Searching for an Experimental Procedure for the Measurement of Alkane-Water Partition Coefficient

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Abstract

The amount and variety of pollutants that are detectable and toxic, even in small concentrations, has been growing. Despite the importance of studying these chemicals and their effect on the environment, there is a lack not only of partition coefficients data of these pollutants between water and alkanes, but also of experimental protocols to measure that information with accuracy. This information is essential to predict their fate once released in the environment.

In this context, the main objectives of this work were to perform a literature review about the methodologies used to measure partition coefficients, to collect and analyze a database of partition coefficients of compounds with diverse chemical structure between water and alkanes and, finally, to implement an experimental procedure to measure the partition coefficients at 298.15 K.

A variant of the shake-flask method was combined with UV-Visible spectroscopy and refractive index methods of analysis to perform the partition coefficients measurements between isooctane and water ($P_{isoo/w}$). Two solutes for which the solubility data are well established, such as toluene and benzoic acid, were selected, covering a wide range of numerical values of the partition coefficients. Hopefully, after, the methodology will be applied on compounds highly relevant from the environmental point of view, but not yet studied. Solubility measurements were also carried out as this type of data are essential for designing the partition coefficients experiments.

In general, the results obtained are in close agreement with the scarce information available in literature. The results of the partition coefficient of toluene was $\log P_{isoo/w} = 3.24 \pm 0.07$ and the distribution ratio of benzoic acid was $\log D_{isoo/w} = 0.26 \pm 0.03$. The errors obtained in the corresponding material balances were 3% and 7% for toluene and benzoic acid, respectively.

Resumo

A quantidade e a variedade de poluentes detetáveis e tóxicos, mesmo em pequenas concentrações, tem vindo a aumentar. Apesar da importância de se estudar estes compostos e os seus efeitos sobre o meio ambiente, faltam dados de coeficientes de partição de poluentes entre água e alcanos, mas também protocolos experimentais para medir essa informação com precisão. Esta informação é essencial para prever o seu destino uma vez libertados no ambiente.

Neste contexto, os principais objetivos deste trabalho foram realizar uma revisão bibliográfica sobre as metodologias utilizadas para medir coeficientes de partição, recolher e analisar uma base de dados de coeficientes de partição entre água e alcanos de compostos com estrutura química diversa e, finalmente, implementar um procedimento experimental para medir os coeficientes de partição a 298.15 K.

Para realizar as medições dos coeficientes de partição entre isooctano e água ($P_{iso/w}$), aplicou-se uma variante do método do frasco agitado, combinada com métodos de análise de espectroscopia UV-Visível e de medição do índice de refração. Selecionaram-se dois solutos para os quais os dados de solubilidade estão bem estabelecidos, o tolueno e o ácido benzóico, cobrindo uma ampla gama de valores de coeficientes de partição. Espera-se que, mais tarde, a metodologia seja aplicada a outros compostos relevantes do ponto de vista ambiental, mas ainda não estudados. Foram também realizadas medições de solubilidade, pois este tipo de dados é essencial para projetar os ensaios dos coeficientes de partição.

Em geral, os resultados obtidos estão de acordo com a reduzida informação disponível na literatura. Os resultados obtidos para o coeficiente de partição do tolueno foram log $P_{iso/w}$ = 3.24 ± 0.07 e a razão de distribuição do ácido benzóico foi log $D_{iso/w}$ = 0.26 ± 0.03. Os erros associados aos balanços de material foram de 3% e 7% para o tolueno e o ácido benzóico, respetivamente.

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List of Symbols and Acronyms:

List of Symbols

P	Partition coefficient
$P_{oct/w}$	Partition coefficient of octanol-water system
$P_{16/w}$	Partition coefficient of hexadecane-water system
$P_{alk/w}$	Partition coefficient of alkane-water system
$P_{cyc/w}$	Partition coefficient of cyclohexane-water system
$P_{iso/w}$	Partition coefficient of isooctane-water system

List of Acronyms

COSMO-RS	COnductor like Screening for Real Solvents
MOSCED	MOdified Separation of cohesive Energy Density
SMD	Solvation Model based on Density
SF	Shake Flask
SS	Slow stirring
RPM	Revolutions Per Minute
Abs	Absorbance
BA	Benzoic acid
UV-vis	UV-visible
HPLC	High Performance Liquid Chromatography

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Chapter 1. Introduction

1.1. Motivation

One of the most important concerns of the scientific community nowadays is the effect of human's activity (industry, transport, chemical weapon…) on the environment. While our activities are expanding and prospering, the waste released in the environment are extending as well, containing substances that can be toxic for the wildlife (animals, vegetables, micro-organisms), thus threatening the natural equilibrium of the whole ecosystem.

To limit and even eliminate these kind of effects, predicting the fate [1] of chemicals released to the environment is a priority. To assess the environmental risk of a substance, the scientific community developed a list of parameters which can show the preferential compartments where a given species will concentrate as well as the effect of a chemical on the environment. It has been consented that the fate of chemicals is firstly controlled by partition coefficients [2,3]. It indicates the extent to which the chemical will concentrate in an aqueous phase, air, soil or an organism.

Measurements for partition coefficient has long been done for n-octanol-water. To avoid issues with water saturated octanol phases, an heterogenous structure (caused by miscibility of n-octanol and water), researchers have been also concentrating on the measurement of alkane-water partition coefficients [4].

In this way, an experimental protocol will be developed and tested, firstly for solutes for which the partition coefficients are well established, covering a wide range of numerical values of these partition coefficients. Hopefully, after, the methodology will be applied on compounds highly relevant from the environmental point of view, but not yet studied.

1.2. Objectives

The main objective of this work is the compilation and analysis of the experimental data published so far of partition coefficients of organic solutes in water-alkane biphasic systems. In addition, a literature review about the experimental methods for the determination of the partition coefficient will be carried out, with the perspective of optimizing and finding a method more suitable to our laboratory conditions and needs. As a complement, a methodology will be also applied to measure the solubility of solid solutes in liquid solvents as this type of data is essential for designing the partition coefficients experiments.

Chapter 2. Literature review

2.1. Distribution and partition coefficients

A substance may distribute itself between two immiscible phases (solvents). This mass transfer is accompanied by very small heat changes, and the migration of molecules from one solvent to the other does not depend on a large positive value of the enthalpy change. Therefore, it is assumed that this phenomena is controlled by an entropic effect [2]. Once the system has reached a state of equilibrium (under specific conditions), the concentration ratio of the compound in the organic phase and the aqueous phase is calculated to have a representative value of the partition of the solute between the two phases. This ratio defines what is called the partition coefficient [5]. Being the quotient between the equilibrium concentration in the organic phase ($C_{\text{organic phase}}$) and the aqueous phase (C_{water}) given by equation (1) [6] and typically measured between 20 and 25 °C. The partition coefficient (*P*) is dimensionless and is usually given in the form of its logarithm to base ten [4].

$$P = C_{\text{organic phase}} / C_{\text{water}} \quad (1)$$

The distribution ratio *D* between an organic phase and water is a measure of *P* that accounts for the pH dependency of an ionizable organic chemical, and is a measure of the distribution of dissociated and non-dissociated species in organic and water as a function of pH. The extent to which an ionizable compound is dissociated across environmentally relevant pH ranges may have a marked effect on properties such as water solubility. It should be noted that neutral form of the species is generally less water soluble and is thus more hydrophobic as compared to the ionized or charged form. The relation between both is given by equation (2), where *Ka* means the acid dissociation constant [30].

$$\log P = \log D + log\left[1 + 10^{(pH - pKa)}\right] \quad (2)$$

2.2. Experimental methods

During 1989, Sangster published an extensive review on experimental methods to measure the partition coefficient. These are briefly summarized in Table 2.1, highlighting their main characteristics and with a brief discussion to point out the most important specification of each technique [8].

