Application of the Dilution Method to Micro-Analysis.

Its Application to the Micro Dumas Method, Eliminating the Use of a Micro-Chemical Balance and Further Studies.

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The dilution method.

Several years of experience in teaching and practicing organic analysis showed that the identification of organic compounds containing nitrogen invariably presented considerable difficulties. A quantitative determination of nitrogen in most cases is highly desirable, but is hampered by the tedious, timeconsuming and somewhat unreliable macro Dumas method.

In the micro method of PREGL[1]) a highly satisfactory method[2—6]) for the quantitative determination of nitrogen has been found. However the dependance of this method on a suitable micro chemical balance constitutes a serious obstacle to a more general and broader application. A micro chemical balance has to be properly cared for to insure reliable results. Most chemical laboratories, however, lack the necessary facilities to insure proper fulfillment of the requirements (vibration-free setting, uniform temperature conditions, etc.) for a satisfactory operation of a micro chemical balance.

It has now been attempted, through the employment of the "dilution method" given below, to utilize the convenient PREGL absolute nitrogen method with the aid of an ordinary analytical balance (sensitivity 0,1 mg) and to eliminate the micro chemical balance completely for this type of analysis.

By using proper concentrations and painstakingly observing certain experimental conditions, satisfactory results were obtained.

For the micro Dumas method samples weighing from 3 to 5 milligrams are required and must be weighed with an accuracy of 0,001 to 0,002 mg. Three decimal places are required; an ordinary macro analytical balance permits weighing to ± 0,1 mg prohibiting immediately any direct weighing of a micro sample (compare A. GLATTFELDER[22]). At once the use of solutions of the samples to be analyzed suggested itself. Careful considerations made the following experimental requirements necessary:

1) the amount of sample ought to be 0,1 g which permits weighing to the required decimal places;

2) the solvent should be volatile, yet safely permit the quantitative transfer of aliquot portions at room temperature. It must be absolutely free of nitrogen, or constituents containing this element and also must not interfere with the combustion process. Of the various organic solvents studied (alcohol, benzene, acetone, ether etc.) carbon tetrachloride has been found highly satisfactory. Its high specific gravity insures a higher accuracy in weighing (for a given volume), it is a good solvent, noninflamable and yet fairly volatile. It contains no hydrogen, its sole combustion products are gases which in no way interfere with the combustion process.

3) A few preliminary experiment showed that a concentration of not more than 1% of the substance to be analyzed, is preferable.

Next the suitability of a gravimetric or volumetric apportioning had to be tested. A number of experiments demonstrated the greater efficiency of the former. The technique of the gravimetric method, under the circumstances given, is simpler than that of the volumetric method, which in this case is hampered by the required volatility of the solvent. It is self evident, that in a combustion apparatus designed for 3 to 5 mg of substance large amounts of solvents cannot be introduced without causing serious trouble in the course of analysis. This makes it necessary to eliminate most of the solvent, as soon as it has served as a diluent, in a suitable manner.

In addition to this, a volumetric quantitative apportioning requires larger amounts of solvents to insure the required accuracy; furthermore, additional washing of the micro pipettes increases the final volume of the samples to an undesirable extent. The gravimetric method is also to be preferred for these reasons since samples of 0,1 g can be weighed with sufficient accuracy, the volume of solvent to be dealt with is only from 0,075 to 0,2 cc depending upon the specific gravity of the liquid, an amount that cannot be handled quantitatively so well otherwise under the experimental circumstances given.

Experimental part.

Preparation of the sample.

As pointed out before a 1% solution by weight has been found the most suitable concentration. A dropping bottle (Fig. 1) of 30 cc volume, that permits hermetic sealing and complete protection against evaporation, is cleaned, dried and weighed accurately. The calculated amount of sample (to give a 1% solution by weight) is carefully introduced and weighed. Then the solvent is added and the mixture carefully shaken to effect complete solution and homogeneity. Carbon tetrachloride so far is the most suitable solvent, but in case of insolubility of the material to

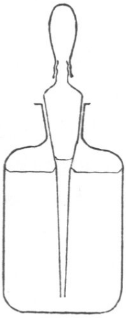

Fig. 1. Dropping bottle.

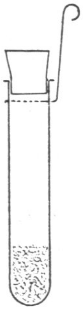

Fig. 2. Mixing tube.

be analyzed, any other non-nitrogenous solvent may be used (water, methyl- and ethyl alcohol, ether, chloroform, benzene, carbon bisulphide etc. or mixtures).

