Idaho State Police
Forensic Laboratory Training Manual
Extraction Techniques

1.0.0   SOLUBILITY

In the field of forensic chemistry, the main purpose of an analysis is the identification of the substance in question. For this reason, each active ingredient of a mixture must be isolated from other sample constituents. There are different ways to achieve this, one of the simplest being by use of the varying solubility properties of chemical compounds. Solubility is the capacity of two or more substances to form spontaneously, without chemical reaction, a homogeneous molecular (or colloidal) dispersion. More specifically, the solubility of a solid in a liquid refers to the concentration that is reached when a fixed amount of liquid has dissolved the entire solid it can hold at equilibrium (at a specific temperature). The solubility of solids in liquids range from very low to very high values. Because of this range of solubilities, the word "soluble" does not have a precise meaning. There is usually an upper limit to the solubility of even the most soluble solid, while even the least soluble would yield a few dissolved crystals per liter of solution. Table 1 below lists descriptive terms for varying degrees of solubility with corresponding (but indefinite) solubility ranges.

Determining the exact solubility of a substance is not required for drug analysis; however, knowing the approximate solubility properties of substances is extremely useful for the separation of constituents in simple drug mixtures.

Descriptive Term	Parts of Solvent Required for 1 Part of Solute
Very Soluble	Less than 1
Freely Soluble	From 1 to 10
Soluble	From 10 to 30
Sparingly Soluble	From 30 to 100
Slightly Soluble	From 100 to 1000
Very Slightly Soluble	From 1000 to 10,000
Practically Insoluble or Insoluble	More than 10,000

Table 1 - Descriptive Solubilities

2.0.0 DRY EXTRACTIONS

Dry extraction (sometimes referred to as direct extraction) is one of the simplest separation techniques known. It is based on the premise that the substance of interest in a sample mixture is soluble in a specific solvent while all other components of the sample are insoluble. A dry extraction procedure is part of the qualitative scheme and involves titration of a portion of a simple powder with a specific solvent, filtration, collection of the extract, and evaporation of the extract to dryness. The resulting residue would be the constituent of interest, in a fairly pure state, available for specific identification studies.

Generally, samples of cocaine (base or HCl salt) intermixed with any sugar and/or inorganic bicarbonates can be dry extracted with chloroform to separate the cocaine from its diluents. Although cocaine is very soluble in methanol, it would not be a suitable extracting solvent for these mixtures. Since sugars are sparingly soluble in methanol, a dry extraction of the sample powder would not only remove the cocaine, but it would dissolve some of the sugar as well and ultimately provide an impure product.

The most common organic solvents used for dry extractions are chloroform, methylene chloride, ethyl ether, and methanol. These solvents must be used in an anhydrous state. Prolonged standing of partially filled solvent containers may allow moisture to accumulate to the point where its use may introduce separation difficulties in the dry extraction process.

3.0.0 CRYSTALLIZATION

Crystallization is a method commonly used for isolation and purification of one or more substances. In crystallization, a substance containing impurities is dissolved in just sufficient solvent to give a saturated or nearly saturated solution close to the boiling point of the solvent. If necessary, the hot solvent is filtered and then cooled rapidly with occasional stirring. Crystals of the desired substance will precipitate out with only a minor amount of impurities (compared to original sample powder) because the cooled saturated solution formed is not saturated with the impurities, which, therefore, remain in solution.

If only one compound is separated from its impurities, the process is called a simple recrystallization. If two or more pure compounds are separated from a mixture, such a process is termed fractional recrystallization. Recrystallization should be repeated as many times as necessary in order to reach the desired level of purity.

The effectiveness of crystallization is determined by two important factors:

1. The solvent power, and

2. The crystallization temperature coefficient.

The power of the solvent is expressed as the mass of solute that can be dissolved in a given mass of pure solvent at a specified temperature. The crystallization

temperature coefficient is an indication of how "powerful" a solvent is at different temperatures. The "power" thus determines the volume of solvent to be used, while the temperature coefficient determines the yield.

