A simple, rapid, microgasometric method for determination of primary aliphatic amine groups in polymers

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Description of a simple apparatus for the gasometric determination of primary aliphatic amines is given. The chemistry is that which is used in the Van Slyke method, i.e. $RCH_2NH_2 + 3HNO_2 \rightarrow RCH_2OH + N_2 + NO + NO_2 + H_2O$. Nitric oxide and nitrogen dioxide are removed from the evolved gas mixture by washing with alkaline permanganate. The nitrogen volume is measured in a glass syringe, then the amine content of the sample is quantitated relative to an amino acid standard, leucine. The method is applied to two water soluble synthetic polymers, one in which primary aliphatic amine is the principle functional group and another in which it is a trace functional group. Relative standard deviations were 2.8% and 4.3%, respectively. The method should have general applicability to synthetic polymers and complex biochemical mixtures.

INTRODUCTION

During the current development of polymeric dyes as nonabsorbable food additives[1] there has been a need for the accurate determination of primary aliphatic amine in both simple and complex synthetic polymers. Several approaches to this measurement were considered, but not used for the following reasons:

(1) *Elemental analysis* by classical microanalysis is accurate only to approximately $\pm 5\%$. If other nitrogen containing groups are present in the polymer, independent data on the number of such groups is needed. Further, elemental analysis cannot be used for measurements in polymers in complex mixtures containing other nitrogen functional groups.

(2) *I.r.*, with the advent of *FT*i.r. and fixed wavelength microprocessor controlled instruments, has become a much more quantitative tool than it has been in the past. Nevertheless, the i.r. signal from primary aliphatic amine groups in complex polymers is often so distorted and unresolved from other signals that quantitation is difficult[2].

(3) *Titration* of protonated primary aliphatic amine with base can be used in some cases. Base titration of protons is such a general method, however, that other functional groups on the polymer or impurities in the sample often interfere.

(4) *Spectrophotometric methods* in which a u.v. absorbing or a fluorescent label reacts with the polymeric primary aliphatic amine, and is then quantitated spectrophotometrically, also have limitations. Generally it is difficult to add these reagents to a polymer quantitatively. This is especially true for trace levels of primary aliphatic amine on a complex polymer. Neighbour–neighbour interactions may also change the spectrum of the fluorophore raising further doubts about quantitation[3]. Even without such complications it is necessary to have a series of polymers with known primary aliphatic amine content to calibrate the method.

(5) ^{13}C *n.m.r.* can be used for certain polymers that exhibit spectra with a fully resolved signal for the primary aliphatic amine carbon. Calibrators of known primary aliphatic amine content, however, are required in order to quantitate. Also the required sample size is large (0.5–1.0 g)[4].

None of the above methods filled the need for a rapid, quantitative method that would apply to small samples of simple and complex polymers.

The chemistry used in the Van Slyke approach to quantitative determination of primary aliphatic amine in biological systems is both simple and specific.

$$RCH_2NH_2 + HNO_2 \rightarrow RCH_2OH + N_2 + H_2O$$

Superimposed on this reaction is the ordinary decomposition equilibrium of nitrous acid to nitric oxide and nitrogen dioxide, making the overall reaction as follows:

$$RCH_2NH_2 + 3HNO_2 \rightarrow RCH_2OH + N_2 + NO + NO_2 + 2H_2O$$

The NO and NO_2 may be removed by washing the gas mixture with alkaline potassium permanganate.

The classical Van Slyke apparatus[5] allows the measurement of nitrogen gas volume from the above reaction. The number of mols of nitrogen may then be calculated using the ideal gas law and measurements of ambient temperature, atmospheric pressure, and vapour pressure.

This classical apparatus is tedious to use, and is subject to experimental error. The approach described in this paper simplifies the apparatus significantly. Also, the measurement is converted from an absolute to a relative one by using an amino acid as a primary amine standard. A comparable approach for the measurement of CO_2 in blood has been described[6].