Then the dropping bottle containing the substance to be analyzed and the solvent is weighed again. The weight must remain constant even over periods of half an hour, to insure no loss of solvent due to leaks. The percentage by weight of the substance to be analyzed in the solvent now can easily be calculated.

Next a mixing tube (Fig. 2) containing 2 cc of powdered copper oxide and provided with a tight fitting stopper is accurately weighed. An aliquot portion of the solution containing from 3 to 5 mg of substance is transferred from the dropping bottle to the mixing tube in the following manner:

Enough solution is drawn into the ground-in-dropper, by pressing and subsequently releasing the elastic rubber end of the dropper. The latter now containing enough solution to yield a micro sample (3 to 5 mg) of the substance to be analyzed, is removed from the flask and its content emptied into the weighed mixing tube, which is then immediately stoppered tightly and weighed again. The stoppered mixing tube thus filled should not lose weight on standing.

In this manner several samples may be prepared for check analysis. Next the solvent must be removed. If carbon tetrachloride was used, it was ascertained that complete removal of the solvent is easily brought about on standing of the opened mixing tubes at room temperature and the sample is left in a finely divided state in the copper oxide powder, which after 24 hours is perfectly dry and ready for analysis. In case of other solvents, ethyl alcohol for instance, evaporation in vacuum might be necessary whereby the time required is shortened. Liquids of low specific gravity have the tendency to creep up the wall of the mixing tube and to deposit there the material to be subjected to analysis, which makes its quantitative transfer into the combustion tube difficult. This objectionable feature might easily be prevented by the addition of some carbon tetrachloride.

The transfer of the solvent from the weighed dropping bottle to the mixing tube is naturally accompanied by loss of solvent due to evaporation. This loss was determined quantitatively and is shown in table below the figures of which indicate, that the resulting change in the concentration of the solution, is negligible if considered for the amount of sample used in the analysis.

Course of analysis.

The analysis may now be performed according to the method of Pregl[1]), Dubsky[10]), Shintaro Ogawa[23]) and others[20]) [26]), or with the use of a gasometer, placed between the Kipp generator and the combustion tube, as described later.

Results.

I. Solute: Azo benzene.

Solvent: Carbon tetrachloride.

Weight of dropping bottle	44,5968 g
Weight of dropping bottle plus sample	44,9840 g
Weight of sample	0,3872 g
Weight of dropping bottle plus solution	89,3892 g
Weight of dropping bottle plus sample	44,9840 g
Weight of solvent	44,4052 g
Percentage of azo benzene	0,8644 %

	Weight of mixing tube	Weight of mixing tube plus solution	Weight of solution	Weight of sample	% nitrogen found	% nitrogen calculated
a)	5,5030 g	6,1550 g	0,6520 g	5,635 mg	15,52 %	15,38 %
b)	5,2354 g	6,1518 g	0,9164 g	7,921 mg	15,55 %	
c)	6,2588 g	6,9618 g	0,7030 g	6,076 mg	15,43 %	
Average:			0,7571 g	6,544 mg	15,50 %	

II. Solute: Benzamide.

Solvent: Alcohol.

Weight of dropping bottle	44,6744 g
Weight of dropping bottle plus sample	44,7676 g
Weight of sample	0,0932 g

Weight of dropping bottle plus solution . . 68,2806 g
Weight of dropping bottle plus sample . . 44,7676 g

Weight of solvent . . . . 23,5130 g

Percentage of benzamide . . . . 0,3948 %

	Weight of mixing tube	Weight of mixing tube plus solution	Weight of solution	Weight of sample	% nitrogen found	% nitrogen calculated
a)	6,2286 g	7,3420 g	1,1134 g	4,396 mg	11,19 %	11,57 %
b)	6,2428 g	7,2862 g	1,0434 g	4,119 mg	11,28 %	
c)	6,1804 g	7,1926 g	1,0122 g	3,996 mg	11,19 %	
d)	7,2514 g	8,3184 g	1,0670 g	4,212 mg	11,30 %	
	Average:		1,0590 g	4,181 mg	11,24 %	

III. Solute: Acetanilide.

Solvent: Ethyl alcohol and carbon tetrachloride mixture.

