Solubility and Extractability in the Pharmaceutical Sciences: A Demonstration to Address These Essential Concepts

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Supplementary materials

1. MATERIALS USED IN LIVE ASSAYS OF SOLUBILITY

For liquid compounds: flasks for the set of alcohols and chloroform (or diethyl ether) plus a sample of acetic acid and glycerol

For solid compounds: 50 mL Falcon tubes for each solid samples (sorbitol, sodium acetate, sodium benzoate, benzoic acid)

Tubes to test the miscibility of alcohols in water (here bottle from gas wash bottles)

Test tubes (volume ~ 16 mL)

A test tube rack

PELD Pasteur pipettes

Micro-powder spatula

A wash bottle containing demineralized water

Absorbent wipes

2. REAGENTS AND COMPOUNDS USED IN LIVE ASSAYS OF SOLUBILITY

Methanol

Ethanol

1-Propanol

1-Butanol

Glycerol (propane-1,2,3-triol)

Sorbitol ((2*S*,3*R*,4*R*,5*R*)-Hexane-1,2,3,4,5,6-hexol)

Acetic acid

Sodium acetate

Benzoic acid

Sodium benzoate

1% aqueous solution of E124

Saturated aqueous solution of sodium chloride

Chloroform (or diethyl ether)

Demineralized water

3. MATERIALS USED IN CONDUCTIVITY ASSAYS

These experiments were prepared before the first demonstration. The measurements take time and it is not possible to do such assays live except if we dedicate all of the time of the demonstration to explain this concept.

An overhead projector is needed during the live session to show the results using transparencies.

- A conductimeter (i.e. HANNA HI8733)
- A beaker low form (150 mL)
- A magnetic stirrer with or without stirring (heating is not necessary)
- A magnetic stirring bar
- A wash bottle containing demineralized water
- A pipette with appropriate tips (whatever the brand)

4. REAGENTS USED IN CONDUCTIVITY ASSAYS

- Demineralized water
- A saturated aqueous NaCl solution
- A 10 % aqueous glucose (or polyol) solution
- Ethanol
- Acetic acid
- A 10 % aqueous sodium acetate solution
- Triethylamine
- A 10% aqueous tetra-n-butylammonium bromide solution

Aqueous solutions are prepared with demineralized water

5. MATERIALS USED DURING EXTRACTION EXPERIMENTS

Similarly to the conductivity assays, these experiments were performed before the first live demonstration. An overhead projector is needed during the live session to show the results using transparencies.

- One Gilson separatory funnel (250 mL) with a PTFE stopcock and a plastic stopper
- One evaporating flask (250 mL)
- One flask ring support
- Two graduated cylinders (100 mL)
- Two glass funnels
- One filter funnel support
- One support ring (with or without bosshead)
- One stand base
- One beaker low form (150 mL)
- Two Erlenmeyer flasks narrow mouth (100 and 150 mL)
- pH indicator paper roll
- glass stick
- Analytical balances
- Rotavapor R-100 Buchi with water batch
- Sartorius paper folded filters qualitative grade 1288

6. REAGENTS AND COMPOUNDS USED DURING EXTRACTION EXPERIMENTS

Six buffers at different values of pH. This sequence, pH 2, 4, 6, 8, 10, 12 is used for the practical session

Diethyl ether (i.e. Thermofischer D/2450/17)

Magnesium sulfate, anhydrous (i.e. ThermoScientific 196850010)

Benzoic acid

Salicylic acid

para-Nitrobenzoic acid

Vanillin

Menthol

para-Toluidine

7. PROTOCOL USED IN CONDUCTIVITY ASSAYS

The demineralized water is poured into a beaker containing a magnetic stirring bar. The volume will depend on the size of the electrode.

With the material mention above, 130 mL of water is used. The electrode is placed and the reading is done after 2-3 minutes of stabilization.

Thereafter, while continually stirring, aliquots of the compounds (pure or solutions) are added. The addition is cumulative. After each addition, the reading will take 2-3 minutes considering the stabilization.

Between each compound, the electrode is rinced with demineralized water and then poured in a new beaker with a volume of demineralized water as previously done. Before the addition of compound, the value is equal to zero or close to.

8. PROTOCOL USED IN EXTRACTABILITY ASSAYS

Historically, the extractability assays were performed using an organic solution of the compound to be extracted. Here, the protocol used will be similar to the one recently introduced in a new laboratory session (Taouba et al. J Chem Educ, submitted for publication). Thus, a separatory funnel is installed in the hood. 100 mL of appropriate buffer is poured in the separatory funnel followed by 50 mL of diethyl ether. 250 mg of the compound is weighed precisely and is directly poured into the separatory funnel. A plastic stopper is placed and the mixture is vigorously agitated for 5 seconds. No trace of the compound should be visible at this step. If some particles remain in suspension, an additional shaking is necessary.