Going through the open literature it was concluded that nowadays the methods that were the most effective, and so to say mostly used, are the shake flask method (SF) and the slow stirring method (SS), which are analyzed in more detail in Table 2.2

Table 2.1 General characteristics of some measurement and estimation methods of *P*.

Method	Optimal log *P* range	Applicability	Strengths	Weaknesses
Shake-flask	-2.5 to 6	General for neutral species	Direct method reliable when precautions taken	Time consuming Requires attention Many details of procedure Less accurate at log $P > 5$
Generator column	2 to 7	General, except for very hydrophilic compounds	Direct method with no danger of emulsion Closed system Efficient temperature control Faster than SF	Column become a strim of solute. New column needed for each solute. Limited to lipophilic compounds. Rather elaborate analytical equipment
Reverse phase high pressure liquid chromatography	0 to 6	Neutral species only	Fast convenient, Impurities does not interfere	Correlational method Single correlation inaccurate
Water solubility correlation	2 to 6	Neutral species of limited water solubility	Convenient	Correlational method Single correlation inaccurate
Henrian activity coefficient	Depends on route to activity coefficient	Neutral Species	Thermodynamically exact	Ease of measurement Accuracy depends on route to activity coefficient
Fragmental constants	Same as SF Useful for extrapolation	Same as SF	Fast convenient reliable for molecules without many polar groups	Requires expertise for complicated molecules

Table 2.2 Comparative table for SS and SF methods.

	Slow stirring method	**Shake flask method**
Log *P* estimated for the chemical	Log $P > 4$	Log $P < 4$
Advantages	No induced miscibility of the organic phase into water	Take way less time than the slow stirring method
Suitable components	With water miscibility	Without water miscibility
System	Octanol-water	Cyclohexane-water

It is essential to indicate that in the case where the chemical species studied are not stable in the pH of the solvent, a buffer most be used depending on the considered system. Being the most used methods [5], the next section will give a general idea about the shake flask and the slow stirring method.

2.2.1. Slow stirring method protocol

In this method, the following conditions should be considered [9]:

- Temperature constant during the experiment.

- Reaction vessel of larger than one liter has to be considered, so that a sufficient volume of water can be obtained for chemical extraction and analysis.

- Purity of octanol at least +99 % (extraction with acid or distillation can be used to prepare the octanol).

- Water should be glass or quartz distilled, or obtained from a purification system, or HPLC-grade water may be used. Filtration through a 0.22 µm filter is required for distilled water.

- Both solvents are mutually saturated prior to the experiment by equilibrating them in a sufficiently large vessel. This is accomplished by slow-stirring the two-phase system for two days.

Regarding the analytical method for quantifying the amount of solute needed, the concentration of the test substance should not exceed 70% of its solubility with a maximum concentration of 0.1 M in either phase.

The volume of the phases should be chosen such that the 1-octanol layer is sufficiently thick (> 0.5 cm) in order to allow the sampling. Typical phase ratios used for the determinations of

compounds with log *P* of 4.5 and higher are 20 experiments: 20 to 50 ml of 1-octanol and 950 to 980 ml of water in one liter vessel for log *P* of 4.5 and higher.

The stirring system

The 1-octanol-water system is stirred until equilibrium is attained. In a pilot experiment the length of the equilibration period is assessed by performing a slow-stirring experiment and sampling the water and organic phases. The sampling time points should be separated by a minimum period of five hours (at least 3 samples).

The stirring rate should be increased slowly. Also it should be adjusted so that a vortex at the interface is formed between water and 1-octanol of 0.5 to maximally 2.5 cm depth.

Equilibration time

The minimum equilibration time is one day before sampling can be started. It is assumed that the equilibrium is achieved when a regression of the concentration ratio against time over a time span of four time points yields a slope that is not significantly different from zero at a p-level of 0.05.

Sampling

The stirrer should be turned off prior to sampling and the liquids should be allowed to stop moving. The organic phase sample volume is close to 100 µL.

2.2.2. Shake flask method protocol

This protocol is a simplified version that aims to give a general idea of the shake flask method that are currently being used [10,11].

-Organic phase:

10 µL of 10 mM compound in dimethyl sulfoxide DMSO.

5 µL of 200 µM propanolol in acetonitrile + 500 µL cyclohexane.

-Shake the mixture for 50 minutes using a plate shaker at 800 rpm.

-Separation of the two solvents by centrifugation for 5 minutes at 3700 RPM in a plate centrifuge.

-Cyclohexane wells: 45 µl of octanol (to avoid accumulation in the HPLC column) + 5 µl cyclohexane from the top phase.

-Water wells: 50 µl of water phase.

The slow stirring method is a modality of the shake flask method. Despite of the fact that they are the most used experimental method, they remain quite complicated and needing permanent checking.

2.3 Partition coefficients database

Abraham and coworkers made one of the most complete compilation of data published until this day (Appendix A) that have been used in most of the subsequent works [12,13].

In what follows, we will use the data collected and provided by of Abraham's works to try to look for a mathematical correlation between $P_{cyc/w}$ values and P of other types to help predicting the $P_{cyc/w}$ values and check for its consistency.

Some clarifications can be given about the data shown in Table A.1 (Appendix A)[12].

- For water-hexadecane, water-alkane and water-cyclohexane some values of P were obtained directly from solubilities in water and organic phase.

- Sulfoxides, alkyl anilines, haloanilines and alkylpyridines were excluded from the water-octanol set, because of a variable basicity problem.

- All aliphatic aldehydes were also excluded from the water-octanol set because of formation of the hydrate, $RCH(OH)_2$.

Correlation between partition coefficients:

In this section, $\log P_{oct/w}$, $\log P_{16/w}$ or $\log P_{alk/w}$ are shown in function of $\log P_{cyc/w}$ in Figures 2.1 , 2.2 and 2.3 to identify if there is a relation between the values that can help do an approximation of $\log P_{cyc/w}$ for unknown components.

