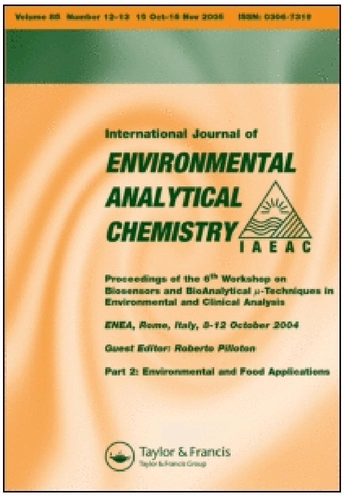

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A. Joshi[a]; P. Tewari[a]; S. K. Khanna[a]; G. B. Singh[a]
[a] Industrial Toxicology Research Centre, Lucknow, India

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A Simple Test to Monitor Aromatic Amine Clearance in Occupational Workers

A. JOSHI, P. TEWARI, S. K. KHANNA† and G. B. SINGH

Industrial Toxicology Research Centre, Post Box 80, Mahatma Gandhi Marg, Lucknow 226001, India

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A simple, quick and sensitive procedure is described to monitor urinary clearance of aromatic amines in industrial workers. Estimation is based on the measurement of coloured Schiff's bases developed with 4-dimethylaminocinnamaldehyde-organic acid reagent. Extraction of amines in water saturated butanol ensured 89–97% recovery from biological samples with a coefficient of variation of less than 8%. Regression analyses of the absorbance-concentration curves with different aromatic amines showed excellent correlation coefficient and a high molar absorptivity with the lowest detection limits ranging between 0.05 0.12 μg ml^{-1}. The proposed reagent has additional use as a spray reagent to detect amine spots on chromatograms. Low final acidity ensures estimates of free amines without causing hydrolysis of conjugated amines.

KEY WORDS: Aromatic amines, urinary clearance, modified DMAC reagent, estimation method.

INTRODUCTION

Aromatic amines find extensive use in the manufacture of several dyes, drugs, pesticides, plastics and as antioxidants in the rubber and cable industry. A number of toxicological symptoms including

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†To whom all the correspondence should be addressed.

incidences of human bladder cancer have been found to be associated with occupational exposure to some aromatic amines.[1-3]

Epidemiological studies on dyes and other aromatic nitro-amino (ANA) plants in Japan have shown a higher urinary excretion rate of aromatic amines in the exposed workers.[4,5] In many cases, estimates of human exposure to aromatic amines have been assessed by the conventional Bratton-Marshall's[6] diazotization reaction. This method is somewhat tedious, involves a number of steps and necessitates fresh reagents each time. Thus the need for a simple but reasonably sensitive monitoring test was felt and the same is being described in this communication.

The proposed method, requiring single step colour development, is several folds more sensitive than the Bratton-Marshall reaction and can detect as low as 0.05–0.12 μg ml^{-1} of aromatic amines.

EXPERIMENTAL

Chemicals

4-Aminodiphenylamine, 3-aminopyrene and 4-dimethylaminocinnamaldehyde (DMAC) were procured from Sigma Chemical Co., St. Louis, Missouri, U.S.A. Benzidine and maleic acid were the respective products of Reanal, Budapest, Hungary and E. Merck, Darmstadt, Germany. 4-Aminoanisole, 1-naphthylamine and 1,2-phenylenediamine were the products of BDH Chemicals, Poole, England. All the other chemicals used were of the analytical reagent grades.

Preparation of DMAC reagent

The DMAC-organic acid reagent was prepared by dissolving 200 mg DMAC and 0.06 *N* either one of the three acids, namely, citric (420 mg), maleic (348 mg) or oxalic (378 mg) in 100 ml of methanol.

Extraction of aromatic amines from biological samples

Aromatic amines were extracted from the fortified urine, serum and tissue homogenates of liver, lungs, kidney, testes, spleen and skin, in two successive steps with 2.5 volumes of freshly prepared water saturated *n*-butanol each time (total 5 volumes). The contents were vertexed thoroughly and centrifuged for the faster separation of aqueous and organic phases. The upper butanolic layer was separated. Non-fortified samples were treated likewise to serve as appropriate control blanks.

