ANALYSIS OF ANESTHETICS BY VAPOR PHASE CHROMATOGRAPHY

Thesis for Ch 80

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Linus Pauling
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Louis Newman

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ABSTRACT

An analysis of a saturated cyclopropane solution of artificial sea water was done. The solubility of the cyclopropane was found to be consistent within 1% for various runs. The value for the solubility was found to be 1.86×10^{-2} moles/liter. The analysis was done using a hydrogen flame vapor phase chromatograph.

INTRODUCTION

This research was connected with the research done by Art Robinson, Ken Manly, and Mike Anthony during the summer of 1963 on the effects of anesthesia on small organisms. The animal finally chosen for their research was the brine shrimp. The idea was to use various concentrations of anesthetics to determine the point at which 50% of the brine shrimp were anesthetized. This was done for several different anesthetics. Most anesthetics were liquids and the concentration was determined by dissolving a known amount of anesthetic in a known amount of brine shrimp life giving solution (Artificial sea water henceforth known as Irving). For cyclopropane, which is a low boiling point gas (b.p. -33° C.), this method was futile and the anesthetic was prepared by saturating a solution of Irving with cyclopropane, and using the literature value for the solubility of cyclopropane in water which was given in several places. Unfortunately, all of these references gave wildly different values. Other problems were the loss of gas into the air upon handling the solution, and accurate saturation of Irving.

During the summer of 1962, many different methods for analyzing anaesthetics had been tried, but all had failed because the concentration of anesthetic was so low. However, gas chromatography offered the most promising possibilities, since the hydrogen flame detector had increased the sensitivity by $10^{?}$. Art Robinson looked into the idea of using gas chromatography and deemed it feasible. He then gave me the problem of analyzing Irving for cyclopropane and, hopefully, other

anesthetics.

I discussed the problem at length with Angelo Lamola, who has a lot of knowledge about the subject. He thought the best idea would be to extract the anesthetic from Irving into an organic solvent such as benzene and using an internal standard in the benzene to calculate the amount of cyclopropane solution injected into the vapor phase chromatograph. He recommended that I use a simple olefin (1-hexene) as the internal standard, and that a good column to use would be a BB..... column. At the time, he did not like using water as the solvent because of the damaging effect on the VPC and the poor results that had been obtained with water.

This report only covers a portion of the research done during the summer of 1963 under the NSF program. The rest of my research is written up in the Report of the Conductivity Studies Subgroup of the Group for Anesthesia Research (unpublished) which was submitted to Professor Pauling in September 1963. I have a copy of this report.

EXPERIMENTAL

Reagents: 1-hexene 99% (Matheson, Coleman, and Bell)
benzene reagent ACS (Allied Chemical)
toluene reagent ACS (Allied Chemical)
cyclopropane (Linde compressed gas cylinder)
Irving (An artificial sea water)

Apparatus: For the preliminary investigations, I used a Loenco Model 15 gas chromatograph with an ionization detector, and no integrator. The quantitative work was done on a Loenco Model 15B gas chromatograph with a hydrogen flame detector (Loenco). He was used as the carrier gas. The recorder was also from Loenco and it was equipped with a mechanical integrator. I used a 25 foot BB..... column prepared by Angelo Lamola, which was in ¼" aluminum tubing. Hamilton syringes were used to measure the 1-hexene into the solution and also to inject the sample into the gas chromatograph. The system as always run at room temperature.

Procedure: The cyclopropane solution in Irving was prepared by Robinson, Manly, and Anthony by bubbling cyclopropane through Irving and keeping the atmosphere at about 1.1 atm of cyclopropane over Irving. A 100 ml. aliquot of this solution was then pipetted or cylindered into a precalibrated 250 ml. iodine flask. A standard solution was made up which consisted of 100 µl. of 1-hexene in 200 ml. of toluene. This solution was then poured over the solution of Irving in the flask and the stopper was placed on carefully so that no air bubbles were in the flask. The flask was stirred magnetically at 20° C. for three to thirty hours. Five µl. of the toluene layer after this

thorough mixing were then injected into the gas chromatograph. Care was taken to avoid air bubbles in the syringe but this was not of prime importance since the volume that actually went through the gas chromatograph was measured by the internal standard, 1-hexene.

A different procedure was used to find out what the gas chromatograph's response to cyclopropane was. A flask was covered with a serum stopper and filled with cyclopropane via syringe needles. Samples of this were run through both of the machines that I used. The Model 15 gave me the percentage of air in the flask, and the Model 15B gave a peak corresponding to the known volume of cyclopropane injected. Thus I could tell how much cyclopropane was in the samples prepared by the above method in toluene.

RESULTS

Preliminary Investigations

Various materials were put into the Model 15 gas chromatograph. I looked for the time it took to elute at room temperature and if there were any impurities where 1-hexene and cyclopropane came out. These times are all relative to one another.

Substance	Time of Elution	Impurities
Air	20 sec.	yes
Cyclopropane	40 sec.	--
1-Hexene	1 min. 15 sec.	--
Pentane	40 sec.	yes
Chloroform	10 min.	yes
Halothane	6 min.	no
Penthrane	46 min.	no
Benzene (reag)	12 min.	yes
Benzene (spectro)	11 min.	yes
Benzene (99.93%)	13 min.	no
Acetone (reag)	14 min.	no
Alcohol (denat)	16 min.	no
Toluene (reag)	30 min.	no

RESULTS

Response of VPC to cyclopropane

Fifteen runs were made using the previous procedure. The attenuation was at x1024. The average of these fifteen runs was 45.7 with a standard deviation $\sigma = \sqrt{\frac{\text{sum residuals squared}}{15 \times 14}} = 1.8$

Pressure (atm) x Volume = moles x R (constant) x Temp.(^{o}K)

$$\frac{739\text{mm}}{760\text{mm}} \qquad 1\ \mu\text{l} \qquad = n \qquad 82.054 \qquad 297\ ^{o}\text{K}$$

$$n = 4 \times 10^{-8} \text{ moles}$$

Since the standard attenuation chosen was x512, the result is that

<u>91.4 (integrated scale) = 1 µl = 4×10^{-8} moles</u>

Response of VPC to 1-hexene

Five microliters of the standard toluene solution with 1-hexene gave a hexene response of 160 on the VPC integral. This had to be calculated at the time the cyclopropane was to prevent changes in the machine response changing the results.

