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# Novel Analytical Approach of Quantitative Spectrophotometry Intended for Quantitation of Absorbance Quenching Analyte Through Measurement of the Absorbing System's Permittance

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This paper proposed a new analytical method for quantitative spectrophotometry. In this study, the analyte's absorbance quenching capability towards the specific light absorbing system was utilized for quantitation of the analyte by measuring the system's transmittance. The absorbing system used was acidic 0.001 M $KMnO_4$ solution and the analytes quantitatively determined at 550 nm were nitrite, thiourea, hydroquinone, thiocyanate and tin(II). Contrary to Beer's law, it was found that for parallel monochromatic radiation that passes through a solution of an absorbing system of constant path length and concentration, the radiant power of transmitted beam increases exponentially as the concentration of absorbance quenching analyte increases arithmetically. The chemical reaction of an analyte with the absorbing system quenches the absorbance, resulting in the increase in the system's transparency which is termed in this paper as clearance, a termed designating the ratio of transmittance of test solution (absorbing system plus analyte) to transmittance of reagent blank solution (absorbing system only), measured at fixed wavelength in a cell of constant path length. The clearance of an absorbing system increases exponentially with arithmetic increase in the concentration of the analyte. The logarithm of clearance termed as permittance in this paper, is plotted *versus* the concentration of analyte for construction of the calibration curve. Though the suggested method employs techniques contrary to Beer's law, yet the efficacy and validity of the method has been demonstrated logically, mathematically and experimentally.

**Key Words: Absorbing system, Clearance, Permittance, Absorbance quenching analyte (Quencher analyte), Test solution, Reagent blank solution and True blank solution.**

## INTRODUCTION

The scientific literature of quantitative spectrophotometry illustrates Beer's law as the direct application for analyte quantitation, producing a coloured system through analyte's reaction with a suitable reagent. The colouring ability of the analyte is used for its quantitative determination, where the transmittance of the coloured system decreases exponentially with arithmetic increase in the concentration of analyte. The negative logarithm of transmittance, *i.e.* absorbance, is plotted *versus*

concentration of analyte for obtaining the calibration or working curve in the first quadrant of Cartesian coordinate system[1-6].

While Beer's law is based on the absorbance enhancing ability of the analyte, this study suggests a different analytical approach where the analyte's ability of quenching the absorbance of a specific light absorbing reagent is considered valuable for determining the analyte's concentration. The present study does not emphasize the decolourization of certain coloured reagent by some analyte but focuses on the decolourizing ability of the analyte for its quantitative determination. When the absorbance of the light absorbing system is quenched by the addition of any analyte, the conventional understanding of the Beer's law does not agree with situations where absorbance decreases with increase in the concentration of analyte. Therefore, to address this contradict situation, certain adjustments need to be made at mathematical and graphical level, so as to formulate the term permittance for obtaining a calibration curve and extending experimentally the applications of quantitative spectrophotometry.

The proposed method uses the same basic instrumentation of photometric measurement and instrument output for per cent transmittance (% T) as in Beer's law. However, it restricts using relationship of absorbance (-log T) with concentration of the analyte, since it generates the relationship of analyte's concentration with the logarithm of the transmittance ratio of the test solution (absorbing reagent plus analyte) and reagent blank solution (absorbing reagent). Therfore, to resolve this dilemma new terms, *viz.*, clearance (Cr) and permittance (Pr) are more useful.

For the verification of the proposed methodology, the absorbance quenching ability of nitrite (analyte) toward the acidic solution of potassium permanganate (absorbing system) served as a model system for the quantitative determination of nitrite. In this quantitative determination a fixed volume aliquots of nitrite solution with increasing concentration (2.0 to 20.0 ppm) were added to fixed and measured volumes (25.0 mL) of acidic 0.001 M $KMnO_4$ reagent. The transmittance measurement at 550 nm of these different solutions showed that the acidic solution of permanganate system of constant path-length and concentration exhibited exponential increase in transmittance with arithmetic increase in nitrite concentration (Fig. 1). Beer's law does not address this contrasting relationship of transmittance with the concentration of the analyte, which is therefore open for further research as this manuscript shows. Following discussion not only addresses this issue but also suggests how to obtain linear proportionality for determination of analyte concentration.

