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Full Length Research Paper

Essence of photoelectric colorimetric assays of alcoholic methyl red dye solution in the purification of azo dye-contaminated waste-water

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The aim of the present research is: to determine the wavelength of maximum absorption of white light by alcoholic methyl red dye solution, to construct/plot the standard concentration calibration curve of the alcoholic methyl red dye solution, to find the Beer-Lambert's concentration limits of alcoholic methyl red dye solution and to determine unknown concentrations of alcoholic methyl red dye in waste water samples, in a simulation model experiment. The wavelength of maximum absorption (1.18), of white light by 0.01% w/v alcoholic methyl red dye solution (pK_a: 4.76, 25°C) was determined by photoelectric colorimetric technique and is in the range of 470-529 nm. A serial dilution of a 0.01% alcoholic methyl red dye stock solution, was prepared and used in constructing a standard concentration calibration curve, whose equation is $A_{470\text{-}529\ nm} = 320C_{\%\ w/v}$. The Beer-Lambert's constant and Beer-Lambert's concentration limits of alcoholic methyl red dye solution are 320 (% w/v. cm)$^{-1}$ and 0.0025% w/v, respectively. The results observed of the simulation model experiment, indicate that the simulated treated-waste water samples of concentrations of alcoholic methyl red dye: 0.0012 ± 0.0002, 0.00047 ± 0.0001 and 0.0003125 ± 0.0001, were significantly lower ($p<0.05$) than a given standard limit low of 0.00125 ± 0.00001% w/v (mean ± standard error, % w/v) of alcoholic methyl red dye solution and thus were purified with an efficiency >90%. The photoelectric colorimetric assay is efficient for use in purification steps of azo dye-contaminated waste-water.

Key words: Waste-water effluents, simulation model, Beer-Lambert's concentration limits.

INTRODUCTION

Spectrophotometry is the measurement of light absorption and transmission and is one of the most valuable analytical techniques, available to biochemists and chemists alike. Unknown compounds can be identified by their characteristic absorption spectra in the ultraviolet, visible or infrared. Concentrations of known compounds in solutions may be determined by measuring the light absorption at the wavelength of maximum absorption. The progress of enzyme-catalyzed reactions frequently can be monitored by measuring spectrophotometrically or colorimetrically, the appearance of a product or the disappearance of a substrate (Segel, 1976; Nelson and Cox, 2008).

Spectrophotometer is an important instrument for detection and investigation of chemicals and is used as a tool in the measurement of the amount of light of a given wavelength that is transmitted or absorbed by a sample solution. A spectrophotometer measures absorbance or transmittance of light by solute/solution in the wavelength range of ultraviolet (100nm-400nm), visible (400nm-800nm), or infrared (800nm-100µm) and is used to determine the concentration of coloured or non-coloured solutes/solution. Colorimeters measure absorbance of light in the wavelength range of the visible spectra and are used in determining the concentration of coloured solutes/solutions (Housecroft and Constable, 2006). Colorimeters use colour-light filters that are complementary to the colour of the solute/solution under

consideration. For example, a red solution uses a blue colour-light filter.

The fraction of incident light that is absorbed by a solution depends on the thickness of the sample, the concentration of the absorbing compound in the solution and the chemical nature of the absorbing compound.

Light absorption follows an exponential law, if transmission is measured at varying concentration of solution or varying sample thickness, in a cuvette. Sample thickness is defined as path length of the sample in the cuvette. The exponential law of light transmission by a solute/solution is given by:

$$\log_{10}\frac{I_o}{I}=acl$$

I_o = amount of light (100%, or unity, 1), that is incident on the solution.

I = amount of light that is transmitted by the solution.

a = absorbance index, or extinction coefficient or Beer-Lambert's constant, which is constant and peculiar to the absorbing compound/solute/solution.

If the concentration of the solution is expressed in Molarity, a = a_m or E = molar absorption coefficient or molar extinction coefficient, but if expressed in g/liter, a = a_s = specific absorption coefficient. a_m = a_s x molecular weight of light absorbing compound.

Light absorption follows a linear law, if absorbance is measured at constant wavelength and constant sample thickness in a cuvette.

$\log_{10}\frac{I_o}{I}$ is a measure of the absorbance, A, and A=acl (the absorbance is directly proportional to the concentration of the absorbing solute). (Nelson and Cox, 2008).