- log $P_{oct/w}$ = f(log $P_{cyc/w}$):

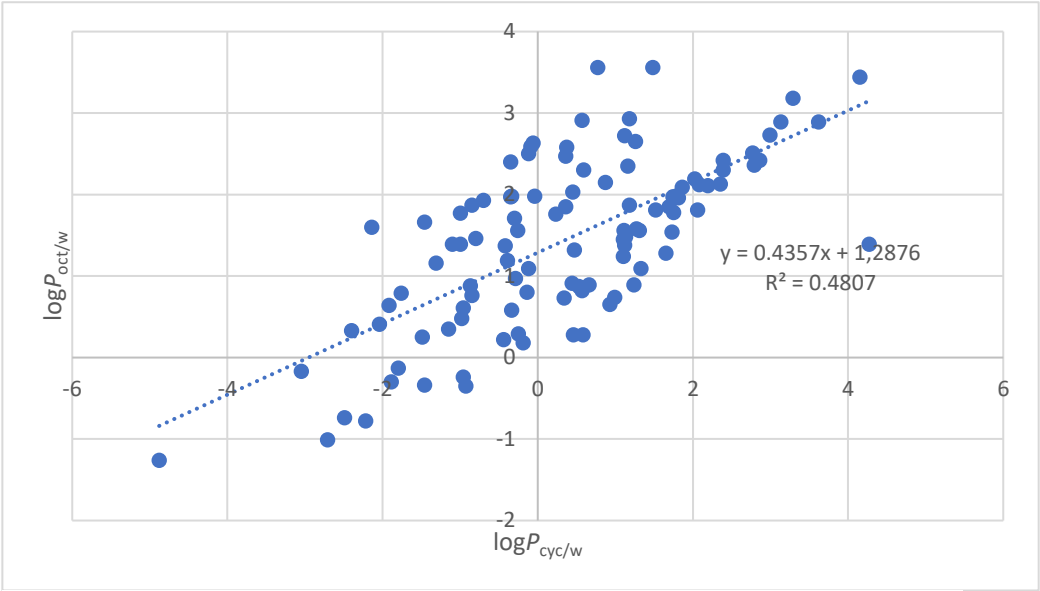

Figure 2.1 Linear relationship between log $P_{oct/w}$ and log $P_{cyc/w}$.

The determination coefficient is $R^2 = 0.4807$, meaning that only 48.7% of change on log $P_{cyc/w}$ can be explained by the change on log $P_{oct/w}$, which make this relation not very valuable, and a big dispersion is observed.

- log $P_{16/w}$ = f(log $P_{cyc/w}$)

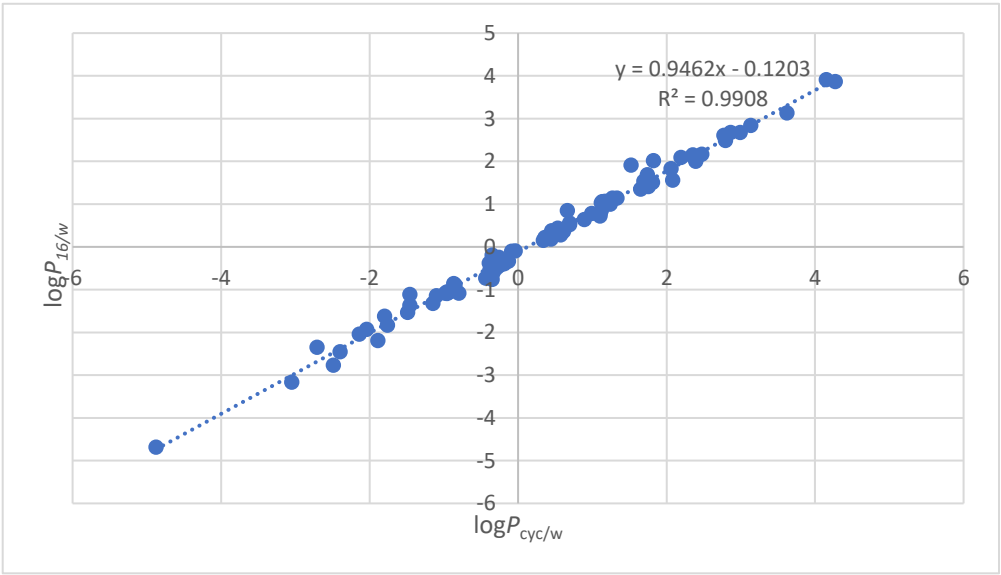

Figure 2.2 Linear relationship between log $P_{16/w}$ and log $P_{cyc/w}$.

The determination coefficient is $R^2 = 0.9908$, meaning that 99.08% of the change on log $P_{cyc/w}$ can be related to the change on log $P_{16/w}$, which make this relation very interesting. The points are almost aligned showing a very small dispersion.

- log $P_{alk/w}$ = f(log $P_{cyc/w}$):

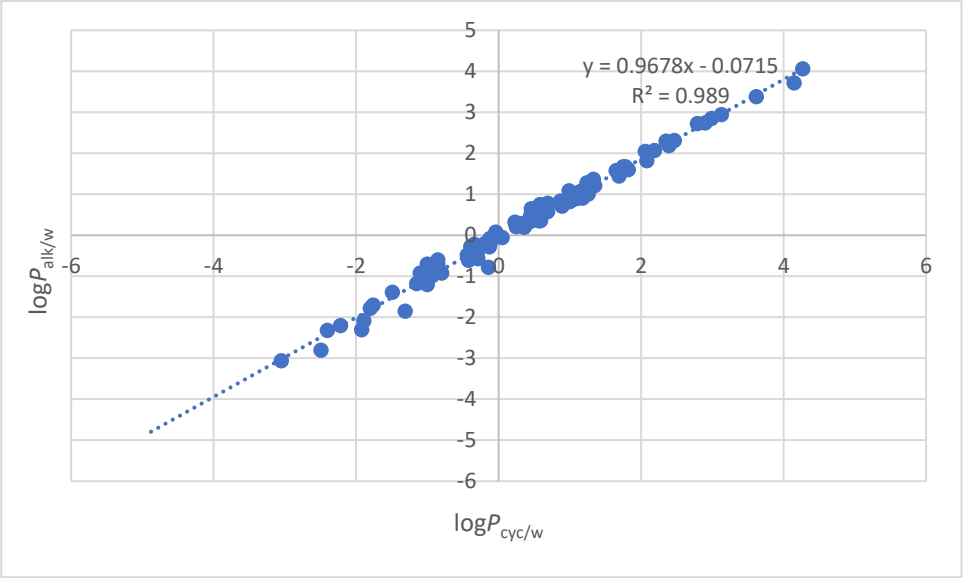

Figure 2.3 Linear relationship between log $P_{alk/w}$ and log $P_{cyc/w}$.

The determination coefficient is $R^2 = 0.989$, meaning that 98.9% of the change on log $P_{cyc/w}$ can be related to the change on log $P_{alk/w}$, which make this also an interesting relation.

Even though a good linear relation has been found, these data need some additional analysis and several approaches should be followed before using them as explained above.

Aiming to predict or approximate the behavior of the value of log *P*, an attempt has been made in the coming graphics to find a potential relationship between the chemical structure of the studied elements and the value of the partition coefficient in the systems. In this way, log *P* has been plotted according to different structural characteristics of a chemical group or families.

Correlation between log *P* and chemical structure.

This aspect is explored from Figures 2.4 to 2.13.