While obtaining the calibration curve for determination of the concentration of analyte (nitrite), the logarithm of transmittance of absorbing system was plotted *versus* concentration. This gave a straight line in the fourth quadrant of the Cartesian coordinate system as in Beer's law. However, it showed intercept on the negative Y-axis. To transfer it to the first quadrant, the logarithm of percent transmittance against the concentration of analyte was plotted, this gave the straight line with intercept on positive Y-axis (Fig. 1).

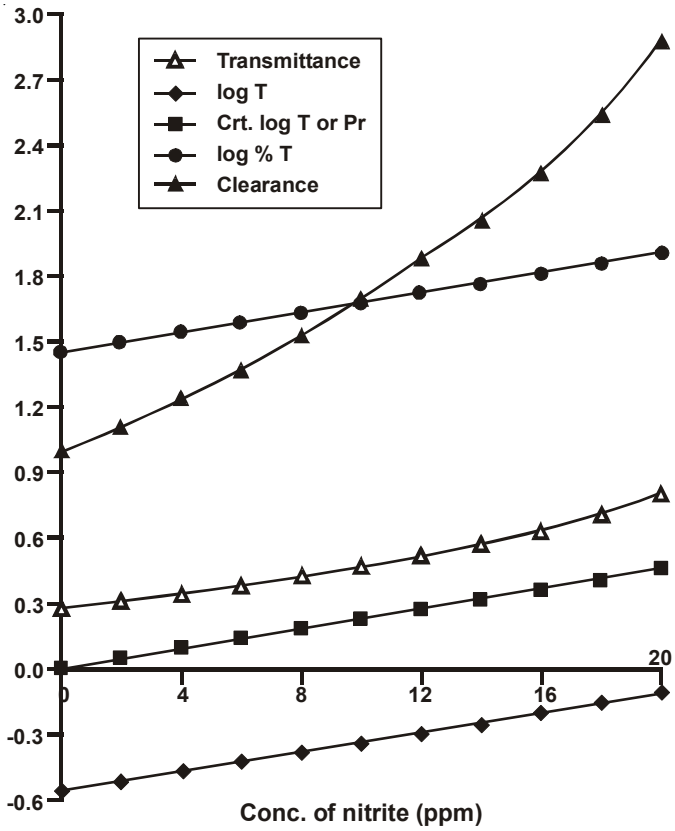


Fig. 1. Development and verification of proposed analytical methodology, the graph obtained in the quantitative determination of nitrite using acidified 0.001 M $KMnO_4$ absorbing system at 550 nm. The graph showing the relationship of clearance, log % T, transmittance, Crt. log T (or Pr) and log T with the concentration of the analyte.

Before going ahead, some things need to be clarified. The solution of absorbing system containing absorbance quenching analyte is termed as the test solution (TS) and that without analyte is termed as the reagent blank (RB). In this new method, the per cent transmittance (% T) of both test solution and reagent blank were measured against the solution of true blank (TB) as a reference. In Beer's law, blank solution without analyte functions as a reference[1-6] to nullify background signal (absorbance). In the proposed method, the clearest solution containing excess of the analyte serves as the blank or reference, showing 100 % T at the wavelength of analysis and is referred to as true blank in order to distinguish it from reagent blank mentioned above.

When the corrected logarithms of transmittance (Crt. log T) of the test solutions were plotted *versus* concentration of analyte, a straight line passing through the origin of the first quadrant was obtained, showing that Crt. log T of the absorbing

system is directly proportional to the concentration of the analyte (Fig. 1). The Crt. log T of each test solution was obtained as shown below:

$$\text{Crt. log T} = [\log \%T_{TS}] - [\log \% T_{RB}] \quad (1)$$

$$\text{Crt.log T} = \log \frac{\%T_{TS}}{\%T_{RB}} \quad (2)$$

The absorbance of the system was quenched because of the oxidation-reduction reaction between analyte and absorbing system. Therefore, % $T_{TS}$ > % $T_{RB}$ and hence the Crt. log T of each test solution is a positive number. For the purpose of usage, a new term permittance (Pr) is introduced for Crt. log T and used throughout the studies.