Serial dilutions follow an arithmetic progression (AP) series, if the consecutive concentration of the dilution series have a common difference. For example, the concentration series (mg/ml): x + 6, x + 4, x + 2, x, have a common difference of n – 2. n is the value of the previous concentration of the solute/solution in the series, in order of consecutive decrease.

Serial dilutions follow a geometric progression (GP) series, if the consecutive concentration of the dilution series have a common ratio. For example, the concentration sequence (mg/ml): x, rx, r^2x, r^3x, has a common ratio, r.

By measuring the absorbance values of the series so formed, a standard calibration curve of the solution/solute can be constructed by plotting a graph of absorbance of the serial dilution of the solute/solution at the wavelength of maximum absorption (on the vertical axis) versus the corresponding concentration of the serial dilution (on the horizontal axis). A straight line curve is usually generated by such an exercise, if the concentration series are dilute enough to fall within the Beer-Lambert's limit of concentration. Very high concentrations among the series deviate from the Beer-Lambert's law. Invariably, a standard calibration curve could be used to determine the Beer-Lambert's limit of a substance/solute/solution, since the Beer-Lambert's limit is a function not only of method of experimentation, but also of the nature of the substance/solute/solution under consideration.

The aims and objectives of the present research include:

i. To determine the wavelength of maximum absorption of white light by alcoholic methyl red dye solution.
ii. To construct/plot the standard calibration curve of the alcoholic methyl red dye solution.
iii. To find the Beer-Lambert's concentration limits of alcoholic methyl red dye solution.
iv. To determine unknown concentrations of alcoholic methyl red dye solution, using the standard calibration curve of the alcoholic methyl red dye solution.

Methyl red [2-(N,N-Dimethyl-4-aminophenyl) azobenzenecarboxylic acid, $(CH_3)_2NC_6H_4NNC_6H_4COOH$)], also called C.I. Acid Red 2, is a dark red powder or violet crystals with a melting point of 180°C; soluble in alcohol, ether and glacial acetic acid, used as an industrial dye and acid-base indicator (pH 4.2-6.3).

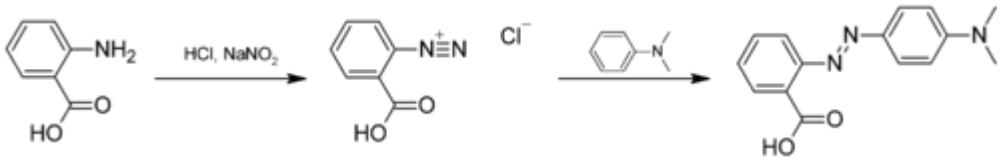
The physical properties of methyl red include: Molar mass, 269.30 g mol^{-1} Density, 0.791 g/cm^3 Melting point, 179-182 °C, 452-455 K, 354-360 °F.

Methyl red may be prepared by diazotization of anthranilic acid, followed by reaction with dimethylaniline as shown in figure 1.

The methyl red test (Kokare, 2008), is used to identify enteric bacteria based on their pattern of glucose metabolism. All enterics initially produce pyruvic acid from glucose metabolism. Some enteric subsequently use the mixed acid pathway to metabolize pyruvic acid to other acids, such as lactic, acetic and formic acids. These bacteria are called methyl-red positive and include *Escherichia coli* and *Proteus vulgaris*. Other enterics use the butylene glycol pathway to metabolize pyruvic acid to neutral end-products and are called methyl-red-negative, which include *Serratia marcescens* and *Enterobacter aerogenes*.

Methyl red are investigated as promising enhancers of sonochemical destruction of chlorinated hydrocarbon pollutants. Waste waters polluted by dyes from textile industries cause severe pollution problems, worldwide (Robinson et al., 2001: Baban et al., 2010). Less than 25% of textile dyes are lost during the dyeing process and an approximately equal amount is directly discharged as aqueous effluents in different environmental components. These azo dyes are carcinogenic and mutagenic to life forms (Suteu et al., 2009: Zaharia et al., 2009).

Ninety-eight (98)% of dyes have a lethal concentration value (LC_{50}) for fishes higher than 1 mg/L and 59% have an LC_{50} value higher than 100mg/L. Without adequate treatment, azo dyes remain in the environment for a long period of time. For instance, the half-life of hydrolysed Reactive Blue 19 is 46 years at pH 7 and 25°C (Hao et al., 2000).