Measurement of colour

An aliquot of 0.1 to 0.5 ml of water saturated butanol containing extracted amines was mixed with 0.5 ml of DMAC reagent. The contents were swirled vigorously for proper mixing, diluted to 3 ml with methanol and kept for 15 minutes at room temperature for final colour development. The intensity of the reddish-brown Schiff's base was measured at 540 nm for routine analysis using a Pye Unicam SP 8-200 UV/VIS double beam spectrophotometer. For precise estimates, however, the absorbance needs to be measured at the wavelength of maximum absorption of the amine in question.

Effect of some variables

To obtain optimum conditions for the proposed method, the effect of some variables on the overall development of coloured base with a typical amine were studied. DMAC concentrations ranging from 2–1000 μg; acid strength between 5×10^{-5} to 1×10^{-1} *N*; choice of acids such as HCl, and organic acids, namely, acetic, boric, citric, maleic, malic, oxalic, succinic and tartaric and contact time of reactants (2–60 min) were tested.

Stability constants and stoichiometric determinants

The apparent stability constant of Schiff's base was determined by the procedure of Harvey and Manning.[7] The stoichiometry of the reaction with monoamine (4-aminodiphenylamine) and diamine (benzidine) was studied by the continuous variation method.[8]

RESULTS

Selection of DMAC concentrations

For a given concentration of 4-aminodiphenylamine (1 μg ml^{-1}), keeping a fixed final acidity of 0.01 *N*, it was found that concentrations of DMAC between 2 to 100 μg per 3 ml of assay system showed a non-linear increase in the absorbance which thereafter formed a plateau between 100 to 1000 μg (Figure 1). In order to prefer a zero order kinetics with a non-limiting zone of DMAC for any amine, a maximum concentration of 1 mg DMAC per reaction tube was chosen.

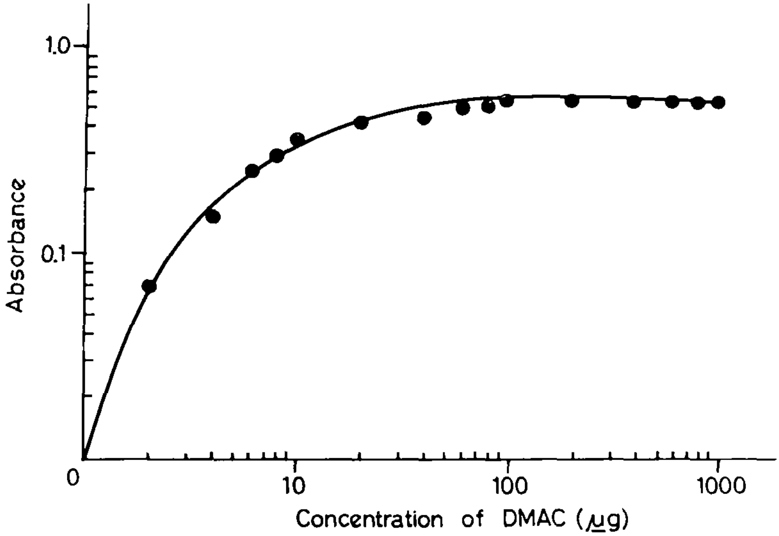

FIGURE 1 Relationship between DMAC concentrations and Schiff's base formation.

Choice of acids and their optimum strength

Keeping a non-limiting concentration of DMAC (1 mg), the effect of HCl and a number of organic acids on the formation of Schiff's base revealed that citric, maleic and oxalic acids gave identical and optimum results (Figure 2). In the case of HCl, concentrations beyond 1×10^{-3} *N* drastically diminished the final colour intensity. Among other organic acids, boric acid was almost ineffective at all the concentrations tested (Figure 2) whereas other acids, though contributed towards colour development, had lower sensitivity.

