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CHM 413
ANALYTICAL CHEMISTRY II

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MODULE 1

Unit 1 The Nature and Scope of Error

Unit 2 Statistical Treatment of Data

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UNIT 1 THE NATURE AND SCOPE OF ERROR

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1.0 INTRODUCTION

Analytical chemistry is a branch of chemistry which seeks ever improved means of measuring the chemical composition of natural and artificial materials. The techniques of this science are used to identify the substances which may be present in a material (qualitative analysis) and to determine the exact amounts of the identified substance (quantitative analysis).

Modern analytical chemistry is overwhelmingly a quantitative science. It may be useful for an analyst to proclaim to have detected some boron in a distilled water sample, but it is much more useful to be able to say how much boron is present. The errors which occur in qualitative studies in any analytical laboratory are of utmost importance.

2.0 OBJECTIVES

By the end of this unit, students should be able to;

- Define the term error
- State various types of errors
- Handle systematic errors

- Plan and design experiments

3.0 MAIN CONTENT

3.1 Definitions of Errors

Error is defined as a difference between a computed, estimated, or measured value and the accepted true, specified, or theoretically correct value. Quantitative results are not of any value unless they are accompanied by some estimate of the errors inherent in them. This principle is applicable to any field of study in which numerical experimental results are obtained.

It is common to perform replicate determinations in the course of a single experiment in order to reveal the presence of random errors. For instance, an analyst performs a titrimetric experiment three times and obtains values of 31.29, 31.16 and 33.29ml. It should be noted that due to variations inherent in the measurements all the three values are different. It is obvious that the third titre is substantially different from the other two. The mean of the two titres is reported as 31.23ml while the third value is rejected.

A second frequent problem involves the comparison of two or more sets of result. Suppose that the vanadium content of steel sample were measured by two different methods. The first method has an average value of 1.04% with an estimated error of 0.07%, and the average value for the second method is 0.95% with an error of 0.04%. Several questions arise from these results. Are the errors in the two methods significant difference? Are the two average values significantly difference? Which of the mean values is closer to the truth? Unit two of this Module discusses these and related questions.

3.2 Types Of Errors

Three types of errors have been identified. These are known as gross, random, and systematic errors.

3.2.1 Gross Errors

Gross errors are defined as errors that are so serious that there is no real alternative to abandoning the experiment and making a fresh start. Complete instrumental breakdown and accidentally dropping of a crucible during the course of experiment exemplify gross errors. These errors occur only occasionally even in the best regulated laboratories.

3.2.2 Random Errors

These are indeterminate errors and cannot be avoided because of the uncertainty in every experiment. These types of errors are also called accidental errors and are due to inherently unpredictable fluctuations in the readings of measurement of apparatus or in the experimenter's interpretation of instrumental reading.

Random errors affects precision and cause replicate results to fall on either side of a mean value. These errors can be estimated using replicate measurements and are minimized by good technique such as averaging of multiple measurements but not eliminated. Random errors are caused by both humans and equipment.

Examples

(a) The type of variation associated with same analyst reading the same absorbance

scale many times.

(b) Variation associated with three or four different analyst reading the same measuring scale or reading the lower measurement of a volumetric flask.

3.2.3 Systematic errors

Systematic errors are also known as determinate errors. These errors are non random and occur when something is wrong with the measurement. In the same given experiment there may be several sources of systemic error, some are positive and others negative. The total systemic error is known as the bias of the experiment, an overall deviation of result from the true value even when random errors are too small. Systematic errors cause all results to be either too high or too low and cannot be detected simply by using replicate measurements, but can be corrected by using standard methods and materials. These errors are caused by humans and equipment. Some commonly encountered determinate errors during the course of laboratory experiments are:

3.2.4 Instrumental Errors

These are errors traced to the use of faulty equipments, as well as uncalibrated or poorly calibrated weights and glass wares during the course of laboratory experiments.

3.2.5 Operative Errors

Basically these are personal (operator) errors attributed to either inexperience on the use of equipment or lack of care by the analyst in the physical manipulation involved. It may be mathematical error in the calculation or prejudice in estimating measurement. Examples of operative errors are incomplete drying of samples, and transfer of solutions.

3.2.6 Methodic Errors

It is a very serious problem for an analyst because these errors are inherent in the method or procedure. These include errors such as co-precipitation with impurities, incomplete reaction, impurities in the reagents used, etc. The methodical error is also correctable by running a reagent blank and standard addition.

Radom errors and systematic errors can occur independently of one another and may arise at different stages of the experiment.

Accuracy is the degree of agreement between a measured value and a true value.

Precision is the degree of agreement between replicate measurements of the same quantity and does not necessarily imply accuracy. Precision describes random error and bias describes systematic error while both precision and accuracy affect accuracy.

3.3 Tackling Systematic Errors

Several procedures are available in handling systematic errors and are evaluated by a wide range of statistical methods.

3.3.1 Foresight

The first precautions is taken at the inception of any laboratory experiment is to identify

the most likely sources of systematic errors. At this stage issues like instrumental functions that need calibrating, the steps of the analytical procedure where errors are inherent, and the checks that can be made during the analysis, such as contamination of reagents, must be identified.

3.3.2 Experimental Design

A thoughtful experimental planning is essential in locating and minimizing the major sources of systematic errors. Weighing by difference can remove some systematic error since they occurred to the same extent in both weighing and subtraction process eliminates them.

Another instance is in the concentration measurement of sample of a single material using spectrometer. Two procedures are available for this analysis; in the first procedure, a sample is placed in 1-cm path-length spectrometer cell at a wavelength of 400nm. Several systematic errors can occur; the wave length might be 405nm instead of 400nm, thus rendering the reference value for ϵ inappropriate, the path length of the cell may not be exactly 1cm, or the molar extinction coefficient might not be correct.

Alternatively, calibration graph approach might be adopted in which case the value of ϵ is not required. The errors due to wavelength shifts, absorbance errors and path length inaccuracies are expected to cancel out, because they occur equally in the calibration and test experiment under the same conditions. Major sources of systematic errors are eliminated by this method.

3.3.3 Standard Reference Material

The third line of action against systematic errors is in the use of standard reference materials and method. Each piece of apparatus is calibrated by an appropriate procedure before the experiment is started. Volumetric equipment can be calibrated by the use of gravimetric methods. Spectrometer wavelength scales is calibrated with the aid of standard light sources which has narrow emission lines at well-established wavelength. Similarly spectrometer absorbance scales can be calibrated with standard solid or liquid filters.

3.3.4 Comparison with Other Methods

The occurrence of systematic errors in any given method is checked by comparing the results with those obtained from different methods. If they two unrelated methods used to perform one analysis consistently yield results showing only random differences, then it can be inferred that no significant systematic errors are present provided each step of the two methods are independent of one another and comparisons made over the whole concentration range for which the procedure is to be used.

4.0 CONCLUSION

Random errors and systematic errors occur independently of one another and may arise at different stage of the experiment.

5.0 SUMMARY

In summary, we have learnt the following in this unit:

- i. The techniques of analytical chemistry are used to identify substances present in

- a material and to determine the exact amounts of the identified substance.
- ii. Errors are variations that naturally accompanied the experiment performed.
 - iii. Three types of errors were identified namely; gross, random, and systematic errors.
 - iv. Random errors affects precision and cause replicate results to fall on either side of a mean values.
 - v. Systematic errors cannot be detected simply by using replicate measurements, but can be corrected by the use of standard methods and materials.

6.0 Tutor Marked Assignment

- 1 Briefly explain why modern analytical chemistry is a quantitative science
- 2 Explain the following terms;
(i) Accuracy (ii) Precision (iii) Methodic error (iv) Instrumental error
- 3 Distinguish between random and systematic errors
- 4 Explain various precautions taken in handling systematic errors

7.0 References and further reading

- 1 John.R.T. (1999). An introduction to Error Analysis: The Study of Uncertainties in Physical Measurement. University Science Books.
- 2 James N.M and Jane C. M.(2005).Statistics and Chemometrics for Analytical chemistry. Pearson Education Limited.
- 3 Skoog W,and Holler C.,(2004) Fundamental of Analytical Chemistry. 8th ed.College Bookstore.

UNIT 2 STATISTICAL TREATMENT OF DATA

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1.0 INTRODUCTION

You will recall that in unit 1, it was learnt that errors are variations that normally accompanied experiments performed and affect precision as well as accuracy result. In this unit you will study statistical treatment of data.

Statistical treatment of data is essential in order to make use of the data in the right form. Raw data collected is only one aspect of any experiment. The organization of data is however very important so that conclusions can be drawn. This is what statistical treatment of data is all about. An important aspect of statistical treatment of data is the handling of errors. All experiments invariably produce errors and noise. Both systematic and random errors need to be taken into consideration.

2.0 OBJECTIVES

By the end of this unit, students should be able to;

- Meaning and standard deviation
- Appreciate the concept of confidence limit
- List appropriate statistical tools available for data handling
- Interpret data and arrive at safe conclusion using various statistical tools

3.0 MAIN CONTENT

3.1 Standard Deviation

The necessity in making repeated measurements in several analytical experiments in order to reveal the presence of random errors was emphasized in unit 1. Some fundamental statistical

concepts in analyzing experimental results shall be applied in units(2 and 3).

Suppose an experimentalist performed five replicates titration experiments and the following results were obtained.

Table 1.1 Titration results

Burette reading(cm ³)	First titre	Second titre	Third titre	Fourth titre	Fifth titre
Final reading(cm ³)	10.08	10.31	15.19	10.12	20.10
Initial reading(cm ³)	0.00	0.20	5.10	0.00	10.00
Volume of acid used(cm ³)	10.08	10.11	10.09	10.12	10.10

Two criteria can be used to compare these results, the average values and the degree of spread. The average value used is otherwise called the arithmetic mean, which is the sum of all the measurements divided by the number of measurements.

Mathematically the mean, \bar{x} , of n measurement is given by
$$\bar{x} = \frac{\sum x_i}{n} \quad (1.1)$$

The spread is also known as the range which is the difference between the highest and the lowest value. A more useful measure of spread which utilizes all values, is the standard deviation.

The standard deviation, s, of n measurements is given by

$$s = \sqrt{\sum_i (x_i - \bar{x})^2 / (n - 1)} \quad (1.2)$$

Example 1.1

Find the mean and standard deviation of the above titration experiments.

Solution

	x_1	$x_1 - \bar{x}$	$(x_1 - \bar{x})^2$
	10.08	-0.02	0.0004
	10.11	0.01	0.0001
	10.09	-0.01	0.0001
	10.12	0.02	0.0004
	10.10	0.00	0.0000
Totals	50.50	0.00	0.0010

$$\bar{x} = \frac{\sum x_i}{n} = \frac{50.50}{5}$$

$$\bar{x} = 10.1 \text{ cm}^3$$

$$s = \sqrt{\sum_i (x_i - \bar{x})^2 / (n - 1)}$$

$$s = \sqrt{\frac{0.001}{4}}$$

$$s = 0.0158 \text{ cm}^3$$

Example 1.2

Calculate the mean and the standard deviation of the following set of analytical results, 15.67, 15.69, and 16.03g.

Solution

Alternatively, equation 1.3 can also be used in solving problems of these nature.

$$s = \sqrt{\frac{\sum (x_i - \bar{x})^2 / N}{N - 1}}$$

(1.3)

X _i	X _i ²
15.67	245.55
15.69	246.18
16.03	256.96
∑ 47.39	∑ 748.69

$$s = \frac{\sqrt{748.69 - (47.39)^2 / 3}}{3 - 1}$$

$$s = 0.210\text{g}$$

The answer to the above example have been arbitrarily given to three significant figures.

3.2 Variance

Variance is a very useful statistics quantity which is the square of the standard deviation, s²

Variance = the square of the standard deviation.

3.2.1 Coefficient of Variation (CV)

Coefficient of variance is also known as the relative standard variation (RSD) which is given by $100s/\bar{x}$ and is a widely used measure of spread. σ

3.3 Confidence Limit of the Mean

For a sample of n measurements, the standard error of mean

$$(\text{s.e.m}) = \frac{\sigma}{\sqrt{n}} \tag{1.4}$$

The confidence interval for the mean is the range of values within which the population mean, μ, is expected to lie with a certain probability. The boundaries are called confidence limit

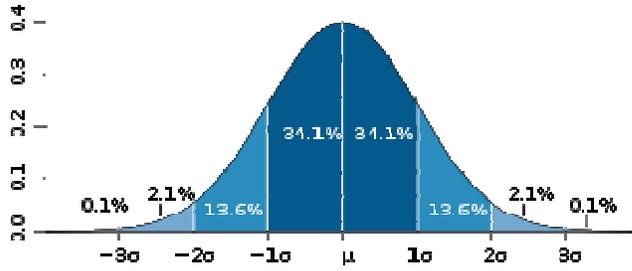


Figure 1.1 The sampling distribution of the mean

Dark blue is less than one standard deviation from the mean. For the normal distribution, this accounts for about 68% of the set, while two standard deviations from the mean (medium and dark blue) account for about 95%, and three standard deviations (light medium, and dark blue) account for about 99.7%. If we assume that the distribution is normal, then 95% of the sample means will lie in the range given by $\mu - 1.96(\sigma/\sqrt{n}) < \bar{x} < \mu + 1.96(\sigma/\sqrt{n})$ (1.5)

The confidence level is the probability that the true mean lies in a certain interval and is often expressed in percentage. The confidence interval for the mean of n measurements can be calculated thus;

$$CI = \bar{x} \pm t_s \sqrt{N} \quad (1.6)$$

Where \bar{x} is the sample mean, s is the standard deviation and t is the t-statistic distribution otherwise known as student's t .

For a single measurement with result x , t is given as,

$$t = \frac{\bar{x} - \mu}{s} \quad (1.7)$$

However, for N measurements, t is given as, $t = \frac{\bar{x} - \mu}{s/\sqrt{N}}$ (1.8)

t depends on the desired confidence level, as well as on the number of degrees of freedom in the calculation of standard deviation. The value of t is found by consulting the t-test table at $N-1$ degrees of freedom. The student t level is shown in Table 1.2

Table 1.2 Values of t for confidence intervals

Degrees of freedom	Values of t for confidence interval of				
	80%	90%	95%	99%	99.9%
1	3.08	6.31	12.7	63.7	637
2	1.89	2.92	4.30	9.92	31.6
3	1.64	2.35	3.18	5.84	12.9
4	1.53	2.13	2.78	4.60	8.60
5	1.48	2.02	2.57	4.03	6.86
6	1.44	1.94	2.45	3.71	5.96
7	1.42	1.90	2.36	3.50	5.40
8	1.40	1.86	2.31	3.36	5.04
9	1.38	1.83	2.26	3.25	4.78
10	1.37	1.81	2.23	3.17	4.59

11	1.36	1.80	2.20	3.11	4.44
12	1.36	1.78	2.18	3.06	4.32
13	1.35	1.77	2.16	3.01	4.22
14	1.34	1.76	2.14	2.98	4.14
∞	1.29	1.64	1.96	2.58	3.29

For large samples, the confidence limits of the mean are given by, $C.I = \bar{x} \pm z s \sqrt{n}$ 1.9

Where the values of z depends on the degree of confidence required. The values for z at various confidence levels for small and large samples can be found in Table 1.3.

Table 1.3 Confidence levels for various values of z

Confidence level, %	z
50	± 0.67
68	± 1.00
80	± 1.29
90	± 1.64
95	± 1.96
96	± 2.00
99	± 2.58
99.7	± 3.00
99.9	± 3.29

Example 1.3

A chemist obtained the following results for the alcohol content of a sample of human blood.

% C₂H₅OH: 0.084, 0.089, and 0.079

Calculate the 95% confidence interval for the mean.

Solution

$$\sum x_1 = 0.084 + 0.089 + 0.079$$

$$= 0.252$$

$$\sum x_1^2 = 0.07056 + 0.007921 + 0.006241$$

$$= 0.021218$$

$$s = \sqrt{\frac{0.021218 - (0.252)^2 / 3}{3 - 1}}$$

$$s = 0.0050\% \text{ C}_2\text{H}_5\text{OH}$$

Hence, $\bar{x} = \frac{0.252}{3}$

$\therefore N=3$, degree of freedom = $N-1=2$

Values of $N-1=2$, at 95% CI from table is 4.30

Thus, 95% CI = $\bar{x} \pm \frac{ts}{\sqrt{N}}$

$$CI = \frac{0.084 + 4.30 \times 0.0050}{\sqrt{3}}$$

$$CI = 0.084 \pm 0.012\% \text{ C}_2\text{H}_5\text{OH}$$

Example 1.4

The sodium ion content of a urine specimen was determined by using an ion-selective electrode. The following values were obtained: 102, 97, 99, 98, 101, 106 Mm.

What are the 95% and 99% confidence limits for the sodium ion concentration?

Solution

The mean and standard deviation of these values are 100.5 Mm and 3.27 Mm respectively.

$\therefore N=6$, degree of freedom, $N-1=5$

From Table 1.1, the values of t for calculating the 95% confidence limits is 2.57 and from equation (1.6) the 95% confidence limits of the mean are given by;

$$CI = 100.5 \pm 2.57 \times 3.27 / \sqrt{6}$$

$$CI = 100.5 \pm 3.4 \text{ mM}$$

Similarly the 99% confidence limits are given by;

$$100.5 \pm 4.03 \times 3.27 / \sqrt{6} = 100.5 \pm 5.4 \text{ mM}$$

3.4 Significance Tests

This technique tests whether the difference between two results is significant, or whether it can be accounted for by virtue of random variation. Several tests which are very useful to analytical chemists are considered below.

3.4.1 Comparison of an experimental mean with a known value

For every significance test employed, the truth of the hypothesis which is known as the null hypothesis, often denoted by H_0 , is tested. The term null is used to imply that there is no difference between the observed and known values other than that which can be attributed to random variation. Let us suppose that this null hypothesis is true, which implies that statistical theory can be used to evaluate the probability that the observed difference between the sample mean, \bar{x} , and the true value, μ , arises mainly due to errors.

Null hypothesis is usually rejected if the probability of such a difference occurring by chance is less than 1 in 20 (i.e. 0.05 or 5%). In other words, the difference is said to be significant at 5% level. Higher levels of significance such as 1% or 0.1% can be used so as to be more certain that the correct decision was made. To test H_0 : the population mean is equal to μ , the statistic t is calculated thus:

$$t = (\bar{x} - \mu) \sqrt{\frac{n}{s}} \quad (1.10)$$

Where \bar{x} = sample mean, s = sample standard deviation and n = sample size. If $|t|$ calculated exceeds a certain critical value then the null hypothesis is rejected.

Example 1.5

In a new method for the determining selenourea in water, the following values were obtained for tap water samples spiked with 50 ng ml^{-1} of selenourea: 50.4, 50.7, 40.1, 49.1, 49.0, 51.1 ng ml^{-1}

Is there any evidence of systematic error?

Solution

$$\sum x = 50.4 + 50.7 + 49.1 + 49.0 + 51.1$$

$$= 250$$

$$\bar{x} = \frac{\sum x_1}{n} = \frac{250.3}{5}$$

$$\bar{x} = 50.06$$

$$\sum x_1^2 = 12533.67$$

$$s = \frac{\sqrt{12533.67 - (250.3)^2 / 5}}{5 - 1}$$

$$s = \sqrt{\frac{3.652}{4}}$$

$$s = 0.956$$

Adopting the null hypothesis that there is no systematic error. Using equation (1.10) and $\mu = 50$

$$t = \frac{(50.06 - 50)\sqrt{5}}{0.956}$$

$$t = 0.14$$

From table 1.2, the critical value is $t_4 = 2.78$ ($P = 0.05$). Since the observed value of $|t|$ is less than the critical values the null hypothesis is retained, there is no evidence of systematic error.

3.4.2 Comparison of two experimental means

The result of a new analytical method may be tested by comparing them with those obtained by using a second method. If the null hypothesis is that the two methods give the same result, in this case $H_0: \mu_1 = \mu_2$. Then we can test whether $(\bar{x}_1 - \bar{x}_2)$ differs significantly from zero. A pooled estimate, s , of a standard deviation can be calculated provided the two samples have standard deviations which are not significantly different.

In order to test the null hypothesis, $H_0: \mu_1 = \mu_2$, the statistical t is evaluated thus:

$$t = \frac{(\bar{x}_1 - \bar{x}_2)}{s \sqrt{\frac{1}{n_1} + \frac{1}{n_2}}} \quad (1.11)$$

Where s is calculated as from:

$$s^2 = \frac{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2}{(n_1 + n_2 - 2)} \quad (1.12)$$

t has $n_1 + n_2 - 2$ degree of freedom.

The basic assumption of this method is that the samples are drawn from populations with equal standard deviations.

Example 1.6

In a comparison of two methods for the determination of chromium in rye grass, the following results ($\text{mg kg}^{-1} \text{Cr}$) were obtained:

Method 1: mean = 1.48; standard deviation 0.28

Method 2: mean=2.33; standard deviation 0.31

Five determinations were made for each method. Do these two methods give results having means which differ significantly?

Solution

The results of the two given by the two methods are equal is the null hypothesis adopted.

From equation 1.12,

$$s^2 = \frac{([4 \times 0.28^2] + [4 \times 0.31^2])}{5 + 5 - 2}$$

$$s^2 = 0.0873$$

$$s = 0.295$$

From equation 1.11

$$t = \frac{2.33 - 1.48}{0.295 \sqrt{\frac{1}{5} + \frac{1}{5}}} = 4.56$$

Degree of freedom = 5 + 5 - 2 = 8

From table (1.2) the critical value $t_8 = 2.31$ (P=0.05). The experimental value of |t| is greater than the critical value, hence the difference between the two results is significant at the 5% level and the null hypothesis is rejected.

3.5 F-TEST

F-test is used to compare the standard deviations in order to detect random errors of two sets of data. In order to test whether the difference between two variances is significant, that is to test

$H_0: \sigma_1^2 = \sigma_2^2$. The statistic F is calculated thus:

$$F = \frac{s_1^2}{s_2^2} \quad (1.13)$$

The number of degrees of freedom of the numerator and denominator are $n_1 - 1$ and $n_2 - 1$ respectively. The test assumes that the populations from which the samples are taken are normal. The null hypothesis is true when the variance ratio is close to 1. If the calculated value of F exceeds a critical value then the null hypothesis is rejected. The values of P=0.05 are given in Table 1.4

Example 1.7

A proposed method for the determination of the chemical oxygen demand of waste water was compared with the standard method. The following results (mg l^{-1}) were obtained for a sewage effluent sample;

Standard method: mean = 72; standard deviation = 3.31

Proposed method: mean = 72; standard deviation = 1.51

Eight determinations were made for each method. Is the precision of the proposed method significantly greater than that of the standard method?

Solution

$$F = \frac{3.31^2}{1.51^2} = 4.8$$

This is an instance of a one-sided test, the critical value is $F_{7,7} = 3.787$ ($P=0.05$). Where the subscripts indicate the degree of freedom of the numerator and denominator respectively. The variance of the standard method is significantly greater than that of the proposed method at the 5% probability level since the calculated value of $F(4.8)$ exceeds the tabulated value (3.787).

Table 1.4 One Tailed Critical Values of F at 5%

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	20	30	40	50	75	100	250	500	∞
1	161.4	199.5	215.7	224.6	230.2	234.0	236.8	238.9	240.5	241.9	243.0	243.9	244.7	245.4	245.9	248.0	250.1	251.1	251.8	252.6	253.0	253.8	254.1	254.3
2	18.51	19.00	19.16	19.25	19.30	19.33	19.35	19.37	19.38	19.40	19.40	19.41	19.42	19.42	19.43	19.45	19.46	19.47	19.48	19.48	19.49	19.49	19.49	19.50
3	10.13	9.552	9.277	9.117	9.013	8.941	8.887	8.845	8.812	8.785	8.763	8.745	8.729	8.715	8.703	8.660	8.617	8.594	8.581	8.563	8.554	8.537	8.532	8.526
4	7.709	6.944	6.591	6.388	6.256	6.163	6.094	6.041	5.999	5.964	5.936	5.912	5.891	5.873	5.858	5.803	5.746	5.717	5.699	5.676	5.664	5.643	5.635	5.628
5	6.608	5.786	5.409	5.192	5.050	4.950	4.876	4.818	4.772	4.735	4.704	4.678	4.655	4.636	4.619	4.558	4.496	4.464	4.444	4.418	4.405	4.381	4.373	4.365
6	5.987	5.143	4.757	4.534	4.387	4.284	4.207	4.147	4.099	4.060	4.027	4.000	3.976	3.956	3.938	3.874	3.808	3.774	3.754	3.726	3.712	3.686	3.678	3.669
7	5.591	4.737	4.347	4.120	3.972	3.866	3.787	3.726	3.677	3.637	3.603	3.575	3.550	3.529	3.511	3.445	3.376	3.340	3.319	3.290	3.275	3.248	3.239	3.230
8	5.318	4.459	4.066	3.838	3.688	3.581	3.500	3.438	3.388	3.347	3.313	3.284	3.259	3.237	3.218	3.150	3.079	3.043	3.020	2.990	2.975	2.947	2.937	2.928
9	5.117	4.256	3.863	3.633	3.482	3.374	3.293	3.230	3.179	3.137	3.102	3.073	3.048	3.025	3.006	2.936	2.864	2.826	2.803	2.771	2.756	2.726	2.717	2.707
10	4.965	4.103	3.708	3.478	3.326	3.217	3.135	3.072	3.020	2.978	2.943	2.913	2.887	2.865	2.845	2.774	2.700	2.661	2.637	2.605	2.588	2.558	2.548	2.538
11	4.844	3.982	3.587	3.357	3.204	3.095	3.012	2.948	2.896	2.854	2.818	2.788	2.761	2.739	2.719	2.646	2.570	2.531	2.507	2.473	2.457	2.426	2.415	2.404
12	4.747	3.885	3.490	3.259	3.106	2.996	2.913	2.849	2.796	2.753	2.717	2.687	2.660	2.637	2.617	2.544	2.466	2.426	2.401	2.367	2.350	2.318	2.307	2.296
13	4.667	3.806	3.411	3.179	3.025	2.915	2.832	2.767	2.714	2.671	2.635	2.604	2.577	2.554	2.533	2.459	2.380	2.339	2.314	2.279	2.261	2.229	2.218	2.206
14	4.600	3.739	3.344	3.112	2.958	2.848	2.764	2.699	2.646	2.602	2.565	2.534	2.507	2.484	2.463	2.388	2.308	2.266	2.241	2.205	2.187	2.154	2.142	2.131
15	4.543	3.682	3.287	3.056	2.901	2.790	2.707	2.641	2.588	2.544	2.507	2.475	2.448	2.424	2.403	2.328	2.247	2.204	2.178	2.142	2.123	2.089	2.078	2.066
20	4.351	3.493	3.098	2.866	2.711	2.599	2.514	2.447	2.393	2.348	2.310	2.278	2.250	2.225	2.203	2.124	2.039	1.994	1.966	1.927	1.907	1.869	1.856	1.843
30	4.171	3.316	2.922	2.690	2.534	2.421	2.334	2.266	2.211	2.165	2.126	2.092	2.063	2.037	2.015	1.932	1.841	1.792	1.761	1.718	1.695	1.652	1.637	1.622
40	4.085	3.232	2.839	2.606	2.449	2.336	2.249	2.180	2.124	2.077	2.038	2.003	1.974	1.948	1.924	1.839	1.744	1.693	1.660	1.614	1.589	1.542	1.526	1.509
50	4.034	3.183	2.790	2.557	2.400	2.286	2.199	2.130	2.073	2.026	1.986	1.952	1.921	1.895	1.871	1.784	1.687	1.634	1.599	1.551	1.525	1.475	1.457	1.438
75	3.968	3.119	2.727	2.494	2.337	2.222	2.134	2.064	2.007	1.959	1.919	1.884	1.853	1.826	1.802	1.712	1.611	1.555	1.518	1.466	1.437	1.381	1.360	1.338
100	3.936	3.087	2.696	2.463	2.305	2.191	2.103	2.032	1.975	1.927	1.886	1.850	1.819	1.792	1.768	1.676	1.573	1.515	1.477	1.422	1.392	1.331	1.308	1.283
250	3.879	3.032	2.641	2.408	2.250	2.135	2.046	1.976	1.917	1.869	1.827	1.791	1.759	1.732	1.707	1.613	1.505	1.443	1.402	1.341	1.306	1.232	1.202	1.166
500	3.860	3.014	2.623	2.390	2.232	2.117	2.028	1.957	1.899	1.850	1.808	1.772	1.740	1.712	1.686	1.592	1.482	1.419	1.376	1.312	1.275	1.194	1.159	1.113
∞	3.841	2.996	2.605	2.372	2.214	2.099	2.010	1.938	1.880	1.831	1.789	1.752	1.720	1.692	1.666	1.571	1.459	1.394	1.350	1.283	1.243	1.152	1.106	1.000

3.6 Outliers

A situation may arise in which one (or more) of the results appears to differ unreasonably from the others in the set. Such a measurement is known as an outlier. The ISO recommended test for outliers is Grubb's test.