Anthranilic acid Methyl red

Figure 1. Industrial preparation of methyl red (Clarke and Kirner, 1941).

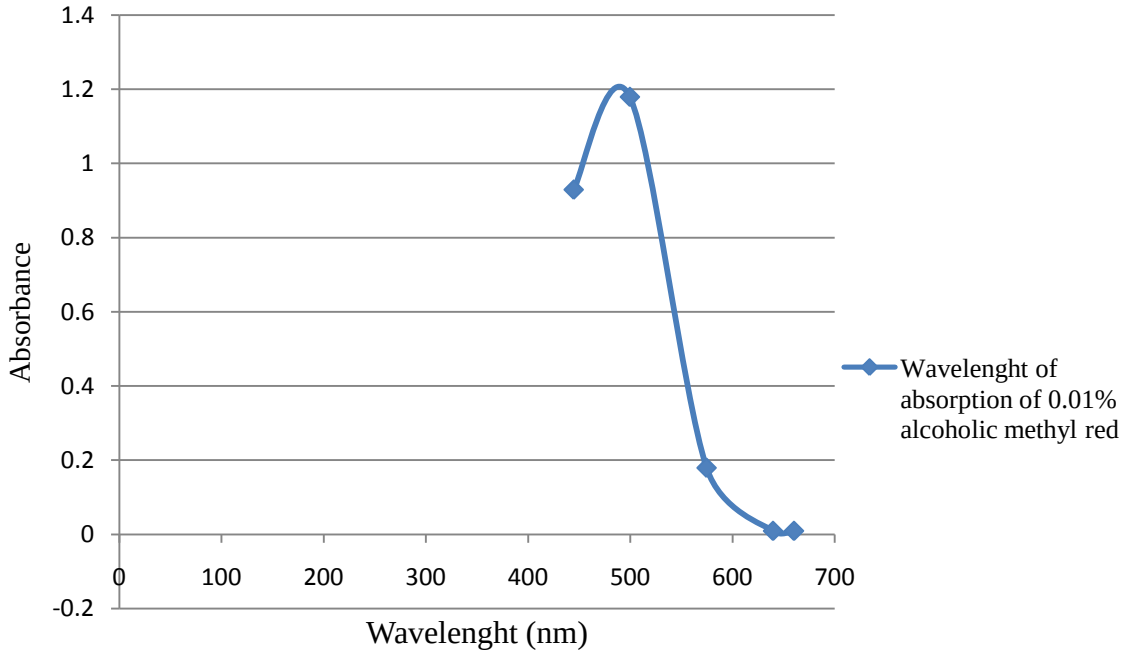


Figure 2. Wave lenght (nm) of absorption of 0.01% alcoholic methyl red dye solution.

Micro-organisms have been used in the purification of azo dye-polluted waste water (Yang et al., 2009). Lignin degrading white rot fungi, *Schizophyllum commune* (50 µm) *and Lenzites eximia* (50 µm), were used, separately, in decolourizing/removal/biodegrading azo dye industrial effluents which included methyl orange/red with 97.57% and 94.79% efficiency, respectively (Selvam et al., 2003: Selvam and Shanmuga Priya, 2012).

Photocatalysis and biological treatment have been employed in a couple, in purification steps to treat industrial azo dye effluents and waste water from textile industries with high degree of efficiency (Jafar et al., 2012).

MATERIALS AND METHODS

The methods employed in the present research are:

Preparation of stock solution of 0.01% alcoholic methyl red dye.

Methyl red stock reagent (0.01grams) was weighed out using a digital balance and added to 62.5ml of 96% v/v alcohol in a 100ml volumetric flask. The volume of the resulting mixture was made up to the 100ml mark with distilled water. The concentration of methyl red in the solution was 0.1 gram methyl red dye/indicator per 1000ml (1L) of solution (pH 6, 25°C). The technique is consistent with the method employed by (Fortune and Mellon, 1938).

Determination of wavelength of maximum absorption of white light by 0.01% alcoholic methyl red dye solution.

