A NEW METHOD OF ESTIMATION OF *m*-DINITROBENZENE BY POTASSIUM CYANIDE REAGENT

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ABSTRACT

m-Dinitrobenzene (*m*-DNB), a toxic chemical, was estimated by potassium cyanide (KCN) reagent in (0.1 to 1 mg) level. It was also assayed in presence of other organic compounds after separating it by thin layer chromatography. Both the assay methods were analysed statistically.

INTRODUCTION

M-DINITROBENZENE, (*m*-DNB) a toxic chemical, belongs to nitro-aromatic compounds[1]. In the *micro-quantitation of this compound Janovsky reaction*[2] is essentially employed wherein alkali acetone mixture is used to develop the colour. The colour complexes of acetone alkali with nitroaromatic compounds have been examined to study their stability [3,4] for qualitative and quantitative[5-8] detection of these compounds. Zimmerman[9] also developed a colorimetric method using 17 keto steroids and *m*-DNB in ethanolic KOH. A close study of these methods reveals that the colour complexes formed in all these are generally unstable and the colour development is rather cumbersome involving use of a number of reagents. Moreover only pure *m*-DNB can be estimated by these methods. Attempts were therefore made to develop a simple colorimetric method to estimate *m*-DNB as a pure compound as well as in the presence of other organic compounds. Following is the report of the investigation.

MATERIALS AND METHODS

A stock solution of *m*-DNB–0.01 g in 100 ml was prepared by boiling for a few minutes.

A stock solution of KCN was prepared by dissolving 10 g KCN in 100 ml distilled water.

An aliquot of *m*-DNB solution (10 ml) and 1 ml of 10% KCN were taken in clean dry test tube and heated in a boiling water bath for 10 min, cooled to room temperature and the intensity of the brown-violet colour was measured at 540 nm, against reagent blank using Spectronic 20 (Bausch and Lamb).

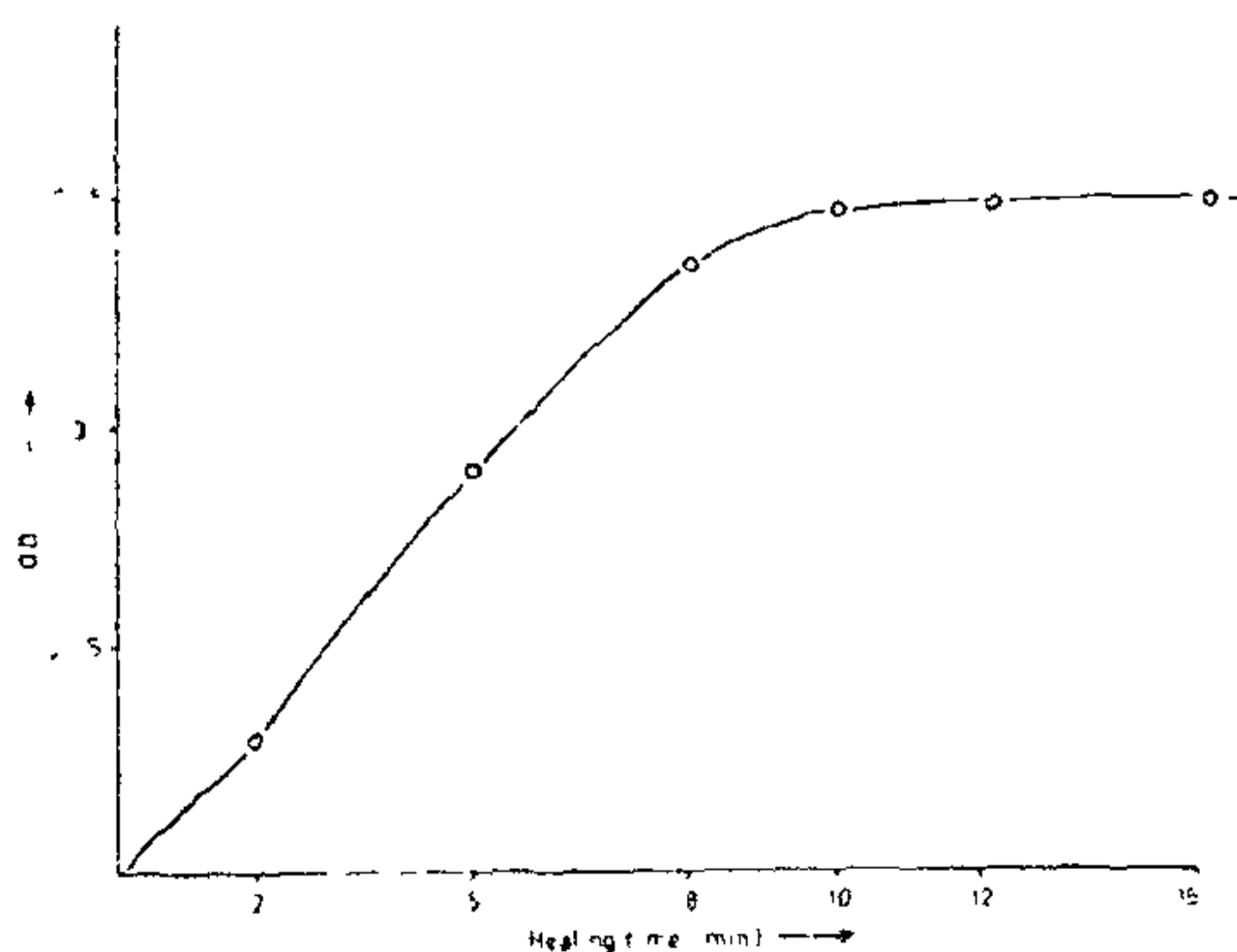The specificity, stability, photosensitivity and maximum absorption band (λ_{max}) of the colour complex was also checked. The sensitivity of the colour was checked at different pH levels namely 5 to 11 and the optimum heating time was also observed. For quantitation, different concentrations of *m*-DNB ranging between 0.1 and 1 mg were treated with 10% KCN and the colour was measured at 540 nm. To ensure the accuracy and precision of the method, different solutions of *m*-DNB of known strength were examined by this method and the data compared with the actual concentrations and analysed statistically by small sample *t* test[10]. To measure *m*-DNB from a mixture of other nitroaromatics and other organic compounds, *m*-DNB was first separated from the other impurities by preparative thin layer chromatography[11] using benzene:ethyl acetate (75:25) as solvent system and then estimated colorimetrically. Sensitivity and reproducibility of the combined method were also compared with direct colorimetric method using different concentrations (5-10 mg) and analysed statistically.