In order to use Grubb's test for an outlier, the null hypothesis is tested: all measurements come from the same population.

Then G is calculated thus;

$$G = (\text{suspect value} - \bar{x}) / s \tag{1.14}$$

Where \bar{x} and s are calculated with the suspected value included. The basic assumption of this test is that the population is normal.

Example 1.8

The following values were obtained for nitrate concentration (mg l^{-1}) in a sample of river water; 0.403, 0.410, 0.401, 0.380, 0.400, 0.413, 0.408.

Should 0.380 be rejected?

The seven values have $\bar{x} = 0.4021$ and $s = 0.01088$

$$G = \frac{|0.380 - 0.4021|}{0.01088} = 2.031$$

The critical value of G(P=0.05) for a sample of seven is 2.020. The suspect measurement is rejected at 5% significance level.

Table 1.5 Critical values of G(P=0.05) for a two-sided test

Sample size	Critical value
3	1.155
4	1.481
5	1.715
6	1.887
7	2.020
8	2.126
9	2.215
10	2.290

Another test for outlier is the Dixon's test. This is also known as the Q-test.

3.6.1 Q-test: Detection of a single outlier

(a) Theory

In a set of replicate measurements of a physical or chemical quantity, one or more of the obtained values may differ considerably from the majority of the rest. In this case there is always a strong motivation to eliminate those deviant values and not to include them in any subsequent calculation (e.g. of the mean value and/or of the standard deviation). This is permitted only if the suspect values can be "legitimately" characterized as outliers.

Usually, an outlier is defined as an observation that is generated from a different model or a different distribution than was the main "body" of data. Although this definition implies that an outlier may be found anywhere within the range of observations, it is natural to suspect and examine as possible outliers only the extreme values.

The rejection of suspect observations must be based exclusively on an objective criterion and not on subjective or intuitive grounds. This can be achieved by using statistically sound tests for "the detection of outliers".

The Dixon's **Q-test** is the simpler test of this type and it is usually the only one described in textbooks of Analytical Chemistry in the chapters of data treatment. This test allows us to examine if one (and only one) observation from a small set of replicate observations (typically 3 to 10) can be "legitimately" rejected or not.

Q-test is based on the statistical distribution of "subrange ratios" of ordered data samples, drawn from the same normal population. Hence, a normal (Gaussian) distribution of data is assumed whenever this test is applied. In case of the detection and rejection of an outlier, Q-test cannot be reapplied on the set of the remaining observations.

(b) How the Q-test is applied

The test is very simple and it is applied as follows:

(1) The N values comprising the set of observations under examination are arranged in ascending order:

$$x_1 < x_2 < \dots < x_N$$

(2) The statistic experimental Q-value (Q_{exp}) is calculated. This is a ratio defined as the difference of the suspect value from its nearest one divided by the range of the values (Q: rejection quotient). Thus, for testing x_1 or x_N (as possible outliers) we use the following Q_{exp} values:

$$Q_{\text{exp}} = \frac{x_2 - x_1}{x_N - x_1} \quad Q_{\text{exp}} = \frac{x_N - x_{N-1}}{x_N - x_1} \quad (1.15)$$

(3) The obtained Q_{exp} value is compared to a critical Q-value (Q_{crit}) found in tables. This critical value should correspond to the confidence level (CL) we have decided to run the test (usually: CL=95%).

(4) If $Q_{\text{exp}} > Q_{\text{crit}}$, then the suspect value can be characterized as an outlier and it can be rejected, if not, the suspect value must be retained and used in all subsequent calculations.

The **null hypothesis** associated to Q-test is as follows: "There is no a significant difference between the suspect value and the rest of them, any differences must be exclusively attributed to random errors".

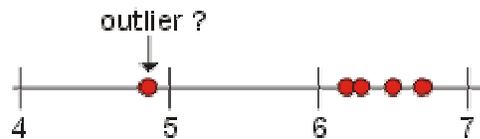
A table containing the critical Q values for CL 90%, 95% and 99% and N=3-10 is given below [from: *D.B. Rorabacher, Anal. Chem. 63 (1991) 139*]

Example 1.9

The following replicate observations were obtained during a measurement and they are arranged in ascending order:

4.85, 6.18, 6.28, 6.49, 6.69.

These values can be represented by the following dotplot:



Can we reject observation 4.85 as an outlier at a 95% confidence level?

Answer: The corresponding Q_{exp} value is: $Q_{\text{exp}} = (6.18 - 4.85) / (6.69 - 4.85) = 0.722$. Q_{exp} is greater than Q_{crit} value (=0.710, at CL:95% for N=5). Therefore we can reject 4.85 and being certain that the probability (p) of erroneous rejection of the null hypothesis (type 1 error) is less than 0.05.

Note: At confidence level 99%, the suspect observation cannot be rejected, hence the probability of erroneous rejection is greater than 0.01.

(c) A general comment on the rejection of outliers

All data rejection tests must be judiciously used. Some statisticians object to the rejection of data from any small size data sample, unless it is solidly known that something went wrong during the corresponding measurement. Other recommend the accommodation of outliers and not their rejection, i.e. they suggest to include deviant values in all subsequent calculations but with reduced statistical weight (Winsorized methods).

It should be also stressed that the use of Q-test is increasingly discouraged in favor of other more robust methods. One such method is the Huber method, which takes into consideration all data present within the set, and not only three as in the case of Q-test.

The test is valid for samples size 3 to 7 and if the calculated value of Q exceeds the critical value, the suspected value is rejected. The critical value of Q for P=0.05 for a two sided test are given in Table(1.6)

Table 1.6 Critical values of Q

N	Q _{crit} (CL.90%)	Q _{crit} (CL.95%)	Q _{crit} (CL.99%)
3	0.941	0.970	0.994
4	0.765	0.829	0.926
5	0.642	0.710	0.821
6	0.560	0.625	0.740
7	0.507	0.568	0.680
8	0.468	0.526	0.634
9	0.437	0.493	0.598
10	0.412	0.466	0.568

Consider the data from the previous example(1.8).Applying Dixon’s test;

$$Q = (0.380 - 0.400) / (0.413 - 0.380)$$

$$Q = 0.606$$

The critical value of Q(P=0.05) for a sample size 7 is 0.570. The suspect value 0.380 is rejected.

3.7 Analysis of Variance

Analysis of variance, abbreviated to ANOVA, is very powerful statistical technique that can be used to separate and estimate the different causes of variation. ANOVA can also be used in situations where there is more than one source of random variation.

Example 1.10

Table 1.7 shall be used to effectively study comparison of several means. It shows the result obtained in an investigation into the stability of a fluorescent reagent stored under different

condition. Anova tests whether the difference between the sample means is too great to be explained by the random error.

Table 1.7

Condition	Replicate measurements	Mean
A Freshly prepared	102,100,101	101
B Stored for 1 hour in the dark	101,101,104	102
C Stored for 1 hour in subdued light	97,95,99	97
D Stored for 1 hour in bright light	90,92,94	92
	Overall mean	98

Within-sample variation; the variance for each sample is estimated by using the formula

$$\sum (x_i - \bar{x})^2 / (n - 1) \quad \text{(see equation 1.2)}$$

Variance of the respective samples are evaluated thus:

$$A = \frac{(102 - 101)^2 + (100 - 101)^2 + (101 - 101)^2}{(3 - 1)} = 1$$

$$B = \frac{(101 - 102)^2 + (101 - 102)^2 + (104 - 102)^2}{(3 - 1)} = 4$$

$$C = \frac{(97 - 97)^2 + (95 - 97)^2 + (99 - 97)^2}{3 - 1} = 4$$

Similarly it can be shown that D has a value of 4.

Averaging these values gives:

$$\text{Within-sample estimate of } \sigma_o^2 = \frac{(1 + 3 + 4 + 4)}{4} = 3$$

The degree of freedom is 8 (since each sample estimate has 2 degree of freedom) and mean square is 3. The sum of squared terms is $3 \times 8 = 24$

Between-sample variation

$$\sigma_o^2 = n \sum_i (\bar{x}_i - \bar{x})^2 / (h - 1).$$

(1.16)

If the null hypothesis is true, the variance of the mean of the samples gives an estimate of σ_o^2 / n

$$\text{Sample mean variance} = \frac{(101 - 98)^2 + (102 - 98)^2 - (97 - 98)^2 - (92 - 98)^2}{(4 - 1)} = \frac{62}{3}$$

$$\text{The estimate of } \sigma_o^2 = \left(\frac{62}{3} \right) \times 3 = 62$$

From calculation above, the mean square is 62 and degree of freedom is 3 therefore the sum of square term is $3 \times 62 = 186$

The statistic $F = \frac{\text{Between-sample mean square}}{\text{Within-sample mean square}}$

$$F = \frac{62}{3} = 20.7$$

The critical value is $F_{3,8} = 4.066$ ($P=0.05$), it can be seen that the calculated value is greater than the critical value, hence the null hypothesis is rejected. This implies that the sample mean differ significantly.

It should be noted that a significant test at $P=0.05$ level involves a 5% risk that a null hypothesis will be rejected even though it is true. This type of error is known as Type I error. The risk of such an error can be reduced by altering the significance level of the test to $P=0.01$ or $P=0.0001$.

However, it is still possible to retain a null hypothesis even when it is false. This is known as the Type II error and the probability of this error can be estimated by the use of alternative postulate (alternative hypothesis, H_1).

4.0 CONCLUSION

Treatment of errors constitute a very important concept that helps in ascertaining the integrity of the values reported in an experiment reported.

5.0 SUMMARY

In this unit we have learnt that:

- i. Standard deviation is not only a useful measure of spread which utilizes all the values but also evaluates the precision in analytical measurement.
- ii. Confidence limit assert with a given degree of confidence that the confidence intervals does include the true value.
- iii. Significance test is employed in order to decide whether the difference between the measured and standard amounts can be accounted for by random error.
- iv. Student t-test, F-test and analysis of variance were the analytical techniques used for the test of significance.
- v. The rejection of suspected observations (outlier) must be based exclusively on an objective criterion and not on subjective or intuitive ground.

6.0 TUTOR MARKED ASSIGNMENT

- 1 Define the following terms:
(i) Standard deviation (ii) Outliers (iii) Type I error (iv) Confidence limit
- 2 Seven measurements of the pH of a buffer solution gave the following results:
5.12 , 5.20, 5.15, 5.17, 5.16, 5.19, 5.15.
Calculate: (i) the 95% and (ii) the 99% confidence limits for the true pH (Assume that there are no systematic errors)
- 3 The following figures refer to the concentration of albumin, in the blood sera of 16 healthy adults;
37, 39, 37, 42, 39, 45, 42, 39, 44, 40, 39, 45, 47, 47, 43, 41.
The first eight figures are for men and the second eight for women. Test whether the mean concentrations for men and women differ significantly.
- 4 The following five values were obtained for the wt% of an organic acid in a sample:
31.2, 32.6, 36.7, 28.9, 30.3.

Determine if the value 36.7 should be rejected at 95% confidence level.

7.0 REFERENCES AND FURTHER READING

1. John.R.T. (1999). An introduction to Error Analysis: The Study of Uncertainties in Physical Measurement. University Science Books.
2. James N.M and Jane C. M.(2005).Statistics and Chemometrics for Analytical Chemistry. Pearson Education Limited.
3. Gordus R.A.(1985). Theory and Problems of Analytical Chemistry.Schaum's Outline Series McGraw-Hill.

UNIT 3 CORRELATION AND REGRESSION

CONTENT

- 1.0 Introduction
- 2.0 Objectives
- 3.0 Main Content
 - 3.1 Product-Moment Correlation
 - 3.1.1 Definition
 - 3.1.2 Understanding and Interpreting the Correlation Coefficient
 - 3.1.3 Variance Interpretation
 - 3.1.4 Further Calculation of the Correlation Coefficient
- 4.0 Conclusion
- 5.0 Summary
- 6.0 Tutor Marked Assignment
- 7.0 References and further reading

1.0 INTRODUCTION

The Pearson Product-Moment Correlation Coefficient (r), or correlation coefficient for short *is* a measure of the degree of linear relationship between two variables, usually labeled X and Y. While in regression the emphasis is on predicting one variable from the other, in correlation the emphasis is on the degree to which a linear model may describe the relationship between two variables. In regression the interest is directional, one variable is predicted and the other is the predictor; in correlation the interest is non-directional, the relationship is the critical aspect.

2.0 OBJECTIVES

At the end of these unit, students should be able to:

- Define Pearson correlation coefficient between two variables.
- Understand and interpret correlation coefficient.
- Appreciate the concept of regression analysis in data treatment.

3.0 MAIN CONTENT

3.1 Product-Moment Correlation

A common method of estimating how well the experimental points fit a straight line is to calculate the product moment correlation.

3.1.1 Definition

Pearson's correlation coefficient between two variables is defined as the covariance of the two variables divided by the product of their standard deviations:

$$\rho_{X,Y} = \frac{\text{cov}(X, Y)}{\sigma_X \sigma_Y} = \frac{E[(X - \mu_X)(Y - \mu_Y)]}{\sigma_X \sigma_Y}, \quad (1.17)$$

The above formula defines the *population* correlation coefficient, commonly represented by the Greek letter ρ (rho). Substituting estimates of the covariances and variances based on a sample gives the *sample correlation coefficient*, commonly denoted r :

$$r = \frac{\sum \{(x_1 - \bar{x})(y_1 - \bar{y})\}}{\left\{ \sum (x_1 - \bar{x})^2 \sum (y_1 - \bar{y})^2 \right\}^{1/2}} \quad (1.18)$$

The correlation coefficient may take on any value between plus and minus one.

$$-1.00 \leq r \leq +1.00$$

The sign of the correlation coefficient (+ , -) defines the direction of the relationship, either positive or negative. A positive correlation coefficient means that as the value of one variable increases, the value of the other variable increases; as one decreases the other decreases. A negative correlation coefficient indicates that as one variable increases, the other decreases, and vice-versa.

Taking the absolute value of the correlation coefficient measures the strength of the relationship. A correlation coefficient of $r = .50$ indicates a stronger degree of linear relationship than one of $r = .40$. Likewise a correlation coefficient of $r = -.50$ shows a greater degree of relationship than one of $r = .40$. Thus a correlation coefficient of zero ($r = 0.0$) indicates the absence of a linear relationship and correlation coefficients of $r = +1.0$ and $r = -1.0$ indicate a perfect linear relationship.

3.1.2 Understanding and Interpreting the Correlation Coefficient

The correlation coefficient may be understood by various means, each of which will now be examined in turn.

The scatterplots presented below perhaps best illustrate how the correlation coefficient changes as the linear relationship between the two variables is altered. When $r = 0.0$ the points scatter widely about the plot, the majority falling roughly in the shape of a circle. As the linear relationship increases, the circle becomes more and more elliptical in shape until the limiting case is reached ($r = 1.00$ or $r = -1.00$) and all the points fall on a straight line.

A number of scatterplots and their associated correlation coefficients are presented below in order that the student may better estimate the value of the correlation coefficient based on a scatterplot in the associated computer exercise.

$$r = 1.00$$

$$r = - 0.54$$

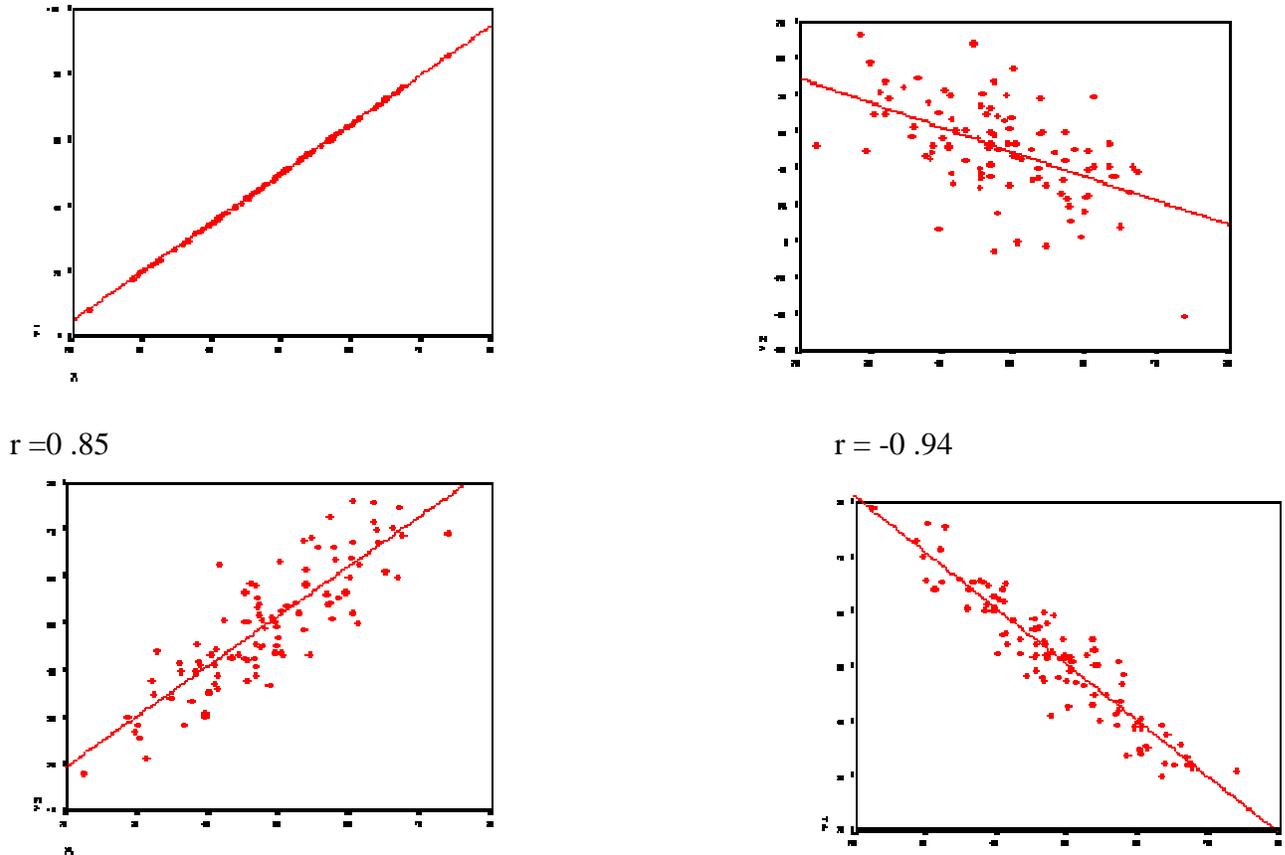


Figure 1.2 Slope of the Regression Line of z-scores

The correlation coefficient is the slope (b) of the regression line when both the X and Y variables have been converted to z-scores. The larger the size of the correlation coefficient, the steeper the slope. This is related to the difference between the intuitive regression line and the actual regression line discussed above.

This interpretation of the correlation coefficient is perhaps best illustrated with an example involving numbers.

Example 1

The raw score values of the X and Y variables are presented below :

X	12	15	19	25	32
Y	33	31	35	37	37

This problem can be solved through two different approach:

In the first method,it can be solved thus;

	x	y	$x_1 - \bar{x}$	$(x_1 - \bar{x})^2$	$y_1 - \bar{y}$	$(y_1 - \bar{y})^2$	$(x_1 - \bar{x})(y_1 - \bar{y})$
	12	33	-8.6	73.96	-1.6	2.56	13.76
	15	31	-5.6	31.36	-3.6	12.96	20.16
	19	35	-1.6	2.56	0.4	0.16	-0.64
	25	37	4.4	19.36	2.4	5.76	10.56
	32	37	11.4	129.96	2.4	5.76	27.36
Sums	103	173	0	257.2	0	27.2	70.81

Applying
$$r = \frac{\sum \{(x_1 - \bar{x})(y_1 - \bar{y})\}}{\left\{ \left[\sum (x_1 - \bar{x})^2 \right] \left[\sum (y_1 - \bar{y})^2 \right] \right\}^{1/2}}$$

$$r = \frac{70.81}{\sqrt{257.2 \times 27.2}} = 0.85$$

Alternatively, the raw score values of the X and Y variables are presented in the first two columns of the following table. The second two columns are the X and Y columns transformed using the z-score transformation.

$$z_X = \frac{X - \bar{X}}{s_X}$$

$$z_Y = \frac{Y - \bar{Y}}{s_Y}$$

(1.19)

That is, the mean is subtracted from each raw score in the X and Y columns and then the result is divided by the sample standard deviation. The table appears as follows:

	X	Y	z_X	z_Y
	12	33	-1.07	-0.61
	15	31	-0.07	-1.38
	19	35	-0.20	0.15
	25	37	0.55	.92
	32	37	1.42	.92
$\bar{X} =$	20.60	34.60	0.0	0.0
	8.02	2.61	1.0	1.0

There are two points to be made with the above numbers: (1) the correlation coefficient is invariant under a linear transformation of either X and/or Y, and (2) the slope of the regression line when both X and Y have been transformed to z-scores is the correlation coefficient.

Computing the correlation coefficient first with the raw scores X and Y yields $r=0.85$. Next computing the correlation coefficient with z_X and z_Y yields the same value, $r=0.85$. Since the z-score transformation is a special case of a linear transformation ($X' = a + bX$), it may be proven that the correlation coefficient is invariant (doesn't change) under a linear transformation of

either X and/or Y. The reader may verify this by computing the correlation coefficient using X and z_Y or Y and z_X . What this means essentially is that changing the scale of either the X or the Y variable will not change the size of the correlation coefficient, as long as the transformation conforms to the requirements of a linear transformation.

The fact that the correlation coefficient is the slope of the regression line when both X and Y have been converted to z-scores can be demonstrated by computing the regression parameters predicting z_X from z_Y or z_Y from z_X . In either case the intercept or additive component of the regression line (a) will be zero or very close, within rounding error. The slope (b) will be the same value as the correlation coefficient, again within rounding error. This relationship may be illustrated as follows:

$$z_Y^i = r z_X^i$$

$$z_X^i = r z_Y^i$$

3.1.3 Variance Interpretation

The squared correlation coefficient (r^2) is the proportion of variance in Y that can be accounted for by knowing X. Conversely, it is the proportion of variance in X that can be accounted for by knowing Y.

One of the most important properties of variance is that it may be partitioned into separate additive parts. For example, consider shoe size. The theoretical distribution of shoe size may be presented as follows:

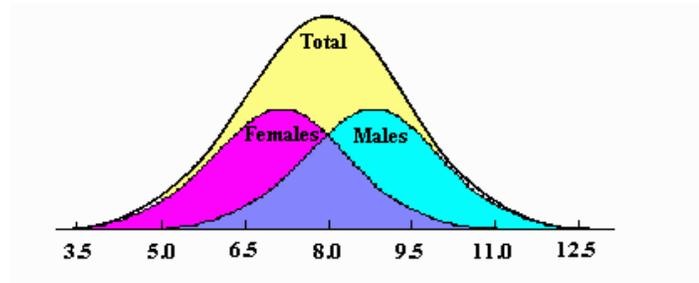


Figure 1.3 The Theoretical distribution of shoe

If the scores in this distribution were partitioned into two groups, one for males and one for females, the distributions could be represented as follows:

If one knows the sex of an individual, one knows something about that person's shoe size, because the shoe sizes of males are on the average somewhat larger than females. The variance within each distribution, male and female, is variance that cannot be predicted on the basis of sex, or error variance, because if one knows the sex of an individual, one does not know exactly what that person's shoe size will be.