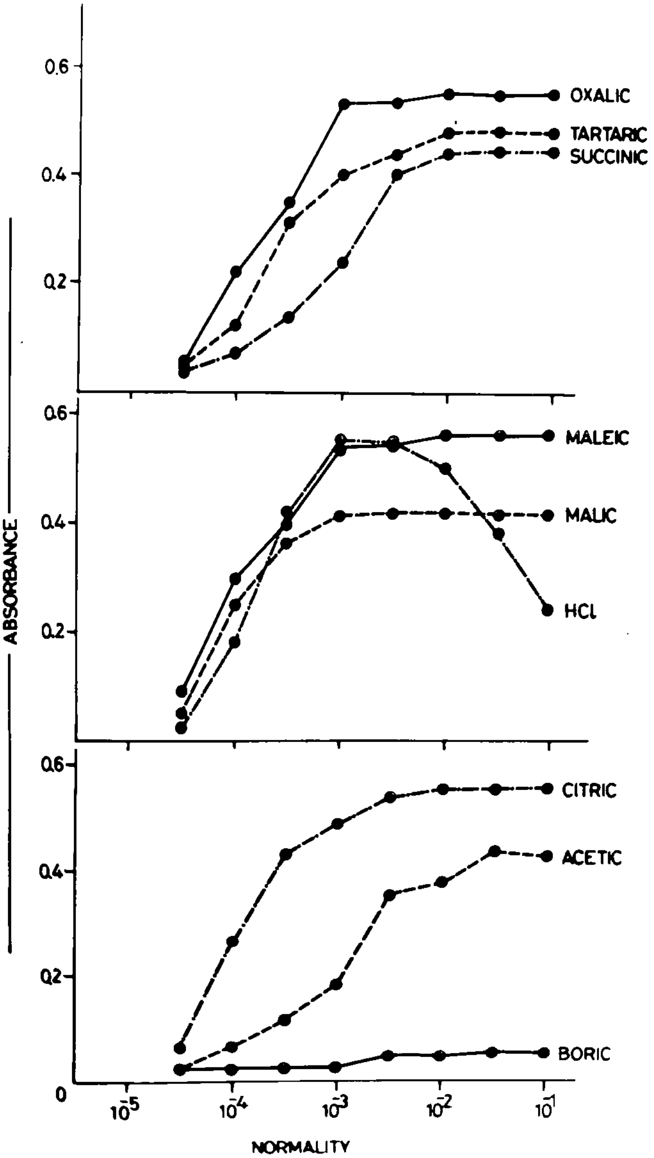

FIGURE 2 Screening of different acids and their strength for optimum colour production.

Stability of the proposed reagent

The shelf-life of the pre-mixed DMAC-organic acid reagent under different storage conditions showed that it keeps well for over one week in an air-tight brown bottle at room temperature (20–25°C) without any difference in its repetitive performance.

Optimum colour development time

The effect of contact time for maximum colour production, maintaining optimum DMAC and acid concentrations, is depicted in Figure 3. The colour intensity increased through first 10 minutes after which time up to 60 min had no influence.

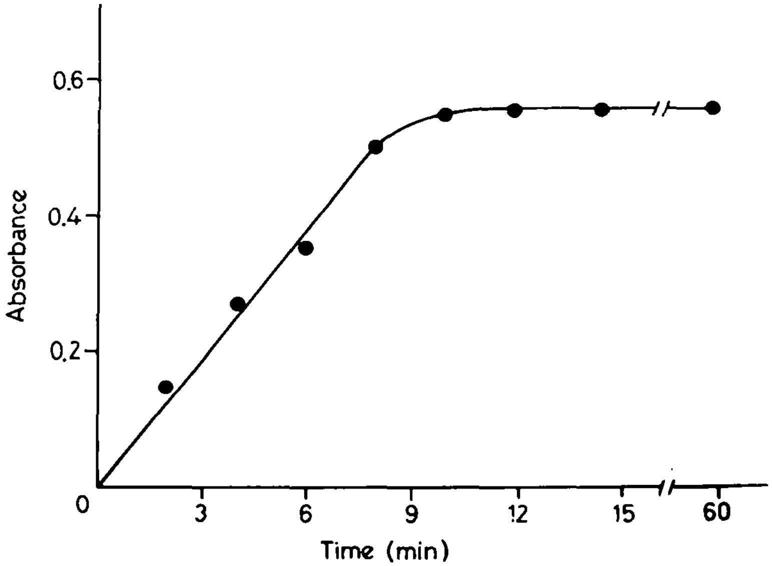

FIGURE 3 Colour development of aromatic amine as a function of time.