The overpressure in the separatory funnel is removed carefully and the mixture is left to settle until both phases are clearly separated. The buffer phase is carefully recovered in a beaker and conserved for a second extraction, while the organic phase is kept in a dry Erlenmeyer. The second extraction is done in the same manner as previously described. Both organic phases are then assembled in the Erlenmeyer and dried with 500 mg - 1 g of anhydrous $MgSO_4$ (i.e. ThermoScientific 196850010). During this step, the Erlenmeyer is covered with a Petri dish or a crystallizing dish. The suspension is stirred for approximately five minutes. Thereafter, the organic solution is filtered using a paper folded filter (i.e. Sartorius folded filter grade 1288) to remove the mineral and then collected in a tare flask. The erlenmeyer is rinced

2 times with 10 mL of fresh diethyl ether and the solvent is also collected in the tare flask. The solvent is evaporated under reduced pressure and the residue is carefully dried. Thereafter, the flask is weighed. The same procedure is then reproduced with the other buffers.

A graph is drawn for each compound using the weight of each residue (in mg). Another way to represent the results would be to convert these weights in percentage of recovery as done in the laboratory session (Taouba et al. J Chem Educ, submitted for publication).

9. TIMING OF THE LIVE DEMONSTRATION (45-50 minutes-session for each group of ~ 30 students)

The intrinsic solubility :

- The preparation of the tubes with demineralized water (~ 50 mL) before students enter the room
- The addition of dye to the water in presence of students.
- The presentation of four alcohols by drawing their chemical structure on the blackboard
- Indicating the polar (in red) and non-polar (in green) region in the molecules
- Mixing the methanol (~ 50 mL) with the water and checking the temperature increase with some students
- Mixing the other three alcohols (same volume) with water
- Conducting the test with brine and 1-propanol (closed tubes circulate in the class). For this test, small tubes are sufficient
- The assay with glycerol and sorbitol (tubes circulate in the class). (same remark)
- The assay with benzoic acid and sodium benzoate (tubes circulate in the class). (same remark)

The potential ionization when solubilized in water:

- A discussion about solubilization and the dissociation of NaCl in water
- A short explanation about conductivity
- A presentation about the conductivity results for NaCl, ethanol and glucose, acetic acid and sodium acetate, triethylamine and tetra-*n*-butylammonium bromide (using transparencies)

Extractability :

- The assay of the solubility with benzoic acid and sodium benzoate in small tubes (tubes circulate in the class). For this test, small tubes are sufficient

- Adding HCl in the tube containing aqueous sodium benzoate solution, to show the precipitation of the acid followed by the addition of the diethyl ether (or chloroform) to extract the non-ionized chemical entity

- A presentation of the experimental approach on how to perform the pH-depending extractions with pure compounds and quantification of the compound extracted

- Thereafter, a presentation of the results obtained for the extractions of benzoic acid, salicylic acid, *para*-nitrobenzoic acid, vanillin, menthol and *para*-toluidine (using transparencies). Potentially, compare the different curves discussed.

10. ADDITIONAL ASSAYS

Assays with alcohols – Preparation of the tubes

To facilitate the visualization of, water is dyed with Ponceau 4R (new coccine or E124), a color additive used for food (Figure S1). Water is added in the tubes before the demonstration while the dye is added just before the assays (Figure S2).