Rather than having just two levels the X variable will usually have many levels. The preceding argument may be extended to encompass this situation. It can be shown that the total variance is the sum of the variance that can be predicted and the error variance, or variance that cannot be predicted. This relationship is summarized below:

$$s^2_{TOTAL} = s^2_{PREDICTED} + s^2_{ERROR}$$

or

$$s^2_{PREDICTED} = s^2_{TOTAL} - s^2_{ERROR}$$

The correlation coefficient squared is equal to the ratio of predicted to total variance:

$$r^2 = \frac{s^2_{PREDICTED}}{s^2_{TOTAL}}$$

This formula may be rewritten in terms of the error variance, rather than the predicted variance as follows:

$$r^2 = \frac{s^2_{TOTAL} - s^2_{ERR}}{s^2_{TOTAL}}$$

$$r^2 = \frac{s^2_{TOTAL}}{s^2_{TOTAL}} - \frac{s^2_{ERR}}{s^2_{TOTAL}}$$

$$r^2 = 1 - \frac{s^2_{ERR}}{s^2_{TOTAL}}$$

The error variance, s^2_{ERROR} , is estimated by the standard error of estimate squared, $s^2_{Y.X}$. The total variance (s^2_{TOTAL}) is simply the variance of Y, s^2_Y . The formula now becomes:

$$r^2 = 1 - \frac{s^2_{YX}}{s^2_Y} \tag{1.20}$$

Solving for $s_{Y.X}$, and adding a correction factor $(N-1)/(N-2)$, yields the computational formula for the standard error of estimate,

$$s_{YX} = \sqrt{\frac{(N-1)}{(N-2)} s^2_Y (1 - r^2)} \tag{1.21}$$

This captures the essential relationship between the correlation coefficient, the variance of Y, and the standard error of estimate. As the standard error of estimate becomes large relative to the total variance, the correlation coefficient becomes smaller. Thus the correlation coefficient is a function of both the standard error of estimate and the total variance of Y. The standard error of estimate is an absolute measure of the amount of error in prediction, while the correlation coefficient squared is a relative measure, relative to the total variance.

3.1.4 Further Calculation of the Correlation Coefficient

The easiest method of computing a correlation coefficient is to use a statistical calculator or computer program. Barring that, the correlation coefficient may be computed using the following formula:

$$r = \frac{\sum_{i=1}^N z_X z_Y}{N-1}$$

Computation using this formula is demonstrated below on some example data: Computation is rarely done in this manner and is provided as an example of the application of the definitional formula, although this formula provides little insight into the meaning of the correlation coefficient.

X	Y	z_X	z_Y	$z_X z_Y$
12	33	-1.07	-0.61	0.65
15	31	-0.07	-1.38	0.97
19	35	-0.20	0.15	-0.03
25	37	0.55	.92	0.51
32	37	1.42	.92	1.31
			SUM =	3.40

$$r = \frac{\sum_{i=1}^N z_X z_Y}{N-1} = \frac{3.40}{4} = .85$$

Example 2

In a laboratory containing polarographic experiment six samples of dust were taken at various distances from the polarograph and the mercury content of each sample was determined. The following results were obtained:

	x	y	$x_1 - \bar{x}$	$(x_1 - \bar{x})^2$	$y_1 - \bar{y}$	$(y_1 - \bar{y})^2$	$(x_1 - \bar{x})(y_1 - \bar{y})$
	1.4	2.4	-6.9	47.2	0.8	0.69	-5.70
	3.8	2.5	-4.5	20.0	0.9	0.87	-4.16
	7.5	1.3	-0.8	0.59	-0.3	0.07	0.21
	10.2	1.3	1.9	3.73	-0.3	0.07	-0.52
	11.7	0.7	3.4	11.8	-0.9	0.76	-2.98
	15.0	1.2	6.7	45.3	-0.4	0.14	-2.49
Sums	49.6	9.4	0	128.62	0	2.60	-15.65

$$r = \frac{-15.65}{\sqrt{128.62 \times 2.60}} = -0.856$$

In order to test for significant correlation, the best way to achieve this is to calculate the t-value, i.e. $H_0 = \text{zero}$, using the equation,

$$t = \frac{|r| \sqrt{n-2}}{\sqrt{1-r^2}}$$

$$t = \frac{0.856 \sqrt{6-2}}{1 - (-0.856^2)} = 3.31$$

The calculated value of t is compared with the tabulated value at the desired significance level, using a two sided t-test and at n-2 degree of freedom. The null hypothesis in this case is that correlation between x and y does not exist. The calculated value of t(3.31) is greater than the tabulated or critical value (P=0.05) of 2.78, so the null hypothesis is rejected. A strong correlation exists and a non-linear relationship is more likely in this case since the closer |r| is to 1 the straight-line relationship becomes stronger.

Example 3

Standard aqueous solutions of fluorescein are examined in a fluorescence spectrometer, and yield the following fluorescence intensities.

Distance:	2.1	5.0	9.0	12.6	17.3	21.0	24.7
Concentration (pg ml ⁻¹):	0	2	4	6	8	10	12

Determine the correlation coefficient, r.

Solution

The data are presented in the table, as follows

	x	y	$x_1 - \bar{x}$	$(x_1 - \bar{x})^2$	$y_1 - \bar{y}$	$(y_1 - \bar{y})^2$	$(x_1 - \bar{x})(y_1 - \bar{y})$
	0	2.1	-6	36	-11.0	121.00	66.0
	2	5.0	-4	16	-8.1	65.61	32.4
	4	9.0	-2	4	-4.1	16.81	8.2
	6	12.6	0	0	-0.5	0.25	0
	8	17.3	2	4	4.2	17.64	8.4
	10	21.0	4	16	7.9	62.41	31.6
	12	24.7	6	36	11.6	134.56	69.6
Sums:	42	91.7	0	112	0	418.28	216.2

$$r = \frac{216.2}{\sqrt{112 \times 418.28}} = \frac{216.2}{216.44} = 0.9989$$

3.2 Regression Analysis

In statistics, **regression analysis** includes many techniques for modeling and analyzing several variables, when the focus is on the relationship between a dependent variable and one or more independent variables. More specifically, regression analysis helps one understand how the typical value of the dependent variable changes when any one of the independent variables is varied, while the other independent variables are held fixed. Most commonly, regression analysis estimates the conditional expectation of the dependent variable given the independent variables — that is, the average value of the dependent variable when the independent variables are held fixed. In regression analysis, it is also of interest to characterize the variation of the dependent variable around the regression function, which can be described by a probability distribution.

Regression analysis is widely used for prediction and forecasting, where its use has substantial overlap with the field of machine learning. Regression analysis is also used to understand which among the independent variables are related to the dependent variable, and to explore the forms of these relationships. In restricted circumstances, regression analysis can be used to infer causal relationships between the independent and dependent variables. However this can lead to illusions or false relationships, so caution is advisable: see correlation does not imply causation. A large body of techniques for carrying out regression analysis has been developed. Familiar methods such as linear regression and ordinary least squares regression are parametric, in that the regression function is defined in terms of a finite number of unknown parameters that are estimated from the data. Nonparametric regression refers to techniques that allow the regression function to lie in a specified set of functions, which may be infinite-dimensional.

3.2.1 Regression analysis: fitting a line to the data

It would be tempting to try to fit a line to the data we have just analyzed - producing an equation that shows the relationship, so that we might predict the body weight of mice by measuring their length, or vice-versa. The method for this is called linear regression.

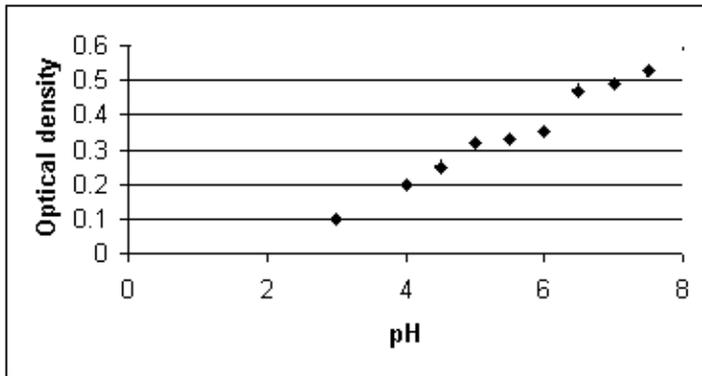
However, this is not strictly valid because linear regression is based on a number of assumptions. In particular, one of the variables must be "fixed" experimentally and/or precisely measurable. So, the simple linear regression methods can be used only when we define some experimental variable (temperature, pH, dosage, etc.) and test the response of another variable to it.

The variable that we fix (or choose deliberately) is termed the independent variable. It is always plotted on the X axis. The other variable is termed the dependent variable and is plotted on the Y axis.

Suppose that we had the following results from an experiment in which we measured the growth of a cell culture (as optical density) at different pH levels.

pH	3	4	4.5	5	5.5	6	6.5	7	7.5
Optical density	0.1	0.2	0.25	0.32	0.33	0.35	0.47	0.49	0.53

We plot these results (see below) and they suggest a straight-line relationship.



Using the same procedures as for correlation set out a table as follows and calculate Σx , Σy , Σx^2 , Σy^2 , Σxy , \bar{x} and \bar{y} (mean of y).

pH (x)	Optical density (y)	x^2	y^2	xy
3	0.1	9	0.01	0.3
4	0.2	16	0.04	0.8
4.5	0.25	20.25	0.0625	1.125
5	0.32	25	0.1024	1.6
5.5	0.33	30.25	0.1089	1.815
6	0.35	36	0.1225	2.1
6.5	0.47	42.25	0.2209	3.055
7	0.49	49	0.240	3.43
7.5	0.53	56.25	0.281	3.975
$\Sigma x = 49$	$\Sigma y = 3.04$	$\Sigma x^2 = 284$	$\Sigma y^2 = 1.1882$	$\Sigma xy = 18.2$

Now calculate $\Sigma d_x^2 = \Sigma x^2 - \frac{(\Sigma x)^2}{n} = 17.22$ in our case.

Calculate $\Sigma d_y^2 = \Sigma y^2 - \frac{(\Sigma y)^2}{n} = 0.1614$ in our case.

Calculate $\Sigma d_x d_y = \Sigma xy - \frac{\Sigma x \Sigma y}{n}$ (this can be positive or negative) = +1.649

Now we want to use regression analysis to find the line of best fit to the data. We have done nearly all the work for this in the calculations above.

The regression equation for y on x is: $y = bx + a$

where b is the slope and a is the intercept (the point where the line crosses the y axis)

We calculate b as:

$$b = \frac{\sum d_x d_y}{\sum d_x^2}$$

$$= 1.649 \times 17.22 = 0.0958 \text{ in our case}$$

We calculate a as:

$$a = \bar{y} - b \bar{x}$$

From the known values of \bar{y} (0.3378), \bar{x} (5.444) and b (0.0958) we thus find a (-0.1837).

So the equation for the line of best fit is: $y = 0.096x - 0.184$ (to 3 decimal places).

To draw the line through the data points, we substitute in this equation. For example: when $x = 4$, $y = 0.384$, so one point on the line has the x,y coordinates (4, 0.384);

when $x = 7$, $y = 0.488$, so another point on the line has the x,y coordinates (7, 0.488).

It is also true that the line of best fit *always* passes through the point with coordinates \bar{x} , \bar{y} so we actually need only one other calculated point in order to draw a straight line.

4.0 CONCLUSION

Having gone through this unit, you have seen that correlation coefficient measures the degree of linear relationship between two variables, x and y . You should also have learnt that a positive correlation coefficient implies that as the value of one variable increases, the value of other variable increases, and vice-versa. A negative correlation coefficient, on the other hand, indicates that one variable increases, the other decreases and vice-versa.

In addition you have learnt that regression analysis focuses on the relationship between a dependent variable and one or more independent variables.

5.0 SUMMARY

In this unit we have learnt that:

- i. The measure of degree of linear relationship between two variables is known as Pearson Product-Moment Correlation Coefficient (r),
- ii. Positive correlation coefficient occurs when the increase in value of one variable brings about a corresponding increase in the value of the other variable and vice-versa.

- iii. Negative correlation coefficient occurs if the increase in the value of one variable decreases the value of the other variable and vice-versa
- iv. The squared correlation coefficient is the proportion of variance in Y that can be accounted for by knowing X
- v. Relationship between a dependent variable and one or more independent variables defines regression analysis.
- vi. Regression analysis has wide application in prediction and forecasting

6.0 TUTOR MARKED ASSIGNMENT

1. In your own words, define correlation coefficient
2. Differentiate between positive and negative correlation
3. List two application of regression analysis
4. The response of a colorimetric test for glucose was checked with aid of standard glucose solutions.
5. Determine the correlation coefficient from the following data.

Glucose concentration, mM	0	2	4	6	8	10
Absorbance	0.002	0.150	0.294	0.434	0.570	0.704

7.0 REFERENCES AND FURTHER READING

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MODULE 2

Unit 1 Potentiometer and pH titrations

Unit 2 Conductometric methods

Unit 3 Electrolytic methods

UNIT 1 POTENTIOMETER AND PH TITRATIONS

CONTENT

- 1.0 Introduction
- 2.0 Objectives
- 3.0 Main Content
 - 3.1 Potentiometric
 - 3.2 PH–Meter
 - 3.3 Potentiometric Measurement
 - 3.4 Glass Membrane Electrode
 - 3.4.1 Solid State Membrane
 - 3.4.2 Liquid-Membrane Electrode
- 4.0 Conclusion
- 5.0 Summary
- 6.0 Tutor Mark Assignment
- 7.0 Reference and Further Reading

1.0 INTRODUCTION

Several Acid-Base titrations are quite difficult to realize ordinarily by the use of visual indicators for one of several reasons. The analyst may be color-blind to a particular indicator color change; it could be that no suitable color change exist for a particular type of titration or the solutions themselves may be colored, opaque or turbid. It may be desired to automate a series of replicate determinations. In such situations, potentiometric titration, using a glass hydronium ion selective electrode, a suitable reference electrode and a sensitive potentiometer (a pH meter) may be advantageous. This study unit is designed to focus on potentiometric measurements and pH titrations. Let us look at what other content you should learn in this study unit as specified in the study unit objectives below.

2.0 OBJECTIVE

By the end of the unit, you should be able to:

- Distinguish between potentiometer and pH meter
- Identify the basic component of a pH meter

- List various interference ions of solid state membrane electrode
- Explain the basic principle of liquid membrane electrode

3.0 MAIN CONTENT

3.1 Potentiometer

Electroanalytical chemistry consists of the field of chemistry that utilizes the relationship between chemical phenomena which involve charge transfer (e.g. redox reactions, ion separation, etc.) and the electrical properties that accompany these phenomena for some analytical determination. The two commonly used instrument for making potential measurement are potentiometer and pH meter

A potentiometer is an instrument for measuring the potential in a circuit. Before the introduction of the moving coil and digital volt meters, potentiometers were used in measuring voltage, hence the '-meter' part of their name. The method was described by Johann Christian Poggendorff around 1841 and became a standard laboratory measuring technique.

Potentiometer is used for measurements of low resistance circuits. The potentiometer operates by connecting a known voltage source to the cell whose voltage is to be measured with a sensitive galvanometer in between, and adjusting the source voltage until it equals the cell voltage. This occurs when no current flows through the galvanometer. This is achieved by means of a sidewire which varies the fraction of the known source voltage applied to the cell. The potential can then be read from the known source voltage. The sensitivity of the potentiometer is related to the sensitivity of the galvanometer and the cell resistance .

3.2 PH- Meter

pH meter is a voltmeter that converts the unknown voltage to current which is later amplified and read out. The pH meter is a null-type device as is the potentiometer. The electrometer draws very small currents and is best suited for irreversible reactions that are slow to established equilibrium. They are also required for high-resistance electrodes, like glass pH or ion-selective

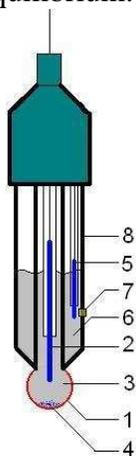


Figure 2.1 Scheme of typical pH glass electrode

Modern pH probe consists of an electrode, which combines both the glass and reference electrodes into one body. The combination electrode consists of the following parts as shown in the figure above:

1. a sensing part of electrode, a bulb made from a specific glass
2. internal electrode, usually silver chloride electrode or calomel electrode
3. internal solution, usually a pH=7 buffered solution of 0.1 mol/L KCl for pH electrodes or 0.1 mol/L MeCl for pMe electrodes
4. when using the silver chloride electrode, a small amount of AgCl can precipitate inside the glass electrode
5. reference electrode, usually the same type as internal electrode
6. reference internal solution, usually 0.1 mol/L KCl
7. junction with studied solution, usually made from ceramics or capillary with asbestos or quartz fiber.
8. body of electrode, made from non-conductive glass or plastics.

The bottom of a pH electrode balloons out into a round thin glass bulb. The pH electrode is best thought of as a tube within a tube. The inside most tube (the inner tube) contains an unchanging 1×10^{-7} mol/L HCl solution. Also inside the inner tube is the cathode terminus of the reference probe. The anodic terminus wraps itself around the outside of the inner tube and ends with the same sort of reference probe as was on the inside of the inner tube. It is filled with a reference solution of 0.1 mol/L KCl and has contact with the solution on the outside of the pH probe by way of a porous plug that serves as a salt bridge.

3.3 Glass Membrane Electrode

Glass membrane electrode is a type of electrode that is commonly used for measuring pH of a given solution. This is classified into solid state membrane and liquid membrane electrode

3.3.1 Solid state membrane

This electrode consists of silver-silver chloride in a reference solution of hydrochloric acid contain in a glass membrane. The membrane is made up of a special glass, usually a hydrated aluminosilicate containing sodium or calcium ions. This electrode is selectively permeable to hydrogen ions and the potential that develops across the membrane hydrogen ions of the tested solution compared with the reference acid solution in the electrode. The potential can be measured against a reference calomel electrode using a high sensitive voltmeter. In pH measurement, calibration of the instrument is important and can be achieved with either buffers whose pH has been previously measured. But for measurement over a range of pH values, it is necessary to standardize the instrument on at least two standard buffer solutions which covers the required range. The simplest solid state membrane are designed to measure the test ions. Alternatively, the test substance may involved in one or two reactions on the surface of the electrode which change the activity of the mobile phase.

Table 2.1 Membrane materials and interfering ions

Test ion	Membrane material	Major interfering ions
Fluoride	LaF	I ⁻ Br ⁻ Cl ⁻
Chloride	AgCl/Ag ₂ S	S ⁻ I ⁻
Bromide	AgBr/Ag ₂ S	S ⁻ I ⁻
Iodide	AgI/ Ag ₂ S	S ⁻
Sulphide	AgI/ Ag ₂ S	Hg ⁺ Ag ⁺
Cupric	Ag ₂ S/CuS	Hg ⁺ Ag ⁺
Lead	Ag ₂ S/PbS	Hg ⁺ Ag ⁺

3.3.2 Liquid-Membrane Electrodes

Liquid membrane electrode is made up of an ion-selective material dissolved in a solvent that is not miscible with water. The liquid is held in a porous inert which allows contact between the test solution on one side and the reference electrolyte on the other. The ions from the reference solution will partition themselves between the two immiscible solvent, giving the electrode a particular potential. The presence of the test ion in the sample affect the activity of the reference ions in the membrane resulting in a change in the potential difference across the membrane.

Example of liquid membrane electrode are:

Test ion	Membrane material
Potassium	Valinomycin in diphenyl ether
Ammonium	Macrotetrolides in tris phosphate
Calcium	Calcium dialkylphosphate

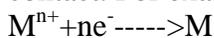
3.4 Potentiometric Measurements

Potentiometric titration is a technique similar to direct titration of a redox reaction. No indicator is used, instead the potential across the analyte, typically an electrolyte solution is measured. To do this, two electrodes are used, an indicator electrode and a reference electrode. The indicator electrode forms an electrochemical half cell with the interested ions in the test solution. The reference electrode forms the other half cell, holding a consistent electrical potential. The overall electric potential is calculated as

$$E_{\text{cell}} = E_{\text{ind}} - E_{\text{ref}} + E_{\text{sol}} \quad (2.1)$$

E_{sol} is the potential drop over the test solution between the two electrodes. E_{cell} is recorded at intervals as the titrant is added. A graph of potential against volume added can be drawn and the end point of the reaction is half way between the jump in voltage.

E_{cell} depends on the concentration of the interested ions with which the indicator electrode is in contact. For example, the electrode reaction may be



As the concentration of M^{n+} changes, the E_{cell} changes correspondingly. Thus the potentiometric titration involve measurement of E_{cell} with the addition of titrant.

For direct potentiometric measurement in which the activity of one ion is to be calculated from

potential of the indicating electrode, the potential of the reference electrode is described by the equation (2.2)

$$E_{cell} = k - \frac{2.303RT}{nF} \log \frac{a_{red}}{a_{ox}} \quad (2.2)$$

Where k is constant determined by measuring the potential of a standard solution in which the activity is known.

If the ionic strength is maintained constant at the same value, the activity coefficients of the test solution remains constant at all concentration of the substance.

Then concentration can be determined from measured cell potentials.

$$E = E^0 - \frac{2.303RT}{nF} \log \frac{C_{red}}{C_{ox}} \quad (2.3)$$

Where E^0 is the standard electrode potential

R is the gas constant

F is the Faraday constant

n is the number of electrons involved

T is the absolute temperature

For measurements made at 25°C the equation is simplified as:

$$E = E^0 - \frac{0.059}{n} \log a \quad (2.4)$$

types of potentiometric titration: acid-base titration (total alkalinity and total acidity), redox titration (HI/HY and cerate), precipitation titration (halides), and complexometric titration

Example 2.1

A solution is 10^{-3} M in $\text{Cr}_2\text{O}_7^{2-}$ and 10^{-2} M in Cr^{3+} . If the pH is 2.0, what is the potential of the half reaction.

Solution



$$E = E^0_{\text{Cr}_2\text{O}_7^{2-}, \text{Cr}^{3+}} - \frac{0.059}{6} \log \frac{[\text{Cr}^{3+}]^2}{[\text{Cr}_2\text{O}_7^{2-}][\text{H}^+]^{14}}$$

$$E = 1.33 - \frac{0.059}{6} \log \frac{(10^{-2})^2}{(10^{-3})(10^{-2})^{14}}$$

$$E = 1.33 - \frac{0.059}{6} \log 10^{27}$$

$$E = 1.33 - \frac{0.059}{6} (1.4314)$$

$$E = 1.33 - [(0.00983)(1.43136)]$$

$$E = 1.31V$$

Example 2.2

Calculate equivalence point potential if 0.02 M $\text{Fe}(\text{CN})_6^{4-}$ is titrated with 0.1 M Ce^{4+} .
 $E_{\text{Ce}^{4+}/\text{Ce}^{3+}}^0 = 1.61 \text{ V}$, $E_{\text{Fe}(\text{CN})_6^{3-}/\text{Fe}(\text{CN})_6^{4-}}^0 = 0.36 \text{ V}$.

This is a straight application of this formula

$$E_{\text{cell}} = \frac{n_A E_A^0 + n_B E_B^0}{n_A - n_B} \quad (2.5)$$

In both half reactions one electron is exchanged, so equivalence point potential is given by

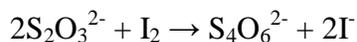
$$E_{\text{eq}} = \frac{1.61 + 0.36}{2} = 0.98 \text{ V}$$

Note, that we can ignore given concentrations of the titrant and titrated substance, as equivalence potential doesn't depend on them.

Example: 2.3

What is equivalence point potential when 0.1 M I_2 solution is titrated with 0.1 M solution of $\text{S}_2\text{O}_3^{2-}$. $E_{\text{I}_2/\text{I}^-}^0 = 0.535\text{V}$, $E_{\text{S}_4\text{O}_6^{2-}/\text{S}_2\text{O}_3^{2-}}^0 = 0.08\text{V}$

Reaction equation for this titration is



obviously we can't use our general formula (neither m_A nor m_B are constant). We have to use the general approach and assume that reaction went to (almost) completion.

We have to mix thiosulfate and iodine in 2:1 ratio. As solutions concentrations are identical that means we will use 2 volumes of thiosulfate and 1 volume of iodine, for the final volume of 3. Thus, taking both stoichiometry and dilution into account, we can write:

$$[\text{S}_2\text{O}_3^{2-}] + 2[\text{S}_4\text{O}_6^{2-}] = \frac{0.2}{3} \text{ M}$$

and

$$[I_2] + \frac{1}{2}[I^-] = \frac{0.1}{3} M$$

However, reaction is shifted so far to the right, that concentrations of bot reactants are (in the mass balance) negligible:

$$[S_4O_6^{2-}] = \frac{0.1}{3} M$$

and

$$[I^-] = \frac{0.2}{3} M$$

We also know from the reaction stoichiometry that

$$[S_2O_3^{2-}] = 2[I_2]$$

Nernst equations for both systems are

$$E_{eq} = E_{0I_2/I^-} + \frac{RT}{2F} \ln\left(\frac{[I_2]}{[I^-]^2}\right)$$

$$E_{eq} = E_{0S_4O_6^{2-}/S_2O_3^{2-}} + \frac{RT}{2F} \ln\left(\frac{[S_4O_6^{2-}]}{[S_2O_3^{2-}]^2}\right)$$

Let's combine Nernst equations with known information listed above, aiming at removing all variables but $[I_2]$:

$$E_{eq} = E_{0I_2/I^-} + \frac{RT}{2F} \ln\left(\frac{[I_2]}{\left(\frac{0.2}{3}\right)^2}\right)$$

$$E_{eq} = E_{0S_4O_6^{2-}/S_2O_3^{2-}} + \frac{RT}{2F} \ln\left(\frac{\frac{0.1}{3}}{(2[I_2])^2}\right)$$

We have two unknowns here. While we need E_{eq} , it is easier to calculate concentration of iodine

first. Besides, that'll let us check if our assumption about the low iodine concentration was right. Subtracting second equation from the first one we get:

$$E_{0I_2/I^-} - E_{0S_4O_6^{2-}/S_2O_3^{2-}} + \frac{RT}{2F} \ln\left(\frac{[I_2]}{\left(\frac{0.2}{3}\right)^2}\right) - \frac{RT}{2F} \ln\left(\frac{0.1}{(2[I_2])^2}\right) = 0$$

After some rearranging and canceling:

$$\frac{RT}{2F} \ln\left(\frac{4[I_2]^3}{\frac{0.1}{3} \left(\frac{0.2}{3}\right)^2}\right) = E_{0S_4O_6^{2-}/S_2O_3^{2-}} - E_{0I_2/I^-}$$

or

$$[I_2] = \sqrt[3]{\frac{\frac{0.1}{3} \left(\frac{0.2}{3}\right)^2 \exp\left(\frac{2F(E_{0S_4O_6^{2-}/S_2O_3^{2-}} - E_{0I_2/I^-})}{RT}\right)}{4}} = 2.49 \cdot 10^{-7} M$$

as iodide concentrations was $0.2/3 = 0.0667$ our assumption that I_2 concentration is much lower was correct. Entering known concentration into Nernst equation we get

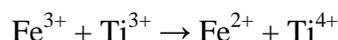
$$E_{eq} = E_{0I_2/I^-} + \frac{RT}{2F} \ln\left(\frac{2.49 \cdot 10^{-7}}{\left(\frac{0.2}{3}\right)^2}\right) = 0.409 V$$

and this is our equivalence potential. Note, that using derived formula we would get 0.308 V, almost 0.1 V off.