The modified Van Slyke apparatus employs three glass syringes and a three-way nylon luer stopcock. The sample is treated with HNO_2 in the first syringe to release N_2 quantitatively, accompanied by NO and NO_2. The gas mixture is transferred into the second syringe containing alkaline permanganate to absorb the NO and NO_2. The

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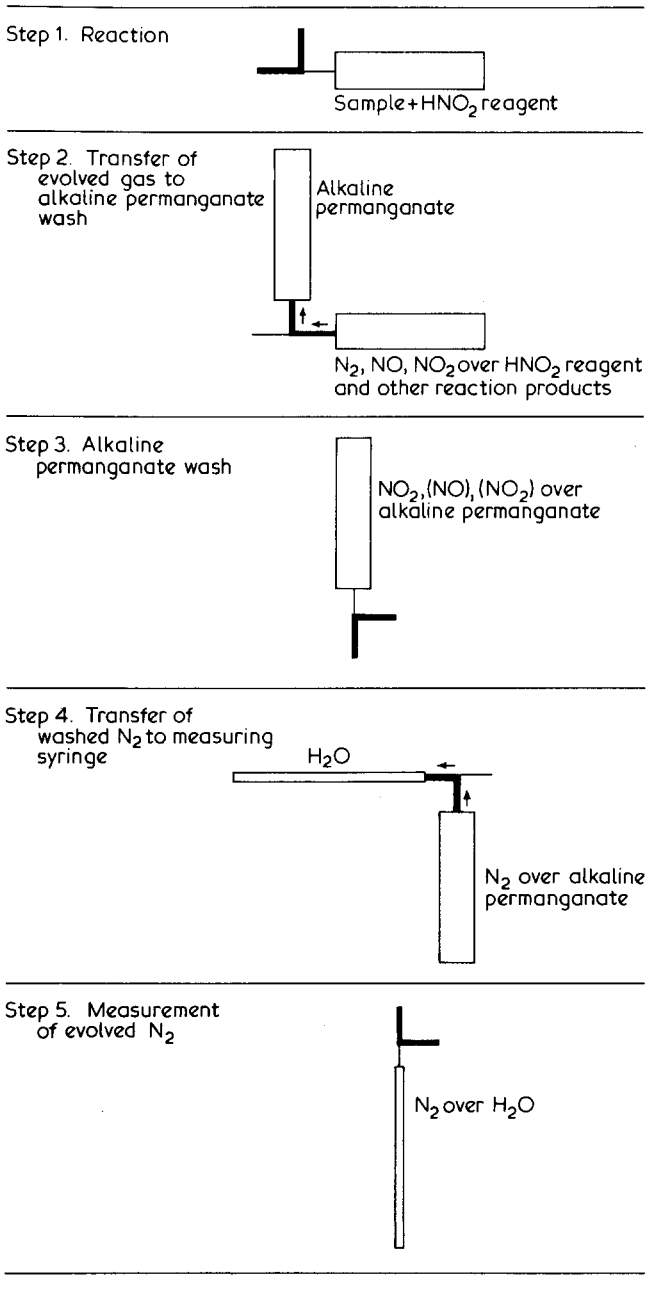

remaining N_2 is then transferred into the third syringe for measurement. The method has been found reliable for the measurement of primary aliphatic amine in synthetic polymers even when the amine is present as a small fraction of the total polymer functional groups. Applicability would be expected to extend to biochemical systems of interest.

EXPERIMENTAL

Apparatus

Two 10 ml glass syringes with glass luer tips, (all syringes must be checked and verified to be gas tight when wet); one ml tuberculin glass syringe with glass luer tip; nylon 3-way luer stopcock, and a wrist action shaker (Burrell, Model 75) were used.

Reagents

Those used were: *Acetic acid* (glacial acetic acid, J. T. Baker reagent grade); *Sodium nitrite* (25 g $NaNO_2$, J. T. Baker reagent grade, in 100 ml deionized water); *Alkaline potassium permanganate* (2.5 g of C.P. sodium hydroxide (Fisher) and 6.0 g potassium permanganate (J. T. Baker reagent grade) in 100 ml water; *L-Leucine*, Calbiochem A. Grade, as a standard; Atoms® 300, liquid Food Emulsifier, mono- and diglycerides with 0.02% butylated hydroxyanisole (BHA) and 0.01% citric acid in propylene glycol (ICI) for use in cases where polymer forms a stable foam.