Absorbance measurements were taken of 0.01% alcoholic methyl red dye/indicator solution using colour-light filters of wavelength range: 420-469nm, 470-529nm, 530-619nm, 620-659nm and 660nm – above (unspecified). A blank solution made of distilled water was used in zeroing the instrument (a photoelectric colorimeter, AE 11 D), before each absorbance reading was taken. The results of the absorbance measurements

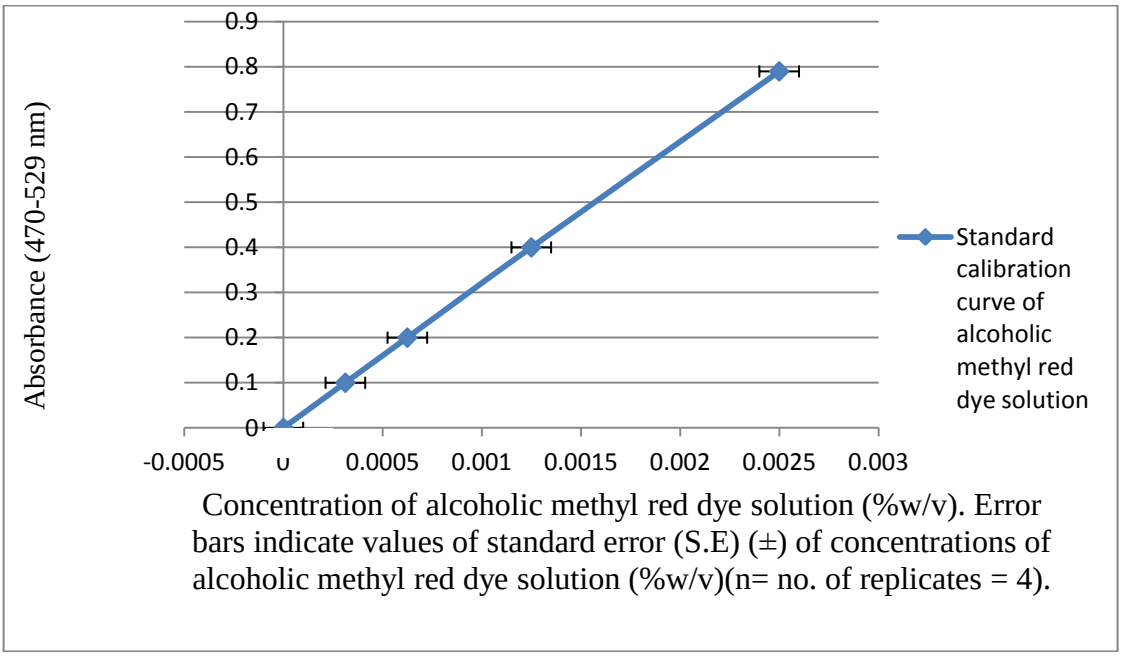


Figure 3. Standard concentration calibration curve of alcoholic methyl red dye solution

made at various wavelengths of the 0.01% alcoholic methyl red dye solution are shown in Figure 2.

Serial dilution of 0.01% alcoholic methyl red dye solution to form a geometric progression (GP) of concentration sequence/series.

The serial dilution was prepared according to a geometric progression concentration sequence/series of term (T_n) $= ar^{n-1}$.

n = the specific term of the concentration in the sequence of the series (e.g n = 1, 2, or 3 corresponding to the first, second or third term, respectively).

a = the value of concentration of the first term of the series = 0.01%.

r = the common ratio = common serial dilution factor =0.5

T_n = the value of concentration of the specific term in the sequence of the series.

$T_1 = 0.01 \times 0.5^{1-1} = 0.01 \times 0.5^0 = 0.01 \times 1 = 0.01\%$.

$T_2 = 0.01 \times 0.5^{2-1} = 0.01 \times 0.5^1 = 0.01 \times 0.5 = 0.005\%$.

$T_3 = 0.01 \times 0.5^{3-1} = 0.01 \times 0.5^2 = 0.01 \times 0.25 = 0.0025\%$.

$T_4 = 0.01 \times 0.5^{4-1} = 0.01 \times 0.5^3 = 0.01 \times 0.125 = 0.00125\%$.

$T_5 = 0.01 \times 0.5^{5-1} = 0.01 \times 0.5^4 = 0.01 \times 0.0625 = 0.000625\%$.

$T_6 = 0.01 \times 0.5^{6-1} = 0.01 \times 0.5^5 = 0.01 \times 0.03125 = 0.0003125\%$.

The Beer-Lambert's constant is given by the slope/gradient of the standard calibration curve of the solute/solution.

