlier results. Furthermore, the toxic effects of MR and MO were deliberated in vitro in order to understand the effect of the structure of each in their analytical and biological properties. The results were indicated, that the MR and MO were harmless, these results were provided using diff. concentrations from each. Our hypothesis that the two indicators can control the process of the inhibition growth of the cancer cells. Therefore, the esophagus (SK-GT-4) cancer cells were tested using MR and MO through using the MTT cell viability assay. The MR and MO, (from low to high concentrations) were revealed high ability to inhibit the growth of cancer cells in contrast with the control (C), (Figures 7 and 8). The inhibition rate was correspondingly calculated using the following equation: PR= B/A*100 and IR= 100- PR.



F[illegible]e [illegible]er cells.

The MR dye was gave high inhibition rates in all selected concentrations, which were equal to around 100% in contrast with the control. But, the MO was giving positive and negative responses in low concentrations and the inhibition rates were increased with rises of the concentration and the inhibition rate was reach to 100% in 1000 µg/ml. Our observation, that the MR and MO had actual significant ability to inhibit the esophagus cancer (SK-GT-4) cells. Due to recommend each of them as a new treatment of esophagus cancer, which can apply directly to the disease.

4. Conclusion

Esophagus cancer (SK-GT-4) cells were effected by MR and MO differently depending of their structure and analytical properties. The MR and MO, which were harmless were gave high inhibition rates towards the cancer disease. These dyes were applied in different concentrations and the best rate was ≈100%, which received by MR in all concentrations that's applied in this study. However, the MO was gave ≈100% in high concentrations, but the low concentration were showed positive and negative responses. Owing to indorse these azo dyes as a new and direct dyes, that can have colored the esophagus cancer cells and inhabited their growth.

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