Procedure

Sample and standard solutions were prepared to approximately 0.015 meq amine/ml water. To reduce foaming, 0.1 ml Atoms® was added to polymer solutions when necessary. Aliquots were drawn into a 10 ml syringe in the following sequence: 0.4 ml acetic acid, 2.0 ml of the sample or standard; 0.1 ml H_2O, and 0.4 ml sodium nitrite solution. The syringe was immediately connected to the closed middle luer of the stopcock and shaken until the plunger reached 10–13 ml, (~45 min). To wash the gas mixture, 5 ml of the alkaline permanganate solution was drawn into the second 10 ml syringe. This syringe was connected to the second luer valve on the stopcock. The gas mixture was transferred from the first syringe into the second. The first syringe was removed and the solution was shaken gently for one minute.

The one ml syringe was connected to the first luer and the nitrogen was transferred into it. A blank solution was run concomitantly. The volume of gas generated by the blank was subtracted form sample and standard volumes. To avoid introduction of air bubbles or loss of gas, luers were filled with water and air bubbles expelled from syringes with water before use.

RESULTS

The modified Van Slyke appratus was evaluated using amino acids. *Table* 1 presents assay values based on theoretical nitrogen content of five amino acids. The modified Van Slyke method is compared with Kjehldahl nitrogen analysis and titration with perchloric acid in acetic acid. Modified Van Slyke values compare well with classical Van Slyke values reported in the literature. Valine, phenylalanine and leucine are reported to react quantitatively, while glycine gives 103% of theoretical due to non-ideal decomposition of the intermediate diazo compound[5]. It appears that γ-aminobutyric acid does not react to completion.

The applicability of the modified Van Slyke method to analysis of polymers was checked using two different water-soluble polymers, one in which amine is a principle functional group and one in which amine is a relatively minor group in terms of mole %. The structure and trivial names of these polymers are as follows:

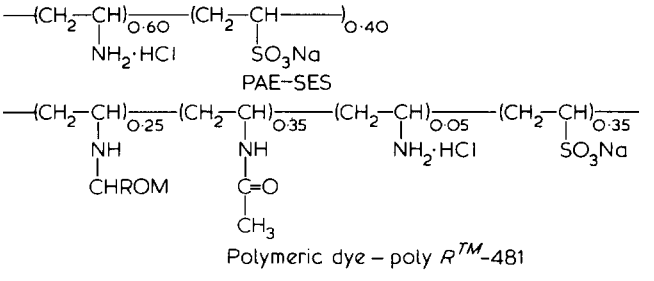

Table 1 Modified Van Slyke analysis of amino acids compared with Kjehldahl and perchloric acid titration. Literature values[1] indicate results obtained by Van Slyke using the classical apparatus

		% Theoretical amine content			
		Modified* Van Slyke	Kjehdahl	Perchloric acid titration	Literature Van Slyke
Substance	Source				
Valine	Sigma	99.7	100.0	99.4	99.9
Phenylalanine	Sigma	98.9	99.7	99.2	100.6
γ-Aminobutyric acid	Calbiochem	94.8	101.7	100.1	–
γ-Aminobutyric acid	Sigma	97.1	97.2	99.8	
Glycine	Calbiochem	103.1	101.7	99.6	103
Glycine	Sigma	104.2	99.8	100.7	
Leucine	Calbiochem	Std.	98.6	100.0	100.0
Leucine	Sigma	Std.	98.9	98.6	

* Values given are an average of 6 determinations. Since the coefficient of variation for this method for a single determination is 2.8%, the uncertainty of these average values as determined by this method is ±3.0% (95% confidence)