Example 2.3

what is potential in 0.01 M Fe^{3+} solution titrated 25% with 0.0112 M Ti^{3+} ? $E_{0Fe^{3+}/Fe^{2+}} = 0.77 V$, $E_{0Ti^{4+}/Ti^{3+}} = 0.130 V$.

This is reduction titration, in which Fe^{3+} is being reduced to Fe^{2+} :



Using approach described above we can immediately write

$$E = E_{0\text{Fe}^{3+}/\text{Fe}^{2+}} + \frac{RT}{nF} \ln\left(\frac{[\text{Fe}^{3+}]}{[\text{Fe}^{2+}]}\right) = E_{0\text{Fe}^{3+}/\text{Fe}^{2+}} + \frac{RT}{nF} \ln\left(\frac{75}{25}\right)$$

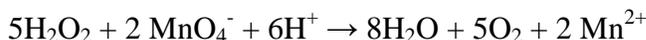
as we know that already 25% of the iron is in the reduced form and so 100%-25%=75% have to be still in the oxidized form. We don't have to care about dilution factor, as it is identical for both forms of iron and cancels out. n is 1. Thus

$$E = 0.77 + \frac{RT}{F} \ln(3) = 0.80 \text{ V}$$

Example 2.4

what is potential in 0.1 M H_2O_2 pH = 1.00 solution titrated 120% with 0.1 M permanganate?
 $E_{0\text{O}_2/\text{H}_2\text{O}_2} = 0.682 \text{ V}$, $E_{0\text{MnO}_4^-/\text{Mn}^{2+}} = 1.51 \text{ V}$.

In this titration hydrogen peroxide is oxidized by permanganate to water and oxygen:



However, this reaction is irrelevant to the question. At 120% solution contains some amount of Mn^{2+} and 20% of that amount of excess permanganate. As both MnO_4^- and Mn^{2+} are present in the reaction quotient in the first power, their real concentrations don't matter, we are interested in the ratio only - and, similarly to the first question, we know enough to calculate this ratio as 20/100.

Nernst equation for the permanganate half reaction is

$$E = E_{0\text{MnO}_4^-/\text{Mn}^{2+}} + \frac{RT}{5F} \ln\left(\frac{[\text{MnO}_4^-][\text{H}^+]^8}{[\text{Mn}^{2+}]}\right)$$

From pH value we know H^+ concentration is 0.1 M:

$$E = 1.51 + \frac{RT}{5F} \ln\left(\frac{20 \cdot 0.1^8}{100}\right) = 1.41 \text{ V}$$

4.0 CONCLUSION

In this study unit, you have learnt about potentiometer as an instrument for measuring the potential of a cell or circuit. You should also have learned the basic components of a pH-meter as well as their respective functions. Lastly, you will have learnt that glass membrane is a type of electrode used for measuring pH of a given solution.

5.0 SUMMARY

In this unit we have learnt that:

- i. The study of the relationship between chemical phenomena which involve charge transfer and the electrical properties that accompanied these phenomena is known as electroanalytical chemistry.
- ii. Potentiometer is an instrument used to measure the potential of a cell whereas a pH meter is a voltmeter which converts unknown voltage to current which is later amplified and read out.
- iii. A typical modern pH probe is a combination of electrode which consists of both glass and reference in one body.
- iv. Glass membrane electrode is classified into solid state membrane and liquid membrane electrode
- v. Solid state membrane is made up of a silver-silver electrode which is selectively permeable to hydrogen ions, and a reference solution of HCl acid contain in a glass membrane.
- vi. The presence of the test ion in the sample, in a liquid membrane electrode, affects the activity of the reference ions which consequently changes the potential difference across the membrane.

6.0 TUTOR MARKED ASSIGNMENT

- i. In what way is pH-meter similar to a potentiometer
- ii. Describe the basic components of a pH-meter
- iii. Explain the basic principle of a liquid membrane electrode
- iv. Hg^+ and Ag^+ constitute the major interfering ions for some test ions in solid membrane electrode. List the three possible test ions.

7.0 REFERENCES AND FURTHER READING

1. Skoog W, and Holler C., Fundamental of Analytical Chemistry. 8th ed.
2. Harris D.C. (1995) Qualitative Chemical Analysis, 4th ed., Freeman W, H. and company, New York.
3. Gary D.C. (1994). Analytical Chemistry. 5th ed. John Wiley and Sons, Inc.

UNIT 2 CONDUCTOMETRIC METHODS

1.0 Introduction

Conductometry means measuring the conductivity and conductometer measures the electrical conductivity of ionic solutions.

This is done by applying an electric field between two electrodes. The ions wander in this field. The anions migrate to the anode and the cations to the cathode. In order to avoid substance conversions and the formation of diffusion layers at the electrodes (polarization), work is carried out with alternating voltage. The rule of thumb is that the frequency of the alternating voltage must be increased as the ion concentration increases. Modern conductometers automatically adapt the measuring frequency to the particular measuring conditions.

2.0 OBJECTIVE

- Define accurately some basic conductometry terms
- Classify substances based on electrical conductance
- Distinguished between equivalent and molar conductivity
- List and explain the factors affecting conductance of electrolyte solution
- State Kohlrausch law of independent migration of ions

3.0 MAIN CONTENT

3.1 Electrical Resistance & Conductance

3.1.1 Resistance (R): The tendency of a material to stop the flow of current is known as resistance. It is measured in ohms (Ω).

According to Ohm's law, The resistance offered by a substance is directly proportional to its length (l), but inversely proportional to its cross sectional area (A).

$$R \propto \frac{l}{A}$$

In case of electrolytic solutions, ' l ' represents the distance between two electrodes and the ' A ' is the cross sectional area of the electrodes.

The above equation can also be written as:

$$R = \rho \frac{l}{A} \tag{2.6}$$

Where ρ is the proportionality constant and is known as specific resistance or resistivity.

If $A = 1 \text{ cm}^2$ and $l = 1 \text{ cm}$, then $R = \rho$.

Therefore, the specific resistance can be defined as follows:

Specific resistance or resistivity (ρ): It is the resistance offered by a material or solution occupying one cm^3 volume.

It is measured in:

ohm. cm (in C.G.S system) or ohm. m (in S.I system). Its unit can be derived as follows:

$$\rho = R \cdot \frac{A}{l} = \text{ohm} \cdot \frac{\text{cm}^2}{\text{cm}} = \text{ohm} \cdot \text{cm}$$

3.1.2 Conductance (G): It is the tendency of a material to allow the flow of current through it. It is the reciprocal of resistance.

It is measured in $\text{ohm}^{-1} = \text{mho} = \text{Siemens}$.

$$\text{Conductance (G)} = \frac{1}{\text{Resistance (R)}}$$

By substituting, R from equation (2.6):

$$G = \frac{1}{\rho} \cdot \frac{A}{l} = \kappa \cdot \frac{A}{l} \quad (2.6)$$

Where

$$\kappa = \frac{1}{\rho} = \text{specific conductance (or) conductivity}$$

From the equation (2.6),

$$\kappa = G \cdot \frac{l}{A}$$

If $A = 1 \text{ cm}^2$ and $l = 1 \text{ cm}$, then $\kappa = G$.

Therefore the specific conductance can be defined as:

specific conductance or conductivity (κ): It is the conductance of a material or solution occupying one cm^3 volume.

It is measured in: $\text{ohm}^{-1} \cdot \text{cm}^{-1} = \text{mho} \cdot \text{cm}^{-1}$ (C.G.S system) or $\text{Siemens} \cdot \text{m}^{-1}$ (S.I system)

The specific conductance depends on the nature of substance or the electrolyte, and it increases with increase in concentration of the electrolytic solution because the number of ions per unit area increases. The ratio of the distance between the electrodes, l to the cross sectional area, A of the electrodes is known as cell constant.

$$\text{cell constant (G}^*) = \frac{l}{A}$$

The cell constant can be determined by using following relations which can be derived easily from expressions discussed above.

$$G^* = \frac{l}{A} = \frac{R}{\rho} = \frac{\kappa}{G} = R\kappa = \frac{1}{G \cdot \rho} \quad (2.7)$$

3.2 Types of Substances Based on Electrical Conductance

Materials are divided into two types based on electrical conductivity :

1) Insulators: The substances which resist the flow of electric current through them are called insulators. They do not have free electrons or freely moving charged particles.

Organic polymers (like plastics), glass, diamond, quartz are just but few examples.

2) Conductors: The substances which allow the flow of electricity through them with little resistance are known as conductors.

Conductors are divided into:

i) Metallic or electronic conductors: These are conductors which conduct the electricity through the electrons, e.g. all metals, graphite etc. In metallic conduction, no chemical reaction occurs during the conduction of electricity, and conductivity decreases with increase in temperature due to vibrational disturbances.

ii) Electrolyte: This is a substance which in aqueous solution or molten form allows electricity to pass through it and decomposes into oppositely charged ions during the process.
E.g :- NaCl, KCl, CH₃COOH, HCl etc.

Free flow of ions towards the oppositely charged electrodes occurs and during conduction of electricity through electrolytes, oxidation occurs at anode whereas reduction occurs at cathode. The conductivity increases with increase in temperature as the extent of ionization increases. The electrolytes undergo dissociation to furnish ions either in molten state or in aqueous solutions. The electrolytes are further divided into two based on the extent of ionization in water:

- a) Strong electrolytes: Undergo complete ionization in water.
E.g. NaCl, KCl, K₂SO₄, HCl, H₂SO₄, NaOH, NaNO₃ etc.
- b) Weak electrolytes: Under go partial ionization in water.
E.g. HF, CH₃COOH, NH₄OH, HCOOH etc.

Non-electrolytes: The substances which do not furnish ions for electrical conduction are called non-electrolytes. e.g urea, glucose, sucrose etc.

3.3 Equivalent Conductance & Molar Conductance

3.3.1 Equivalent conductivity (Λ)

The conductance of that volume of solution containing one equivalent of an electrolyte is known as equivalent conductivity. It is denoted by Λ .

Let us consider the $V \text{ cm}^3$ of solution containing one equivalent of an electrolyte. Its conductance is equal to equivalent conductance, Λ .

Also we know that the conductance shown by 1 cm^3 solution containing this electrolyte is called specific conductance, κ .

i.e.,

the conductance of $V \text{ cm}^3$ ----- Λ

the conductance of 1 cm^3 ----- κ

Therefore:

$$\Lambda = \kappa \cdot V \tag{2.8}$$

We know that the normality (N) of a solution is given by the equation:

$$N = \frac{n_e}{V(\text{in cc})} \times 1000$$

For above electrolytic solution, no. of equivalents, $n_e = 1$.

Hence

$$V(\text{in cc}) = \frac{1000}{N}$$

By substituting the above value in the equation (2.8), we can now write:

$$\Lambda = \kappa \cdot \frac{1000}{N} \tag{2.9}$$

Units of Λ :

$$\begin{aligned} &= \frac{\text{Ohm}^{-1} \cdot \text{cm}^{-1}}{\text{equivalents} \cdot \text{cm}^{-3}} \\ &= \text{cm}^2 \cdot \text{ohm}^{-1} \cdot \text{equiv}^{-1} = \text{cm}^2 \cdot \text{mho} \cdot \text{equiv}^{-1} \text{ or } \text{m}^2 \cdot \text{Siemens} \cdot \text{equiv}^{-1} \end{aligned}$$

3.3.2 Molar conductivity (Λ_m or μ)

The conductance of that volume of solution containing one mole of an electrolyte is known as molar conductivity. It is denoted by Λ_m or μ .

It is related to specific conductance, κ as:

$$\mu = \kappa \cdot V \text{ or}$$

$$\mu = \kappa \cdot \frac{1000}{M} \quad (2.10)$$

Where M = molarity of the electrolytic solution.

Units of μ : $= \text{cm}^2 \cdot \text{ohm}^{-1} \cdot \text{mol}^{-1} = \text{cm}^2 \cdot \text{mho} \cdot \text{mol}^{-1}$ or $\text{m}^2 \cdot \text{Siemens} \cdot \text{mol}^{-1}$

The relation between equivalent conductance, Λ and molar conductance, μ can be given by:

$$\mu = \Lambda \times \text{equivalent factor of the electrolyte}$$

The equivalent factor of the electrolyte is usually the total charge on either anions or cations present in one formula unit of it. It may be equal to basicity in case of acids or equal to acidity in case of bases.

3.4 The Factors Affecting the Conductance of Electrolyte Solutions

a) **Temperature:** The conductance of an electrolyte solution increases with increase in the temperature due to increase in the extent of ionization.

b) **Nature of electrolyte:**

The strong electrolytes undergo complete ionization and hence show higher conductivities since they furnish more number of ions.

Whereas weak electrolytes undergo partial ionization and hence show comparatively low conductivities in their solutions.

c) **Ionic size & mobility:**

The ionic mobility decreases with increase in its size and hence conductivity also decreases.

E.g. In molten state, the conductivities of lithium salts are greater than those of cesium salts since the size of Li^+ ion is smaller than that of Cs^+ ion.



smaller and moves faster larger and moves slowly

In aqueous solutions the extent of hydration affects the mobility of the ion, which in turn affects the conductivity. Heavily hydrated ions show low conductance values as a result of larger size.

For instance, in aqueous solutions Li^+ ion with high charge density is heavily hydrated than Cs^+ ion with low charge density. Hence hydrated Li^+ is bigger than hydrated Cs^+ . As a result, lithium salts show lower conductivities compared to those of cesium salts in water.

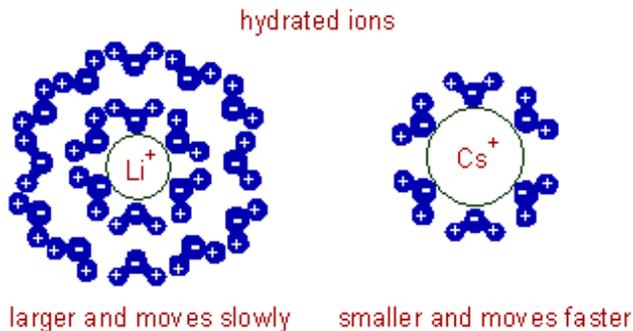


Figure 2.2 Hydration of Lithium and Cesium ions in aqueous solution

d) The Viscosity

The ionic mobility is reduced in more viscous solvents, that is, the ease at which ions migrate in aqueous solution decreases as viscosity increases. Hence the conductivity decreases.

e) Concentration

The specific conductance (κ) increases with increase in concentration of solution as the number of ions per unit volume increases.

Whereas, both the equivalent conductivity and molar conductance increase with decrease in concentration (i.e. upon dilution) since the extent of ionization increases.

This is because the concentration decreases, one can expect decrease in equivalent conductivity due to decrease in available number of ions per unit volume. However the increase in volume (V) factor more than compensates this effect. The volume must be increased in order to get one equivalent of electrolyte since the concentration is decreased. Hence the net effect is increase in equivalent conductivity.

3.5 Limiting equivalent conductivity (Λ_0)

The equivalent conductivity reaches a maximum value at certain dilution and does not change upon further dilution (i.e., by adding solvent further). This concentration is also termed as infinite dilution.

The equivalent conductivity at infinite dilution is known as the limiting equivalent conductivity (Λ_0). At this dilution, the ionization of even the weak electrolyte is complete.

However at infinite dilution (i.e., when concentration approaches zero) the conductivity of the solution is so low that it cannot be measured accurately. Therefore the limiting equivalent conductivity of an electrolyte is calculated by using Debye-Huckel-Onsager equation as explained below.

Conductance ratio (α) : The ratio of the equivalent conductance at given concentration, Λ_c to that at infinite dilution, Λ_0 is called conductance ratio, α .

$$\alpha = \frac{\Lambda_c}{\Lambda_0} \quad (2.11)$$

For weak electrolytes, the ' α ' is also called as degree of ionization.

It is possible to determine the equivalent conductivities of electrolytes in water at given concentration by using Debye-Huckel-Onsager equation.

$$\Lambda_c = \Lambda_o - A\sqrt{c} \quad (2.12)$$

Where

Λ_c = equivalent conductivity at given concentration.

Λ_o = equivalent conductivity at infinite dilution.

c = concentration

$$A = \text{a constant} = \frac{82.4}{(DT)^{\frac{1}{2}}} + \frac{8.2 \times 10^5}{(DT)^{\frac{3}{2}}} \Lambda_o \quad (2.13)$$

D = Dipole moment of water

T = Absolute temperature

A straight line with negative slope is obtained when the equivalent conductivity values (Λ_c) of strong electrolytes are plotted against square roots of different concentrations (\sqrt{c}). The equivalent conductivity at infinite dilution (Λ_o) can be determined by extending this straight line to zero concentration.

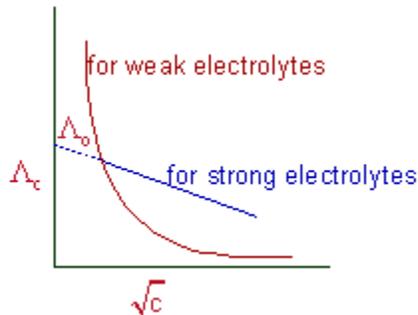


Figure 2.3 A plot of equivalent conductance versus square root of concentration.

However the equivalent conductivity of weak electrolytes increases steeply at very low concentrations (as in the above graph) and hence their limiting values (Λ_o) cannot be determined by extrapolating the Λ_c to zero concentration.

Therefore, Λ_o for weak electrolytes is obtained by using Kohlrausch law of independent migration of ions, which is described in below.

3.6 Kohlrausch Law of Independent Migration of Ions

In 1874, Kohlrausch formulated the law of independent migration of ions based on the experimental data of conductivities of various electrolytes. This law can be stated as follows:

At infinite dilution, the dissociation of the electrolyte is complete and hence each ion makes definite contribution to the equivalent conductivity of the electrolyte irrespective of the nature of other ions associated with it.

Therefore the limiting equivalent conductivity of an electrolyte is the algebraic sum of limiting equivalent conductivities of its constituent ions.

i.e., The limiting equivalent conductivity of an electrolyte, $\Lambda_o^{\text{electrolyte}}$

$$\Lambda_o^{\text{electrolyte}} = \lambda_o^+ + \lambda_o^- \quad (2.14)$$

Where λ_o^+ and λ_o^- are the limiting equivalent conductivities of cation and anion respectively.

However the Kohlrausch law can also be stated in terms of molar conductivities as:

The limiting molar conductivity of an electrolyte is the sum of individual contributions of limiting molar conductivities of its constituent ions.

i.e., The molar equivalent conductivity of an electrolyte, $\mu_0^{\text{electrolyte}}$

$$\mu_0^{\text{electrolyte}} = n_+ \mu_0^+ + n_- \mu_0^- \quad (2.15)$$

Where μ_0^+ and μ_0^- are the limiting molar conductivities of cation and anion respectively.

And n_+ and n_- are the stoichiometric numbers of positive and negative ions formed during the dissociation of electrolyte.

3.6.1 Experimental Basis and Theoretical Explanation of Kohlrausch Law

Kohlrausch observed that at infinite dilutions, the difference between the conductivities of sodium and potassium salts is constant irrespective of the associated anions, as tabulated below.

Salt pair	Conductivity (mho cm ² equiv)	Difference
NaCl KCl	108.90 130.10	21.20
NaNO ₃ KNO ₃	105.33 126.50	21.17
NaBr KBr	111.10 132.30	21.20

Kohlrausch argued that the constant difference in the conductivities of above pairs can be ascribed to the fact that the mobility of sodium and potassium ions at infinite dilution is not influenced by the nature of counter ions. The ions at such a low concentration migrate in the electric field as they are independent i.e., they show same ionic conductance irrespective of the nature of counter ion.

3.5.2 Applications of Kohlrausch Law

1) Calculation of limiting conductivities of weak electrolytes: The Kohlrausch law can be used to calculate the limiting conductivities of weak electrolytes.

E.g., The calculation of limiting equivalent conductance of acetic acid, a weak electrolyte is illustrated below.

According to Kohlrausch law, the limiting equivalent conductance values of CH₃COOH, CH₃COONa, HCl and NaCl can be written as follows:

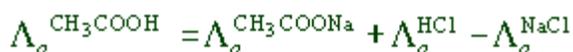
$$\Lambda_0^{\text{CH}_3\text{COOH}} = \lambda_0^{\text{CH}_3\text{COO}^-} + \lambda_0^{\text{H}^+}$$

$$\Lambda_0^{\text{CH}_3\text{COONa}} = \lambda_0^{\text{CH}_3\text{COO}^-} + \lambda_0^{\text{Na}^+}$$

$$\Lambda_0^{\text{HCl}} = \lambda_0^{\text{H}^+} + \lambda_0^{\text{Cl}^-}$$

$$\Lambda_0^{\text{NaCl}} = \lambda_0^{\text{Na}^+} + \lambda_0^{\text{Cl}^-}$$

Therefore



2) Determination of degree of ionization (α) of weak electrolyte: The degree of ionization of a weak electrolyte at a particular concentration is equal to the ratio of actual number of ions formed due to partial ionization to the expected number of ions formed upon complete dissociation.

$$\alpha = \frac{\text{Actual no. of ions formed due to partial dissociation}}{\text{Expected no. of particles formed due to complete dissociation}}$$

Since the conductance is proportional to the number of ions in the solution, the degree of ionization is equal to the conductance ratio as given below.

$$\alpha = \frac{\Lambda_c}{\Lambda_o} = \frac{\Lambda_c}{\lambda_o^+ + \lambda_o^-}$$

Where

Λ_c = equivalent conductivity at given concentration.

Λ_o = limiting equivalent conductivity.

λ_o^+ = limiting equivalent conductivity of cation.

λ_o^- = limiting equivalent conductivity of anion.

Calculations on Conductivity

Example 1

A conductivity cell is constructed of platinum electrodes in the form of squares 4 cm on a sides the electrodes being spaced 2 cm apart. When the cell is filled with 0.1M HCl, the resistance of the cell is found to be $R = 3.194 \Omega$. What is the molar conductance Λ of the HCl?

Solution

Since the resistance is proportional to the area and inversely proportional to the distance between the electrodes, the resistance reduced to 1-cm² plates separated by 1 cm is $(4^2/2)(3.194) = 25.652 \Lambda$. Since $\Lambda = \sigma / c$ and $\sigma = k/R$, $\Lambda = [(25.552) (0.0001)]^{-1} = 391.3 \text{ ohm}^{-1} \text{ cm}^2 \text{ mol}^{-1}$

Example 2

Some 0.05M NaCl is placed in the same cell as that described in Example 2. The resistance with the NaCl solution is 22.502Ω . Find Λ_{NaCl} without using the dimensions of the cell.

Solution

We can find Λ from the known value for the 0.1M HCl. Equation (3) can be written as $\Lambda = \sigma / c = 1/(Rc)$, or $R\Lambda = 1$. Thus $(Rc\Lambda)_{\text{HCl}} = (Rc\Lambda)_{\text{NaCl}}$ and

$$\Lambda_{\text{NaCl}} = \frac{(391.3)(3.194)(0.0001)}{(22.502)(0.00005)} = 111.1 \text{ ohm}^{-1} \text{ cm}^2 \text{ mol}^{-1}$$

Note that this is the way Λ is usually measured experimentally; the conductivity cell is first calibrated against a solution of known Λ , and for the unknown is found by comparison.

Example 3

For acetic acid (HAc), $\Lambda^{\circ} = 390.6 \text{ ohm}^{-1} \text{ cm}^2 \text{ mol}^{-1}$, while at a concentration of $0.003441 \text{ mol dm}^{-3}$, $\Lambda = 27.19 \text{ ohm}^{-1} \text{ cm}^2 \text{ mol}^{-1}$. What is the degree of dissociation, the concentration of each species, and the equilibrium constant?

Solution

The degree of dissociation can be found by using Equation (12) $\alpha = \Lambda / \Lambda^{\circ} = 27.19 / 390.6 = 0.0696$

The concentrations of H^+ and R^- are therefore;

$$[\text{H}^+] = [\text{R}^-] = (0.0696)(0.003441) = 2.40 \times 10^{-4} \text{ M}$$

and the concentration of HAc is $[\text{HAc}] = 0.003441 - 0.000240 = 0.003201 \text{ M}$

The equilibrium constant is then $(2.4 \times 10^{-4})^2 / 0.00320 = 1.8 \times 10^{-5}$

Example 4

have the specific conductance of a decinormal solution of tribasic acid which is 0.33 mS/cm . This is the only detail I have and I need to calculate molar conductance.

Solution

decinormal = 0.10 N $1 \text{ M} = 3 \text{ N}$ so molar conductivity would be conductivity of a 1 molar sol. or 30 times the conductivity of 0.10 N $0.10 \text{ N} \times 10 = 1 \text{ N} \times 3 = 1 \text{ M}$
 $0.33 \text{ mS/cm} \times 30 = 9.9 \text{ mS/cm}$

Example 5

A solution of salt (1.0 N) surrounding two platinum electrodes 2.1 cm apart and 4.2 cm^2 in area was found to offer a resistance of 50 ohms . Calculate the equivalent conductivity of the solution.