RESULTS

Analysis for Cyclopropane

The recorder gave a peak and an integral. The peak for cyclopropane was off-scale at an attenuation of x512 so x1024 was used for it and x512 was used for 1-hexene which had a broader peak. The attenuator is accurate to within .5% so the integral for cyclopropane was doubled. Several runs were made out of the same flask and averaged. All the information that I needed is the ratio of cyclopropane to 1-hexene. This information was put into the following formula.

Volume (Irving) x Formality (Irving) =
Volume (toluene) x Formality (toluene)

$$\text{Vol. (Irv)} \times \text{F (Irv)} = \text{Vol. (tol)} \times \frac{\text{VPC cyc}}{\text{VPC hex}} \frac{\text{moles cyc}}{\text{cyc resp.}} \frac{\text{hex resp}}{\text{vol. injected}}$$

(see previous page)

$$100 \text{ ml.} \times \text{F (Irv)} = \text{Vol (tol)} \times \frac{\text{VPC cyc}}{\text{VPC hex}} \frac{4 \times 10^{-8}}{91.4} \frac{160}{5 \mu\text{l.}}$$

about 160ml

RESULTS

Analysis for Cyclopropane

Run No.	Date	Flask	Vol. Flask	Ratio $\frac{cyc}{hex}$	Formality $\frac{moles}{liter}$	
BS 45	9-14	1pipet before	260.60 ml	3X .822	1.85×10^{-2}	
		2pipet before	259.95 ml	2X .823	1.84×10^{-2}	
BS 48	9-17	1pipet before	260.60 ml	4X .825	1.85×10^{-2}	
		2pipet before	259.95 ml	4X .857	1.92×10^{-2}	
		3cyldr after	270.05 ml	4X .795	1.89×10^{-2}	
	9-19	1pipet before	260.60 ml	4X .878	1.97×10^{-2}	}
	(redone)	2pipet before	259.95 ml	3X .858	1.92×10^{-2}	} not in avg.
		3cyldr after	270.05 ml	4X .739	1.76×10^{-2}	}
BS 50	9-21	2cyldr before	259.95 ml	6X .825	1.83×10^{-2}	
		3cyldr before	270.05 ml	6X .780	1.85×10^{-2}	
			Average	29X	1.86×10^{-2} $\frac{moles}{liter}$	

DISCUSSION

The main purpose of the experiment was to insure a consistent cyclopropane solution. The results show that these solutions were indeed all identical with one another within the limits of the accuracy of the experiment. Some of the solutions were pipetted and some were cylindered. VPC shows that there is no difference between these two methods. Also one run was taken from a cyclopropane solution after it had been used to anesthetize the brine shrimp and had been standing for about one-half hour. There did not seem to be any appreciable loss of the cyclopropane into the air. As another check, one set of flasks was left sealed after the original run for two days and run again. This caused the concentrations to change markedly as both the cyclopropane and the 1-hexene evaporated leaving about the same ratio but this can not be taken as true data. Thus all the results compare favorably with one another and show that the method for comparing them is valid.

The absolute value of the solubility in Irving has many more errors involved. The volume of the solutions was no problem but the standard responses of cyclopropane and 1-hexene were very poor values. Ideally, for this type of analysis, a known solution should be made which contains a known amount of 1-hexene and cyclopropane. I could not think of a way to do this, so I resorted to doing them separately. The 1-hexene was not a great problem as it is a liquid and can be handled easily. The cyclopropane standardization is the largest source of error in the calculation. For this reason many runs were made. The error in this standardization is about 5% and I think that this

value limits the accuracy of the value for the cyclopropane concentration. Undoubtedly, an accuracy of 1% can be obtained easily with this system of analysis and different standardization technique.

The work was based on several assumptions regarding the chemistry. I do not know if the assumptions are valid and no investigation was made into them. The solubility of cyclopropane was assumed to be the same in pure H_2O and Irving. The separation constants of cyclopropane, toluene, and 1-hexene between water and toluene were assumed to be small enough as not to affect the results. However, syringing was not accepted as a valid method for placing a known amount of material into the VPC.

The preliminary investigations indicated that other solvents could be used, but the problem with many of them is that they are water soluble. Various anesthetics were also tried. Pentane also worked well. The others would be a little more time consuming but the practice of injecting one sample after another would cut down on the time. An analysis for halothane was done and it gave consistent results. The standardization problem with the liquid anesthetics would be very easy as a known solution could be made. In general, there are no decent literature values for solubility of anesthetics.

My conclusion regarding this work is that VPC is an excellent method for analyzing small concentrations of anesthetics and for determining their solubilities.

EPILOGUE

The brine shrimp apparatus was dismantled and the experiment discontinued. Further experiments on anesthetics were done using goldfish by Dr. Arthur Cherkin and Mr. Chuck Leonard. They used water solutions of anesthetics for analysis. I do not know what the results were of their extensive research into this subject. Water solutions appeared to work fine for them.