Selection of the proper solvent is based on several conditions. For inorganic substances, water is the ideal solvent since it is inexpensive and readily available. For organic substances, a variety of liquid ketones, ethers, chlorinated hydrocarbons, or aromatics can be used. A mixture of these can be used when a substance is highly soluble in one and only slightly soluble in the other.

Other factors to consider for proper solvent selection are volatility, viscosity, flammability, toxicity, flash point, carcinogenic properties, availability, reactivity of solvent/solute, and purity. Purity is extremely important since a contaminated solvent can inhibit a substance from crystallizing or impart an undesirable coloring to the crystals.

The development of different crystalline forms (needles, rods, etc.) is caused by the formation of different crystal habits or by polymorphism. Different crystal habits are produced when the environment of the growing crystal affects its external shape without changing its internal structure. This phenomenon is caused by such circumstances as super-saturation at different points in the solution, proximity of one crystal to another, cooling rate, degree of agitation, size and number of nuclei.

Polymorphism occurs when the same compound exists in one or more crystalline and/or amorphous forms. Different polymorphic forms are produced, depending on such factors as:

1. The surface on which the crystals are formed

2. The type of nuclei introduced

3. The temperatures at which spontaneous crystallization occurs

4. The type of solvent used.

Polymorphic forms of a compound's crystals can be distinguished by melting points, light microscopy, X-ray diffraction, infrared spectroscopy, and other techniques.

Although crystallization is an excellent method for the production of pure substances, it is a tedious and time-consuming procedure. The success of this technique depends largely on the care taken when scratching the surface, adding the proper crystalline form of the nuclei (also known as "seed," "germ," or "submicron"), controlling the cooling rate, etc. There are three common techniques of inducing crystallization:

1. Leaving the sample open to the environment (dust sometimes induces crystallization)

2. Placing the sample in a refrigerator (cooling)

3. Placing the sample in a desiccator (hygroscopic reasons).

When time and sample size permit, the sample should be divided into three different portions, and all three techniques attempted simultaneously. Then, the portion exhibiting the best crystallization can be selected for final use. Once crystallization begins, the surface of the container can be scratched (and the "germs" dispersed).

4.0.0 PARTITIONING AND DISTRIBUTION

The extraction or removal of a constituent of a solid mixture by contact with a suitable solvent or by transferring the substance from one liquid to another (immiscible) liquid is called distribution. The distribution of a solute between two immiscible liquids is called partitioning.

The distribution law governs the partitioning of a solute between two immiscible solvents. This law is expressed in terms of solute concentrations (C) in each liquid phase, independent of the total amount of solute (A) present. If the solute A is allowed to distribute itself between water and an organic phase, the resulting equilibrium can be expressed as:

$$A_W \leftrightarrow A_O$$
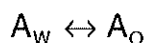
At equilibrium, the ratio of phase concentrations is constant and is termed the partition coefficient or distribution coefficient (K).

$$K = \frac{(A\ organic)}{(A\ aqueous)} \quad \text{or} \quad K = \frac{C_O}{C_W}$$

Where C_O = concentration of A in organic and C_W = concentration of A in aqueous phase.

The values of C can be determined experimentally and plotted as C_O vs. C_W, in which the slope equals K. This plot is called the partition or distribution isotherm and should be linear. (However, there are exceptions; e.g., the formation of a dimer between the solute and one of the solvents.)

For a simple or single extraction in which K is known for a particular system, one can determine the fraction of the solute in each phase at equilibrium, since:

p = fraction of the solute in the organic phase
q = fraction of the solute in the aqueous phase

so that,

$$p = \frac{amount\ of\ solute\ in\ the\ organic\ phase}{total\ amount\ of\ solute}$$

Solute amounts (moles, etc.) can be expressed in terms of concentration and volumes, as follows:

Solute in the organic phase = $(C_O)(V_O)$, where V_O = volume of organic phase

Total solute = $[(C_O)(V_O)] + [(C_W)(V_W)]$, where V_W = volume of aqueous phase.