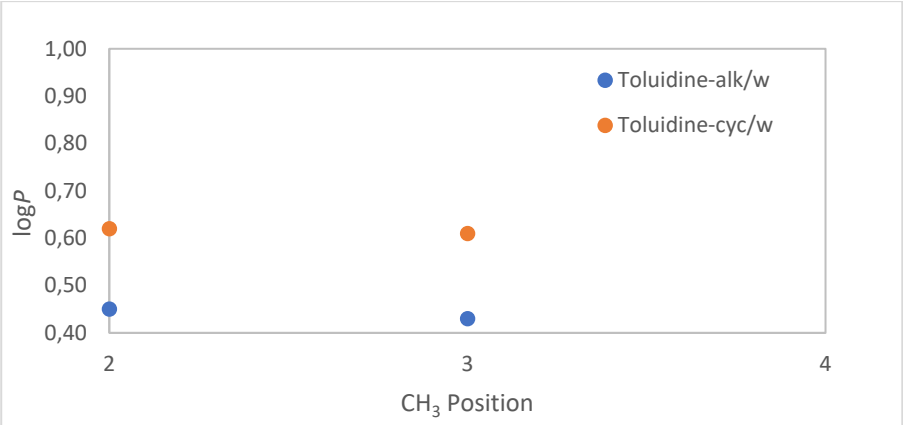

Figure 2.4 Evolution of log *P* of toluidine according to the position of substituents.

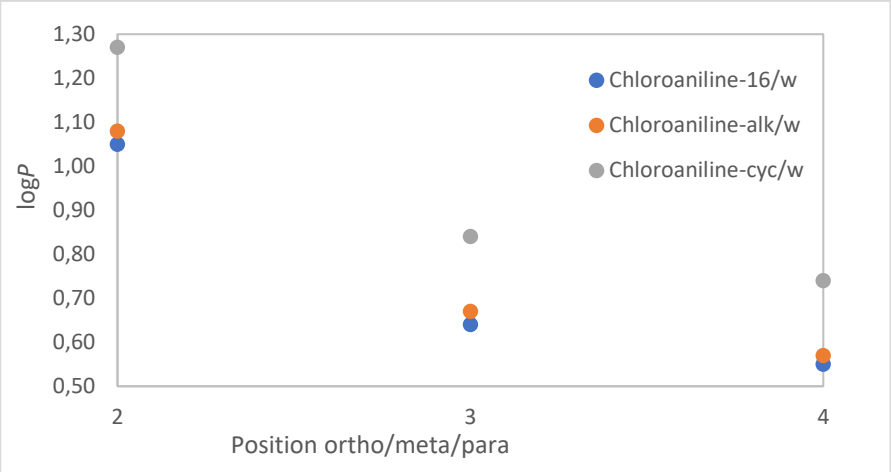

Figure 2.5 Evolution of log *P* of chloroaniline according to the position of substituents.

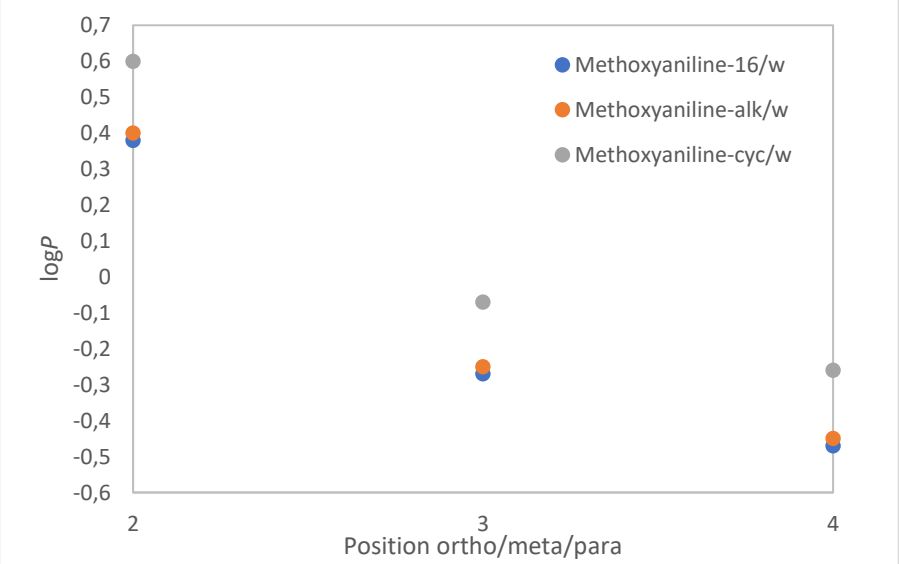

Figure2.6 Evolution of log *P* of methoxyaniline according to the position of substituents.

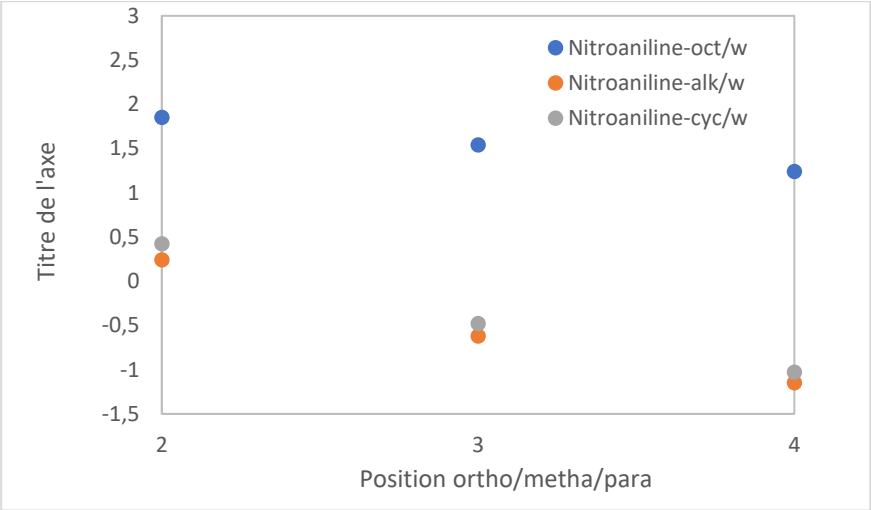

Figure 2.7 Evolution of log *P* of nitroaniline according to the position of substituents.

There is an interesting similarity between Figures 2.4 to 2.10 in which the log *P* of these solutes follow the same behavior for the alkane-water and cyclohexane-water systems. The log *P* is decreasing respectively from the *ortho*, *meta* to *para* disposition for all the compounds.

In the following Figures we will continue to study aniline but with one and then two methyl groups on the functional group. Log *P* is significantly higher for a dimethyl than for a methylaniline.