Weight of dropping bottle . . . . 44,6104 g
Weight of dropping bottle plus sample . . 44,8332 g

Weight of sample . . . . 0,2228 g

Weight of dropping bottle plus solution . 64,5178 g
Weight of dropping bottle plus sample . . 44,8332 g

Weight of solvent . . . . 19,6846 g

Percentage of acetanilide . . . . 1,1192 %

	Weight of mixing tube	Weight of mixing tube plus solution	Weight of solution	Weight of sample	% nitrogen found	% nitrogen calculated
a)	5,7504 g	5,9792 g	0,2288 g	2,260 mg	10,75 %	10,37 %
b)	5,3224 g	5,6696 g	0,3472 g	3,886 mg	10,42 %	
c)	5,2266 g	5,6580 g	0,4314 g	4,828 mg	10,35 %	
d)	6,4304 g	6,7300 g	0,2996 g	3,353 mg	10,36 %	
	Average:		0,3267 g	3,582 mg	10,47 %	

IV. Solute: Toluamide.

Solvent: Ethyl alcohol and carbon tetrachloride.

Weight of dropping bottle	47,0990 g
Weight of dropping bottle plus sample	47,3050 g
Weight of sample	0,2060 g
Weight of dropping bottle plus solution	68,2990 g
Weight of dropping bottle plus sample	47,3050 g
Weight of solvent	20,9940 g
Percentage of toluamide	0,9717%

	Weight of mixing tube	Weight of mixing tube plus solution	Weight of solution	Weight of sample	% nitrogen found	% nitrogen calculated
a)	5,6196 g	5,7824 g	0,1628 g	1,582 mg	10,62%	10,37%
b)	7,4026 g	7,8198 g	0,4172 g	4,054 mg	10,04%	
c)	5,4318 g	5,7640 g	0,3322 g	3,228 mg	10,29%	
d)	6,4030 g	6,9323 g	0,5293 g	5,143 mg	10,13%	
e)	6,2004 g	6,5476 g	0,3472 g	3,373 mg	10,21%	
f)	5,9867 g	6,4116 g	0,4249 g	4,128 mg	10,07%	
	Average:		0,3689 g	3,584 mg	10,23%	

Change in concentration due to evaporation of the solvent during transfer.

The change in concentration due to evaporation of the solvent can be found in the following manner:

The dropping bottle with its contents is weighed before and after the transfer of the aliquot portion of the solution to the mixing tube, giving so the amount of solution removed. The weight of the solution in the mixing tube will always be lower and the difference is the loss of solvent due to evaporation. As shown in the following table the change in concentration is between 0,0001 and 0,0006%, depending on the nature of solvent, technique in the transfer and the concentration of the solute; this change, however, if calculated for the amount of sample used in the actual analysis is so small, that it comes within the weighing error of even the best micro-chemical balance (KUHLMANN's) at our disposal at present. Only if a large number of samples are being prepared and the dropping bottle is opened too often is a reweighing of the solution and subsequent recalculation of the percentage necessary.

		Loss of solvent	Change in concentration	Increase in weight of sample
Experiment # I:	a)	0,0288 g		
	b)	0,0086 g		
	c)	0,0102 g		
Average:		0,0158 g	0,0002 %	0,0015 mg
Experiment # II:	a)	0,0106 g		
	b)	0,0056 g		
	c)	0,0060 g		
	d)	0,0052 g		
Average:		0,0068 g	0,0001 %	0,0011 mg
Experiment # III:	a)	0,0188 g		
	b)	0,0090 g		
	c)	0,0086 g		
	d)	0,0066 g		
Average:		0,0108 g	0,0006 %	0,0019 mg
Experiment # IV:	a)	0,0028 g		
	b)	0,0050 g		
	c)	0,0060 g		
	d)	0,0047 g		
	e)	0,0054 g		
	f)	0,0049 g		
Average:		0,0048 g	0,0002 %	0,0007 mg

The gasometer method.

Numerous discussions in the literature deal with certain phases of Pregl's method and with the fact that too high results[7]) are usually obtained, in cases where unknown substances are subjected to analysis and where the nitrogen content of a given compound cannot be made a function of the somewhat obscurely stated length of time alloted to the sweeping and washing in the latter part of the analysis.