Recovery from biological samples

The recovery of different aromatic amines from spiked urine and serum was between 89 and 96% with a coefficient of variation (CV) of less than 8% (Table I). Similarly, recovery from spiked tissue homogenates of liver, lung, kidney, testes, spleen and skin was over 90%.

TABLE I

Recovery of aromatic amines from biological samples

Aromatic amine	Amount added (μg)	Urine % recovery	Urine CV[a]	Serum % recovery	Serum CV
4-Aminoanisole	25	95.2	2.4	91.2	2.8
	50	92.6	1.6	90.8	3.4
	100	92.9	0.9	90.2	1.9
4-Aminodiphenylamine	25	96.0	3.7	92.8	4.9
	50	92.0	3.8	90.8	5.1
	100	90.9	6.7	92.9	4.9
3-Aminopyrene	25	94.8	1.9	93.6	3.3
	50	90.0	2.5	90.6	5.3
	100	89.6	2.0	91.7	1.9
Benzidine	25	92.8	0.9	93.2	0.8
	50	91.6	0.8	90.4	0.8
	100	90.8	2.1	91.3	1.4
1-Naphthylamine	25	92.2	3.9	92.0	1.2
	50	89.6	1.2	90.4	3.9
	100	89.2	2.8	89.7	1.2
1,2-Phenylenediamine	25	92.4	2.9	92.4	2.9
	50	92.4	7.5	90.9	5.0
	100	89.4	7.8	89.4	7.7

[a] Coefficient of variation.

Stoichiometry and absorbance characteristics

It is apparent from Figure 4 that one mole of DMAC condenses with one mole of 4-aminodiphenylamine to form a Schiff's base. This 1:1 stoichiometric relationship remains unaltered even with benzidine which has two functional amino groups.

The Schiff's bases formed with different amines have different λ max, stability constants and high molar extinction coefficient (Table II).

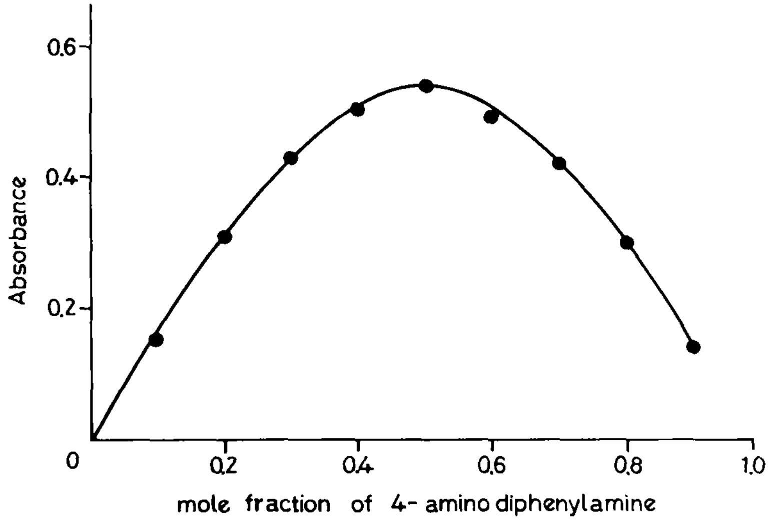

FIGURE 4 Continuous variation plot of aromatic amine and DMAC-organic acid reagent versus the absorbance of the condensation product.