Solution:

$$l = 2.1 \text{ cm} \quad C = 1.0 \text{ N}$$

$$a = 4.2 \text{ cm}^2$$

$$R = 50 \text{ ohm}$$

Example 6

Specific conductance of a decinormal solution of KCl is $0.0112 \text{ ohm}^{-1} \text{ cm}^{-1}$.

The resistance of a cell containing the solution was found to be 56 . What is the cell constant?

$$K = 0.0112 \text{ ohm}^{-1} \text{ cm}^{-1}$$

$$R = 56 \text{ ohm}$$

Cell constant = Specific conductance \times Resistance

$$= K \times R$$

$$= 0.0112 \times 56 = 0.6272 \text{ cm}^{-1}$$

4.0 CONCLUSION

In this unit, you have learnt that conductometry is the study of the conductivity measurement made possible by the use of an instrument known as conductometer.

You should also have learned that insulators resist the flow of electric current whereas metallic conductors and electrolyte allow electricity to flow through electrons and mobile oppositely charged ions respectively.

It was also learnt that conductivity of conductors and electrolytes depends on temperature; at higher temperature the conductivity of conductors decreased due to vibrational disturbance whereas in electrolyte, conductivity increases with increase in temperature.

Finally, you will have learnt that conductivity of the electrolyte is depended on other factors such as nature of electrolyte, ionic size and mobility, viscosity, and concentration. Kohlrausch law of independent migration, its applications as well as some calculations were studied.

5.0 SUMMARY

In this unit we have learnt that:

- i. Resistance is the tendency of a material to stop the flow of current, and is directly proportional to the length of the conductor and inversely proportional to its cross sectional area. On the other hand, the tendency of a material to allow the flow of current through it is called conductance.
- ii. Insulators resist the flow of electricity but conductors allow the flow of electricity through them.
- iii. Strong electrolytes undergo complete ionization in water unlike weak electrolytes which ionize partially in water.
- iv. The conductance of that volume of solution containing one mole of an electrolyte and that containing one equivalent of an electrolyte are called molar and equivalent conductivity respectively.
- v. Factors such as temperature, nature of electrolyte, ionic mobility, viscosity as well as concentration affect the conductance of electrolyte solution. Ionic mobility decreases with increased ionic size which consequently decreases conductivity. The ease with which ions migrate in aqueous solution decreases as viscosity increases, this in turn decreases conductivity.
- vi. Strong electrolytes exhibit higher conductivity while weak electrolytes show lower conductivity. The conductance of an electrolyte solution increases with increased temperature.

6.0 TUTOR MARK ASSIGNMENT

- 1 Explain briefly the following terms:
 - (i) Resistance
 - (ii) specific conductance
 - (iii) molar conductance
- 2 List the factors that affect the conductivity of electrolyte solution.
- 3 State Kohlrausch law of independent migration
- 4 The equivalent conductance of NaCl, HCl and CH₃COONa at infinite dilution are 126.45, 426.16 and 91.0 ohm⁻¹ cm² equiv⁻¹, respectively at 25°. Calculate the

equivalent conductance of acetic acid at infinite dilution.

7.0 REFERENCE AND FURTHER READING

- 1 Skoog W, and Holler C., Fundamental of Analytical Chemistry. 8th ed.
- 2 Harris D.C.(1995) Qualitative Chemical Analysis ,4th ed.,Freeman W,H.and company, New York.
- 3 Gary D.C.(1994).Analytical Chemistry.5th ed.John Wiley and Sons,Inc.

UNIT 3 ELECTROLYTIC METHODS

1.0 Introduction

In the last unit you learnt about conductivity of conductors and electrolytes. In this unit we discuss electrolytic method of analysis. Electrolytic methods consist of the most accurate and most sensitive instrumental techniques. The analyte is oxidized or reduced at an appropriate electrode and the amount of electricity involved in the electrolysis is related to the amount of analyte. These methods are best suited for large quantities of analyte, for instance, millimole amount. Smaller amount of samples can sometimes be measured in these methods. Selectivity can easily be achieved in electrolytic method by appropriate choice of electrolysis potential since different analytes have different potential at which they are oxidized or reduced.

2.0 OBJECTIVE

- Discuss the principle of voltammetry
- List and explain the types of voltammetry
- Distinguish between chronoamperometry from other voltammetric techniques

3.0 MAIN CONTENT

3.1 Voltammetry

Voltammetry is essentially a current-voltage techniques in which electrolysis is done on a microscale using a micro working platinum or any suitable electrode. This is a category of electroanalytical chemistry and various industrial processes.

3.2.1 Basic Principle of Voltammetry

Voltammetry is the study of current as a function of applied potential. These curves $I = f(E)$ are called voltammograms. The potential is varied arbitrarily either step by step or continuously, and the actual current value is measured as the dependent variable. The shape of the curves depends on the speed of potential variation (nature of driving force) and on whether the solution is stirred or quiescent (mass transfer). Most experiments control the potential (volts) of an electrode in contact with the analyte while measuring the resulting current (amperes).

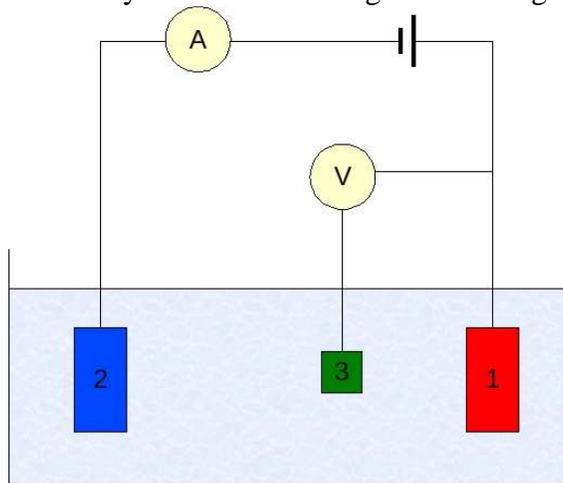


Figure 2.- Voltammetric cell

[Three-electrode setup: (1) working electrode; (2) auxiliary electrode; (3) reference electrode]

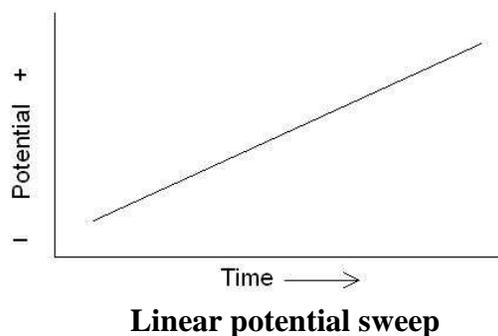
To conduct such an experiment requires at least two electrodes. The working electrode, which makes contact with the analyte, must apply the desired potential in a controlled way and facilitate the transfer of charge to and from the analyte. A second electrode acts as the other half of the cell. This second electrode must have a known potential with which to gauge the potential of the working electrode, furthermore it must balance the charge added or removed by the working electrode. While this is a viable setup, it has a number of shortcomings. Most significantly, it is extremely difficult for an electrode to maintain a constant potential while passing current to counter redox events at the working electrode.

To solve this problem, the role of supplying electrons and referencing potential has been divided between two separate electrodes. The reference electrode is a half cell with a known reduction potential. Its only role is to act as reference in measuring and controlling the working electrodes potential and at no point does it pass any current. The auxiliary electrode passes all the current needed to balance the current observed at the working electrode. To achieve this current, the auxiliary will often swing to extreme potentials at the edges of the solvent window, where it oxidizes or reduces the solvent or supporting electrolyte. These electrodes, the working, reference, and auxiliary make up the modern three electrode system. In practice it can be very important to have a working electrode with known dimensions and surface characteristics. As a result, it is common to clean and polish working electrodes regularly. The auxiliary electrode can be almost anything as long as it doesn't react with the bulk of the analyte solution and conducts well. The reference is the most complex of the three electrodes, there are a variety of standards used and its worth investigating elsewhere. For non-aqueous work, IUPAC recommends the use of the ferrocene/ferrocenium couple as an internal standard. In most voltammetry experiments, a bulk electrolyte (also known as a supporting electrolyte) is used to minimize solution resistance. It is possible to run an experiment without a bulk electrolyte, but the added resistance greatly reduces the accuracy of the results.

3.2 TYPES OF VOLTAMMETRY

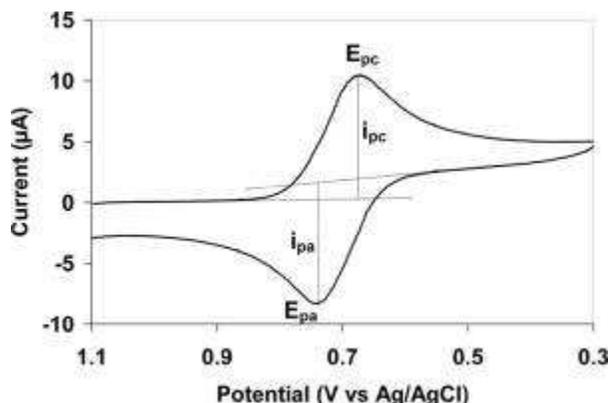
a) Linear sweep voltammetry

Voltammetric method in which the potential between the working electrode and a reference electrode is swept linearly in time while the current at a working electrode is measured is known as linear sweep voltammetry. Oxidation or reduction of species is registered as a peak or trough in the current signal at the potential at which the species begins to be oxidized or reduced.



b) Cyclic voltammetry

Cyclic voltammetry is a type of potentiodynamic electrochemical measurement. In a cyclic voltammetry experiment the working electrode potential is ramped linearly versus time like linear sweep voltammetry. Cyclic voltammetry takes the experiment a step further than linear sweep voltammetry which ends when it reaches a set potential. When cyclic voltammetry reaches a set potential, the working electrode's potential ramp is inverted. This inversion can happen multiple times during a single experiment. The current at the working electrode is plotted versus the applied voltage to give the cyclic voltammogram trace. Cyclic voltammetry is generally used to study the electrochemical properties of an analyte in solution.



Experimental setup

The method uses a reference electrode, working electrode, and counter electrode which in combination are sometimes referred to as a three-electrode setup. Electrolyte is usually added to the test solution to ensure sufficient conductivity. The combination of the solvent, electrolyte and specific working electrode material determines the range of the potential.

Electrodes are static and sit in unstirred solutions during cyclic voltammetry. This "still" solution method results in cyclic voltammetry's characteristic diffusion controlled peaks. This method also allows a portion of the analyte to remain after reduction or oxidation where it may display further redox activity. Stirring the solution between cyclic voltammetry traces is important as to supply the electrode surface with fresh analyte for each new experiment. The solubility of an analyte can change drastically with its overall charge. Since cyclic voltammetry usually alters the charge of the analyte it is common for reduced or oxidized analyte to

precipitate out onto the electrode. This layering of analyte can insulate the electrode surface, display its own redox activity in subsequent scans, or at the very least alter the electrode surface. For this and other reasons it is often necessary to clean electrodes between scans.

Common materials for working electrodes include glassy carbon, platinum, and gold. These electrodes are generally encased in a rod of inert insulator with a disk exposed at one end. A regular working electrode has a radius within an order of magnitude of 1 mm. Having a controlled surface area with a defined shape is important for interpreting cyclic voltammetry results.

To run cyclic voltammetry experiments at high scan rates a regular working electrode is insufficient. High scan rates create peaks with large currents and increased resistances which result in distortions. Ultramicroelectrodes can be used to minimize the current and resistance.

The counter electrode, also known as the auxiliary or second electrode, can be any material which conducts easily and won't react with the bulk solution. Reactions occurring at the counter electrode surface are unimportant as long as it continues to conduct current well. To maintain the observed current the counter electrode will often oxidize or reduce the solvent or bulk electrolyte.

c) Adsorptive stripping voltammetry

Adsorptive stripping voltammetry is similar to anodic stripping voltammetry and cathodic stripping voltammetry except that the preconcentration step is not controlled by electrolysis. The preconcentration step in adsorptive stripping voltammetry is accomplished by adsorption on the working electrode surface, or by reactions with chemically modified electrodes.

d) Differential Pulse Voltammetry

Differential Pulse Voltammetry (Differential Pulse Polarography or DPP) is often used to make electrochemical measurements. It is considered as a derivative of linear sweep voltammetry or staircase voltammetry, with a series of regular voltage pulses superimposed on the potential linear sweep or stair steps. The current is measured immediately before each potential change, and the current difference is plotted as a function of potential. By sampling the current just before the potential is changed, the effect of the charging current can be decreased.

By contrast, in normal pulse voltammetry the current resulting from a series of ever larger potential pulses is compared with the current at a constant 'baseline' voltage. Another type of pulse voltammetry is squarewave voltammetry, which can be considered a special type of differential pulse voltammetry in which equal time is spent at the potential of the ramped baseline and potential of the superimposed pulse.

The system of this measurement is usually the same as that of standard voltammetry. The potential between the working electrode and the reference electrode is changed as a pulse from an initial potential to an interlevel potential and remains at the interlevel potential for about 5 to 100 milliseconds; then it changes to the final potential, which is different from the initial potential. The pulse is repeated, changing the final potential, and a constant difference is kept between the initial and the interlevel potential. The value of the current between the working electrode and auxiliary electrode before and after the pulse are sampled and their differences are

plotted versus potential

Uses

These measurements can be used to study the redox properties of extremely small amounts of chemicals because of the following two features:

- (1) In these measurements, the effect of the charging current can be minimized, so high sensitivity is achieved.
- (2) Faradaic current is extracted, so electrode reactions can be analyzed more precisely.

Characteristics

Differential pulse voltammetry has these characteristics:

- (1) Reversible reactions show symmetrical peaks, and irreversible reactions show asymmetrical peaks.
- (2) The peak potential is equal to $E_{1/2}^r - \Delta E$ in reversible reactions, and the peak current is proportional to the concentration.
- (3) The detection limit is about 10^{-8} M.

e) **Cathodic stripping voltammetry**

Cathodic stripping voltammetry is a voltammetric method for quantitative determination of specific ionic species. It is similar to the trace analysis method anodic stripping voltammetry, except that for the plating step, the potential is held at an oxidizing potential, and the oxidized species are stripped from the electrode by sweeping the potential positively. This technique is used for ionic species that form insoluble salts and will deposit on or near the anodic, working electrode during deposition. The stripping step can be either linear, staircase, squarewave, or pulse

f) **Anodic stripping voltammetry**

Anodic stripping voltammetry is a voltammetric method for quantitative determination of specific ionic species. The analyte of interest is electroplated on the working electrode during a deposition step, and oxidized from the electrode during the stripping step. The current is measured during the stripping step. The oxidation of species is registered as a peak in the current signal at the potential at which the species begins to be oxidized. The stripping step can be either linear, staircase, squarewave, or pulse.

Electrochemical Cell Set-Up

Anodic stripping voltammetry usually incorporates three electrodes, a working electrode, auxiliary electrode (sometimes called the counter electrode), and reference electrode. The solution being analyzed usually has an electrolyte added to it. For most standard tests, the working electrode is a mercury film electrode. The mercury film forms an amalgam with the analyte of interest, which upon oxidation results in a sharp peak, improving resolution between analytes. The mercury film is formed over a glassy carbon electrode. A mercury drop electrode has also been used for much the same reasons. In cases where the analyte of interest has an

oxidizing potential above that of mercury, or where a mercury electrode would be otherwise unsuitable, a solid, inert metal such as silver, gold, or platinum may also be used.

Anodic stripping voltammetry usually incorporates 4 steps if the working electrode is a mercury film or mercury drop electrode and the solution incorporates stirring. The solution is stirred during the first two steps at a repeatable rate. The first step is a cleaning step; in the cleaning step, the potential is held at a more oxidizing potential than the analyte of interest for a period of time in order to fully remove it from the electrode. In the second step, the potential is held at a lower potential, low enough to reduce the analyte and deposit it on the electrode. After the second step, the stirring is stopped, and the electrode is kept at the lower potential. The purpose of this third step is to allow the deposited material to distribute more evenly in the mercury. If a solid inert electrode is used, this step is unnecessary. The last step involves raising the working electrode to a higher potential (anodic), and stripping (oxidizing) the analyte. As the analyte is oxidized, it gives off electrons which are measured as a current.

Stripping analysis is an analytical technique that involves (i) preconcentration of a metal phase onto a solid electrode surface or into Hg (liquid) at negative potentials and (ii) selective oxidation of each metal phase species during an anodic potential sweep.

Stripping analysis has the following properties:

Very sensitive and reproducible (RSD<5%) method for trace metal ion analysis in aqueous media.

Concentration limits of detection for many metals are in the low ppb to high ppb range (S/N=3) and this compares favorably with AAS or ICP analysis.

Field deployable instrumentation that is inexpensive.

Approximately 12-15 metal ions can be analyzed for by this method.

The stripping peak currents and peak widths are a function of the size, coverage and distribution of the metal phase on the electrode surface (Hg or alternate)

Sensitivity

Anodic stripping voltammetry can detect $\mu\text{g/l}$ concentrations of analyte. This method has an excellent detection limit (typically 10^{-9} - 10^{-10} M)

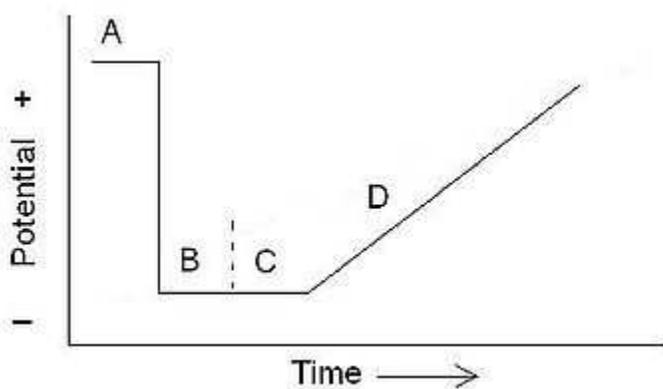


Figure 2.5; A: Cleaning step, B: Electroplating step, C: Equilibration step, D: Stripping step

Amperometry

Amperometry is the application of voltammetric measurements at a fixed potential to detect changes in currents as a function of concentration of electroactive species.

Amperometric is in principle similar to voltammetric, refers to a class of titrations in which the equivalence point is determined through measurement of the electric current produced by the titration reaction. It is a form of quantitative analysis.

Chronoamperometry is an electrochemical technique in which the potential of the working electrode is stepped and the resulting current from faradic processes occurring at the electrode is monitored as a function of time. Limited information about the identity of the electrolyzed species can be obtained from the ratio of the peak oxidation current versus the peak reduction current. However, as with all pulsed techniques, chronoamperometry generates high charging currents, which decay exponentially with time as any RC circuit. The Faradaic current--which is due to electron transfer events and is most often the current component of interest--decays as described in the Cottrell equation. In most electrochemical cells this decay is much slower than the charging decay--cells with no supporting electrolyte are notable exceptions. Since the current is integrated over relatively longer time intervals, chronoamperometry gives a better signal to noise ratio in comparison to other amperometric technique.

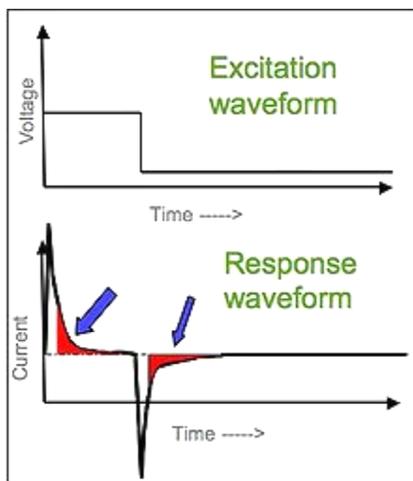


Figure 2.6 Double-pulsed chronoamperometry waveform showing integrated region for charge determination.

Anthracene in deoxygenated dimethylformamide (DMF) will be reduced ($An + e^- \rightarrow An^-$) at the electrode surface that is at a certain negative potential. The reduction will be diffusion-limited, thereby causing the current to drop in time (proportional to the diffusion gradient that is formed by diffusion).

You can do this experiment several times increasing electrode potentials from low to high. (In between the experiments, the solution should be stirred.) When you measure the current $i(t)$ at a certain fixed time point τ after applying the voltage, you will see that at a certain moment the current $i(\tau)$ does not rise anymore; you have reached the mass-transfer-limited region. This means that anthracene arrives as fast as diffusion can bring it to the electrode.

4.0 CONCLUSION

This unit can be concluded by observing that electrolytic methods consist of the most accurate and sensitive instrumental techniques. The half cell reactivity of analytes can be accurately investigated by voltammetry, where current is studied as a function of applied potential.

5.0 SUMMARY

- i. Voltammetric cell consists of the working electrode, reference electrode and auxiliary electrode.
- ii. Working electrode must apply the desired potential in a controlled way and facilitate the transfer of charge to and from the analyte.
- iii. The reference electrode acts as a reference in the measuring and controlling the working electrodes potential.
- iv. The auxiliary electrode passes all the current needed to balance the current observed at the working electrode quite unlike the reference electrode.
- v. Types of voltammetry studied are linear sweep voltammetry, differential pulse voltammetry, cathodic stripping voltammetry and amperometry.
- vi. Cathodic stripping voltammetry is similar to anodic stripping voltammetry, except that for the plating step, the potential is held at an oxidizing potential and the oxidizing species are stripped from the electrode by sweeping.

6.0 TUTOR MARKED ASSIGNMENT

1. Describe the principle of voltammetry.
2. Distinguish between cathodic stripping voltammetry and anodic stripping voltammetry.

7.0 REFERENCES/FURTHER READING

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MODULE 3

- Unit 1 Basic Chromatography Techniques
- Unit 2 Calorimetry
- Unit 3 Radiochemical Method
- Unit 4 Radioanalytical Separation Techniques

UNIT 1 Chromatography Techniques

CONTENT

- 1.0 Introduction
- 2.0 Objectives
- 3.0 Main Content
 - 3.1 History of Chromatography
 - 3.2 Basic Chromatography Terms
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 - 3.3.2.1 Preparation of Column
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- 4.0 Conclusion
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1.0 INTRODUCTION

Chromatography (from Greek *chroma* "color" and *graphein* "to write") is the collective term for a set of laboratory techniques for the separation of mixtures. The mixture is dissolved in a fluid called the "mobile phase", which carries it through a structure holding another material called the "stationary phase". The various constituents of the mixture travel at different speeds, causing them to separate. The separation is based on differential partitioning between the mobile and stationary phases. Subtle differences in a compound's partition coefficient result in differential retention on the stationary phase and thus changing the separation.

Chromatography may be preparative or analytical. The purpose of preparative chromatography is to separate the components of a mixture for further use (and is thus a form of purification). Analytical chromatography is done normally with smaller amounts of material and is for measuring the relative proportions of analytes in a mixture.

2.0 OBJECTIVES

By the end of this unit, students should be able to:

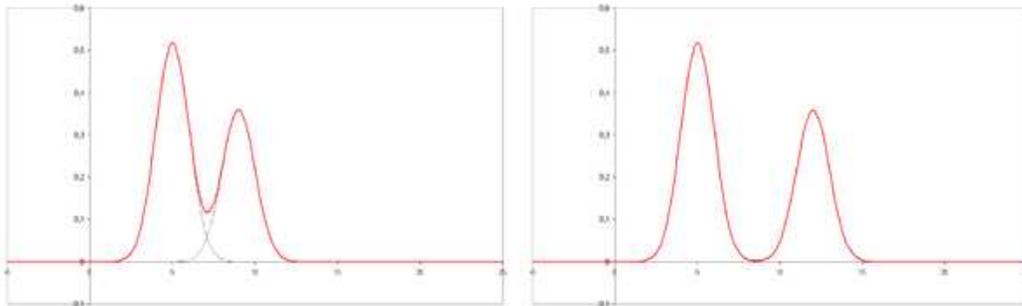
- Appreciate the origin of chromatography
- Define some basic chromatography terms
- Describe basic chromatographic techniques
- State the application of various chromatographic methods

3.1 History of Chromatography

Chromatography, literally "color writing", was first employed by Russian scientist Michael Tswett in 1900. He continued to work with chromatography in the first decade of the 20th century, primarily for the separation of plant pigments such as chlorophyll, carotenes, and xanthophylls. Since these components have different colors (green, orange, and yellow, respectively) they gave the technique its name. Between 1930s and 1940s new types of chromatography emerged and the technique became useful for many separation processes. Archer John Martin Porter and Richard Laurence Millington Synge during the 1940s and 1950s worked extensively on chromatography technique that led to the establishment of the principles and basic techniques of partition chromatography, and their work encouraged the rapid development of several chromatographic methods: paper chromatography, gas chromatography, and what would become known as high performance liquid chromatography. The technology has since then advanced rapidly. Researchers found that the main principles of Tswett's chromatography could be applied in many different ways, resulting in the different varieties of chromatography described below. Separation of increasingly similar molecules is made possible due to persistence improvement in the technical performance of chromatography.

3.2 Basic Chromatography Terms

- The analyte is the substance to be separated during chromatography.
- Analytical chromatography is used to determine the existence and the concentration of analyte(s) in a sample.
- A bonded phase is a stationary phase that is covalently bonded to the support particles or to the inside wall of the column tubing.
- The visual output of the chromatograph is a chromatogram which consists of different peaks that correspond to different components of the separated mixture.



Plotted on the x-axis is the retention time and plotted on the y-axis a signal (for example obtained by a spectrophotometer, mass spectrometer or a variety of other detectors) corresponding to the response created by the analytes exiting the system. The signal is proportional to the concentration of the analyte separated.

- A chromatograph is equipment that enables a sophisticated separation e.g. gas

chromatographic or liquid chromatographic separation.