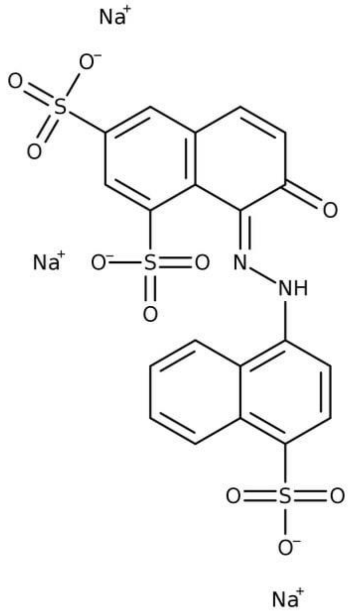


Figure S1. Chemical structure of the dye

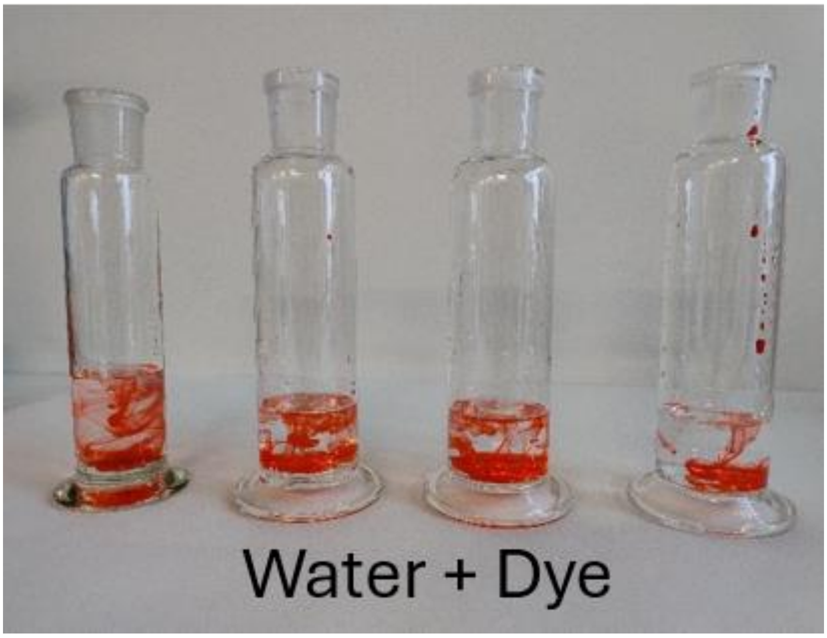


Figure S2. A set of tubes used in the assay of the miscibility of short-carbon chain alcohols in water. The dye is added during the live session.

The four alcohols used in the assay of water solubility are presented in a similar flask to show their macroscopic similarities (Figure S3).

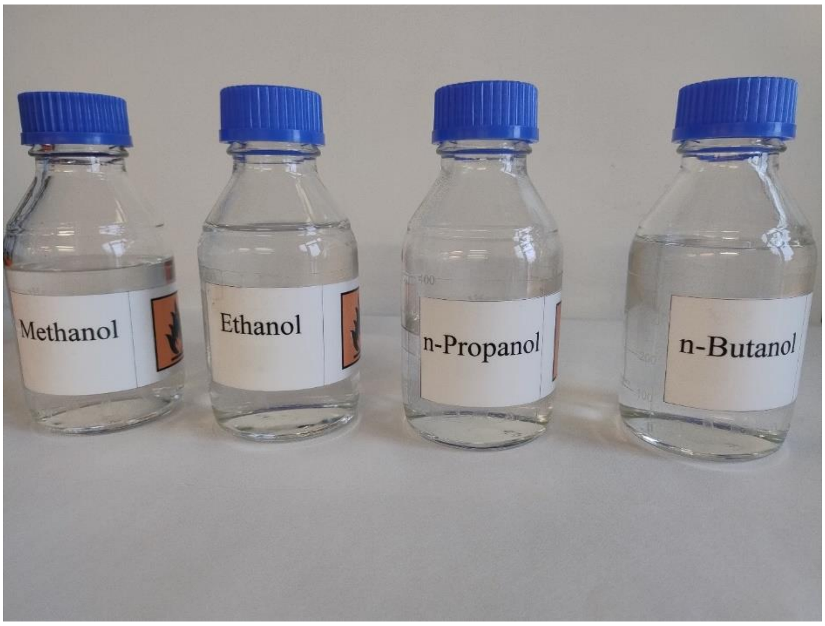


Figure S3. Set of flasks containing short-carbon chain alcohols.

Assays with polyhydroxylated compounds

The assay is conducted with propane-1,2,3-triol (glycerol) and sorbitol (glucose would be fine as used in the conductivity measurements) (Figure S4). These compounds are frequently used in the pharmaceutical field.

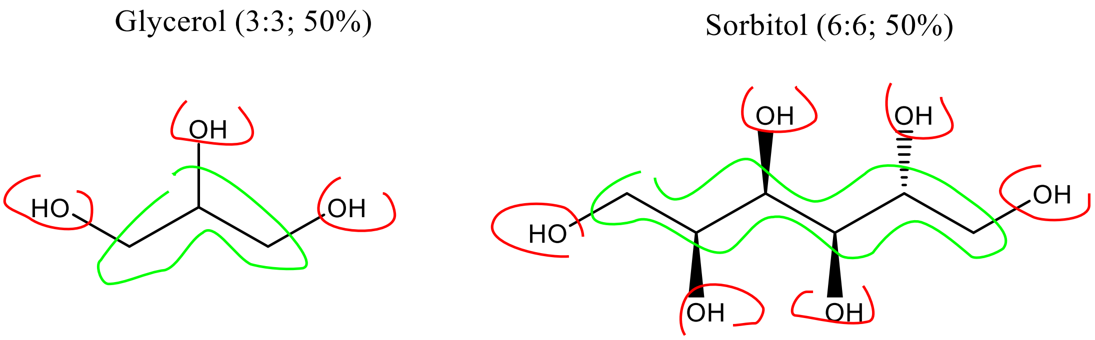
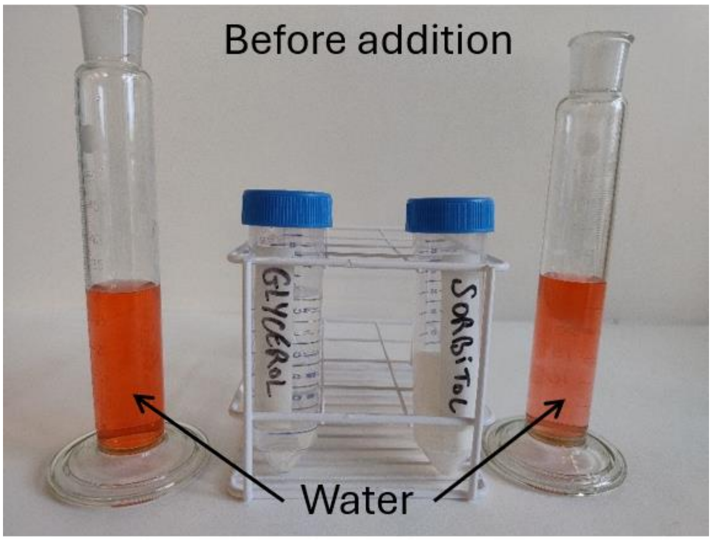