Table 2 Modified Van Slyke analysis of PAE–SES solutions compared with Kjehldahl nitrogen and base titration in DMSO. Kjehldahl analyses were performed by Stoner Laboratories, Santa Clara, CA. Samples **A**–D are crude reaction mixtures of the polymer. Samples E–H are dilute purified solutions of PAE–SES having different amine–sulfonate ratios

Sample	meq NH_2/g by Modified Van Slyke	meq N/g by Kjehldahl Analysis	meq H^+/g by Titration in DMSO
A	1.49	1.57	1.70
B	1.57	1.65	1.75
C	1.40	1.44	1.37
D	1.36	1.34	1.37
E	0.07	–	0.08
F	0.43	0.44	0.45
G	0.34	0.34	0.37
H	0.13	0.12	0.14

Table 3 Summary of regression analysis for linearity study

	Y-Intercept meq Amine	Slope meq amine/g	r^2
Poly *R*^{TM}–481	-8×10^{-5}	0.318	0.999
PAE–SES solution	-7×10^{-6}	1.43	0.999

Where CHROM is an organic chromophore group with non-interfering functional groups.

Comparison with other methods

Table 2 presents a comparison of meq/g amine values determined by modified Van Slyke, Kjehldahl and base titration in DMSO on different PAE–SES solutions. These results indicate that values for the modified Van Slyke method compare well with reference method values.

A reliable reference method to determine the trace amine functional group on the polymeric dye is not available. Van Slyke values do agree, however, with amine determined by subtracting meq chromophore, determined spectrophotometrically, from the sum of amine and chromophore, determined by base titration in DMSO.

Reproducibility

Reproducibility of the polymer analyses was evaluated by duplicate analysis of two solutions of the sample prepared daily on five different days. This gave a total of twenty analyses. The data was evaluated by analysis of variance.

The relative standard deviation for the analysis of PAE-SES is 2.8%. Day-to-day differences account for 70% of the variance, while 20% of the variance is due to sample preparation and 10% due to replicate analysis of the same sample solution.

The relative standard deviation for the analysis of the polymeric dye is 4.3%. Day-to-day differences account for 40% of the variance, while 50% of the variance is due to sample preparation and 10% due to replicate analysis of the same solution.

Linearity

Linearity studies were performed for analysis of both the polymeric dye and PAE-SES. Solutions were prepared to be 0.003–0.018 $\frac{\text{meq amine}}{\text{ml}}$. Five dilutions were prepared and analysed in triplicate for each polymer. The volumes of nitrogen evolved ranged from 0.16–0.88 ml when corrected for the 0.07 ml blank obtained. These values represent the range of volumes suitable for measurement with the 1 ml syringe. Regression analysis of this data indicates excellent linearity and is summarized in *Table* 3.

DISCUSSION

The specificity of the chemistry used in the Van Slyke approach to amine determination has always been attractive. However, the tediousness of and potential errors in the gasometric measurement have been a deterrent to its use. An improvement in the original Van Slyke method was made in 1962 by Hoffman and Lysyj[7], in which the evolved gas mixture is analysed by gas chromatography thereby eliminating some of the difficulties in the original method. The practical, simple apparatus described in this paper allows use of the specific chemistry to obtain sure and quantitative results.

Some experiments have been carried out in our work using various cosolvents along with water as a vehicle for the sample in an effort to make the approach described here more generally applicable to other synthetic polymers. Results have been encouraging. In particular, DMSO does not seem to interfere with the assay. No validation studies, however, have been carried out with this solvent.

It has been noted in the literature[8] that some primary aliphatic amines react slowly with nitrous acid. Therefore,

before application of this method to other amine-containing polymers, completeness of the reaction should be checked by variation of sample concentration and variation of allowed time for reaction.

Further, the specificity of the method would make possible determination of primary aliphatic amine in biopolymers or in complex biochemical mixtures.

The modified Van Slyke method has been shown to give results comparable to the classical apparatus. Furthermore, the applicability of the method to water soluble synthetic polymer analysis has been demonstrated.

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