- Chromatography is a physical method of separation in which the components to be separated are distributed between two phases, one of which is stationary (stationary phase) while the other (the mobile phase) moves in a definite direction.
- The eluate is the mobile phase leaving the column.
- The eluent is the solvent that will carry the analyte.
- An eluotropic series is a list of solvents ranked according to their eluting power.
- An immobilized phase is a stationary phase which is immobilized on the support particles, or on the inner wall of the column tubing.
- The mobile phase is the phase which moves in a definite direction. It may be a liquid (LC and Capillary Electrochromatography (CEC)), a gas (GC), or a supercritical fluid (supercritical-fluid chromatography, SFC). The mobile phase consists of the sample being separated/analyzed and the solvent that moves the sample through the column. In the case of HPLC the mobile phase consists of a non-polar solvent(s) such as hexane in normal phase or polar solvents in reverse phase chromatography and the sample being separated. The mobile phase moves through the chromatography column (the stationary phase) where the sample interacts with the stationary phase and is separated.
- Preparative chromatography is used to purify sufficient quantities of a substance for further use, rather than analysis.
- The retention time is the characteristic time it takes for a particular analyte to pass through the system (from the column inlet to the detector) under set conditions. The sample is the matter analyzed in chromatography. It may consist of a single component or it may be a mixture of components. When the sample is treated in the course of an analysis, the phase or the phases containing the analytes of interest is/are referred to as the sample whereas everything out of interest separated from the sample before or in the course of the analysis is referred to as waste.
- The solute refers to the sample components in partition chromatography.
- The solvent refers to any substance capable of solubilizing other substance, and especially the liquid mobile phase in LC.
- The stationary phase is the substance which is fixed in place for the chromatography procedure. Examples include the silica layer in thin layer chromatography.

3.3 Techniques by chromatographic bed shape

3.3.1 Planar chromatography

Planar chromatography is a separation technique in which the stationary phase is present on a plane. The plane can be a paper, or the paper may be impregnated by a substance as the stationary bed (paper chromatography). It could also be a layer of solid particles spread on a support such as a glass plate (thin layer chromatography). Various components of the sample mixture migrate at different rates according to how strongly they interact with the stationary phase as compared to the mobile phase. The specific Retention factor (R_f) of each chemical can be used to aid in the identification of an unknown substance.

3.3.1.1 Paper Chromatography

Paper chromatography is a technique used for separating and identifying mixtures that are either coloured or can be coloured. The secondary or primary colors in ink can easily be separated by this technique. This method is a powerful teaching tool but has been greatly replaced by thin layer chromatography. Complex mixtures of similar compounds such as amino acids can be separated by using a two-way paper chromatograph otherwise known as two-dimensional chromatography. In this method, two solvents are used and the paper is rotated at 90° in between.

The retention factor (R_f) is defined as the ratio of the distance traveled by the substance to the distance traveled by the solvent. R_f values are usually expressed as a fraction of two decimal places. If R_f value of a solution is zero, the solute remains in the stationary phase and thus it is immobile. If R_f value = 1, this implies that the solute has no affinity for the stationary phase and travels with the solvent front. To calculate the R_f value, take the distance traveled by the substance divided by the distance traveled by the solvent. For example, if a compound travels 1.5 cm and the solvent front travels 2.2 cm, ($1.5/2.2$) the R_f value = 0.68

Paper chromatography is one method for testing the purity of compounds and identifying substances. Paper chromatography is a useful technique because it is relatively quick and requires small quantities of material.

In paper chromatography, like thin layer chromatography, substances are distributed between a stationary phase and a mobile phase. The stationary phase is usually a piece of high quality filter paper. The mobile phase is a developing solution that travels up the stationary phase, carrying the samples with it. Components of the sample will separate readily according to how strongly they adsorb on the stationary phase versus how readily they dissolve in the mobile phase.

When a colored chemical sample is placed on a filter paper, the colors separate from the sample by placing one end of the paper in a solvent. The solvent diffuses up the paper, dissolving the various molecules in the sample according to the polarities of the molecules and the solvent. If the sample contains more than one color, that means it must have more than one kind of molecule. Because of the different chemical structures of each kind of molecule, the chances are very high that each molecule will have at least a slightly different polarity, giving each molecule a different solubility in the solvent. The unequal solubilities cause the various color molecules to leave solution at different places as the solvent continues to move up the paper. The more soluble a molecule is, the higher it will migrate up the paper. If a chemical is very nonpolar it will not dissolve at all in a very polar solvent. This is the same for a very polar chemical and a very nonpolar solvent.

It is important to note that when using water (a very polar substance) as a solvent, the more polar the colour, the higher it will rise on the paper.

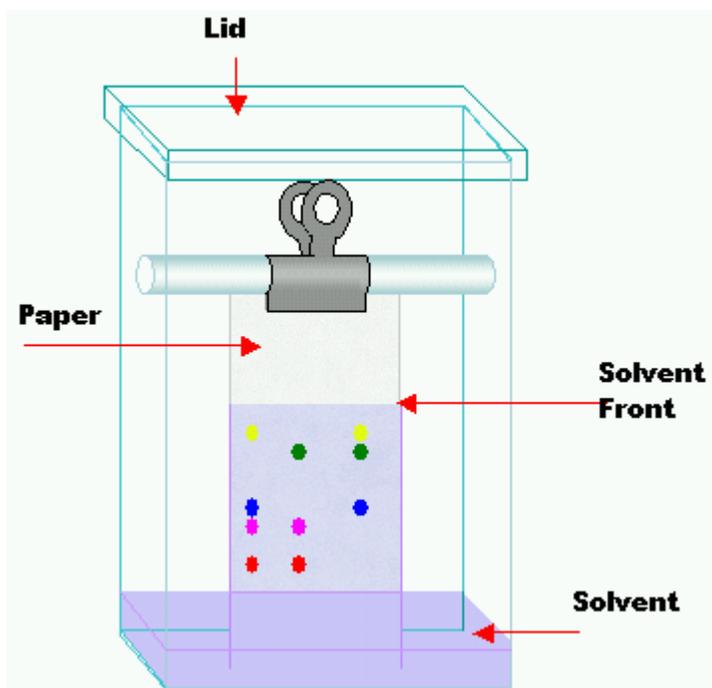


Figure 3.1 Chromatography Tank(Paper Chromatography)

3.3.1.2 Thin layer chromatography (TLC) is a chromatography technique used to separate mixtures. Thin layer chromatography is performed on a sheet of glass, plastic, or aluminum foil, which is coated with a thin layer of adsorbent material, usually silica gel, aluminium oxide, or cellulose (blotter paper). This layer of adsorbent is known as the stationary phase.

After the sample has been applied on the plate, a solvent or solvent mixture (known as the mobile phase) is drawn up the plate via capillary action. Because different analytes ascend the TLC plate at different rates, separation is achieved.

Thin layer chromatography can be used to monitor the progress of a reaction, identify compounds present in a given mixture, and determine the purity of a substance. Specific examples of these applications include: analyzing ceramides and fatty acids, detection of pesticides or insecticides in food and water, analyzing the dye composition of fibers in forensics, assaying the radiochemical purity of radiopharmaceuticals, or identification of medicinal plants and their constituents

A number of enhancements can be made to the original method to automate the different steps, to increase the resolution achieved with TLC and to allow more accurate quantization. This method is referred to as HPTLC, or "high performance TLC".

Plate preparation

TLC plates are usually commercially available, with standard particle size ranges to improve reproducibility. They are prepared by mixing the adsorbent, such as silica gel, with a small amount of inert binder like calcium sulfate (gypsum) and water. This mixture is spread as a thick slurry on an unreactive carrier sheet, usually glass, thick aluminum foil, or plastic. The resultant plate is dried and *activated* by heating in an oven for thirty minutes at 110 °C. The thickness of the adsorbent layer is typically around 0.1 – 0.25 mm for analytical purposes and around 0.5 – 2.0 mm for preparative TLC.

Development of a TLC plate, a purple spot separates into a red and blue spot.

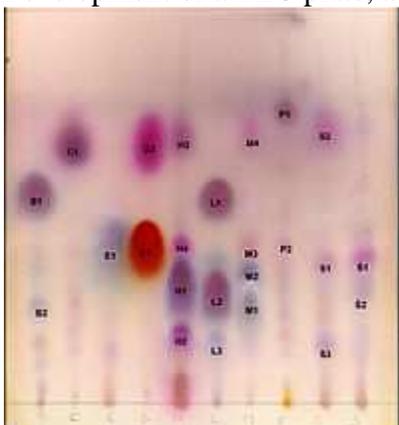


Figure 3.2 Chromatogram of 10 essential oils coloured with vanillin reagent.
(<http://en.wikipedia.org/wiki/file:TLC-Essential-Oil.jpg>)

The process is similar to paper chromatography with the advantage of faster runs, better separations, and different stationary phases are available for use. Because of its simplicity and speed TLC is often used for monitoring chemical reactions and for the qualitative analysis of reaction products.

Thin layer chromatography can be run easily if the following procedure is carried out:

A small spot of solution containing the sample is applied to a plate, about 1.5 centimeters from the bottom edge. The solvent is allowed to completely evaporate off, otherwise a very poor or no separation will be achieved. The plate should be dried in a vacuum chamber especially when a non-volatile solvent was used to apply the sample.

A small amount of an appropriate solvent is poured in to a suitable transparent container to a depth of less than 1 centimeter. A strip of filter paper is put into the chamber, so that its bottom touches the solvent, and the paper lies on the chamber wall and reaches almost to the top of the container. The container is closed with a cover glass or any other lid and is left for a few minutes to let the solvent vapors ascend the filter paper and saturate the air in the chamber. The TLC plate is then placed in the chamber so that the spots of the sample do not touch the surface of the solvent in the chamber, and the lid is closed. The solvent moves up the plate by capillary action, meets the sample mixture and carries it up the plate (elutes the sample). When the solvent front reaches no higher than the top of the filter paper in the chamber, the plate should be removed and dried .

Separation Process

Different compounds in the sample mixture travel at different rates due to the differences in their attraction to the stationary phase, and because of differences in solubility in the solvent. By changing the solvent, or perhaps using a mixture, the separation of components (measured by the R_f value) can be adjusted. Also, the separation achieved with a TLC plate can be used to estimate the separation of a flash chromatography column.

Separation of compounds is based on the competition of the solute and the mobile phase for binding places on the stationary phase. For instance, if normal phase silica gel is used as the stationary phase it can be considered polar. Given two compounds which differ in polarity, the

more polar compound has a stronger interaction with the silica and is therefore more capable to dispel the mobile phase from the binding places. Consequently, the less polar compound moves higher up the plate (resulting in a higher R_f value). If the mobile phase is changed to a more polar solvent or mixture of solvents, it is more capable of dispelling solutes from the silica binding places and all compounds on the TLC plate will move higher up the plate. It is commonly said that "strong" solvents (elutants) push the analyzed compounds up the plate, while "weak" elutants barely move them. The order of strength/weakness depends on the coating (stationary phase) of the TLC plate. For silica gel coated TLC plates, the elutant strength increases in the following order: Perfluoroalkane (weakest), Hexane, Pentane, Carbon tetrachloride, Benzene/Toluene, Dichloromethane, Diethyl ether, Ethylacetate, Acetonitrile, Acetone, 2-Propanol/n-Butanol, Water, Methanol, Triethylamine, Acetic acid, Formic acid (strongest). For C18 coated plates the order is reverse. Practically this means that if you use a mixture of ethyl acetate and hexane as the mobile phase, adding more ethyl acetate results in higher R_f values for all compounds on the TLC plate. *Changing the polarity of the mobile phase will normally not result in reversed order of running of the compounds on the TLC plate.* An eluotropic series can be used as a guide in selecting a mobile phase. If a reversed order of running of the compounds is desired, an apolar stationary phase should be used, such as C18-functionalized silica.

Analysis

As the chemicals being separated may be colorless, several methods exist to visualize the spots: Often a small amount of a fluorescent compound, usually manganese-activated zinc silicate, is added to the adsorbent that allows the visualization of spots under a blacklight (UV₂₅₄). The adsorbent layer will thus fluoresce light green by itself, but spots of analyte quench this fluorescence.

Iodine vapors are a general unspecific color reagent

Specific color reagents exist into which the TLC plate is dipped or which are sprayed onto the plate:

Potassium permanganate – oxidation, iodine and bromine.

In the case of lipids, the chromatogram may be transferred to a polyvinylidene difluoride (PVDF) membrane and then subjected to further analysis, for example mass spectrometry, a technique known as Far-Eastern blotting.

Once visible, the R_f value, or retardation factor, of each spot can be determined by dividing the distance the product traveled by the distance the solvent front traveled using the initial spotting site as reference. These values depend on the solvent used and the type of TLC plate and are not physical constants.

Applications

As an example the chromatography of an extract of green leaves (for example spinach) in 7 stages of development. Carotene elutes quickly and is only visible until step 2. Chlorophyll A and B are halfway in the final step and lutein the first compound staining yellow.

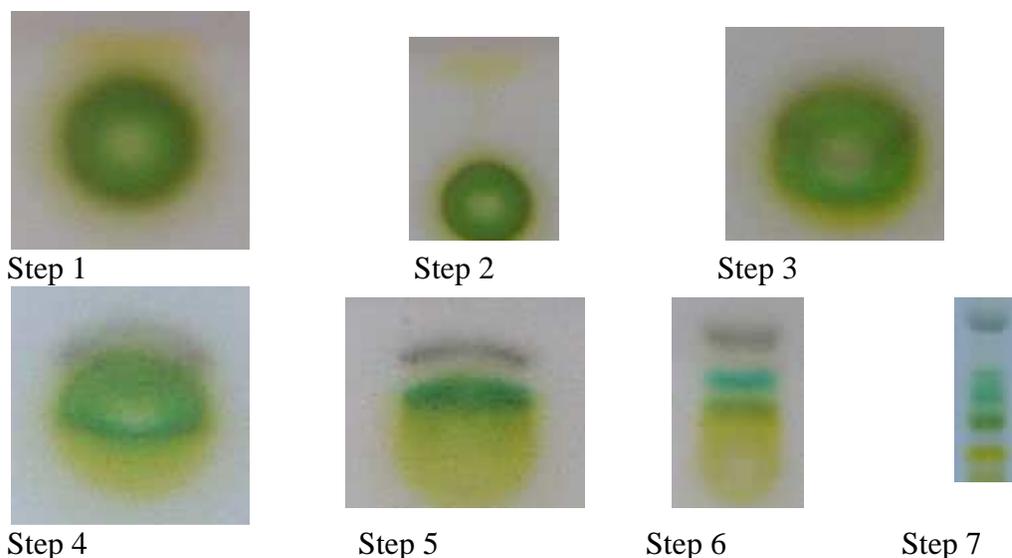


Figure 3.3 *Developmental Stages of Chlorophyll Extract*

In one study TLC has been applied in the screening of organic reactions for example in the fine-tuning of BINAP synthesis from 2-naphthol. In this method the alcohol and catalyst solution (for instance iron(III) chloride) are placed separately on the base line, then reacted and then instantly analyzed.

3.3.2 Column Chromatography

Separation of a mixture and isolation of the components in larger amounts is made possible by chromatography on a column than by TLC. The column is made of glass and is packed with particles, which constitute the stationary phase. The mixture under test is placed on top of a layer of sand on the column, and a slow stream of solvent, the eluant, washes the mixture through it. The function of sand is to prevent the particles being disturbed by the liquid. The substance that is the least attracted into the stationary phase is washed out at the bottom of the column first, followed by the remaining components over a period of time.

3.3.2.1 Preparation of Column

The column is prepared in the following steps:

- Place a wad of glass wool in the bottom of the tube and pour a layer of sand over this. The sand retains fine particles and also provides a flat horizontal base for the adsorbent column.
- Fill the tube with the first solvent to be used and then add the dry adsorbent in a fine stream shaking or tapping the tube to dislodge air bubbles, and draining solvent out at the bottom to make room as needed, but keeping the solvent level above the adsorbent
- When the adsorbent has settled in to a compact column, add another layer of sand at the top of the adsorbent to prevent disturbance of the surface where solvent is added
- Permit the solvent to drain down to just above the top sand layer. Note that the adsorbent column must be kept covered with solvent during the chromatography, otherwise, channels and cracks will develop
- Dissolve the sample in a minimum volume of solvent and add the solution to the

solution to the column with a pipet and bulb. Allow the solution to drain onto the column and immediately add more solvent.

In 1978, W. C. Still introduced a modified version of column chromatography called flash column chromatography (flash). The technique is very similar to the traditional column chromatography, except for that the solvent is driven through the column by applying positive pressure. This allowed most separations to be performed in less than 20 minutes, with improved separations compared to the old method. Modern flash chromatography systems are sold as pre-packed plastic cartridges, and the solvent is pumped through the cartridge. Systems may also be linked with detectors and fraction collectors providing automation. The introduction of gradient pumps resulted in quicker separations and less solvent usage.

In expanded bed adsorption, a fluidized bed is used, rather than a solid phase made by a packed bed. This allows omission of initial clearing steps such as centrifugation and filtration, for culture broths or slurries of broken cells.



Figure 3.4 Typical set up for manual column chromatography.

3.3.2.2 Column Chromatogram Resolution Calculation

Typically, column chromatography is set up with peristaltic pumps, flowing buffers and the solution sample through the top of the column. The solutions and buffers pass through the column where a fraction collector at the end of the column setup collects the eluted samples. Prior to the fraction collection, the samples that are eluted from the column pass through a detector such as a spectrophotometer or mass spectrometer so that the concentration of the separated samples in the sample solution mixture can be determined.

For example, if you were to separate two different proteins with different binding capacities to the column from a solution sample, a good type of detector would be a spectrophotometer using a wavelength of 280 nm. The higher the concentration of protein that passes through the eluted solution through the column, the higher the absorbance of that wavelength.

Because the column chromatography has a constant flow of eluted solution passing through the

detector at varying concentrations, the detector must plot the concentration of the eluted sample over a course of time. This plot of sample concentration versus time is called a chromatogram. The ultimate goal of chromatography is to separate different components from a solution mixture. The resolution expresses the extent of separation between the components from the mixture. The higher the resolution of the chromatogram, the better the extent of separation of the samples the column gives. This data is a good way of determining the column's separation properties of that particular sample. The resolution can be calculated from the chromatogram. The separate curves in the diagram represent different sample elution concentration profiles over time based on their affinity to the column resin. To calculate resolution, the retention time and curve width are required.

The time from the start of signal detection by the detector to the peak height of the elution concentration profile of each different sample is called retention time while the width of the concentration profile curve of the different samples in the chromatogram in units of time is known as curve width.

A simplified method of calculating chromatogram resolution is to use the plate model. The plate model assumes that the column can be divided into a certain number of sections, or plates and the mass balance can be calculated for each individual plate. This approach approximates a typical chromatogram curve as a Gaussian distribution curve. By doing this, the curve width is estimated as 4 times the standard deviation of the curve, 4σ . The retention time is the time from the start of signal detection to the time of the peak height of the Gaussian curve.

From the variables in the figure above, the resolution, plate number, and plate height of the column plate model can be calculated using the equations:

Resolution (R_s)

$$R_s = 2(t_{RB} - t_{RA}) / (w_B + w_A)$$

Where:

t_{RB} = retention time of solute B, t_{RA} = retention time of solute A

w_B = Gaussian curve width of solute B and w_A = Gaussian curve width of solute A

Plate Number (N):

$$N = (t_R)^2 / (w/4)^2$$

Plate Height (H):

$$H = L/N$$

Where L is the length of the column.

3.3.2.3 Column Adsorption Equilibrium

For an adsorption column, the column resin (the stationary phase) is composed of microbeads. Even smaller particles such as proteins, carbohydrates, metal ions, or other chemical compounds are conjugated onto the microbeads. Each binding particle that is attached to the microbead can be assumed to bind in a 1:1 ratio with the solute sample sent through the column that needs to be purified or separated.

Binding between the target molecule to be separated and the binding molecule on the column beads can be modeled using a simple equilibrium reaction $K_{eq} = [CS] / ([C][S])$ where K_{eq} is the equilibrium constant, [C] and [S] are the concentrations of the target molecule and the binding molecule on the column resin, respectively. [CS] is the concentration of the complex of the target molecule bound to the column resin.

Using this as a basis, three different isotherms can be used to describe the binding dynamics of a column chromatography: linear, Langmuir, and Freundlich.

The linear isotherm occurs when the solute concentration needed to be purified is very small relative to the binding molecule. Thus, the equilibrium can be defined as:

$$[CS] = K_{eq}[C].$$

For industrial scale uses, the total binding molecules on the column resin beads must be factored in because unoccupied sites must be taken into account. The Langmuir isotherm and Freundlich isotherm are useful in describing this equilibrium. Langmuir Isotherm:

$$[CS] = (K_{eq}S_{tot}[C])/(1 + K_{eq}[C]),$$
 where S_{tot} is the total binding molecules on the beads.

Freundlich Isotherm:

$$[CS] = K_{eq}[C]^{1/n}$$

The Freundlich isotherm is used when the column can bind to many different samples in the solution that needs to be purified. Because the many different samples have different binding constants to the beads, there are many different K_{eq} 's. Therefore, the Langmuir isotherm is not a good model for binding in this case.

3.4 Displacement chromatography

A molecule with a high affinity for the chromatography matrix will compete effectively for binding sites, and thus displace all molecules with lesser affinities. There are distinct differences between displacement and elution chromatography. In elution mode, substances typically emerge from a column in narrow, Gaussian peaks. Wide separation of peaks, preferably to baseline, is desired in order to achieve maximum purification. The speed at which any component of a mixture travels down the column in elution mode depends on many factors. But for two substances to travel at different speeds, and thereby be resolved, there must be substantial differences in some interaction between the biomolecules and the chromatography matrix. Operating parameters are adjusted to maximize the effect of this difference. In many cases, baseline separation of the peaks can be achieved only with gradient elution and low column loadings. Thus, two drawbacks to elution mode chromatography, especially at the preparative scale, are operational complexity, due to gradient solvent pumping, and low throughput, due to low column loadings. Displacement chromatography has advantages over elution chromatography in that components are resolved into consecutive zones of pure substances rather than "peaks". Because the process takes advantage of the nonlinearity of the isotherms, a larger column feed can be separated on a given column with the purified components recovered at significantly higher concentrations. Historically, displacement chromatography was applied to preparative separations of amino acids and rare earth elements and has also been investigated for isotope separation.

3.5 Techniques by physical state of mobile phase

3.5.1 Gas chromatography (GC)

Gas chromatography (GC) is a common type of chromatography used in analytical chemistry for separating and analyzing compounds that can be vaporized without decomposition. Typical uses of GC include testing the purity of a particular substance, or separating the different components of a mixture (the relative amounts of such components can also be determined). In some situations, GC may help in identifying a compound. In preparative chromatography, GC can be used to prepare pure compounds from a mixture.

In gas chromatography, the *mobile phase* (or "moving phase") is a carrier gas, usually an inert

gas such as helium or an unreactive gas such as nitrogen. The *stationary phase* is a microscopic layer of liquid or polymer on an inert solid support, inside a piece of glass or metal tubing called a column (an homage to the fractionating column used in distillation). The instrument used to perform gas chromatography is called a *gas chromatograph* (or "aerograph", "gas separator").

The gaseous compounds being analyzed interact with the walls of the column, which is coated with different stationary phases. This causes each compound to elute at a different time, known as the *retention time* of the compound. The comparison of retention times is what gives GC its analytical usefulness.

Gas chromatography is in principle similar to column chromatography (as well as other forms of chromatography, such as HPLC, TLC), but has several notable differences. Firstly, the process of separating the compounds in a mixture is carried out between a liquid stationary phase and a gas mobile phase, whereas in column chromatography the stationary phase is a solid and the mobile phase is a liquid. (Hence the full name of the procedure is "Gas-liquid chromatography", referring to the mobile and stationary phases, respectively.) Secondly, the column through which the gas phase passes is located in an oven where the temperature of the gas can be controlled, whereas column chromatography (typically) has no such temperature control. Thirdly, the concentration of a compound in the gas phase is solely a function of the vapor pressure of the gas.

Gas chromatography is also similar to fractional distillation, since both processes separate the components of a mixture primarily based on boiling point (or vapor pressure) differences. However, fractional distillation is typically used to separate components of a mixture on a large scale, whereas GC can be used on a much smaller scale (i.e. microscale).

Gas chromatography is also sometimes known as vapor-phase chromatography (VPC), or gas-liquid partition chromatography (GLPC). These alternative names, as well as their respective abbreviations, are frequently found in scientific literature. Strictly speaking, GLPC is the most correct terminology, and is thus preferred by many authors.

GC analysis

A gas chromatograph is a chemical analysis instrument for separating chemicals in a complex sample. A gas chromatograph uses a flow-through narrow tube known as the *column*, through which different chemical constituents of a sample pass in a gas stream (carrier gas, *mobile phase*) at different rates depending on their various chemical and physical properties and their interaction with a specific column filling, called the *stationary phase*. As the chemicals exit the end of the column, they are detected and identified electronically. The function of the stationary phase in the column is to separate different components, causing each one to exit the column at a different time (*retention time*). Other parameters that can be used to alter the order or time of retention are the carrier gas flow rate, column length and the temperature.

In a GC analysis, a known volume of gaseous or liquid analyte is injected into the "entrance" (head) of the column, usually using a microsyringe (or, solid phase microextraction fibers, or a gas source switching system). As the carrier gas sweeps the analyte molecules through the column, this motion is inhibited by the adsorption of the analyte molecules either onto the column walls or onto packing materials in the column. The rate at which the molecules progress along the column depends on the strength of adsorption, which in turn depends on the type of molecule and on the stationary phase materials. Since each type of molecule has a different rate of progression, the various components of the analyte mixture are separated as they progress

along the column and reach the end of the column at different times (retention time). A detector is used to monitor the outlet stream from the column; thus, the time at which each component reaches the outlet and the amount of that component can be determined. Generally, substances are identified (qualitatively) by the order in which they emerge (elute) from the column and by the retention time of the analyte in the column.