Figure S4. Chemical structures of polyhydroxylated compounds. The polar regions are surrounded in red while the nonpolar regions are surrounded in green. In parentheses, the number of each group in the molecule and then the percentage of the polar group.

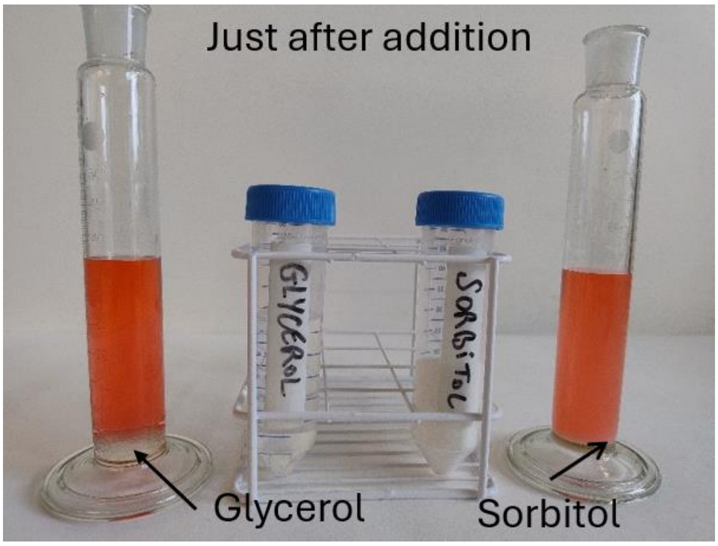
Pure glycerol (Figure S4) presents a viscous aspect similar to liquid honey (Figure S5A). This viscosity is related to the higher number of hydrogen bonds in the pure compound which delays the solubility in water. When glycerol is added to water, it sinks in the bottom of the tube (Figure S5B). Progressively, by gentle stirring, the glycerol is solubilized (Figure S5C). During the live demonstration, a tube with the mixture circulates among the students to show this progressive solubilization.

Afterwards, a six carbon chain and six hydroxyl group molecule, or in other words sorbitol (Figure S4), was tested. In its pure form it is a white solid due to its higher molecular weight (Figure S5A). Although the carbon chain is bigger, the solubility of the compound in water remains important due to the presence of these six hydroxyl groups which induce a high hydrophilicity. After adding it to water, the product disperses (Figure S3B) and then gradually dissolves (Figure S5C). Both compounds have a high polar/nonpolar ratio which explains their high solubility in water.



A

B



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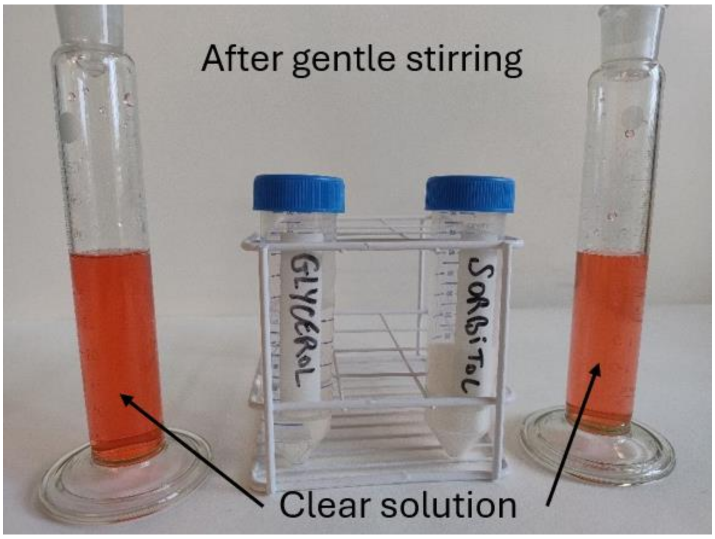


Figure S5. Assay of the solubilization of glycerol (left tube) and sorbitol (right tube) in water. A. Before (both samples of pure compound are presented to show the macroscopic differences) B. After addition. C. After stirring.