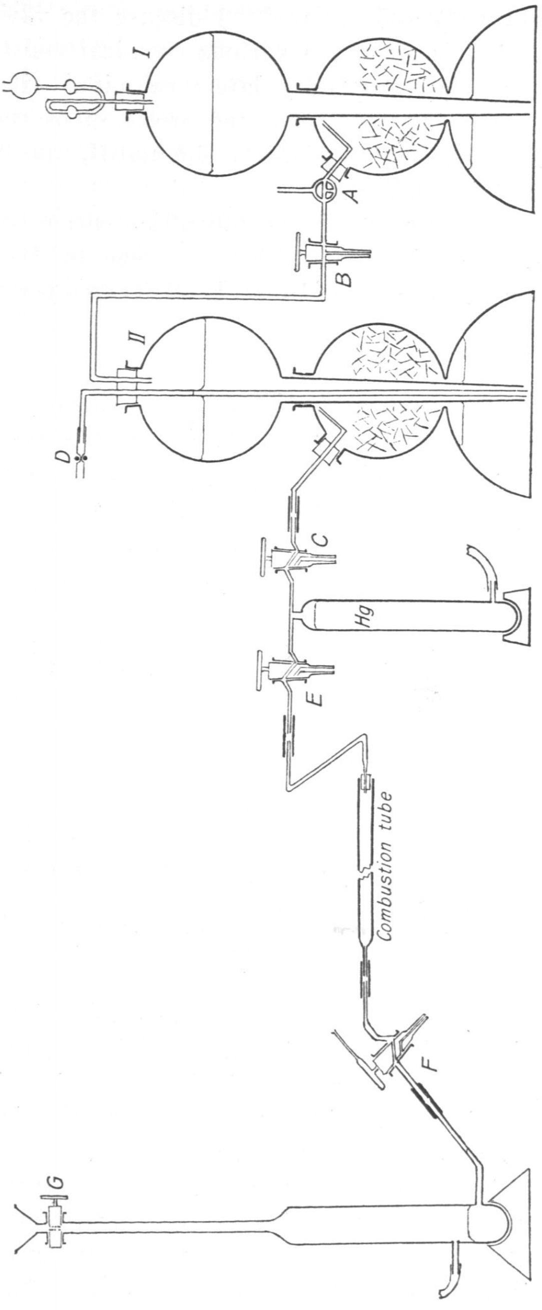

Fig. 3.

Arrangement of the double Micro Dumas apparatus. (showing the gasometer and Kipp generator connections).

While F. HALLA8) and others 9—19) discuss the various phase equilibria existing between the various chemical constituents in the combustion tube, TRAUTZ20) lately examined other sources of error and their influence upon the precision of the analysis and showed how such errors might be eliminated, thus raising the dependability of the method.

In a practical way some of the difficulties encountered by the various authors can be successfully overcome by the use of a suitable gasometer (suggested by R. H. MÜLLER) placed between the Kipp generator (see Fig. 3) and the combustion tube. The combustion of the substances to be analyzed is always carried out with a standard amount of carbon dioxide the purity of which can easily be determined. In this manner uncertainties in regard to the end of the analysis are at once eliminated.

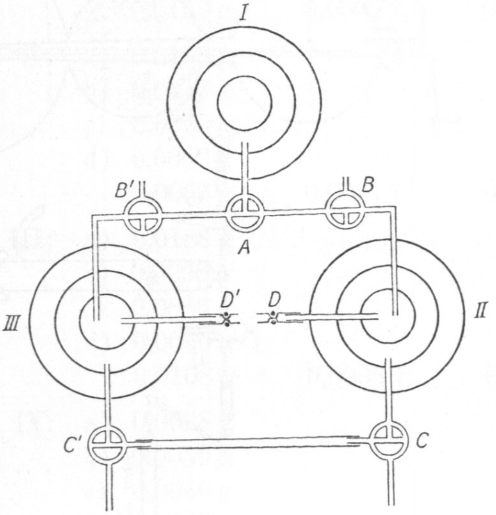

Fig. 4.

Arrangement of the 3 Kipp generators. (for hermetic sealing and automatic removal and renewal of the acid with the exclusion of air.

In laboratories where large numbers of quantitative nitrogen determinations have to be carried out the arrangement of apparatus as given in figure 3 may prove to be of advantage. Two sets of micro Dumas apparatus, (compare DUBSKY and

GRANACHER[21]) each one having the gasometer spoken of before, are put in parallel. The two Kipp generators are connected to a third one (Fig. 4), thus preventing the hydrochloric acid from coming in contact with air.