The Beer-Lambert's linear relationship between the absorbance (A) of white light by the solute/solution, at the wavelength of maximum absorption and the concentration of the solute/solution is given by A = acl. a = Beer-Lambert's constant. If l is 1cm, the Beer-Lambert's linear relationship is given by A = ac and is the equation of the standard calibration curve (a straight line, that passes through the origin).

The intensity of colour of alcoholic methyl red is a function and measure of its concentration and is directly proportional to its absorbance of white light, according to the Beer-Lambert's law.

Materials were dispensed according to the scheme shown in Table 1. The measurement of the absorbance of each of the solutions in the series was carried out by introducing the solutions into separate cuvettes and measuring the absorbance values using the 470-529nm colour-light filter, at which wavelength range, the alcoholic methyl red dye solution, absorbs white light, maximally.

A blank solution made of distilled water was used in zeroing the photoelectric colorimeter (AE 11 D), before each reading was taken. Ethanol contributed, insignificantly to the absorption of white light by the alcoholic methyl red, because ethanol absorbs very weakly at most wavelengths (Wikipedia, 2013).

Table 1. Schematic for the preparation of geometric progression (GP) dilution series of alcoholic methyl red dye solution.

Test tube	1	2	3	4	5	6	7 (blank)
Stock solution : 0.01% alcoholic methyl red dye /indicator solution (mls)	2	2	2	2	2	2	-
Distilled water (mls)	-	2	6	14	30	62	2
Final volume of solution (mls)	2	4	8	16	32	64	2
Concentration of alcoholic methyl red dye solution (%w/v)	0.01 ± 0.0001	0.005 ± 0.0001	0.0025 ± 0.0002	0.00125 ± 0.00001	0.000625 ± 0.00001	0.0003125 ± 0.00001	0.0 ± 0.0

Values of concentration of alcoholic methyl red dye solution are expressed as mean ± standard error (S.E) (%w/v)(n= no of replicates = 4).

Table 2. Absorbance and corresponding concentration of simulated treated waste water samples.

Sample absorbance ($A_{470\text{-}529\ nm}$)	**Sample concentration** (C) **%w/v**.
1± 0.01	0.00313± 0.0001
0.95± 0.01	0.003± 0.0001
0.96± 0.01	0.003± 0.0001
0.90± 0.01	0.0028± 0.0001
0.85± 0.02	0.0027± 0.0002
0.83± 0.01	0.0026± 0.0001
0.38± 0.03	0.0012^{a} ± 0.0002
0.41± 0.01	0.00128 ± 0.0001
0.15± 0.01	0.00047^{a} ± 0.0001
0.1± 0.02	0.0003125^{a} ± 0.0001

Values are mean ± standard error (S.E) (n = 4).
Mean values labelled with the superscript, a, are significantly lower ($p<0.05$) than 0.00125 ± 0.00001% w/v of alcoholic methyl red dye solution.

Application of the photoelectric colorimetric assay of alcoholic methyl red dye solution in a simulation model experiment.

The photoelectric colorimetric assay of alcoholic methyl red dye solution developed in the present study were used in determining the concentration of alcoholic methyl red dye solution present in ten (10) different simulations of treated waste water samples. The concentrations of the ten samples were simulation models of the concentration of waste-water, purified by means of decolorization of toxic azo dyes according to the technique employed by (Adebayo et al., 2004), who used bacterial mixed-cultures, namely, *Vibrio logei* and *Pseudomonas nitroreducens,* to degrade a toxic azo dye (methyl red), in a wastewater treatment plant by decolourization. Complete decolourization using the culture was achieved at pH 6, 30°C within 6 h at 5 mg/l methyl red concentration and 16 h at 20—30 mg/l methyl red. The decolourized dye was not toxic to a monkey kidney cell line (COS-7) at a concentration of 250 µM.

RESULTS

It was observed, as shown in figure 2, that the absorbance measurements on 0.01% alcoholic methyl red dye solution (pK_a: 4.76, 25°C), made at various wavelength ranges, had a peak value of 1.18. Invariably, the wavelength of maximum absorption of white light by alcoholic methyl red dye solution is in the range of 470-529 nm.