Assays with alcohols – Hydrogen bonds

In the case of methanol being added to water, it is possible to record the temperature during the demonstration (Figure S6). The temperature is measured for both compounds before and after mixing.

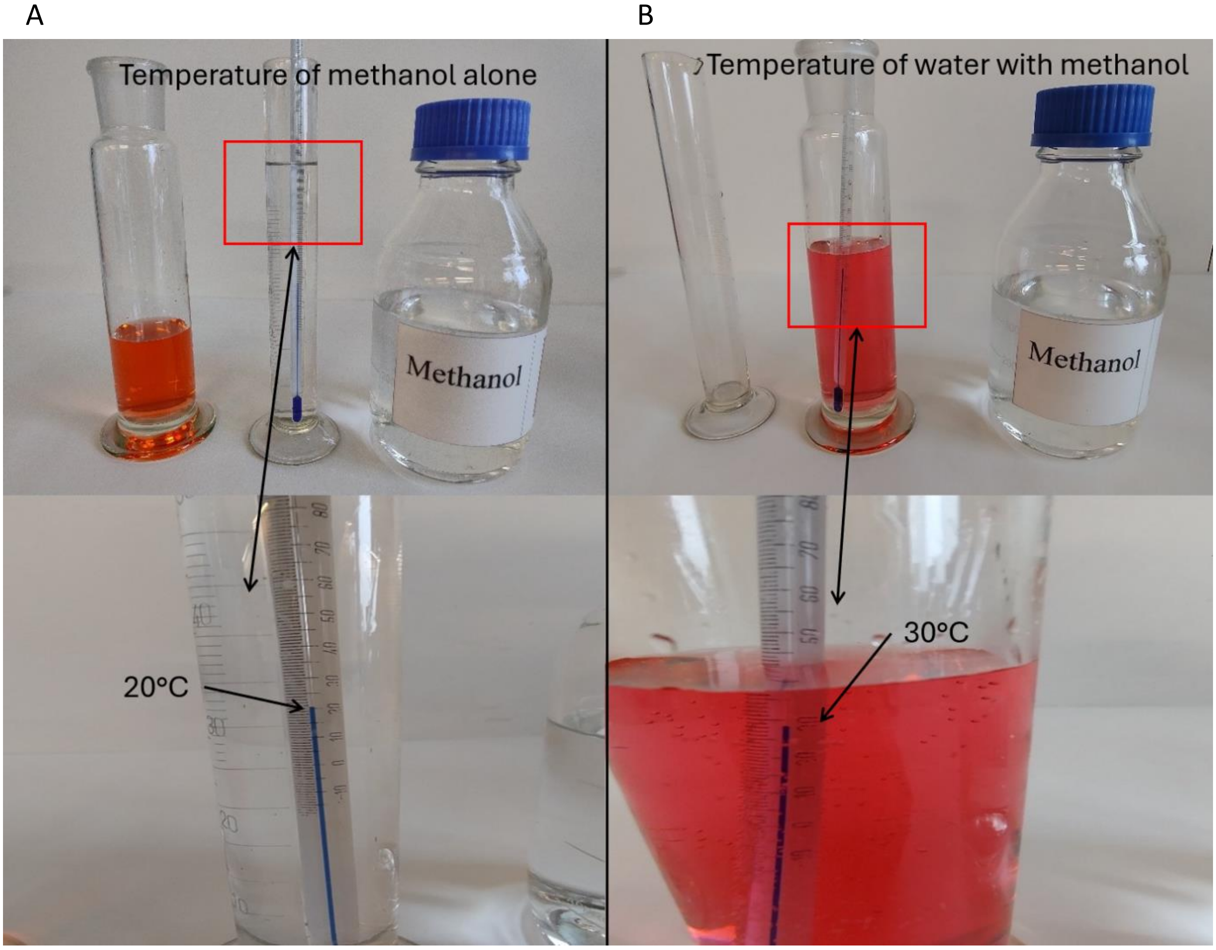


Figure S6. Assay showing the addition of methanol to water to demonstrate the significant increase in temperature following its addition. Measuring methanol temperature before (A) and after (B) its addition to water.

Regarding the assay with 1-propanol and sodium chloride, it is better to use a 50% saturated sodium chloride. When using a saturated sodium chloride solution, an important precipitate of the mineral quickly appears besides the presence of the biphasic mixture.

Assay with acetic acid and sodium acetate (conjugate base)

This assay (Figure S7) is not performed during the live demonstration due to the strong smell of the pure acid. More importantly, the pure samples are shown to the students to demonstrate the macroscopic differences between each entity.