Eqn. 2 can further be simplified as below.

$$\text{Pr} = \log \frac{T_{TS}}{T_{RB}} \quad (3)$$

The permittance of the absorbing system can thus be defined as the logarithm of the ratio of the transmittance of the test solution and to the transmittance of the reagent blank solution measured at a fixed wavelength in a cuvette of constant path-length. The ratio $T_{TS}$ to $T_{RB}$ is termed as clearance (Cr).

The transmittance[1-6] in Beer's law is given as below:

$$T = \frac{P_{Sample}}{P_{oBlank}} \quad (4)$$

In the new method the transmittance of test solution (TS) and reagent blank (RB) were measured using true blank (TB) as a reference. Therefore eqn. 3 is further written for permittance (Pr) as below.

$$\text{Pr} = \log \frac{P_{TS} / P_{o\,TB}}{P_{RB} / P_{o\,TB}} \quad (5)$$

That is,

$$\text{Pr} = \log \frac{P_{Ts}}{P_{RB}} \quad (6)$$

Now permittance can also be defined as the logarithm of the ratio of radiant power in a beam of radiation after it has passed through the test solution ($P_{TS}$) to the power in a beam that has passed through reagent blank ($P_{RB}$). The ratio of $P_{TS}$ to $P_{RB}$ is termed here as clearance (Cr) of the absorbing system which may also be defined as the ratio of radiant power in a beam of radiation after it has passed through the test solution to the power in a beam after it has passed through the reagent blank solution measured at fixed wavelength in a cell of constant path length. All the equations which involve the logarithmic functions are written with base-10 logarithm.

The clearance of the absorbing system orignates at the value of 1.0 on the Y-axis and increases exponentially with arithmetic increase in the concentration of quencher analyte (Fig. 1). Therefore the logarithm of clearance *i.e.* permittance, shows the direct relationship with concentration of the analyte given as below:

$$Pr = a\ b\ c_{g/L} \quad \text{or} \quad Pr = \varepsilon\ b\ c_{mol/L} \tag{7}$$

Eqn. 7 is used for quantitative determination of the analyte that quenches the absorbance of the absorbing system. This equation imitates and yet differs a lot from the following eqn. 8 that represents Beer's law.

$$A = a\ b\ c_{g/L} \quad \text{or} \quad A = \varepsilon\ b\ c_{mol/L} \tag{8}$$

In both eqn. 7 and 8, the proportionality constants, absorptivity 'a' or molar absorptivity 'ε' and path-length of the absorbing system 'b' are constant under the condition of experiment. The value of 'a' or 'ε' of the absorbing system is governed by the wavelength selected for analysis and is greatest at that wavelength where the system shows maximum absorbance ($\lambda_{max}$). For quantitative estimation of analyte the $\lambda_{max}$ of absorbing system is preferred in the proposed methodology as well since even a small amount of the analyte that quenches the absorbance may be detected for quantitation of trace concentration of the analyte.

## EXPERIMENTAL

The percentage transmittance was measured using Shimadzu Pharmaspec UV-1700 double beam UV-Visible spectrophotometer with 1 cm quartz cuvettes.

**Preparation of standard stock solutions:** (i) 1.0 L of 0.01 M $KMnO_4$ solution was prepared by dissolving accurately weighed quantity of AR grade $KMnO_4$ in distilled water. (ii) 2.0 L of 1.0 M $H_2SO_4$ solution was prepared by diluting the accurate volume of AR grade concentrated $H_2SO_4$ in distilled water. Solutions (i) and (ii) were used for preparation of absorbing system/reagent.