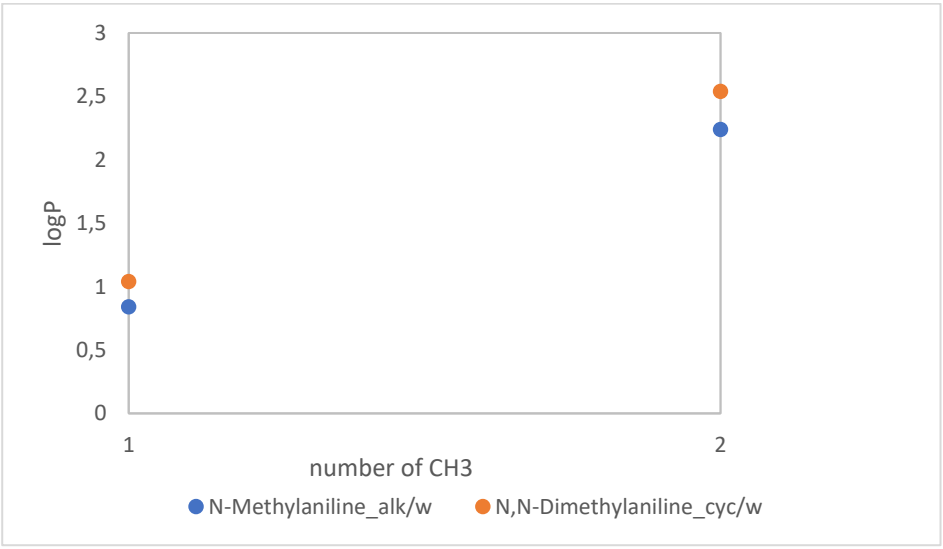

Figure 2.8 Evolution of log *P* according to number of methyl group in aniline.

The following two figures show the variation of log *P* for phenol substituted with halogens (Cl or Br) in the *ortho*, *meta* and *para* positions.

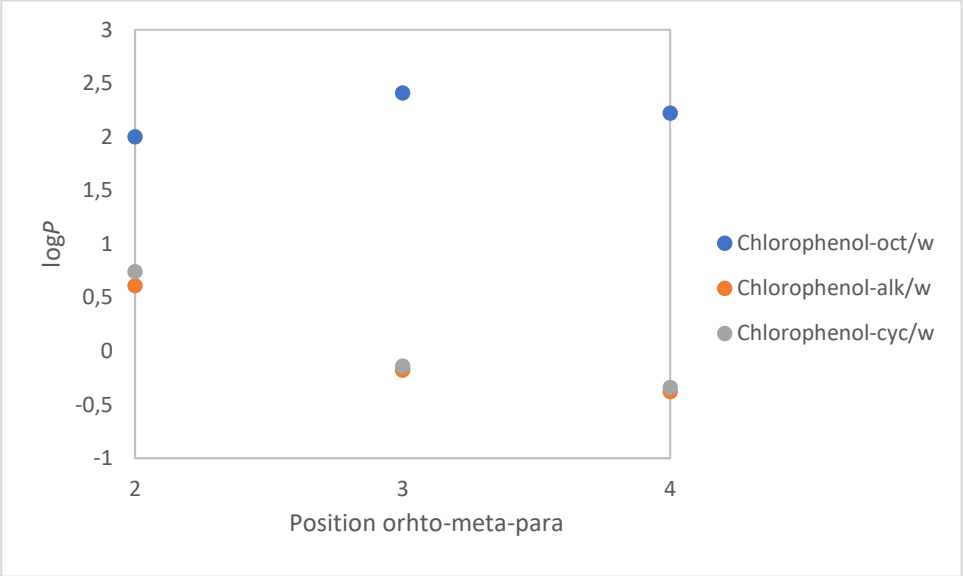

Figure 2.9 Evolution of log *P* of chlorophenol according to the positions of substituents.

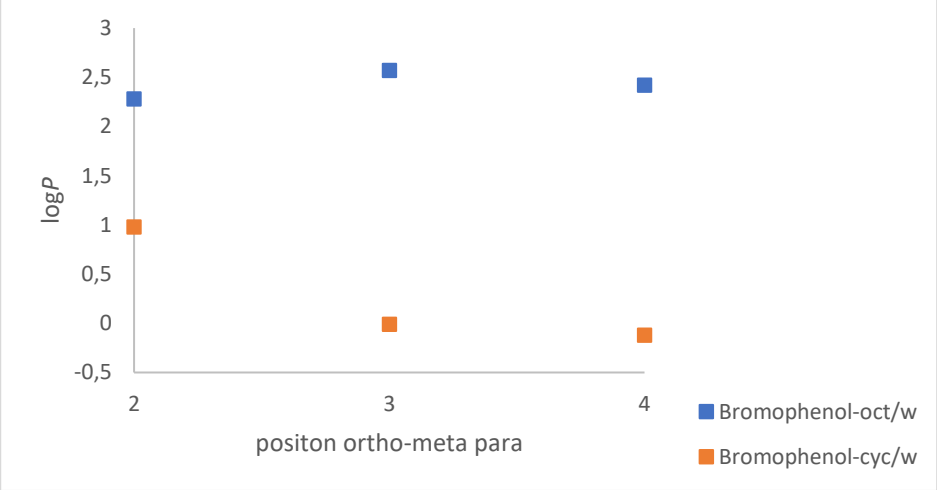

Figure 2.10 Evolution of log *P* of bromophenol according to the positions of substituents.

For phenols, there is a different behavior of log *P* in the octanol-water system on the other hand for cyclohexane-water and alkane-water, log *P* is higher respectively in the *ortho*, *meta* and *para* positions.

Figure 2.11 shows the variation of log $P_{cyc/w}$ of benzaldehyde substituted with a methyl group:

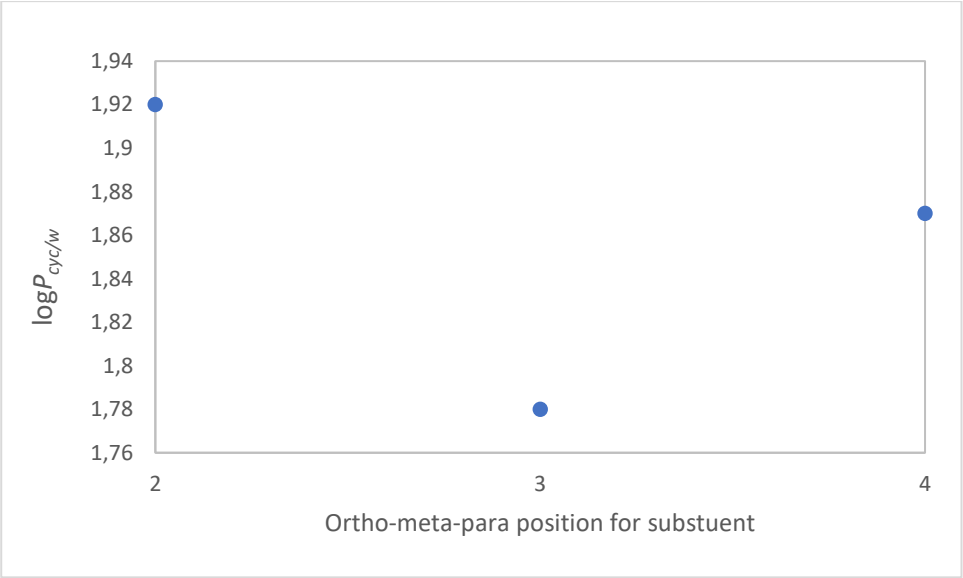

Figure 2.11 Evolution of log $P_{cyc/w}$ of methylbenzaldehyde according to the positions of the substituent.