To simplify, substitute volume ratios expressed in terms of U, as follows:

$$U = \frac{V_O}{V_W}$$

Since $p + q = 1$ by definition and $U = V_O/V_W$, one can express p and q in terms of K and U:

$$p = \frac{KU}{KU+1} \qquad \text{and} \qquad q = \frac{1}{KU+1}$$

5.0.0 LIQUID - LIQUID EXTRACTIONS

The selective power of the extracting process for separating and purifying drugs is vastly expanded when two immiscible solvents are employed simultaneously in a liquid-liquid partitioning or "shake-out" procedure. In this process, a drug dissolved in one solvent (usually the aqueous phase) may be separated from accompanying impurities by shaking out with a second immiscible solvent (the organic phase) in which the substance sought is quite soluble, but the impurities are not. Removal of the organic phase containing the desired compound with impurities retained in the aqueous layer isolates the desired component in a purified solution. In other instances, the impurities may be in the organic phase that is initially removed and the compound of interest retained in the aqueous layer. The classical apparatus used in accomplishing this segregation of immiscible phases is the separatory funnel. Other extracting devices such as the mixer-extractor-separators, liquid-liquid extractors, super-critical fluid extractors, etc., are commercially available.

The shakeout procedure has been widely exploited for the qualitative and quantitative separation of drugs. Its use is dependent upon the availability of two convenient immiscible solvents in which the substance sought shows a distribution coefficient considerably different from that of accompanying impurities or secondary products.

Theoretically, a single extraction for the quantitative recovery of a substance is feasible when K is 10 or greater (and accompanying components partition coefficient values are small). In practice, however, even if the distribution coefficient is extremely large, a single extraction will not quantitatively extract the substance of interest. What causes this deviation from the theoretical is that part of the organic phase (containing some of the substance) remains in the aqueous phase within the separatory funnel because of the impossibility to achieve complete segregation of the separatory phases. Also, another contributing factor is that the organic phase will adhere to the surface of the separatory funnel.

To achieve a quantitative recovery, the extraction process of the original phase (usually the aqueous phase) must be repeated with fresh portions of the organic phase (after removing the first extract and washing with solvent as needed). The extracts and washings are combined for quantitative measurement.

One can determine the number of extractions needed by determining p and q values. If one calculates the progress of the extractions, the following conclusions are reached in the Nth extraction:

1. Fraction of the total extracted in the Nth extraction = Fraction of the total left after (N-1) extraction times p; written as: $pq^{(N-1)}$

2. Total fraction extracted = $\sum_{N=1}^{N} pq^{(N-1)}$

3. Fraction remaining = q^N

A more efficient extraction is achieved by performing several extractions rather than a single one utilizing the same total volume of extractant.

The factor determining the success of a separation by extraction if the separability factor B, where $B = K_1 / K_2$.

The more B deviates from unity, the more feasible the separation. Although the most important factor in the separation of two substances is the difference between their distribution ratios, there are other factors to consider:

1. Nature of the solvent -- solubility of solute in it (polar, non-polar, etc.)
2. Ease of recovery of solute from solvent
3. Miscibility of the two phases
4. Relative specific gravity
5. Viscosity
6. Tendency to form emulsions
7. Toxicity and flammability

Acid-base extractions are commonly employed in liquid-liquid partitioning to isolate a compound (or a group of similar compounds) from a complex matrix and to retrieve the isolated compound in the form of a concentrate for further study. While performing the extraction procedure, the retention of a compound in acid or basic solution provides some general clues about the compound. For example, most basic drugs are soluble (and retained) in acid solution and insoluble in basic solution. Most acidic drugs are soluble (and retained) in basic solutions and insoluble in acid solution. Neutral drugs (as well as a few basic drugs) are soluble, as well as extractable, from either acid or basic solutions. There are a number of qualitative schemes devised for the extraction of different types of drugs. These schemes should only be considered as a guide since a forensic

chemist must usually modify a selected scheme in order to accommodate the nature of the sample and the resolution of a specific problem.