Such an arrangement no longer requires the flushing of the Kipp generator before the analysis, as practiced heretofore. Even the refilling of the Kipp apparatus with hydrochloric acid solution with the exclusion of air can be done quite easily in this type of set-up as shown later, without the inconvenience of dismantling the apparatus.

Apparatus:

A Kipp generator (No. II) which is connected to another Kipp generator (No. I) provides the necessary carbon dioxide. A gasometer (150 to 200 cc volume) is connected with the Kipp generator by means of the stop cock B. The combustion tube, which is filled according to PREGL, is connected with the gasometer by means of the two-way stop cock C. Finally connection is made with the micro nitrometer through double capillary stop cock F.

The method:

It is self evident, that in any of the gasometric nitrogen methods known at present PREGL's[1]), DUBSKY's[10]), FLASCHENTRAEGER's[19]) and others) a gasometer as shown in Figure 3 may be employed with advantage. All the above mentioned methods show the following steps in the procedure:

1) the sweeping-out of the combustion tube with carbon dioxide;
2) the actual combustion and
3) the washing-out of the combustion gases.

The operation of the apparatus in the sweeping-out process (compare PREGL[1]), SHINTARO OGAWA[23]), BERL[24]), BANNISTER[25]) and FLASCHENTRAEGER[19]) is, by allowing the carbon dioxide from Kipp generator (No. II) pass through the combustion tube into the open by proper adjustment of stop cocks C, E and F.

The actual combustion can be carried out as recommended by PREGL, or in the modified form of other authors, by closing stop cock E and connecting the combustion tube with the nitrometer through stop cock F.

While the combustion is in progress the gasometer is being filled with carbon dioxide from the Kipp generator through stop cock C. The bulb containing the mercury is lowered until the mercury in the gasometer reaches the mark. (The mark is usually placed for 100 cc of carbon dioxide, which amount has been found to be sufficient for any combustion.) Then stop cock C is closed and the levelling bulb raised to its original position.

At the end of the combustion stop cock E is opened carefully so as to give the desired flow of the gas. From here on the combustion does not have to be watched any more as the flow of the gas will cease automatically as soon as the gasometer is exhausted due to the absence of pressure. In this way, there can never be any doubt when the combustion is finished, removing automatically a difficulty that has been discussed repeatedly in the literature.

Since even the purest carbon dioxide obtained in the before mentioned experimental conditions still contains air (see Trautz[20]) the amount of air present in a gasometer filling can easily be determined by a blank experiment carried out in an exactly similar manner.

Renewal of the acid in the Kipp generators with exclusion of air.

Kipp generator No. II for instance may be emptied as follows:

Connection is made between all the three Kipp generators through stop cocks A, B, B′, C, C′ (Fig. 4); then suction is applied to D (a glass tubing extending down to the bottom of the Kipp generator) and the desired amount of hydrochloric acid can be removed without the introduction of air into the system. Kipp generator No. III may be emptied in a similar manner.

Kipp generator No. II for example may be filled as follows:

Stop cocks C and D (Fig. 4) are closed and glass tubing D immersed in hydrochloric acid solution. The Kipp generator is evacuated through stop cock B. Now stop cock or pinch cock D is opened, the hydrochloric acid enters the apparatus. As soon as enough of the acid has been introduced stop cock B is turned to form connection between Kipp No. I and the upper bulb of Kipp

No. II. Kipp generator No. III naturally may be refilled in a similar manner.

Such a removal and renewal of the hydrochloric acid used for the generation of the carbon dioxide from marble, aside from the immediate production of carbon dioxide of highest purity (in this manner „micro-bubbles“ may be obtained, that require 2 minutes to rise in the nitrometer) without lengthy flushing and washing of the Kipp generator, as heretofore practiced, insures continuous and satisfactory operation of the apparatus (compare LINDNER[27]), DIEPOLDER[28]), HEIN[29]).

Summary.

1. PREGL's, DUBSKY's, or any other modified micro DUMAS nitrogen apparatus and method can be made use of without a micro-chemical balance;

2. the use of a gasometer between the Kipp generator and the combustion tube standardizes the method and has several advantages;

3. removal and renewal of the acid in the initial Kipp generators may be brought about with the exclusion of air.

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