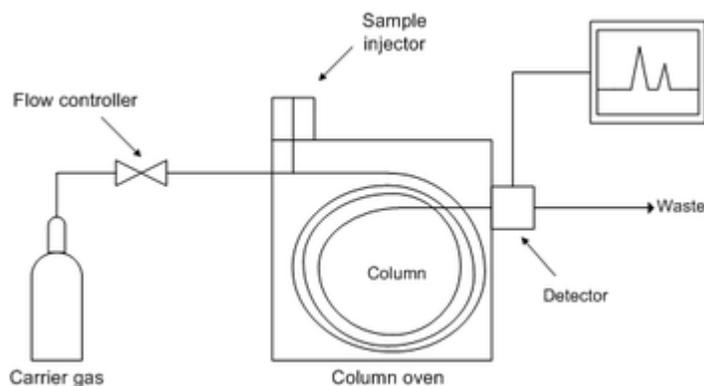


Figure 3.5 Schematic diagram of a gas chromatograph

Component of a gas chromatograph

The **choice of carrier gas** (*mobile phase*) is important, with hydrogen being the most efficient and providing the best separation. However, helium has a larger range of flow rates that are comparable to hydrogen in efficiency, with the added advantage that helium is non-flammable, and works with a greater number of detectors. Therefore, helium is the most common carrier gas used.

Detectors

Detectors are the flame ionization detector (FID) and the thermal conductivity detector (TCD). Both are sensitive to a wide range of components, and both work over a wide range of concentrations. While TCDs are essentially universal and can be used to detect any component other than the carrier gas (as long as their thermal conductivities are different from that of the carrier gas, at detector temperature), FIDs are sensitive primarily to hydrocarbons, and are more sensitive to them than TCD. However, an FID cannot detect water. Both detectors are also quite robust. Since TCD is non-destructive, it can be operated in-series before an FID (destructive), thus providing complementary detection of the same analytes.

Other detectors are sensitive only to specific types of substances, or work well only in narrower ranges of concentrations. Some gas chromatographs are connected to a mass spectrometer which acts as the detector. The combination is known as GC-MS. Some GC-MS are connected to an NMR spectrometer which acts as a backup detector. This combination is known as GC-MS-NMR. Some GC-MS-NMR are connected to an infrared spectrophotometer which acts as a backup detector. This combination is known as GC-MS-NMR-IR. It must, however, be stressed this is very rare as most analyses needed can be concluded via purely GC-MS.

3.5.2 Liquid chromatography

Liquid chromatography (LC) is a separation technique in which the mobile phase is a liquid. Liquid chromatography can be carried out either in a column or a plane. Present day liquid chromatography that generally utilizes very small packing particles and a relatively high pressure is referred to as high performance liquid chromatography (HPLC).

In HPLC the sample is forced by a liquid at high pressure (the mobile phase) through a column that is packed with a stationary phase composed of irregularly or spherically shaped particles, a porous monolithic layer, or a porous membrane. HPLC is historically divided into two different sub-classes based on the polarity of the mobile and stationary phases. Methods in which the stationary phase is more polar than the mobile phase (e.g. toluene as the mobile phase, silica as the stationary phase) are termed normal phase liquid chromatography (NPLC) and the opposite (e.g. water-methanol mixture as the mobile phase and C18 = octadecylsilyl as the stationary phase) is termed reversed phase liquid chromatography (RPLC). Ironically the "normal phase" has fewer applications and RPLC is therefore used considerably more.

3.6 Affinity Chromatography

Affinity chromatography is a method of separating biochemical mixtures and based on a highly specific interaction such as that between antigen and antibody, enzyme and substrate, or receptor and ligand.

Principle

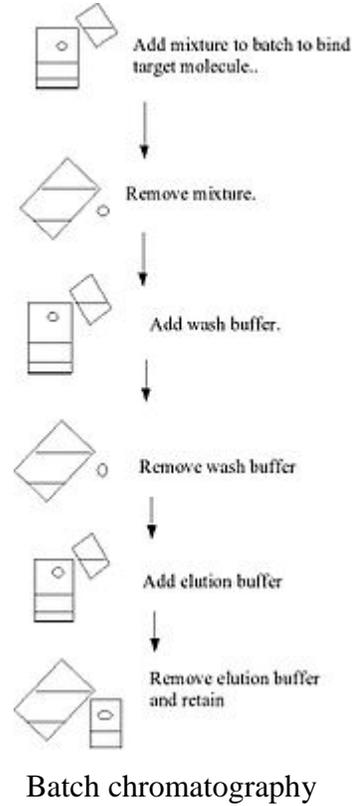
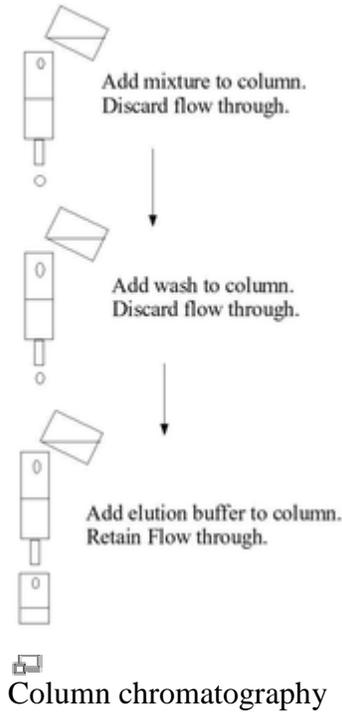
The immobile phase is typically a gel matrix, often of agarose; a linear sugar molecule derived from algae. Usually the starting point is an undefined heterogeneous group of molecules in solution, such as a cell lysate, growth medium or blood serum. The molecule of interest will have a well known and defined property which can be exploited during the affinity purification process. The process itself can be thought of as an entrapment, with the target molecule becoming trapped on a solid or stationary phase or medium. The other molecules in solution will not become trapped as they do not possess this property. The solid medium can then be removed from the mixture, washed and the target molecule released from the entrapment in a process known as elution. Possibly the most common use of affinity chromatography is for the purification of recombinant proteins.

Batch and column setup

Binding to the solid phase may be achieved by column chromatography whereby the solid medium is packed onto a column, the initial mixture run through the column to allow setting, a wash buffer run through the column and the elution buffer subsequently applied to the column and collected. These steps are usually done at ambient pressure. Alternatively binding may be achieved using a batch treatment, by adding the initial mixture to the solid phase in a vessel, mixing, separating the solid phase (for example), removing the liquid phase, washing, re-centrifuging, adding the elution buffer, re-centrifuging and removing the eluate. Sometimes a hybrid method is employed, the binding is done by the batch method, then the solid phase with the target molecule bound is packed onto a column and washing and elution are done on the column.

A third method, expanded bed adsorption, which combines the advantages of the two methods

mentioned above, has also been developed. The solid phase particles are placed in a column where liquid phase is pumped in from the bottom and exits at the top. The gravity of the particles ensure that the solid phase does not exit the column with the liquid phase. Affinity columns can be eluted by changing the ionic strength through a gradient. Salt concentrations, pH, pI, charge and ionic strength can all be used to separate or form the gradient to separate.



Affinity chromatography can be used to:

- Purify and concentrate a substance from a mixture
- Reduce the amount of a substance in a mixture
- Discern what biological compounds bind to a particular substance
- Purify and concentrate an enzyme solution.

3.7 Size-exclusion chromatography (SEC)

This is a chromatographic method in which molecules in solution are separated by their size, and in some cases molecular weight. It is usually applied to large molecules or macromolecular complexes such as proteins and industrial polymers. Typically, when an aqueous solution is used to transport the sample through the column, the technique is known as **gel-filtration chromatography**, versus the name Gel permeation chromatography, which is used when an organic solvent is used as a mobile phase. SEC is a widely used polymer characterization

method because of its ability to provide good molar mass distribution results for polymers.

Applications

The main application of gel-filtration chromatography is the fractionation of proteins and other water-soluble polymers, while gel permeation chromatography is used to analyze the molecular weight distribution of organic-soluble polymers. Either technique should not be confused with gel electrophoresis, where an electric field is used to "pull" or "push" molecules through the gel depending on their electrical charges.

Advantages

The advantages of this method include good separation of large molecules from the small molecules with a minimal volume of eluate, and that various solutions can be applied without interfering with the filtration process, all while preserving the biological activity of the particles to be separated. The technique is generally combined with others that further separate molecules by other characteristics, such as acidity, basicity, charge, and affinity for certain compounds. With size exclusion chromatography, there are short and well-defined separation times and narrow bands, which lead to good sensitivity. There is also no sample loss because solutes do not interact with the stationary phase. Disadvantages are, for example, that only a limited number of bands can be accommodated because the time scale of the chromatogram is short, and, in general, there has to be a 10% difference in molecular mass to have a good resolution.

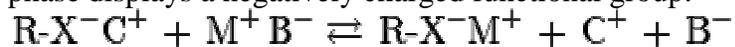
3.8 Ion-exchange chromatography

Ion-exchange chromatography (or *ion chromatography*) is a process that allows the separation of ions and polar molecules based on their charge. It can be used for almost any kind of charged molecule including large proteins, small nucleotides and amino acids. The solution to be injected is usually called a *sample*, and the individually separated components are called *analytes*. It is often used in protein purification, water analysis, and quality control.

Principle

Ion-exchange chromatography retains analyte molecules on the column based on coulombic (ionic) interactions. The stationary phase surface displays ionic functional groups (R-X) that interact with analyte ions of opposite charge. This type of chromatography is further subdivided into cation exchange chromatography and anion exchange chromatography. The ionic compound consisting of the cationic species M⁺ and the anionic species B⁻ can be retained by the stationary phase.

Cation exchange chromatography retains positively charged cations because the stationary phase displays a negatively charged functional group:



Anion exchange chromatography retains anions using positively charged functional group:



Note that the ion strength of either C⁺ or A⁻ in the mobile phase can be adjusted to shift the equilibrium position and thus retention time.

The ion chromatogram shows a typical chromatogram obtained with an anion exchange column.



Figure 3.6 *Metrohm 850 Ion chromatography system*

A sample is introduced, either manually or with an autosampler, into a sample loop of known volume. A buffered aqueous solution known as the mobile phase carries the sample from the loop onto a column that contains some form of stationary phase material. This is typically a resin or gel matrix consisting of agarose or cellulose beads with covalently bonded charged functional groups. The target analytes (anions or cations) are retained on the stationary phase but can be eluted by increasing the concentration of a similarly charged species that will displace the analyte ions from the stationary phase. For example, in cation exchange chromatography, the positively charged analyte could be displaced by the addition of positively charged sodium ions. The analytes of interest must then be detected by some means, typically by conductivity or UV/Visible light absorbance.

In order to control an IC system, a chromatography data system (CDS) is usually needed. In addition to IC systems, some of these CDSs can also control gas chromatography (GC) and HPLC

Preparative-scale ion exchange column used for protein purification.

Proteins have numerous functional groups that can have both positive and negative charges. Ion exchange chromatography separates proteins according to their net charge, which is dependent on the composition of the mobile phase. By adjusting the pH or the ionic concentration of the mobile phase, various protein molecules can be separated. For example, if a protein has a net positive charge at pH 7, then it will bind to a column of negatively-charged beads, whereas a negatively charged protein would not. By changing the pH so that the net charge on the protein is negative, it too will be eluted.

Elution by changing the ionic strength of the mobile phase is a more subtle effect - it works as ions from the mobile phase will interact with the immobilized ions in preference over those on the stationary phase. This "shields" the stationary phase from the protein, (and vice versa) and allows the protein to elute.

3.9 High-Performance Liquid Chromatography (HPLC)

Introduction

A form of column chromatography in which the mobile phase is a liquid material is called High-performance liquid chromatography (HPLC). Separation of mixture is achieved by

differential distribution of the sample components between the stationary and the mobile phase. HPLC is now a highly developed technique and a wide range of stationary phases are available. These enable partition, gel permeation, affinity and ion exchange chromatography to be performed in conjunction with the HPLC. For effective separation, it is essential to have a very small and regular shaped support media, a supply of mobile phase pumped at a pressure that is adequate to give suitable constant flow rate through the column and a convenient efficient detector system. HPLC instruments consist of a reservoir of mobile phase, a pump, an injector, a separation column, and a detector. Compounds are separated by injecting a plug of the sample mixture onto the column. The different components in the mixture pass through the column at different rates due to differences in their partitioning behavior between the mobile liquid phase and the stationary phase.

Instrumentation

The solvent reservoir, pump, chromatographic column and oven, detector unit, and amplifier and signal processing unit recorder are the five major components of HPLC instrument. Solvents must be degassed to eliminate formation of bubbles. The pumps provide a steady high pressure with no pulsating, and can be programmed to vary the composition of the solvent during the course of the separation. The liquid sample is introduced into a sample loop of an injector with a syringe. When the loop is filled, the injector can inject the sample into the stream by placing the sample loop in line with the mobile phase tubing. The presence of analytes in the column effluent is recorded by detecting a change in refractive index, UV-VIS absorption at a set wavelength, fluorescence after excitation with a suitable wavelength, or electrochemical response. Mass spectrometers can also be interfaced with liquid chromatography to provide structural information and help identify the separated analytes.

Choice of column packing

A wide range of column materials have been developed. In selecting a column material for the separation of a specific substance it is necessary to consider the physical characteristic of the molecule. The column packing for an ionic species should be an ion exchange resin, while for a molecule with moderate polarity adsorption column packing is the right choice. But for the separation of large molecules, gel permeation should be considered.

The mobile phase

The liquid which can be used for HPLC separation may consist of water, aqueous buffer solutions, organic solvents such as methanol, acetonitrile, etc. All solvents should be of utmost purity, dust free and above all, should be free from any gaseous impurity.

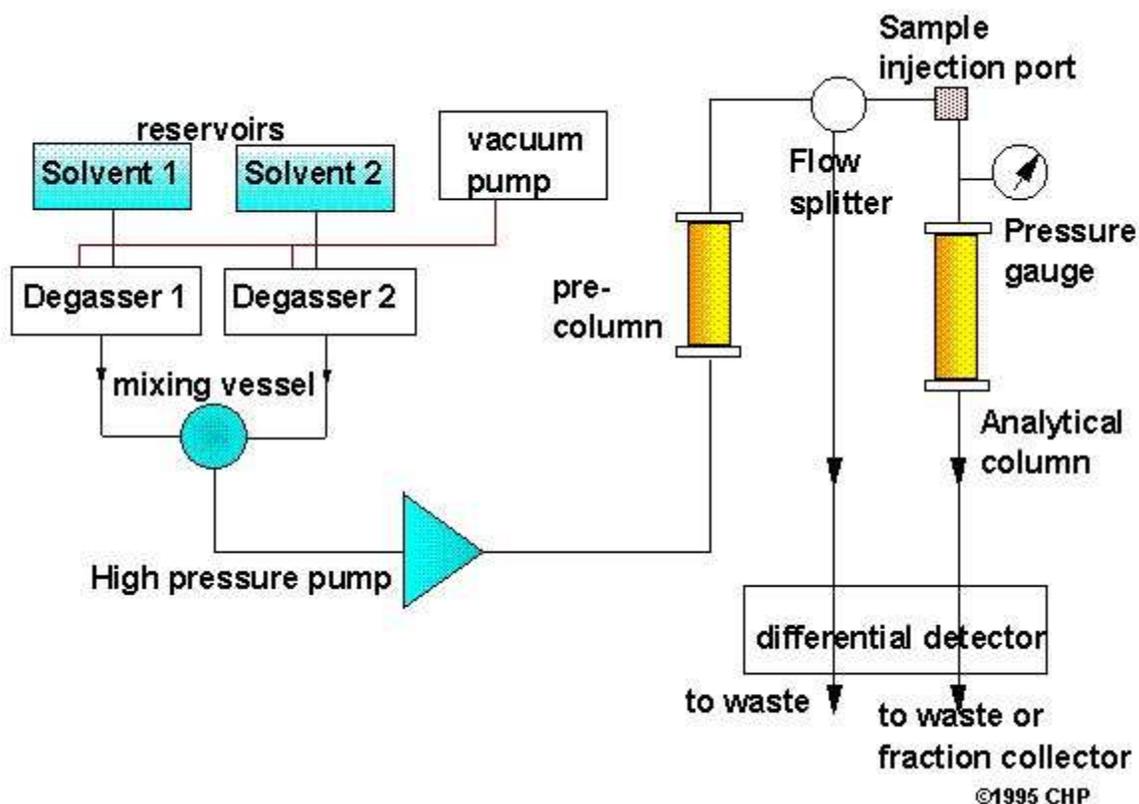


Figure 3.7; Schematic of an HPLC instrument

Column

The column for analytical HPLC are typically 10-25cm long and 4-6mm internal diameter. The columns are made of stainless steel to cope with the operating high pressure. It also lined with glass internally to prevent metal catalysis of the solvent-solute reactions.

Detectors

The choice of detector is governed by the properties of the solute and sensitivity required for the analysis. Various types of detectors are used in HPLC for the detection of eluted solute. These are classified as:

- Detectors which monitors a specific property of the solute, e.g. UV absorbance and fluorescence.
- Detecting system which monitors a bulk property of the eluant, e.g. refractive index.
- Detectors which function by separating the solvent from the eluant, e.g. flame ionization (FID) or mass spectrometry (MS) detectors.

Application

HPLC is used for both qualitative and quantitative analysis of variety of substances, such as drugs, pesticides, herbicides, vitamins, natural products, etc.

4.0 CONCLUSION

In conclusion, we can say the differential rate of migration of components of a mixture on an

adsorbent material over the influence of the mobile phase is the basic principle of chromatographic separation.

5.0 SUMMARY

- i. Chromatography is a separation technique which is based on the differential rate of migration of components of a mixture on an adsorbent over the influence of the mobile phase. Separation is based on differential partitioning between the mobile and stationary phase.
- ii. Chromatography may be preparative or analytical. Preparative if it is designed for separation/purification of components of a mixture for further use whereas analytical chromatography involves the use of smaller amounts of material purely for measuring the relative proportions of analytes.
- iii. Various types of chromatographic techniques were studied ranging from planar, column, displacement, affinity, size –exclusion, ion exchange and gas chromatography.
- iv. In displacement chromatography, molecules with high affinity for the chromatography matrix competes effectively for binding sites, and thus displace all molecules with lesser affinities.
- v. Chromatography on a column of adsorbent provides a means of separation of mixture and isolation of components in larger amount than is possible in TLC.
- vi. Displacement chromatography has advantages over elution chromatography in that components are resolved into consecutive zones of pure substances rather than peaks.
- vii. Substances that can be vaporized without decomposition are separated and analyzed using gas chromatography.
- viii. Ions and polar molecules are separated based on their charge in ion-exchange chromatography.

6.0 TUTOR MARKED ASSIGNMENT

1. Write short notes on the followings:
i) Chromatography ii)Chromatogram iii)Retention time iv) Stationery phase
2. Describe the basic principle of ion-exchange chromatography
3. Differentiate between thin layer chromatography and column chromatography.
4. List any four uses of affinity chromatography

7.0 REFERENCEES AND FURTHER READING

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UNIT 2 CALORIMETRY

1.0 INTRODUCTION

The science of measuring the heat of chemical reactions or physical changes using a calorimeter is known as calorimetry. Scottish physician and scientist Joseph Black, who was the first to recognize the dissimilarity between heat and temperature, is said to be the founder of calorimetry. In this unit, we discuss calorimetric techniques and their applications.

2.0 OBJECTIVE

- Define the term calorimetry
- Explain the principle of differential scanning calorimetry
- Describe the principle of differential thermal analysis
- Enumerate the applications of differential scanning calorimetry

3.0 MAIN TEXT

3.1 CALORIMETRY

Calorimetry is the quantitative measurement of the heat absorbed or evolved during a chemical process. Calorimetry as the name implies is derived from two English loan words: color (Latin word) meaning heat, and Greek metry meaning to measure. All calorimetric techniques are primarily based on the measurement of heat that may be generated (exothermic process), consumed (endothermic) or simply dissipated by a sample.

There are numerous techniques that have been developed for heat measurement ranging from simple thermometric (temperature measurement) methods to a more recently advanced in electronics and control, which enables users to collect data and maintain samples under conditions that were previously not possible.

3.2 Calorimeter

The property of an object when heat is transferred to it is that of temperature increase, similarly when heat is removed from an object, the temperature of such object decreases. The relationship between heat transferred and change in temperature can be appreciated by the equation below:

$$q = CDT = C(T_f - T_i)$$

Where q is the heat that is transferred

DT is the change in temperature

C is the proportionality constant otherwise known as heat capacity

Calorimeter is an instrument used to measure the heat of reaction during a well defined process. It can be simple and cheap or sophisticated and expensive. The calorimeter consists of a well insulated container and reaction initiated and temperature difference before and after reaction measured.

It is important to note that a calorimeter can be operated under constant pressure, or constant volume.

The heat capacity of a calorimeter is defined as the amount of heat required to change the temperature of the entire calorimeter by one degree. The heat capacity of the calorimeter is determined by transferring a known amount of heat into it and measuring its temperature rise. The temperature differences are usually very small consequently extreme sensitive thermometers are required for these measurements.

3.3 Differential Scanning Calorimetry

Differential scanning calorimetry(DSC) is a thermoanalytical technique in which the difference in the amount of heat required to increase the temperature of a sample and reference is measured as a function of temperature. Both the sample and reference are maintained at nearly the same temperature throughout the experiment. Generally, the temperature program for a DSC analysis is designed such that the sample holder temperature increases linearly as a function of time. The reference sample should have a well-defined heat capacity over the range of temperatures to be scanned.

The first adiabatic differential scanning calorimeter that could be used in biochemistry was developed by P.L. Privalov and D.R. Monaselidze in 1964 The term DSC was coined to describe this instrument which measures energy directly and allows precise measurements of heat capacity.

3.3.1 Detection of phase transitions

The basic principle of this technique is that when the sample undergoes a physical transformation such as phase transitions, more or less heats flow to it than the reference to maintain both at the same temperature. The amount of heat flows into the sample essentially depends on whether the process is exothermic or endothermic. By comparing the difference in heat flow between the sample and reference, differential scanning calorimeters are able to measure the amount of heat absorbed or released during such transitions. For example, a melting solid sample requires more heat flow through it so that its temperature increases at the same rate as the reference. This is an endothermic phase transition from solid to liquid hence heat is absorbed. Similarly, as the sample undergoes exothermic processes (such as crystallization) less heat is required to raise the sample temperature. It is widely used in industrial settings as a quality control instrument due to its applicability in evaluating sample purity and for studying polymer curing.

3.3.2 Differential Thermal Analysis (DTA)

Another technique, which is closely related to DSC, is differential thermal analysis (DTA). The heat flow to the sample and reference is the same rather than the temperature. Identically phase changes and other thermal processes brings about a difference in temperature between the sample and reference as soon as the sample and reference are heated.It important to note that DSC measures the energy required to keep both the reference and the sample at the same temperature whereas DTA measures the difference in temperature between the sample and the reference when they are both put under the same heat.

A graph/curve of heat flux versus temperature or time is plotted with data generated from a DSC experiment.This curve is useful in calculating enthalpies of transitions by integrating the peak corresponding to a given transition.Entalpy of transition is given by the equation below:

$$\Delta H = KA$$

where ΔH is the enthalpy of transition,
 K is the calorimetric constant, and

A is the area under the curve. The calorimetric constant can be determined by analyzing a well-characterized sample with known enthalpies of transition.

Applications

Differential scanning calorimetry can be used to monitor fusion and crystallization events as well as glass transition temperatures. DSC can also be used to study oxidation and other chemical reactions.

As the temperature of an amorphous solid is increased glass transition may occur and is in the real sense not a phase change but essentially due to change in heat capacity.

An amorphous solid tends to become less viscous as the temperature increases. At a certain temperature (otherwise known as the crystallization temperature, T_c) the molecules become more mobile which consequently led to a spontaneous arrangement into crystalline form.

This transition from amorphous solid to crystalline solid is an exothermic process, and results in a peak in the DSC signal. As the temperature increases the sample eventually reaches its melting temperature (T_m). The melting process results in an endothermic peak in the DSC curve. The ability to determine transition temperatures and enthalpies makes DSC a valuable tool in producing phase diagrams for various chemical systems

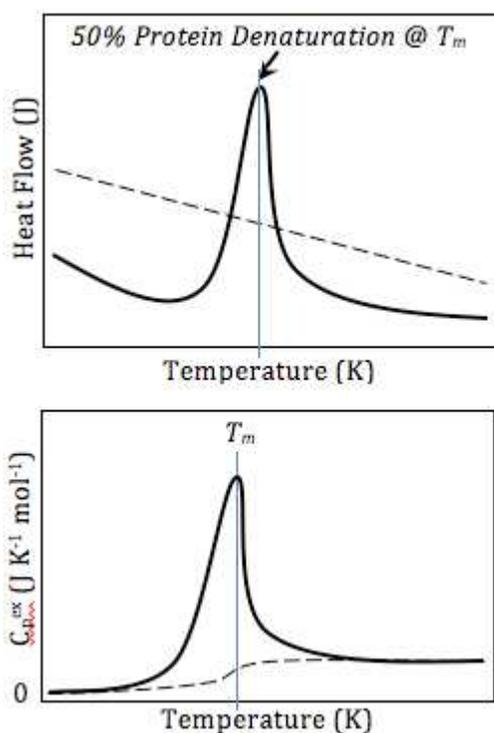


Figure 3.8 Top: A schematic DSC curve of amount of energy input (y) required to maintain each temperature (x), scanned across a range of temperatures. Bottom: Normalized curves setting the initial heat capacity as the reference. Buffer-buffer baseline (dashed) and protein-buffer variance (solid).

Polymers

The composition of a polymer can be examined using DSC.

Melting points and glass transition temperatures for most polymers are available from standard compilations, The degradation of a polymer is indicated by the lowering of the expected melting point, T_m , and hence the experimental melting point and glass transition temperature

for any polymer is compared with those available in a chart of standard compilations. T_m depends on the molecular weight of the polymer which implies that lower grades will have lower melting points than expected. The percentage crystallinity of a polymer can be found from the crystallization peak of the DSC graph since the heat of fusion can be calculated from the area under an absorption peak. Impurities in polymers can be determined by examining thermograms for anomalous peaks, and plasticisers can be detected at their characteristic boiling points.

Liquid Crystals

Matter may change from solid to liquid through a third state in which the properties of both phases are well displayed. This anisotropic liquid is known as a liquid crystalline or mesomorphous state. The energy changes (though small) that occur as matter converts from a solid to liquid crystal and from a liquid crystal to an isotropic liquid can be monitored using DSC. Liquid crystals therefore are a state of matter with properties between those of conventional liquid and those of solid crystals. Liquid crystals depend markedly on temperature, concentration as well as inorganic-organic composition ratio.