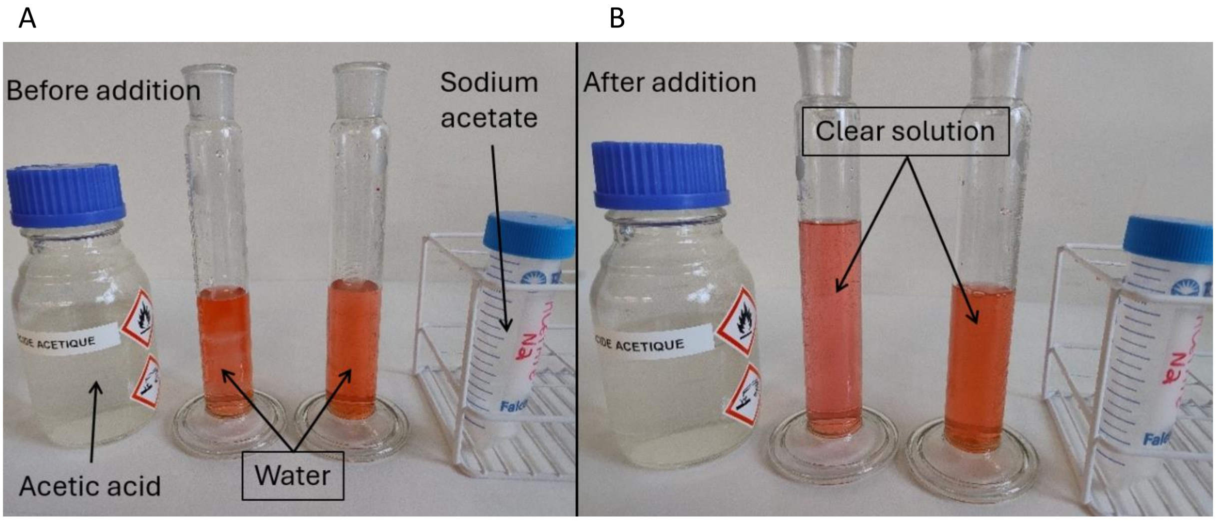


Figure S7. Assay of the solubility of a short-carbon side chain acid (acetic acid) and its conjugate base (sodium acetate) in water. The tubes before the addition of the substances (A) and after the addition (B).

11. ASSESSMENT

As mentioned, in the course curriculum, this organic chemistry course is proposed to second-year in pharmaceutical sciences. The concept of solubility and extractability are extensively described, explained during the course and seminars, during this demonstration and also applied during different practical sessions. For a while, the last sessions of the ten in the program is dedicated to the characterization of an unknown product. During this session, each student analyzes his own product through an elemental analysis procedure and then regarding the solubility and extractability before to conclude by the possible compound found in a list. Besides the experimental exercise, each student will have also a theoretical and similar exercise on a precise compound.

- Example of laboratory report:

Applied Organic chemistry to drug analysis and drug design (CHIM0714)

Characterization of unknown organic compounds – Elemental analysis – Acid/base character – Hydrophilicity-Lipophilicity

REPORT **Session 10** **Table : SURNAME, First Name :**

Sample 1 : Number and color code = ……

Sodium fusion test :

Assays :

Results : ……..

Elemental analysis : CH … **To be corrected = Pré-rapport A**

Solubilities : **Water :** **pH of the aqueous solution :**

Water + HCl :

Water + NaOH :

Organic solvent (specify which one) :

UV absorption: Yes / No

Extractabilities : (specify the detection approach: UV or residue following the evaporation)

	CH_2Cl_2	Diethylether
Water :		
Water + HCl :		
Water + NaOH :		

Acid/Base character of the compound :

Justification or explanation:

Halogen (specificy which one), sulphur (if present) : ionized or not ?

Nitrogen if present : ionized or not ? Justify.

CONCLUSION : the compound is …………… **To be checked = Pré-rapport B---------**

POSSIBLE COMPOUND : circle it in the document. If there are several possibilities, choose one to determine the molecular formula and its elemental composition.

Molecular formula :

Molecular weights (atomic masses rounded to the nearest unit) :

Elemental composition (indicate only the values for the atoms mentioned below):

C %, N %, S %, H %

After the analysis, return the sample

Sample 2 (theoretical exercice)

Here, the compound to be characterized is indicated in the list that the students received

Sodium fusion test :

Assays :

Results : ……..