RESULTS AND DISCUSSION

Different nitro compounds produce different shades with KCN solution which are given in table 1 except for TNT which gives similar shade as of *m*-DNB but differs in λ_{max} and in reaction with 1 N HCl[12]. The identification limit for *m*-DNB is 5 μg. It is evident from figures 1 and 2 that addition of 1 ml of KCN and 10 min of heating is optimum for colour development. The colour complex is not photosensitive, and is stable at 4°C and 26 ± 2°C for 48 hr and over a wide range of pH (table 2). Figure 3 shows that maximum absorption occurs at 540 nm. The linear curve seen in figure 4 suggests that the intensity of the colour formed at 540 nm is concentration-dependent indicating that the reaction obeys Beer Lambert's law. The results of the quantification of standard solutions of *m*-DNB by this method are given in table 3 and figure 4 which show

Table 1 *Colour complexes formed by different nitroaromatic compounds with KCN*

Name of the Compounds	Solvent	Limit of identification μg	Colour	Maximum absorption band (λ_{max})
m-Dinitrobenzene	Water Acetone	5	Brown Violet	540 nm
o-Nitrophenol	Water	5	Yellow (low) Orange (high)	410 nm
p-Nitrophenol	Water	5	Lemon yellow	400 nm
Dinitrotoluene	Acetone	2	Blue	620 nm
Picric acid	Acetone	5	Pink	525 nm
Trinitrotoluene	Acetone	5	Dark brownish violet	No sharp peak
m-Nitrophenol	Water	—	No colour	—
p-Dinitrobenzene	Acetone	—	No colour	—
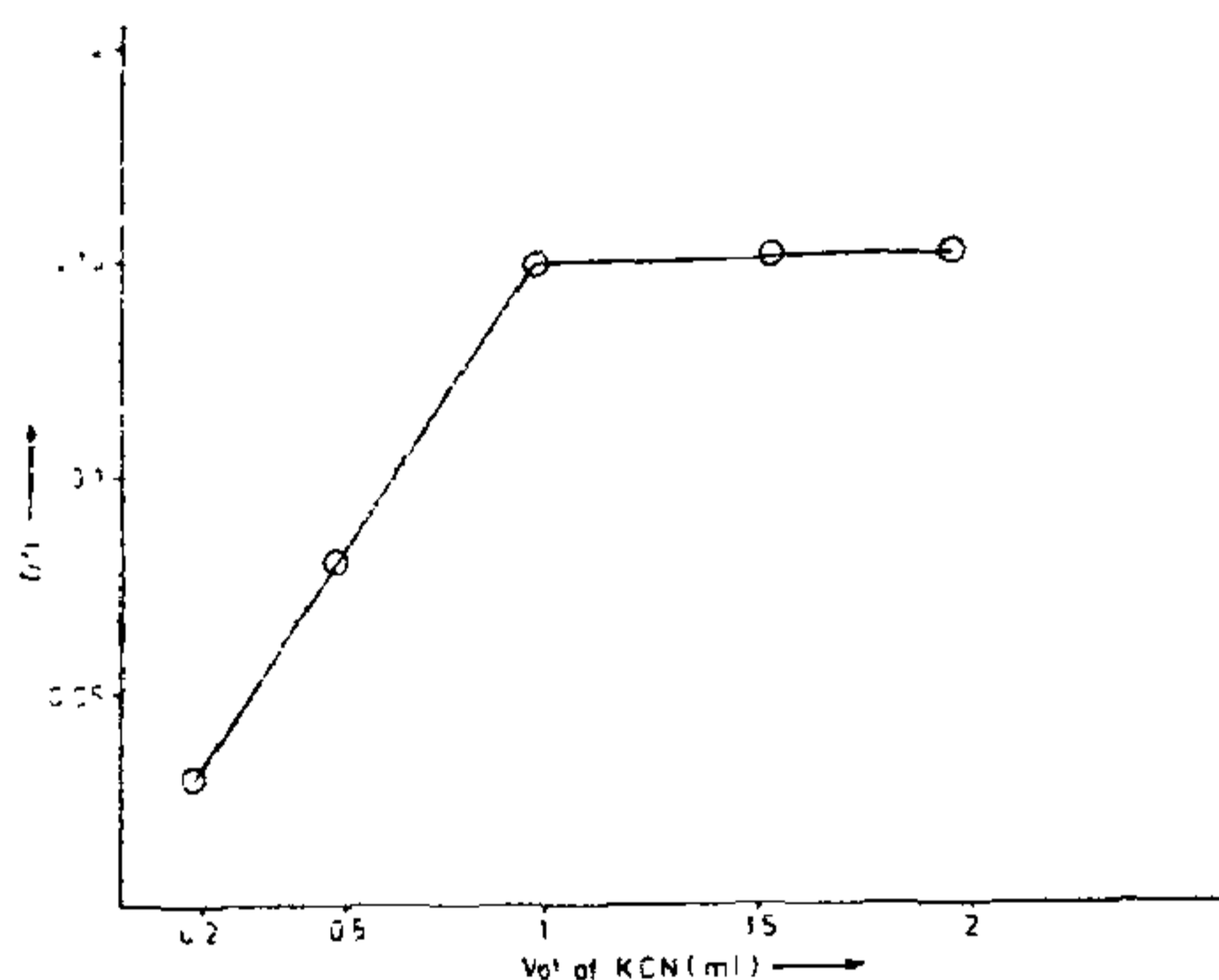
Figure 1. Effect on colour intensity of a variation in KCN concentration