For methylbenzaldehyde, log *P* position meta < log *P* position para, and only complete data is available for cyclohexane-water partition system.

Tables 2.3 and 2.4 show the collected results for tri and tetrasubstituted phenols, respectively.

Table 2.3 Log $P_{cyc/w}$ and log $P_{alk/w}$ for position isomers of dimethylphenol.

	log $P_{cyc/w}$	**log $P_{alk/w}$**
2,6-Dimethylphenol	0.99	0.81
2,3-Dimethylphenol	0.56	0.42
2,4-Dimethylphenol	0.43	0.29
2,5-Dimethylphenol	0.33	0.21
3,4-Dimethylphenol	0.20	0.08

Table2.4 log $P_{cyc/w}$ of different position isomers of trimethylphenol.

	log $P_{cyc/w}$
2,3,5-Trimethylphenol	0.92
2,4,5-Trimethylphenol	0.86
3,4,5-Trimethylphenol	0.61

According to the data, in general, the value of log *P* is greater when the CH_3 groups are closer to the functional group OH, log *P* decreases with the increase of the distance between CH_3 substituent and the main functional group.

In order to find a correlation between the number of carbons in a family of compounds and its partition coefficient, the log *P* of alcohols or carboxylic acids is presented in Figures 2.12 and 2.13, respectively, according to the length of the hydrocarbon chain. It is easy to conclude that all the partition coefficients increase with the number of carbons in the structure of alcohols or carboxylic acids, and octanol-water partition coefficients are always larger than all the other partition coefficients.

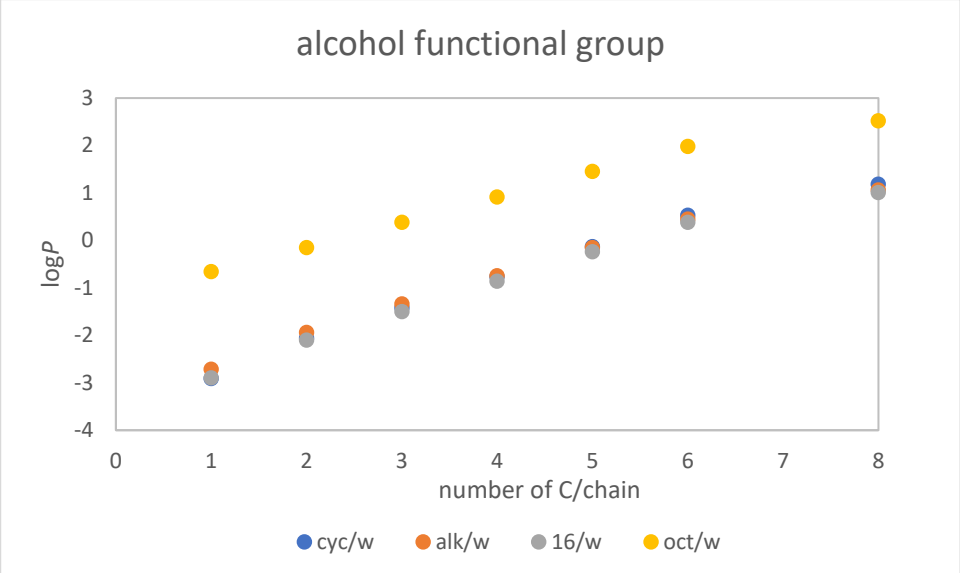

Figure 2.12 Evolution of log *P* according to the number of carbons in the alcohols series.

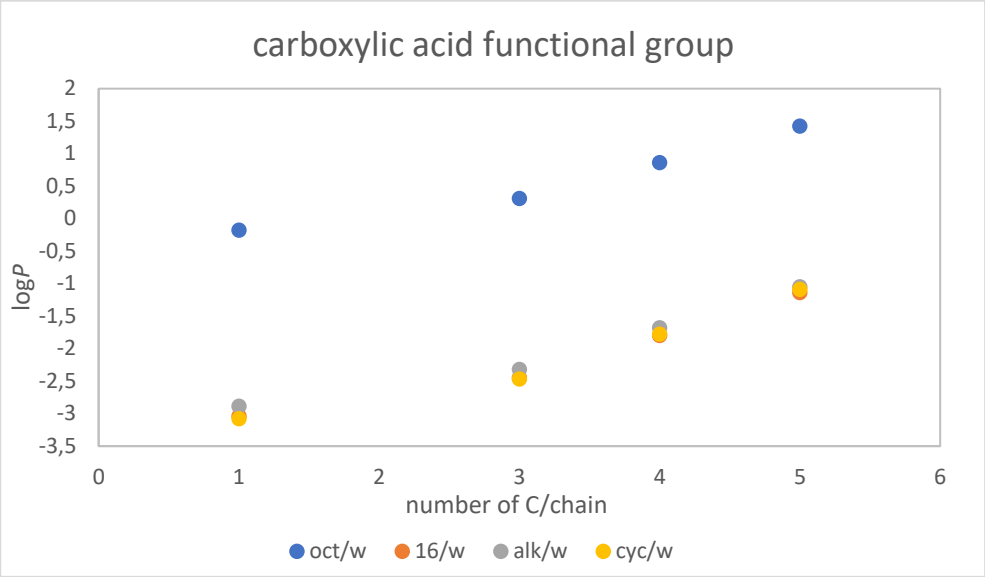

Figure 2.13 Evolution of log *P* according to the number of carbon in the carboxylic acids series.

2.4. Prediction methods

Predicting models are ways to calculate a physical parameter by means of mathematical equations, and correlations with other variables that represent physico-chemical properties such as: charge densities, molecular surface [14], volume and free energy of the molecule we are interested in. The advent, speed and low cost of computing technologies facilitated the development of fundamental quantum mechanical methods of calculating solute–solvent free energy interactions leading to “first principles” estimation methods that require no experimental data, except for validation [12].

Prediction models for partition coefficient are based on an indirect path of calculation. Hence partition coefficient values are calculated from parameters that are related to.

Figure 2.14 illustrates the relation between variables and why it is possible to calculate one from the other. In the following illustration, Mackay and coworker consider octanol as the organic phase and not cyclohexane but it does not affect the accuracy of the reasoning.

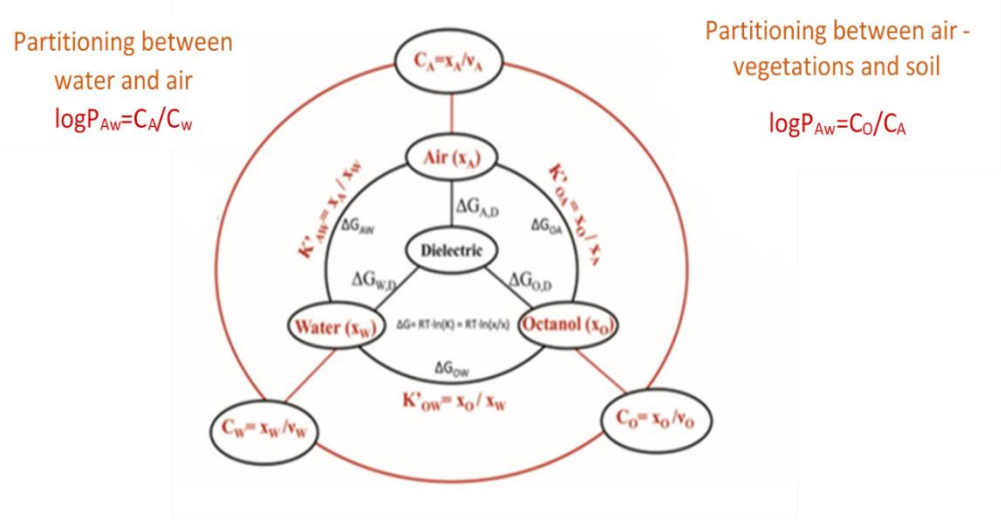

Figure 2.14 A representation showing related variables relevant to environmental partitioning. The central quantities in black font are the fundamental thermodynamic parameters, those in red are the measured physico-chemical properties. Figure adapted from [15].