TABLE II

Wavelengths, stability constants and molar absorptivities of Schiff's base complexes of aromatic amines with DMAC reagent

Aromatic amine	Wavelength max (nm)	Stability constant (litre mole^{-1})	Molar absorptivity (litre mol^{-1} cm^{-1})
4-Aminoanisole	505	9.95×10^5	1,22,471
4-Aminodiphenylamine	552	7.95×10^5	92,605
3-Aminopyrene	550	2.76×10^4	1,04,065
Benzidine	582	4.07×10^5	2,95,734
1-Naphthylamine	512	9.19×10^4	1,26,437
1,2-Phenylenediamine	520	9.18×10^5	80,168

Lowest detectable limits and comparative sensitivity

Under the optimum experimental conditions, the absorbance [A] was found to be linearly related to amine concentrations [C μg ml^{-1}] up to 1.5 μg ml^{-1} at 95% confidence limits. Beer's plot showed an excellent correlation coefficient with the minimum detectable limits ranging between 0.05–0.12 $\mu g\,ml^{-1}$, taking 0.05 as the lowest measurable absorbance (Table III).

Comparison of linear regression equations revealed that the proposed DMAC method, depending on the nature of amines, is 5 to 40 folds sensitive than the conventional Bratton–Marshall procedure (Table III).

DISCUSSION

Among the occupational surveillance techniques, workers themselves prove the best sampler of their work environment. Urinary analysis at times offer the simplest of such monitoring programmes to warn against the excessive exposure to industrial chemicals.[9] Widespread use of aromatic amines and their associated toxicity threats,[10] demand regular monitoring to safeguard the health of exposed workers.[11] The conventional Bratton–Marshall's (B–M) method has found application in a number of the epidemiological surveys aimed at measuring the urinary clearance of amines *vis à vis* their industrial exposure.[4] [5] The method suffers from some of the drawbacks attendant to diazotization and subsequent coupling reactions.

Use of dimethylaminocinnamaldehyde in conjugation with HCl, as a spray reagent for the detection of amines on paper chromatograms was suggested by Harley-Mason and Archer.[12] An attempt has been made to convert this spray reagent into a quantitative method. Acidity was found to play a critical role in this reaction since the final colour got diminished at higher acid concentrations. Substitution of HCl with organic acids such as citric, maleic or oxalic, even at a much less acidity (0.06 *N*) led to the development of the present method which worked out to be several folds sensitive than the B–M procedure. The test is simple, quick and detects as low as 0.05–0.12 $\mu g\,ml^{-1}$ of amines giving a stable final colour. In contrast to B–M, the proposed DMAC-organic acid method utilises a single step colour development and the pre-mixed reagent stores well for over a week. Lower acidity in the present case has added advantage that only free amines are estimated as against methods with higher acidity where possibility of conjugated amines getting hydrolysed and indicated as free amines cannot be overruled.

TABLE III

Comparative sensitivity[a] of the proposed method to Bratton–Marshall procedure

Aromatic amine	Proposed DMAC method		Bratton Marshall method		Fold sensitivity of DMAC method
	LRE	r	LRE	r	
4-Aminoanisole	$A = 0.997\,C - 0.0013$	0.9995	$A = 0.024\,C + 0.001$	0.9983	39.8
4-Aminodiphenylamine	$A = 0.503\,C - 0.0007$	0.9994	$A = 0.080\,C - 0.0003$	0.9989	6.3
3-Aminopyrene	$A = 0.487\,C - 0.008$	0.9996	$A = 0.017\,C + 0.0055$	0.9950	21.8
Benzidine	$A = 1.133\,C \quad 0.017$	0.9980	$A = 0.209\,C + 0.023$	0.9989	5.0
1-Naphthylamine	$A = 0.875\,C + 0.0079$	0.9995	$A = 0.066\,C - 0.008$	0.9995	15.2
1,2-Phenylenediamine	$A = 0.755\,C - 0.0127$	0.9995	$A = 0.021\,C + 0.006$	0.9989	27.5

[a] Based on Linear Regression Equation [LRE] and Correlation Coefficient [r] between Concentration [C μg ml^{-1}] and Absorbance [A] relationship.

The method has additional scope for application as an amine spray reagent on thin layer or paper chromatograms. On the overall, the procedure is simple, less time consuming, sensitive and can serve as a monitoring tool to determine the amine exposure levels in occupational workers.

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