Table 2 *Effect on colour intensity on variation of pH*

pH	m-DNB
5	0.12
6	0.13
7	0.12
8	0.12
9	0.13
10	0.13
11	0.12
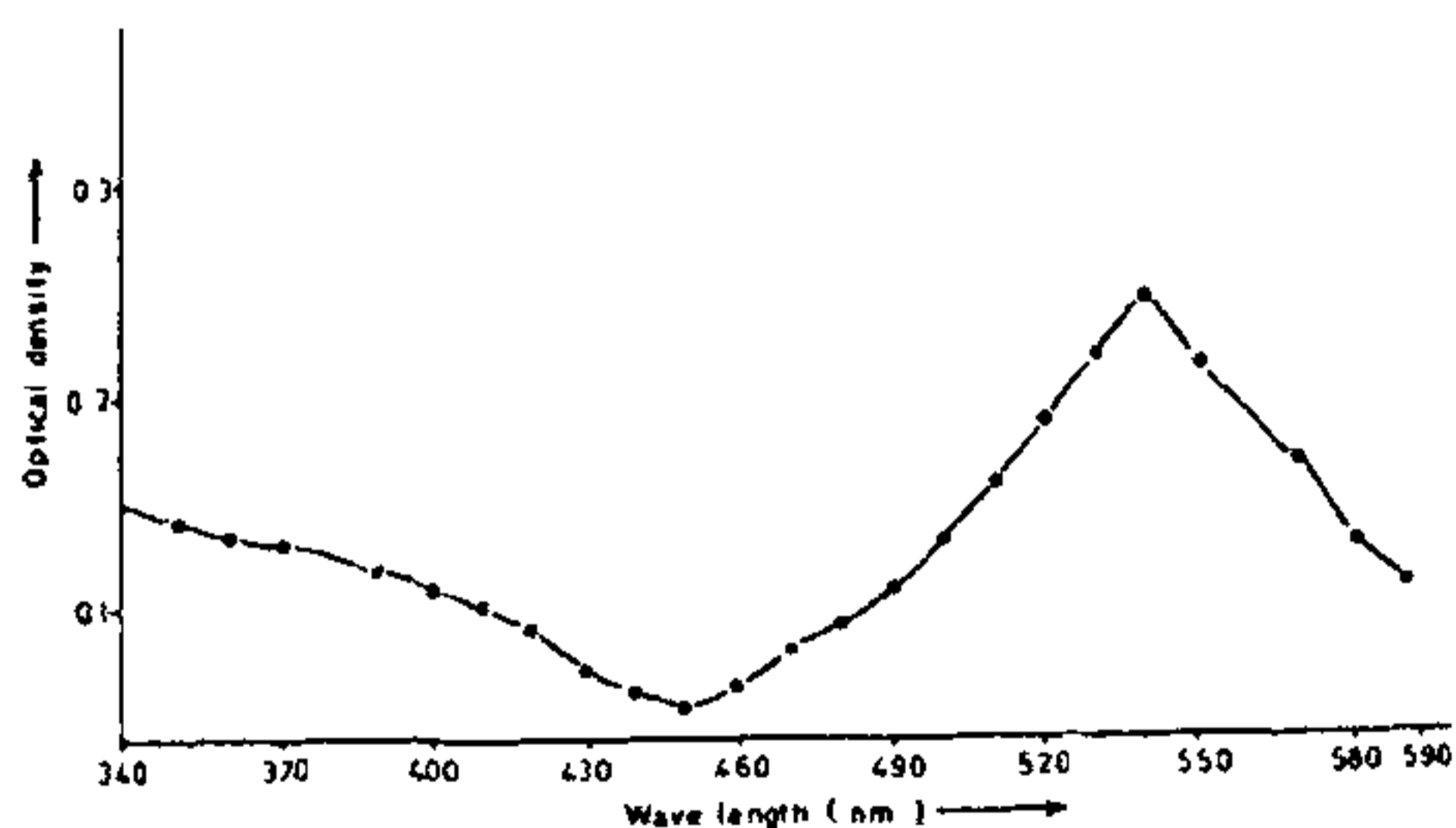
Figure 3. The absorbance values of m-DNB-KCN complex at different wave lengths.