Oxidative Stability

Differential scanning calorimetry can be used to study the stability to oxidation of samples in an airtight sample chamber. The sample is first brought to the desired test temperature under an inert atmosphere, usually nitrogen. Subsequently, oxygen is added to the system and oxidation is expected to occur. If this happens, it is viewed as a deviation in the baseline. This analysis has been used to determine the stability and optimum storage conditions for a material or compound.

Drug Analysis

DSC is extensively used in the polymer and pharmaceutical industries. DSC is a versatile tool for a polymer chemist in studying curing processes, which is deliberate efforts aimed at fine tuning of polymer properties. The polymer molecules cross-linked exothermically during the curing process resulting in a positive peak in the DSC curve that usually appears soon after the glass transition.

It is desirable to have well-characterized drug compounds in order to define processing parameters in the pharmaceutical industry. For example, drugs should be delivered in the amorphous form, it is necessary to process the drug at temperatures below those at which crystallization can occur.

General Chemical Analysis

Freezing-point depression can be used as a purity analysis tool when analysed by Differential scanning calorimetry. This is possible because the temperature range over which a mixture of compounds melts is dependent on their relative amounts. Consequently, less pure compounds will exhibit a broadened melting peak that begins at lower temperature than a pure compound.

Food Science

In food science research, DSC is used in conjunction with other thermal analytical techniques to determine water dynamics. Changes in water distribution may be correlated with changes in

texture. Similar to materials science studies, the effects of curing on confectionery products can also be analyzed.

4.0 CONCLUSION

In conclusion we can say that the heat change accompanying chemical reactions is studied using a calorimeter and differential scanning calorimetry find usefulness in the determination of water dynamics in food science, oxidation stability of reactions and in drug analysis.

5.0 SUMMARY

- i. The quantitative measurement of heat loss or gain accompanying chemical reaction or physical process highlights the concept of calorimetry.
- ii. The heat capacity of a calorimeter is defined as the amount of heat energy required to change the temperature of a calorimeter by one degree.
- iii. Differential scanning calorimetry is a thermoanalytical technique that uses the differences in the amount of heat energy required bring about a rise in temperature of a sample reference which is measured as a function of temperature.
- iv. Liquid crystals are a state of matter with properties between those of conventional liquid and those of solid crystals. Liquid crystals depend on temperature, concentration as well as inorganic-organic composition ratio.
- v. Differential scanning calorimetry has various applications ranging from studying oxidation stability of reactions, polymer composition, curing processes of polymers, drug analysis, and determination of water dynamics amongst others.

6.0 TUTOR MARKED ASSIGNMENT

- 1 Define the term calorimetry
- 2 Briefly explain the principle of differential scanning calorimetry
- 3 Enumerate three applications of differential scanning calorimetry

7.0 REFERENCES/FURTHER READING

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UNIT 3: RADIOCHEMICAL METHODS

CONTENT

- 1.0 Introduction
- 2.0 Objectives
- 3.0 Main content
 - 3.1 History and Application
 - 3.2 Interaction of Radiation with Matter
 - 3.3 Radiation Detectors
 - 3.3.1 Gas Ionization Detectors
 - 3.3.2 Solid-State Detectors
 - 3.3.3 Scintillation Detectors
 - 3.4 Radioanalytical Chemical Principles
 - 3.4.1 Sample Loss by Radiocolloidal Behavior
 - 3.4.2 Carrier or Tracer Addition
- 4.0 Conclusion
- 5.0 Summary
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1.0 INTRODUCTION

Radioanalytical chemistry focuses on the analysis of sample for their radionuclide content. Various methods are employed to purify and identify the radioelement of interest through chemical methods and sample measurement techniques.

2.0 OBJECTIVES

At the end of this unit, students should be able to:

- describe the history and application of radioanalytical chemistry
- differentiate between alpha, beta and gamma rays
- explain basic principles of some radiation detectors
- describe radioanalytical chemical principles

3.0 MAIN CONTENT

3.1 History and Application

Marie Curie originally developed the field of radioanalytical chemistry, however, Ernest Rutherford and Frederick Soddy made tangible contribution to this field of study. They developed separation and radiation measurement techniques on terrestrial radioactive substances. Since Curie's time, the applications of radioanalytical chemistry have since proliferated and recently, researchers have applied chemistry as well as nuclear procedures to elucidate nuclear properties and reactions, used radioactive substances as tracers, and measure radionuclides in many different types of samples.

The applications of radioanalytical chemistry include: forming and characterizing new

elements, determining the age of materials, and creating radioactive reagents for specific tracer use in tissues and organs.

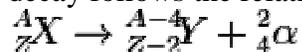
3.2 Interaction of radiation with matter

The detection and measurement of radionuclides is mainly based on observing the emitted rays or particles. Some basic understanding of the interaction of these emissions with other materials is important not only for dealing with radiation detectors but for discussing problems of radiation protection.

Interaction with matter involves a transfer of energy to atoms and molecules. This interaction can cause ionization or excitation or both. The interaction depends on the type of particles or photons and energy of radiation as well as on the properties of matter. The thickness of a radiation absorbing material is of interest and is given in surface density or weight per unit area (mg/cm^2).

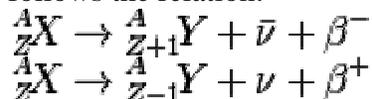
Alpha-particles, having a rather high mass and a double electrical charge lose their energy rapidly as they pass through matter and leave a dense trail of ionized material along their way. The range of alpha particles in matter is small (a few centimeters in air), but the energy transfer is very high, because all the energy is released within a short distance. Alpha-particles from a given radionuclide have a definite energy and a definite range in matter. A certain thickness of a shielding material is just able to stop alpha-particles of a corresponding energy.

Alpha decay is characterized by the emission of an alpha particle, a ${}^4\text{He}$ nucleus. The mode of this decay causes the parent nucleus to decrease by two protons and two neutrons. This type of decay follows the relation:



Beta particles with a single electrical charge and a much lower mass than the alpha particles are much more readily deflected by collisions with atoms and molecules. Beta-particles have a maximum range in matter. For beta particles of a given initial energy, there is always a defined thickness of shielding material which can stop these beta-particles completely. The range of a beta-particle of 1MeV initial energy is approximately 3m in air or 4mm in water or animal tissue.

Beta decay is characterized by the emission of a neutrino and a negatron which is equivalent to an electron. This process occurs when a nucleus has an excess of neutrons with respect to protons, as compared to the stable isobar. This type of transition converts a neutron into a proton; similarly, a positron is released when a proton is converted into a neutron. These decays follow the relation:

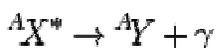


Gamma-and x-ray photons are much more penetrating than particulate radiations of the same energy, because of the rather low interaction with matter. The absorption of gamma or x-ray photons is a complex process and the mechanism depends on the photon energy, atomic number

and the density of the absorbing materials. The most important aspect of interaction with matter at low energy is what is called photo effect. The energy of a photon is converted into the kinetic of an orbital electron which is ejected from an atomic shell by interaction of the photon. The kinetic energy of this photo electron corresponds to the energy of the gamma-photon minus the binding energy with which the electron was held initially within the electron structure of the molecules. The photoelectron will dissipate its energy in to matter similar to a beta-particle.

Compton scattering is another mechanism by which gamma-photons lose their energy. Only a part of the photon energy in this case, is converted to kinetic energy of an electron. The rest remains in the form of a scattered gamma-photon of lower energy which may undergo interactions with different atomic shells, producing a photo electron or another Compton electron. The Compton electrons will interact with matter similar to a beta particle .Compton scattering is a more important mechanism at higher photon energy.

This decay follows the relation:



3.3 Radiation Detection

3.3.1 Gas Ionization Detectors

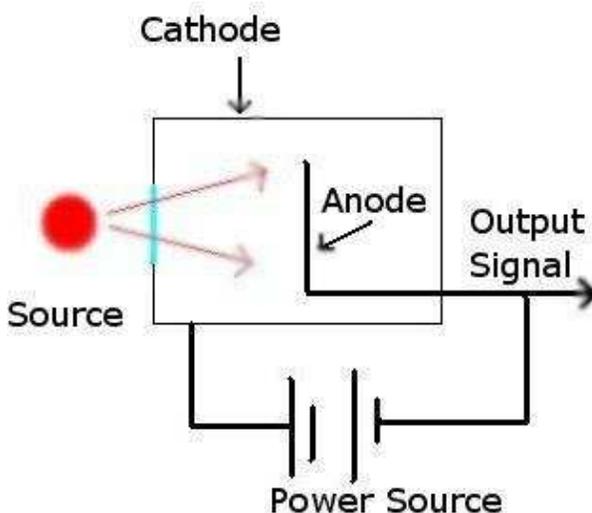


Figure 3.9 Schematic of a ionization detector
http://en.wikipedia.org/wiki/File:Gas_det.JPG

Gaseous ionization detectors collect and record the electrons freed from gaseous atoms and molecules by the interaction of radiation released by the source. A voltage potential is applied between two electrodes within a sealed system. Since the gaseous atoms are ionized after they interact with radiation they are attracted to the anode which produces a signal. The applied

voltage is varied such that the response falls within a critical proportional range.

3.3.2 Solid-State Detectors

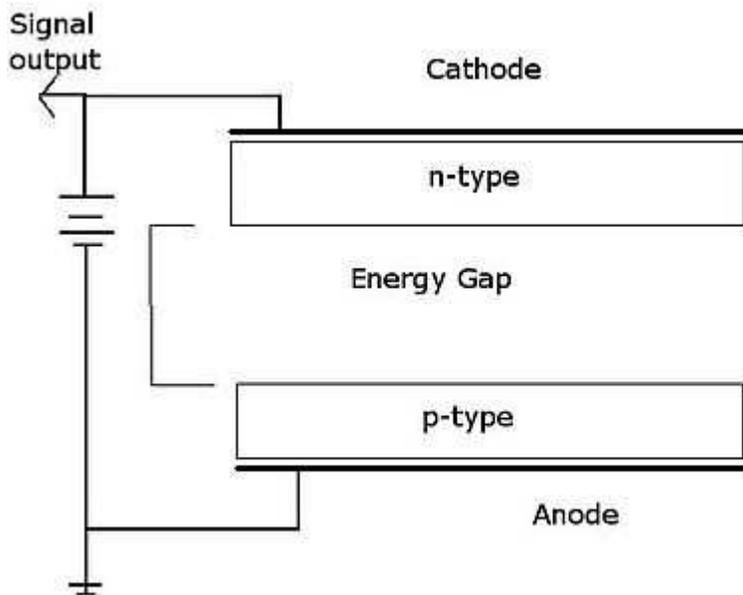


Figure 3.10 Schematic of a solid-state detector
(http://wikipedia.org/wiki/File:Solid_det.JPG)

The operating principle of Semiconductor detectors is similar to gas ionization detectors except instead of ionization gas atoms, free electrons and holes are produced which create a signal at the electrodes. The advantage of solid state detectors is the greater resolution of the resultant energy spectrum. Usually NaI(Tl) detectors are used; for more precise applications Ge(Li) and Si(Li) detectors have been developed. For extra sensitive measurements high-pure germanium detectors are used under a liquid nitrogen environment.

3.3.3 Scintillation Detectors

Scintillation detectors use a photo luminescent source (such as ZnS) which interacts with radiation. When a radioactive particle decays and strikes the photo luminescent material a photon is released. This photon is multiplied in a photomultiplier tube which converts light into an electrical signal. This signal is then processed and converted into a channel. By comparing the number of counts to the energy level (typically in keV or MeV) the type of decay can be determined.

3.4 Radioanalytical Chemistry Principles

3.4.1 Sample Loss by Radiocolloidal Behaviour

Samples with very low concentrations are difficult to measure accurately due to the radioactive atoms unexpectedly depositing on surfaces. Sample loss at trace levels may be due to adhesion to container walls and filter surface sites by ionic or electrostatic adsorption, as well as metal foils and glass slides. Sample loss is an ever present concern, especially at the beginning of the

analysis path where sequential steps may compound these losses.

Various solutions are known to circumvent these losses which include adding an inactive carrier or adding a tracer. Research has also shown that pretreatment of glassware and plastic surfaces can reduce radionuclide sorption by saturating the sites.^[7]

3.4.1 Carrier or Tracer Addition

Due to the inherent nature of radionuclides yielding low concentrations a common technique to improve yields is the addition of carrier ions or tracers. Isotope dilution involves the addition of a known amount of radionuclide tracer to the sample that contains a known stable element. This is done at the start of the analysis procedure so once the final measurements are taken, sample loss is considered. This procedure avoids the need for any quantitative recovery which greatly simplifies the analytical process.

Carrier addition is the reverse technique of tracer addition. Instead of isotope dilution, a known mass of stable carrier ion is added to radionuclide sample solution. The carrier reagent must be calibrated prior to addition to the sample. To verify the resultant measurements, the expected 100% yield is compared to the actual yield. Any loss in yield is analogous to any losses in the radioactive sample. Typically the amount of carrier added is conventionally selected for the ease of weighing such that the accuracy of the resultant weight is within 1%. For alpha particles, special techniques must be applied to obtain the required thin sample sources.

Commonly measured long lived cosmogenic isotopes

element	mass	half-life (years)	typical source
helium	3	- stable -	air, water, and biota samples for bioassays
carbon	14	5,730	dating of organic matter, water
iron	55	2.7	produced in iron and steel casings, vessels, or supports for nuclear weapons and reactors
Strontium	90	28.8	common fission product
Technetium	99	214,000	another common fission product
iodine	129	15.7 million	groundwater tracer
Cesium	137	30.2	nuclear weapons and nuclear reactors (accidents)
Promethium	147	2.62	naturally occurring fission product
Radon	226	1,600	rain and groundwater, atmosphere

Uranium	232, 233, 234, 235, 236, 238	Varies	terrestrial element
Plutonium	238, 239, 240, 241, 242	Varies	nuclear weapons and reactors
Americium	241	433	result of neutron interactions with uranium and plutonium

4.0 CONCLUSION

We conclude this unit by observing that interaction of radiation with matter depends on factors such as types of particles, energy of radiation and the properties of matter. Gamma and x-ray photons have much more penetrating than alpha and beta radiation due to low interaction with matter.

5.0 SUMMARY

In this unit we studied the following:

- i. Analysis of sample for their radioactive for their radionuclide content is the base principle of radioanalytical chemistry
- ii. Interaction of radiation with matter can cause ionization or excitation but depends on the type of particles, energy of radiation and the properties of matter. Alpha particles have very small range in air and, the range of energy transfer is very high, but can easily be stopped by a certain thickness of shielding material.
- iii. Beta particles have maximum range in matter but much more readily deflected by collisions with atoms and molecules.
- iv. Gamma and X-ray photons have low interaction with matter, consequently have much more penetrating than particulate radiations of the same energy.
- v. The inherent nature of radionuclide yielding low concentrations is often times circumvented by pretreatment of glasswares and by addition of inert carrier or by tracer addition.

6.0 TUTOR MARKED ASSIGNMENT

1. Explain briefly radioanalytical chemistry.
2. Describe the basic principle of gas ionization detectors.
3. Enumerate the basic differences between particulate radiation and electromagnetic radiation.
4. State how low concentration yielding of radionuclide could be circumvented.

7.0 REFERENCES/FURTHER READING

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UNIT 3 RADIOCHEMICAL SEPARATION TECHNIQUES

CONTENT

- 1.0 Introduction
- 2.0 Objectives
- 3.0 Main content
 - 3.1 Precipitation
 - 3.2 Distillation
 - 3.3 Solvent extraction
 - 3.4 Ion exchange
 - 3.5 Electrodeposition
- 4.0 Conclusion
- 5.0 Summary
- 6.0 Tutor Marked Assignment
- 7.0 References/Further Reading

INTRODUCTION

In the last unit you studied the interaction of radiation with matter and basic properties of particulate radiation and X-rays.

Similar analytical separation techniques can be used for radioactive nucleotides because they have properties similar to their stable, inactive, counterparts. In this unit you will be studying the radioanalytical separation techniques. These separation methods include precipitation, ion exchange, liquid liquid extraction, solid phase extraction, distillation, and electrodeposition.

2.0 OBJECTIVES

At the end of this unit, students should be able to:

- recognise the importance of precipitation as a radioanalytical techniques.
- describe briefly the basic principles of distillation.
- explain solvent extraction as a radioanalytical separation method.
- Appreciate ion exchange as a veritable separation method in radioanalytical studies.
- Explain the term electrodeposition in relation to a separation method.

3.0 MAIN CONTENT

3.1 Precipitation

Solids can be formed in a solution or inside another solid during a chemical reaction. This process is called precipitation and the solid formed in a liquid is known as precipitate. The liquid remaining above the solid is called the supernate or supernatant. Precipitation occurs in chemical reaction when an insoluble substance is introduced into a solution.

In soluble substances, precipitation is enhanced as the solution becomes supersaturated. The relative supersaturation is proportional to $(Q-S)/S$. Where Q is the total concentration of the solute at any instant, S is the equilibrium solubility. It should be noted also that that the particle

size of a precipitate varies inversely as the relative supersaturation. Precipitates are formed by nucleation and by particle growth. Nucleation is a process in which a minimum number of atoms, ions, or molecules join together to give a stable solid. Nucleation increases with increase with in relative supersaturation. If nucleation predominates, a large number of fine particles results. If particles growth predominates, a smaller number of larger particles is obtained.

As a practical matter, precipitation is usually carried out in hot, dilute aqueous solutions to allow the slow formation of large crystals. The pH of the solution is chosen to minimize colloid formation. After precipitation, the precipitate is washed carefully to remove impurities, dissolved and re-precipitated to cause further purification. The precipitate is collected by filtration

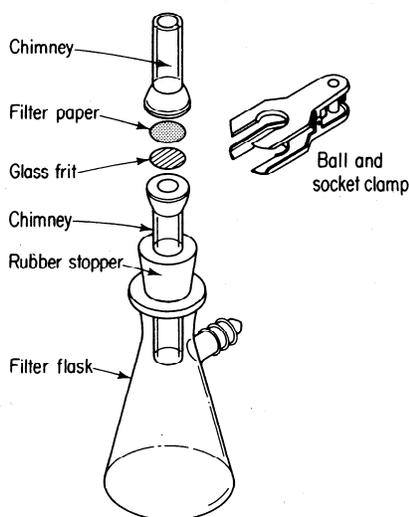


Figure 3.11 Schematic diagram of a filtration apparatus used in radiochemistry. (From Wang, Willis, and Loveland)

The filter paper is supported by a glass frit clamped between two glass tubes. The precipitate is washed finally with acetone or alcohol to dry it. The precipitate is chosen to have a known stoichiometry to allow calculation of the yield of the separation and should not absorb water or CO_2 so that an accurate weight can be obtained. (The filter paper used in the filtration must be treated with all the reagents beforehand, dried and weighed so that any material loss in filtration is minimized.)

3.2 Distillation

Distillation is a technique of separating mixtures based on differences in volatilities of components in a boiling liquid mixture. Distillation is a physical separation process and not a chemical reaction. Distillation allows the separation of components in a mixture whose partition coefficients between solution and vapour phases differ significantly. The separation process is simple if one species has a partition coefficient that is large compared with the other components of the mixture. A fractional distillation procedure is required for those systems in which the difference in partition coefficient is small.

3.3 Solvent Extraction

A solute can be separated from a solution by shaking a solution with solvent in which the solute is more soluble. The extent to which solutes distribute themselves between two immiscible liquids differs greatly and these differences have been used to achieve separations of chemical species. The distribution ratio otherwise known as the partition coefficient which is equal to the concentration of a solution in the organic phase divided by its concentration in the aqueous phase. The distribution ratio depends on temperature, the concentration of chemical species in the system.

Separation by liquid-liquid extraction (solvent extraction) has played an important role in radiochemical separations.

Multistage countercurrent continuous processes are used for metals such as the lanthanides, mainly because the separation factors between the lanthanides are so infinitesimal and many extraction stages are required. It is possible to extract uranium, plutonium, or thorium from acid solutions. Organophosphate tri-n-butyl-phosphate is a very common solvent that could be used for this extraction.

Ether extraction of uranium was used in early weapons development, and the use of tri-butyl phosphate (TBP) as an extractant for U and Pu was recognized in 1946, resulting in the commercial PUREX process for reprocessing spent reactor fuel. In recent years, there has been a good deal of development

3.4 Ion Exchange

Ion exchange is one of the radiochemical separation techniques, which has high selectivity and better ability to perform separations rapidly. Ion exchange is also a process of purification and decontamination of ion-containing solutions using solid polymers or mineralic ion exchangers. In ion exchange, a solution containing the ions to be separated is brought into contact with a synthetic organic resin containing specific functional groups that selectively bind the ions in question. Organic ion exchangers have reactive groups such as $-\text{OH}$, $-\text{COOH}$, or $-\text{SO}_3\text{H}$, and are insoluble in water as well as organic solvents. These exchangers should have an open, permeable molecular structure so that ions and solvent molecules can move freely in and out of the molecular structure.

The ions in question can be removed from the resin by elution with a suitable solution that differs from the initial solution. Characteristically the solution containing the ions is run through a column packed with resin beads. The resins are usually cross-linked

polystyrenes with attached functional groups. Most cation exchangers (such as Dowex 50) contain free sulfonic acid groups, SO_3H , where the cation displaces the hydrogen ion. Anion exchangers (such as Dowex 1) contain quaternary amine groups, such as $\text{CH}_2\text{N}(\text{CH}_3)_3\text{Cl}$

A group of ions can be absorbed on the column material and then selectively eluted. Eluants are complexing agents which form complexes of varying solubility with the absorbed ions. Competition usually exists between the complexing agent and the resin for each ion and each ion will be exchanged between the resin and the complexing agent in succession as it moves down the column. Spatial separation between "bands" of different ions occurs due to the different rates at which various ions migrate down the column. The ions can be collected separately in successive eluant fractions (see Figure below)

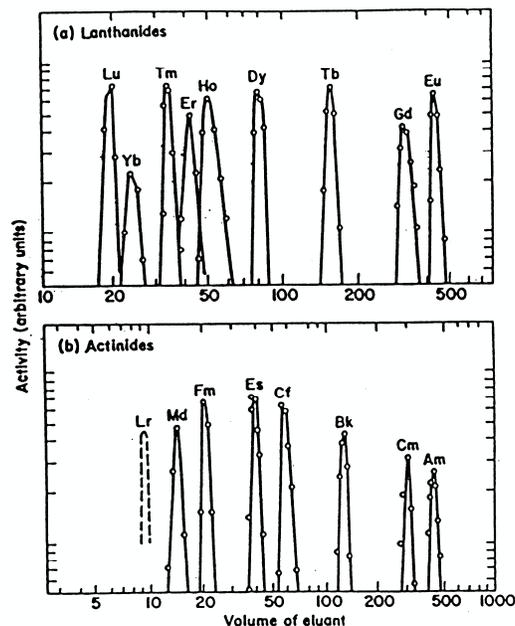


Figure 3.12 Elution of tripositive lanthanide and actinide ions on Dowex-50.

The most widely cited application of ion exchange techniques is the separation of the rare earths or actinides from one another. This is done with cation exchange using a complexing agent of α -hydroxyisobutyric acid (“ α -but”). The order of elution of the ions from a cation exchange column is generally in order of the radii of the hydrated ions with the largest hydrated ions leaving first; thus lawrencium elutes first and americium last among the tri-positive actinide ions (see Figure). In the case of the data of Figure, the separation between adjacent cations and the order of elution is derived from the comparative stability of the aqueous actinide or lanthanide complexes with α -hydroxyisobutyrate. As shown in Figure 19-6, there is a strikingly analogous behavior in the elution of the actinides and lanthanides that allowed chemists to prove the identity of new elements in the discovery of elements 97-102 (Bk-No). For cation exchange, the strength of absorption goes as $M^{4+} > M^{3+} > MO_2^{2+} > M^{2+} > MO_2^+$. A mixture of Mn (II), Co (II), Cu (II), Fe (III), and Zn (II) can be separated by being placed on a Dowex 1 column using 12 M HCl, followed by elutions with 6M HCl (Mn),

Some inorganic ion exchangers, such as the zeolites, have been very useful and are used in situations where heat and radiation might prevent the use of organic resins although the establishment of equilibria may be slow.

More selective resins have been developed, among these are the chelating resins (such as Chelex-100) that contain functional groups that chelate metal ions. Most common functional groups include iminodiacetic acids, 8-hydroxyquinoline or macrocyclic units such as the crown ethers, calixarenes or cryptands. The bifunctional chelating ion exchange material, Diphonix® resin-a substituted diphosphonic acid resin, shows promise in treating radioactive waste. Important newer resins include those with immobilized phosphorus ligands.

3.5 Electrochemical deposition

It is a process by which a film of solid metal is deposited from a solution of ions onto an

electrically conducting surface. The deposited film usually has dimensions within the nanoscale and hence the resulting product has gone through a process of nanomanufacturing. Electrolysis or electrochemical deposition can be used to plate out active material of interest as the case may be or plate out other substances thereby leaving active material in solution.

4.0 CONCLUSION

We conclude this unit by pointing out that radioactive nucleotides have chemical properties similar to their stable counterparts, consequently similar analytical separation techniques are employed.

5.0 SUMMARY

- i. Precipitates are formed by nucleation and by particle growth and precipitates have very low solubilities.
- ii. Separation of components in a mixture whose partition coefficients between solution and vapour phases differ greatly is made possible through distillation.
- iii. Solutes distribute themselves between two immiscible liquids at different rates and this physical property is very vital in chemical separations of chemical substances such as lanthanides via solvent extraction technique.
- iv. Ion exchange is a method of separation, purification and decontamination of ion containing solution using mineral exchangers or solid polymers.
- v. Substances can be plated out thereby leaving active material in solution through a radioanalytical technique known as electrochemical deposition.

6.0 TUTOR MARKED ASSIGNMENT

1. Explain briefly the concept of precipitation as radioanalytical chemical technique.
2. Describe the basic principle of solvent extraction
3. Enumerate two differences between distillation and ion exchange technique
4. Explain electrochemical deposition.

7.0 REFERENCES/FURTHER READING

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