**Preparation of standard stock solutions of analytes:** A 100 ppm stock solutions for thiourea, hydroquinone, thiocyanate (from KSCN), nitrite (from $NaNO_2$), tin(II) (from $SnCl_2$) were prepared by accurately weighing the required quantity of each compound and dissolving it in distilled water and further diluting to 1.0 L with distilled water. The solution of tin(II) was prepared by adding 10 mL conc. $H_2SO_4$ in $SnCl_2$ salt just before it was required. Similarly 10 mL acetone was used for dissolving hydroquinone. Both tin(II) and hydroquinone solutions were diluted to 1.0 L using distilled water. All the solutions were stored in airtight flasks and protected from light.

**Preparation of absorbing system/reagent:** A 0.001 M potassium permanganate absorbing system was prepared by mixing 100 mL of 0.01 M $KMnO_4$ solution with 400 mL of 1.0 M $H_2SO_4$ and then diluted to 1.0 L using distilled water.

**Analytical methodology:** The proposed method was first applied for quantitative determination of nitrite using acidic permanganate absorbing system. The standard or test solutions (TS) of nitrite within the concentration range 2.0 to 20.0 ppm were prepared with 25.0 mL acidified 0.001 M $KMnO_4$ solution. During the preparation

of standard solutions different aliquots of 100 ppm nitrite solutions were added drop wise into 100 mL graduated flasks, each containing 25.0 mL of permanganate absorbing system. The reaction mixtures were mixed thoroughly and kept for 15 min at room temperature and further diluted to 100 mL using distilled water. The reagent blank (RB) solution was also prepared by diluting 25.0 mL acidic 0.001 M permanganate absorbing system to 100 mL in a graduate flask. For preparation of true blank (TB) solution the excess of nitrite solution (50 ppm in 100 mL dilution) was added to 25.0 mL of absorbing system till its complete decolourization and then diluted to 100 mL using distilled water. The % T of each test solution and also reagent blank was measured only once at 550 nm against true blank as a reference. The % T of the reagent blank was used for obtaining clearance of each solution. The graphs of clearance and pemittance *versus* concentration of analyte were prepared for testing the validity and linearity of the proposed method.

Furthermore the suggested method was pursued for quantitative determination of hydroquinone, thiourea, thiocyanate and tin(II) using 25.0 mL of 0.001 M acidic permanganate absorbing system at 550 nm in a 50 mL dilution.

## RESULTS AND DISCUSSION

Most of the times, in Beer's law, the analyte is quantitatively determined by producing a colour system through its reaction with a suitable reagent. The absorbance enhancing ability of the analyte determines the magnitude of optical density or absorbance which is directly proportional to the concentration of the analyte. However, in the suggested method the analyte's absorbance quenching capability towards a specific light absorbing system was utilized for analyte quantitation, where the analyte's chemical reaction with the absorbing system decreases absorbance. The extent of decrease in absorbance of the absorbing system is measured as permittance for quantitative determination of quencher analyte.

The absorbing system is the specific reagent that absorbs in the visible or ultraviolet spectrum of electromagnetic radiation and whose absorbance is quenched by addition of an analyte through chemical reaction. Essentially the $\lambda_{max}$ of the absorbing system must be identical before and after the reaction with analyte. It should be tested first that the absorbance of the system has not been quenched by the acids and other reagents if they are used during sample preparation as well as by the other sample matrix present along with the analyte. The concentration of the absorbing system was chosen by considering the concentration of analyte in the sample solution.

An acidic solution of permanganate acts as a powerful oxidizing reagent[6] and therefore it is a good absorbing system at 550 nm ($\lambda_{max}$) for quantitative determination of reducing analytes such as nitrite, thiocyanate, thiourea, hydroquinone and tin(II). The overall determination of these analytes depends on the course of redox reaction, in which the purple coloured permanganate solution is reduced to colourless manganese solution. The extent of quenching of absorbance (or the fading of the colour of permanganate system) is associated with the concentration of analyte. At room

temperature, these redox reactions do not take place instantaneously. Therefore, the reaction mixtures were left for 15 min at room temperature, then diluting with distilled water the per cent transmittance was measured only once at 550 nm.