Figure 2. Effect on colour intensity of a variation of heating time

that the strengths of m-DNB solutions obtained through estimation were nearly the same as the original concentrations. The marginal difference in value is found to be insignificant statistically. The Rf value of m-DNB was 0.51 while the other nitroaromatics had a lower value except for orthonitrophenol which show higher R_f value (0.81). Table 4 shows that both the methods are quite sensitive and
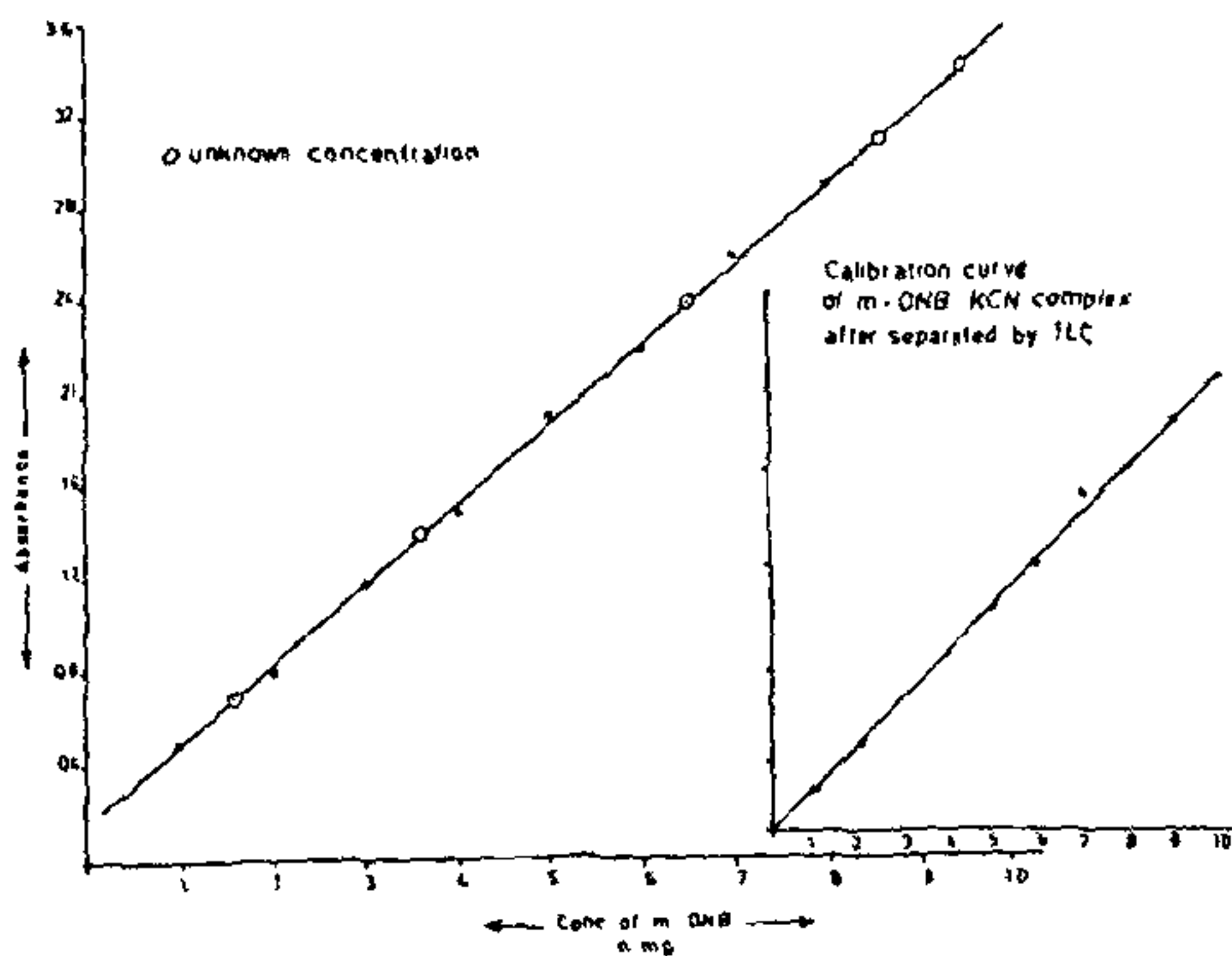
Figure 4. Spectro colorimetric estimation of m-DNB (0.1 to 1 mg) and (inset) of same amount of m-DNB after separation by TLC.

1
2 } Samples of DNB waste

Figure 5. Separation of m-DNB from wastes containing other nitro aromatics.

Table 3 *Absorbance values of known concentrations of m-DNB from the curve and statistical analysis*

Actual conc. (mg/ml)	Absorbance	Conc. from curves (mg/ml)
0.15	0.07	0.16
0.35	0.14	0.36
0.65	0.24	0.65
0.85	0.31	0.86
0.95	0.34	0.95
0.59		0.596

Small sample t value = 0

accurate. The present method will be useful for the rapid assay of m-DNB at milligram level in water as pure compound as well as in presence of other organic compounds.

The constitution of the colour complex is partly known [12]. Presumably this is either substituted phenyl hydroxylamine which in turn changes to alkali salts of a dibasic orthoquinoidal nitrogen acid[13,14].