Elemental analysis : CH …

Solubilities : **Water :** **pH of the aqueous solution :**

Water + HCl :

Water + NaOH :

Organic solvent (specify which one) :

UV absorption: Yes / No

Extractabilities : (specify the detection approach: UV or residue following the evaporation)

	CH_2Cl_2	**Diethylether**
Water :		
Water + HCl :		
Water + NaOH :		

Based on solubilities and extractabilities indicate the acid/base character and hydro/lipophilic character of the compound :

Justification or explanation:

Calcination to identify the cation : YES / NO

Analysis of the calcination residue. Assays to identifiy the cation :

Molecular formula :

Molecular weights (atomic masses rounded to the nearest unit) :

Elemental composition (indicate only the values for the atoms mentioned below):

C %, N %, S %, H %

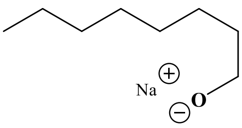
- Intermediate and final evaluations:

Questions regarding solubility and extractability of compound are asked especially regarding the curves of extractability based on the examination of the compounds in terms of functional groups. Students must learn precisely most of functional groups with their acid/base characteristic and train to discriminate their behaviour either in the organic chemistry context or the pharmaceutical one (aqueous media). The question regarding extractability curves can be proposed alone or integrated in a sequence of reaction. Examples of these questions are reported below.

Applied Organic chemistry to drug analysis and drug design (CHIM0714) : Example of questions for the intermediate examination

A. Indicate the acid/base character and hydro/lipophilic character of these five compounds in relation to pH. Justify your answer.

1



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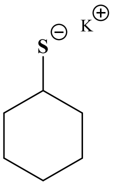

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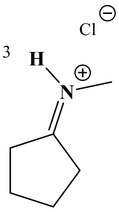

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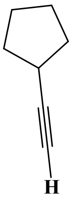

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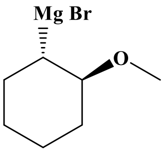
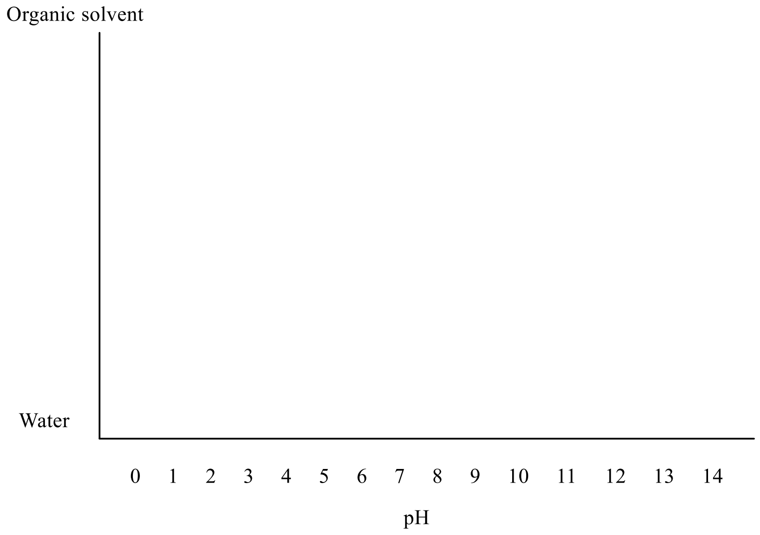


B. Draw on the graph the theoretical curves of extractability for the five compounds.



Answers :

A.

1. The compound contains a sodium salt of an alkoxide : this is the conjugate base of the alcohol (the acid form with a pKa ~ 15). This strong base will react with water in an acid/base reaction to form the alcohol. Thus, in this context, the alcohol is a neutral entity. Due to its long carbon side chain it is lipophilic.

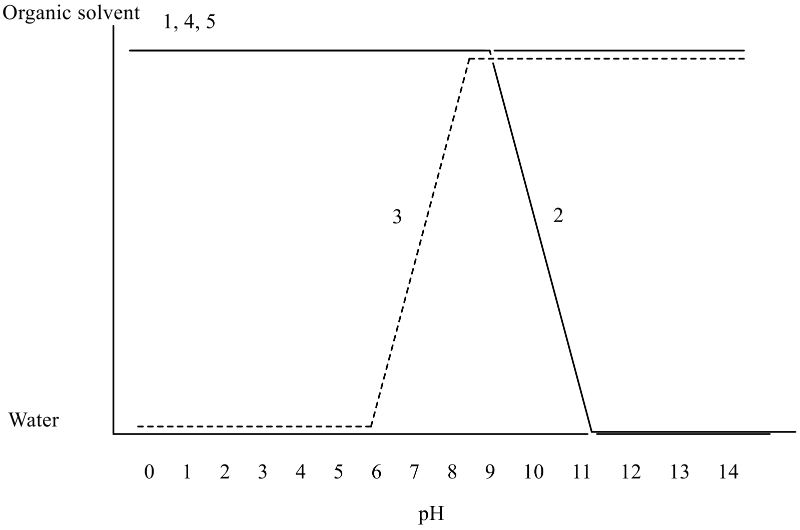
2. The compound contains a potassium salt of a thioalkoxide: this is the conjugate base of the thiol (acid form) with a pKa ~ 10. The compound is a weak base which is stable in water. At a pH inferior to the pKa value, the thiol is mainly present and non-ionized, the molecule being lipophilic. At a pH superior to the pKa, the conjuage base is mainly present, ionized and the entity is hydrophilic.