TABLE-1
RESULTS OBTAINED IN THE IMPLEMENTATION OF SUGGESTED ANALYTICAL METHODOLOGY IN THE QUANTITATIVE DETERMINATION OF NITRITE, AT 550 nm USING ACIDIC 25.0 mL OF 0.001 M $KMnO_4$ ABSORBING SYSTEM IN 100 mL DILUTION

| ppm conc. of nitrile (Crt. log T) | % T | T | log % T | log T | Cr | Pr |
|---|---|---|---|---|---|---|
| 0.0 RB | 27.82 | 0.2782 | 1.4444 | -0.5557 | 1.000 | 0.0000 |
| 2.0 | 30.91 | 0.3091 | 1.4901 | -0.5099 | 1.111 | 0.0457 |
| 4.0 | 34.51 | 0.3451 | 1.5379 | -0.4621 | 1.240 | 0.0935 |
| 6.0 | 38.21 | 0.3821 | 1.5822 | -0.4178 | 1.374 | 0.1378 |
| 8.0 | 42.42 | 0.4242 | 1.6275 | -0.3724 | 1.524 | 0.1831 |
| 10.0 | 47.12 | 0.4712 | 1.6732 | -0.3268 | 1.694 | 0.2288 |
| 12.0 | 52.21 | 0.5221 | 1.7178 | -0.2823 | 1.877 | 0.2734 |
| 14.0 | 57.32 | 0.5732 | 1.7583 | -0.2417 | 2.060 | 0.3139 |
| 16.0 | 63.12 | 0.6312 | 1.8001 | -0.1998 | 2.268 | 0.3557 |
| 18.0 | 70.61 | 0.7061 | 1.8489 | -0.1512 | 2.538 | 0.4045 |
| 20.0 | 79.81 | 0.7981 | 1.9021 | -0.0979 | 2.869 | 0.4577 |

The acidic permanganate solution oxidizes nitrite to nitrate through self decolourization and nitrate does not decolourize the permanganate solution[7]. The hydroquinone is also oxidized to quinone by the acidic solution of permanganate[8]. It was tested before hand that acetone does not decolourize the permanganate solution; hence acetone was used for dissolution of hydroquinone during the sample preparation. Likewise the acidified solution of permanganate oxidizes Sn(II) to Sn(IV) and thiocyanate to HCN at room temperature with decolourization of absorbing system. Thiourea is sulphur containing organic carcinogenic substance[9] oxidizes to urea and $H_2S$ by the acidic permanganate solution. The evolution of $H_2S$ was confirmed by moist lead acetate paper and it was also tested that urea does not react with acidified permanganate solution at these conditions of the experiment. These underlying concepts were utilized for quantitation of analytes.

Though each of these redox reactions are not instantaneous and can not be quantitatively completed within the period of 15 min, yet each of the analyte was determined quantitatively since spectrophotometric methods are a result of comparative study. The acidified $KMnO_4$ absorbing system with the volume 25.0 mL and the concentration 0.001 M does not deviate from its linearity when analyte concentration was measured within the range of 2.0 to 20.0 ppm.

The quantitative determination of analyte is based on the reduction of permanganate solution; therefore in each determination, interference is produced if the sample matrix contains the reducing substances.

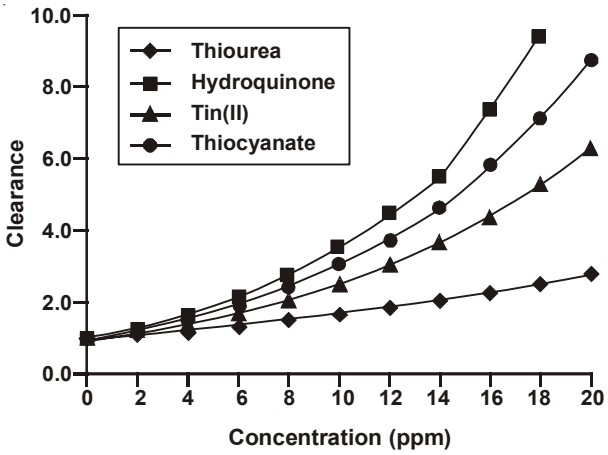


Fig. 2. Proposed methodology was pursued for quantitative determination of hydroquinone, thiourea, thiocyanate and tin(II) using 0.001 M permanganate absorbing system at 550 nm. The clearance was determined experimentally and plotted against the concentration of the analyte