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Table 4 *Percentage recovery of m-DNB by two methods and statistical data on the comparison between two methods*

Sample No.	Conc. of m-DNB mg	Conc of m-DNB mg estimated by Col	Conc of m-DNB mg estimated by TLC+Col	% recovery Col	% recovery TLC+Col	Mean difference d	Std. error of difference	t Theoretical	1% Exp.
1	5	49	4.5	98	90	—	—	—	—
2	5	5.0	4.7	100	94			3.25	2.659
3	6	5.9	5.9	98.3	98 3				
4	6	59	58	98.3	96 6	3 89	4.389		
5	8	7.8	7.2	97.5	90				ie
6	8	8 0	76	100	95				insignifi-cant
7	9	8.9	8.8	98.7	97.7				
8	9	89	8.6	98.7	95.5				
9	10	9.7	9.5	97	95				
10	10	10.0	9.6	100	90				

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The Indian National Science Academy invites applications from deserving scientists for extending them partial financial support to participate in important international scientific conferences/symposia abroad during 1986–87. Scientists interested to avail of financial support from the Academy for international conferences to be held during 1986–87 are requested to apply in the prescribed proforma which can be obtained from the Office of the Executive Secretary, Indian National Science Academy, Bahadur Shah Zafar Marg, New Delhi-110 002 by sending a stamped self addressed envelope size (9″ × 4″).

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(a) His/Her age should be 35 years or below on the date of commencement of the conference.

(b) His/Her paper should be accepted.

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