3. The compound contains an iminium as hydrochloride (salt of imine) : it is the conjugate acid (ionized thus hydrophilic) with a pKa ~ 7. The entity is hydrophilic. At a pH inferior to pKa value, the imine is mainly protonated thus ionized and the molecule hydrophilic. At a pH superior to the pKa value, the imine is mainly non-ionized and thus the molecule is lipophilic due to the carbon side chain.

4. In this compound there is a terminal alkyne meaning that the distal carbon as an hydrogen attached to triply bonded carbon. This functional group is a weak acid with a pKa ~ 25 so there is no acid/base reaction with water. The molecule is neutral and lipophilic.

5. The compound contains two functional groups. One is an organomagnesium reagent (or a Grignard reagent) which is a very strong base. In water, it will have immediately an acid/base reaction leading to the formation of the corresponding hydrocarbon moiety. The other one is an ether which is a neutral group. Thus, in this context, the molecule is neutral and lipophilic.

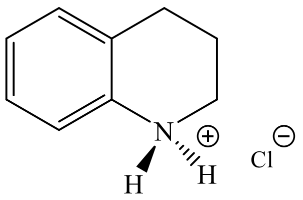
B.



Applied Organic chemistry to drug analysis and drug design (CHIM0714): Example of questions for the final examination

A. For these molecules, indicate precisely the functional groups and then indicate their acid/base character and their hydro/lipophilic character in relation to the pH. Justify your answer.

1



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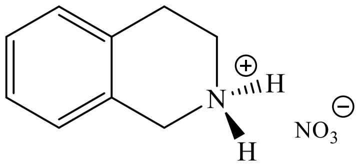

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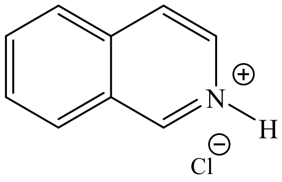

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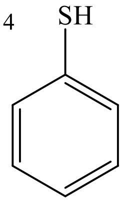

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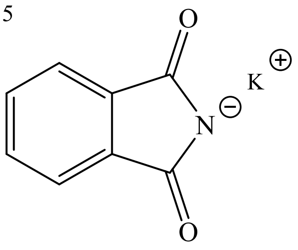
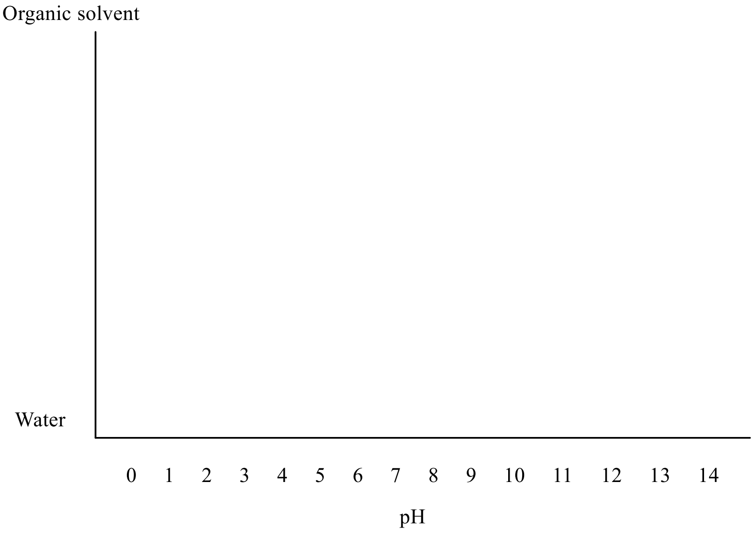


B. Draw on the graph the theoretical curves of extractability for the five compounds.



Answers :

A.

1. The compound contains the hydrochloride of a secondary aromatic amine (the conjugate acid with a pKa ~ 5). The compound is an acid and is hydrophilic.

2. The compound contains the salt (nitrate) of a secondary aliphatic amine (the conjugate acid with a pKa ~ 10). The compound is an acid and is hydrophilic.

3. The compound contains the hydrochloride of an aromatic nitrogen-containing heterocycle (the conjugate acid with a pKa ~ 5). The compound is an acid and is hydrophilic.

4. The compound contains a thiophenol, a protonated form non-ionized, which is a weak acid with a pKa ~ 5-7. This compound is an acid and is lipophilic.

5. The compound contains the potassium salt of an imide (the conjugate base of the imide). The imide is a weak acid with a pKa ~ 8. The compound is a base and is hydrophilic

B.