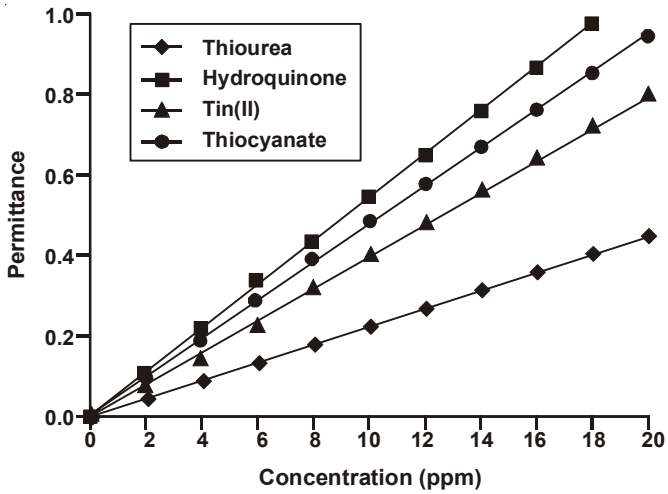


Fig. 3. Calibration curves of permittance *versus* concentration show that the permittance of the absorbing system increases linearly with the concentration of analyte

## Conclusion

The main goal of the proposed analytical methodology is to extend and or enhance the methods for quantitative spectrophotometry. This method measures the absorbance quenching capability of the analyte towards a specific light absorbing system for analyte quantitation. The extent of decrease in absorbance (because of

the chemical reaction of analyte with absorbing system) of the absorbing system is determined as permittance for quantitative determination of quencher analyte. Thus, the suggested analytical method attempts to broaden the scope of quantitative spectrophotometry. It works well for those analytes which cannot be quantitatively determined by forming a chromophoric system using Beer's law.

In Beer's law, the analyte's absorbance enhancing ability with the specific reagent determines the magnitude of optical density or absorbance which is directly proportional to the concentration of the analyte (eqn. 8). However, in the proposed method the analyte's absorbance quenching capability towards the specific light absorbing reagent determines the magnitude of permittance. Permittance is the inability of test solution to absorb the light with respect to reagent blank solution which is directly proportional to the concentration of the quencher analyte (eqn. 7).

The absorbance in Beer's law and permittance in the suggested method are contrasting parameters used for analyte quantitation. The former measures the concentration of absorbance enhancing analyte while the latter quencher analyte. This novel analytical approach of photometric analysis uses the already formed absorbing system and deals with quantitation of analyte by the measurement of clearance and permittance through the measurement of the system's transmittance, rather than its absorbance. For the absorbing system, the clearance is closely related with the transmittance (in Beer's law) and graphically shows the contrasting relationship with the concentration of analyte. Furthermore permittance is a logarithm of clearance and absorbance is the negative logarithm of transmittance.

In Beer's law the 'ε' value for the blank solution or reference is zero since it does not absorb at the wavelength selected for measurement and it is maximum for the solution with the greatest concentration of the absorbance enhancing analyte. While in the proposed method the 'ε' value is maximum for the absorbing system *i.e.* the reagent blank (RB) solution though its permittance is zero. The 'ε' value decreases with increase in the concentration of the quencher analyte in the absorbing system and it becomes zero for true blank (TB) solution containing highest concentration of quencher analyte.

Because of all these contrasts with Beer's law the suggested method may be called as Anti-Beer's Law, though both the laws evaluate the equivalent instrumentation outcome and are associated with similar types of merits and demerits.

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