To bring two immiscible solvents into close contact and to establish the equilibrium distribution of the solute sought, one must shake the solvents in a separatory funnel. The purpose of the shaking is to temporarily emulsify the phases so as to increase the interface at which the exchange or partitioning of the solute occurs. However, long or violent agitation may cause the two phases to form a stable emulsion.

Stable emulsions are one of the main problems encountered when conducting acid-base extractions. As a rule, alkaline solutions emulsify more readily than acidic solutions. For an emulsion to break, sedimentation and coalescence of the droplets of the dispersed phase must occur. The settling will decrease as the viscosity decreases, the density difference decreases, and the drop size decreases.

In systems where emulsions tend to occur, one may use one or more of the following techniques to limit emulsion formation or stability:

1. Give special attention to the method of agitation;
2. Choose solvents which will not react chemically with the solutes;
3. Choose liquids with large interfacial tensions;
4. Filter to remove any solid material before extraction;
5. Increase the relative volume of the organic solvent;
6. Filter the emulsion through some porous substance to induce coalescence.

6.0.0 READINGS

1. <u>Microgram</u>, Vol. XVI, No. 1, Jan 1983, "The ANOR Extraction Procedure"

7.0.0 EXERCISES

1. Obtain a phenobarbital and lactose mixture from your training instructor. Extract half the mixture with ether and water. In which solvent do you expect to find the phenobarbital? Evaporate the correct solvent and verify the phenobarbital by IR. Extract the remaining portion of the mixture with chloroform and water. Again, isolate the phenobarbital and verify by IR. Explain any differences you may find in the IR spectrums.

2. Obtain a methamphetamine HCl, secobarbital, caffeine and sugar mixture from your training instructor. Devise and conduct a separation of all four individual components. Obtain an IR of each individual component. Run the three organic soluble compounds on the GC/MS to verify completeness of isolation.

8.0.0 QUESTIONS

1. List some methods used to differentiate crystal habits from polymorphic forms.

2. Compound Y has a partition coefficient of 4 in a water/ether system. Show that for a 10 mL aqueous solution of Y a more efficient extraction can be attained by using four 10 mL portions of ether rather than by using a single 40 mL portion of ether.

3. Predict whether or not the Cocaine HCl, Cocaine Base, Phenobarbital and Phenobarbital Sodium will dissolve in the following solvents. For here we will say that dissolve means at least sparingly soluble.

a. H_2O
b.   1M $NaHCO_3$
c. $CHCl_3$
d.   Ethyl ether
e.   Acetone (predict for only Cocaine HCl and Cocaine base)

4. Devise a dry powder extraction scheme to separate the following mixtures:

a.   Cocaine HCl and Mannitol
b.   Cocaine Base, Cocaine HCl and Inositol
c.   Cocaine HCl and Nicotinamide
d.   Amphetamine Sulfate and Lactose
e.   Caffeine, Heroin HCl and Lactose

5. Devise a liquid-liquid extraction scheme to separate the following mixtures:
a.   Cocaine HCl and Mannitol (will cocaine still be present as the HCl salt?
b.   Cocaine Base, Cocaine HCl and Inositol
c.   Cocaine HCl and Nicotinamide
d.   Amphetamine Sulfate and Lactose
e.   Methamphetamine and Phenylacetone from a two-layered liquid

6. Devise a method of your choice to separate the following mixtures:
a.   Phenobarbital and Stearic acid
b.   Methamphetamine HCl, Amobarbital, and Starch

7. You are given a sample from a clandestine methamphetamine laboratory. It consists of 2-layers. The bottom layer is aqueous with a pH of 12. How would you extract and analyze this sample with respect to methamphetamine?