There are several calculation model such as COSMO-RS [16], MOSCED [16], OPLS-AA [17], among others. Most of these models, or an improved version of them, have been used for the SAMPL5 (Statistical Assessment of the Modeling of Proteins and Ligands) challenge which is a blind predictive challenge and one of the most important works that have been done on the cyclohexane-water partition coefficient until now [18]. This project gave interesting results that helped to figure the relevance of the calculation models that have been used. A brief presentation of two of these models will be given in the following sections.

COSMO-RS (COnductor-like Screening MOdel for Real Solvents)

Principle

Conventionally, the non-interacting gas phase state of the molecule was used as a thermodynamic reference state. The COSMO model is based on solvation theory in which quantum-mechanical calculations are used to predict, by energy minimization, the preferred configuration and electron distribution of a molecule within an electrostatically responsive solvation environment. The “COSMO” approach is much closer to a realistic solvation state, so the error in using it as a reference for real solvents is much smaller than that associated with the gas phase. Continuing the same path to get closer to the real state; to determine the relative Gibbs free energies of the actual solvents one needs to regulate the solvent response in the COSMO-RS calculation on the ideal or perfect solvent (i.e infinite dielectric constant). COSMO-RS theory then calculates the relative energy of interaction of the molecule with its

surrounding “solvent” with respect to this ideal COSMO state [19]. That is where the approach gets its name COSMO-Real Solvent [20]. To have a general definition of COSMO-RS: it is a solvation theory based on the determination of solvation energy in a realistic solvent, thermodynamically referenced to a perfect or ideal solvation environment.

Accuracy of the model

We can notice from Figure 2.15that the dispersion of the predicted and experimental values of $P_{oct/w}$ is very small in such a way that the linear line passes through almost all the points. The following graph is adapted from Mackay [15].

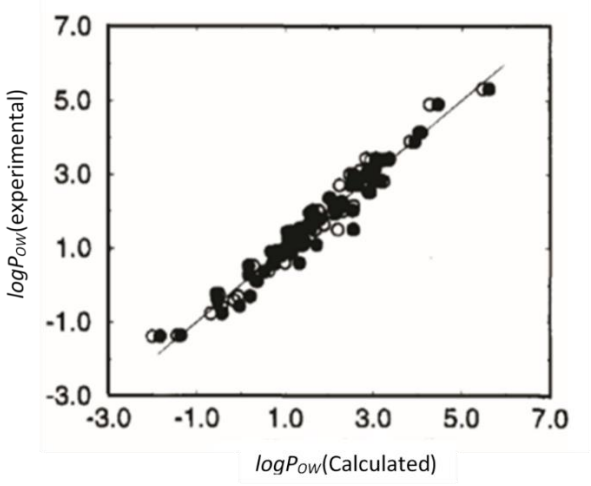

Figure 2.15 Diagram of experimental and COSMO-RS log $P_{oct/w}$ adapted from [15].

MOSCED model (Modified Separation of cohesive Energy Density)

Solubility is an important parameter in the study and the prediction of the partition coefficient. MOSCED (Modified Separation of cohesive Energy Density) [16] is one of several solubility parameter methods used nowadays. With only knowledge of solute chemical structure, the parameters for the MOSCED method can be obtained by performing electronic structure calculations with the SMD continuum solvent model. The specificity of this model is that it can predict infinite dilution activity coefficients as a function of temperature which means that it is not limited to 298 K. In addition, once parameterized, MOSCED calculates the infinite dilution activity coefficient of the solute in the solvent, in addition to the infinite dilution activity coefficient of the solvent in the liquid solute (hypothetical).

Despite the advantages cited above the accuracy of the model has not yet been confirmed since it was used for the SAMPL5 challenge which gave the following data: correlation coefficient = 0.6 ± 0.1 (error), root mean square of 2.8 ± 0.3 [21].

2.5. Selected system and solutes

Despite of the lack of effectiveness of the experimental procedures and the calculation models available, partition coefficients are very important in toxicology and environmental chemistry [22]. Figure 2.16, adapted from a work within our research group [23], explains the importance of partitioning and the capacity of chemicals to contaminate water, soil and air. On the other hand, it highlights the gap between the experimental and calculated values and confirms the weakness of the available data.

The main objective in this work is to implement an experimental methodology to measure the partition coefficients using the following model solutes: toluene and benzoic acid. The experimental methodology developed in this work, will be applied in future work to important compounds such as nitrophenol (and isomers), hydroquinone, p-benzoquinone, catechol, paracetamol, acetamide, acetic acid and oxalic acid. The isooctane-water system will be used for the partition phases. The choice of the partition system was not done randomly; knowing that phenol, hydroquinone, p-benzoquinone, catechol and carboxylic acids are the oxidized intermediates produced by the peroxidation of 2-NP (2-nitrophenol) in aqueous phase considering them in a biphasic mixture of isooctane-water is in fact a simulation of contaminated oily streams.

In addition, considering the importance of knowing the solubility data of the solutes in water to design the partition assays, an experimental methodology to measure solubility data will be also developed.