The standard concentration calibration curve of alcoholic methyl red dye solution is shown in figure 3 and is a straight line graph from the origin, whose equation is $A_{470\text{-}529\ nm} = 320C_{\%\ w/v}$, which is the Beer-Lambert's linear relationship between absorbance value ($A_{470\text{-}529\ nm}$) of alcoholic methyl red dye solution and its concentration ($C_{\%\ w/v}$), measured in % w/v (grams/100ml) solution. The value, 320 is the coefficient of $C_{\%\ w/v}$, which is the Beer-Lambert's constant and is the slope/gradient of the standard calibration curve of alcoholic methyl red dye solution.

The values of absorbance of ten (10) different simulated treated waste water samples, measured at 470-529 nm, using

the photoelectric colorimetric assay of alcoholic methyl red dye solution, is shown in Table 2, along with the corresponding concentration of alcoholic methyl red dye solution in the treated waste water samples and are derived from the equation of the standard calibration curve of alcoholic methyl red dye solution, which is $A_{470\text{-}529\ nm} = 320C_{\%\ w/v}$.

DISCUSSION

Alcoholic methyl red dye solution absorbs white light maximally at a wavelength range of 470-529nm as shown in figure 1. This finding is corroborated by those of Zhang et al. (2012), who postulated that UV-visible electronic absorption spectra of methyl red aqueous solutions are characterized by the overlap of a principal peak at λ_{max} (520±15) nm with a shoulder peak at λ_{max} (435±20) nm, which are assigned to acidic species (HMR) and basic species (MR^-) of methyl red, respectively. The standard concentration calibration curve of alcoholic methyl red dye solution shown in figure 3 is a straight line graph, plotted, using the serial dilution of 0.01% alcoholic methyl red dye solution, prepared according to a geometric progression sequence/series of values of concentration.

An essential deduction that can be made from the graph is that the Beer-Lambert's concentration limits of alcoholic methyl red dye solution, beyond which the Beer-Lambert's linear law ceases to apply to the alcoholic methyl red dye solution, is 0.0025% w/v. This is so because at values of concentration of alcoholic methyl red dye solution greater than 0.0025% w/v, the curve deviates from the Beer-Lambert's linear law of light absorbance by dilute solutions.

Application of the photoelectric colorimetric assay of alcoholic methyl red dye solution in a simulation model experiment.

If the efficiency of a given purification/water treatment step of a waste water effluent of a textile industry, polluted by alcoholic methyl red/methyl red, is measured as a function of a significant difference ($p < 0.05$), between the mean value of the concentration of the treated waste water samples and a given standard value of very dilute concentration of alcoholic methyl red dye solution, the photoelectric colorimetric assay of alcoholic methyl red dye solution developed as shown in the present study, could be employed in the qualitative analysis of the treated waste water.

For example, treated waste water samples from an effluent of a textile industry, polluted by alcoholic methyl red/methyl red, obtained after a given purification step, may be considered to be treated with at least, 90% efficiency, only if their values of concentration are significantly lower ($p<0.05$) than 0.00125 ± 0.00001% w/v of alcoholic methyl red dye solution (expressed as mean ± standard error). This postulate is comparable with the capacity of lignin degrading white rot fungi to remove methyl red from azo dye polluted waste water with greater than (>) 90% efficiency (Selvam et al., 2003: Selvam and Shanmuga Priya, 2012).

Results observed of the simulation model experiment, shown in Table 2, indicate that the treated waste water samples of alcoholic methyl red dye solution concentration: 0.0012 ± 0.0002, 0.00047 ± 0.0001 and 0.0003125 ± 0.0001 (mean ± standard error, % w/v), were considered to be treated with at least, 90% efficiency.

Furthermore, the photoelectric colorimetric assay of alcoholic methyl red dye solution could be used in determining the unknown concentration of bench alcoholic methyl red indicators, used in routine analysis, in laboratories.

CONCLUSION

The wavelength of maximum absorption of white light by alcoholic methyl red dye solution is in the range of 470-529 nm. The standard calibration curve of alcoholic methyl red dye solution is a straight line graph from the origin, whose equation is $A_{470\text{-}529\ nm} = 320C_{\%\ w/v}$. The Beer-Lambert's concentration limits of alcoholic methyl red dye solution is 0.0025% w/v.

The photoelectric colorimetric assay of alcoholic methyl red dye solution can be used in qualitative analysis of treated waste-water, polluted by methyl red dye solution and also in determining the unknown concentration of bench alcoholic methyl red indicators, used in routine analysis, in laboratories.

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