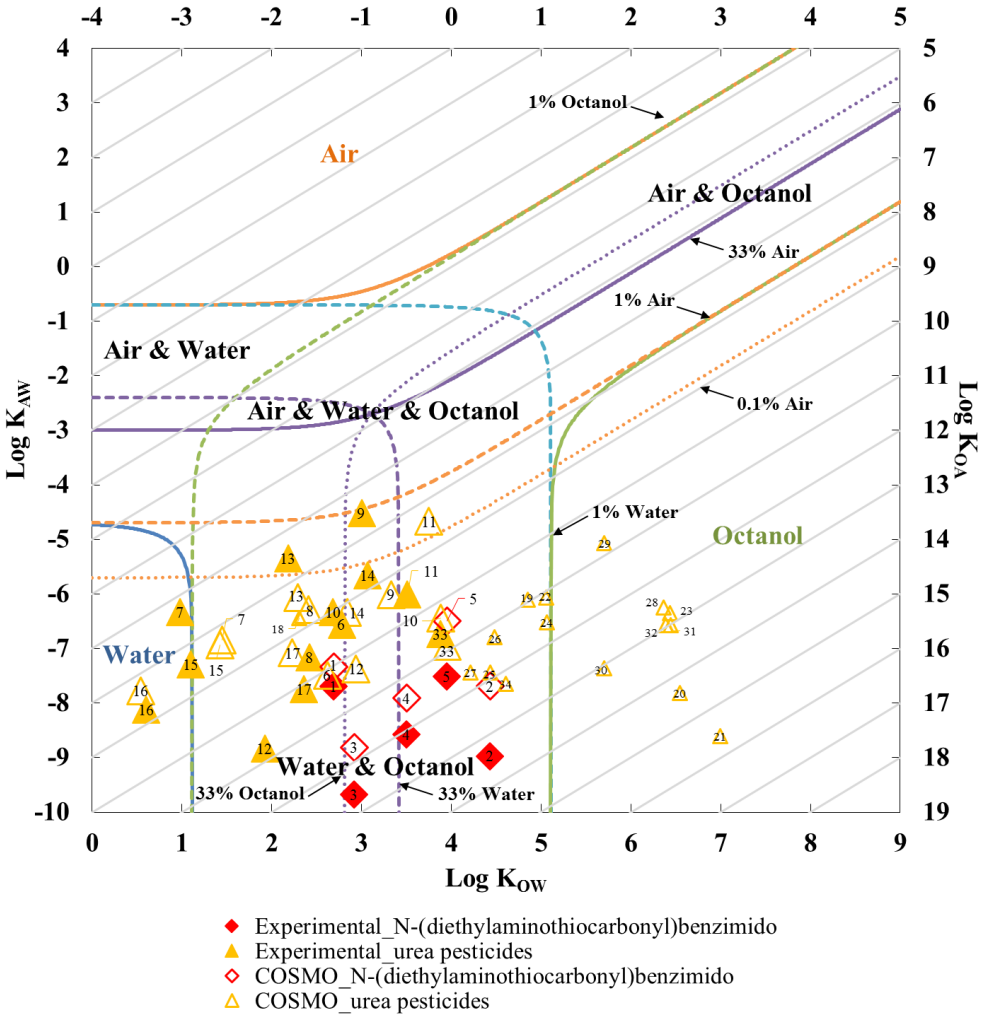

Figure 2.16 Partitioning of N-propylbenzamide and urea based pesticides in water, octanol and air.

Chapter 3. Experimental work

3.1 Materials

All the compounds described in Table 3.1 were used as received, and the solids kept in a desiccator to avoid water contamination. Ultra-pure water (distilled twice) was used for the solubility and the partition coefficient measurements.

Table 3.1 Properties of the compounds used in this work.

Compound	CAS number	Purity (weight fraction)	Supplier
Oxalic acid 2-hydrate	6153-56-6	0.995	Applichem Panreac
Isooctane	540-84-1	0.999	Acros Organics
Toluene	108-88-3	0.9985	Acros Organics
Benzoic Acid	65-85-0	0.99	Acros Organics

3.2 Methodologies

3.2.1 Solubility measurement

The solubility experiments were carried out by the analytical isothermal stirring method using the experimental setup in Figure 3.1. A saturated solution was prepared mixing a small excess of solid solute with about 50 ml of solvent. To reach equilibrium, the solution was continuously stirred for at least 30 hours, and placed in a thermostatic bath at 25 °C (Lauda Instruments, model E20, Ecoline 025). Later, the solution was allowed to settle at least 14 h before sampling [25]. From preliminary experiments, it was found that after equilibrium at 25 ºC, and for temperatures between 30 and 40 ºC, a stirring period of 20 hours followed by a period of 3 hours of settling time was adequate.

Samples (at least 1 ml) of the saturated liquid phase were after collected using plastic syringes coupled with polypropylene filters (0.45 μm), previously heated, in order to avoid any precipitation. The gravimetric method was chosen for the quantitative analysis. Therefore, the samples were placed into pre-weighed glass vessels and immediately weighed (±0.1 mg). The

Figure 3.1 Experimental set-up for the solubility experiments.

next step was to evaporate all the solvent, first under the hood until there is no more liquid then into the oven at 70 °C. The drying procedure was followed by taking weight measurements every 48 h until a constant value was reached.

3.2.2 Partition coefficient measurement

The partition coefficient experiments were carried out by a variant of the shake flask procedure. The methodology developed in this work was divided in three parts as follows:

Part 1: Preparation of the tubes

- Preparation of the mother solution containing the solute (either organic or aqueous, depending on the solubility of the solute in each phase) by weighing exactly the right amount of solute used.

-Transfer the mother solution to the glass tube (Figure 3.2A) with a glass pipette.

- Addition of the other phase to the tubes, with the same volume of the mother solution phase. To minimize the amount of air above the liquid, around 7 ml of each phase were prepared to fill the 15 ml tubes.

Part 2: Establishment of the equilibrium and phase separation

-To help transfer solute from one phase to another, once closed, the tubes were placed in a thermostatic shaker (ThermoMixer C, Eppendorf 15ml) at 25 °C (Figure 3.2B) and with shaking rate of 300 rpm for 6 hours.

- The solutions were allowed to settle one week, at 25 ºC, before the composition analysis of both phases.

A) B)

Figure 3.2 A) Glass graduated tubes; B) Thermostatic and shaking equipment (ThermoMixer C, Eppendorf)

Part3: Chemical analysis of the phases in equilibrium

Preferably, the concentrations of the solute in both phases should be evaluated.

- Considering that the aqueous phase (lower phase) should be sampled by a procedure that minimizes the risk of contamination from the upper phase, first the organic phase was sampled with a glass pipette, and after removed, letting just a thin layer (interface layer). After a syringe with removable needle goes through this layer while expelling air gently [4] to sample the aqueous phase.

Depending on the order of magnitude of the concentrations the analysis were carried out by UV spectrophotometry (model Jasco V-730) or by measuring the refractive index (Abbemat 500, Anton Paar) with a reproducibility within $\pm 1 \times 10^{-5}$. Figure 3.3 shows the refractometer equipment used. Adequate dilutions were made to have proportions fitting those of the calibration curves. Once the analysis was done, the total amount of solute present in both phases could be estimated and compared with the quantity originally introduced, assuming both phases to be totally immiscible.

A calibration curve for toluene in isooctane was prepared in few steps (Appendix B):

- measurement of the refractive index of water (reference substance).

- measurement of the refractive index of the standards. First, the prism was washed with the standard itself to avoid contaminations with previous substances. The filling height should be at least 1 mm above prism surface, which means minimum of 1ml of sample.

-As a final measurement, the water reference value is again checked.

A) B)

Figure 3.3 A) Refractometer, B) prism surface of the refractometer

Calibration curves (Appendix B) were also prepared for UV measurements with a maximum wavelength of 262 nm for toluene and 273 nm for benzoic acid: preparation of six to seven standards covering an adequate range of concentrations, using the mixed solvents water + ethanol (50:50 in weight) or isooctane + ethanol (50:50 in weight).

3.3 Results and discussion

3.3.1 Solubility data

The solubility measured for oxalic acid at 298.2 K was 11.28 ± 0.08 g/100 g of water which is in closer agreement with the values obtained by Hyva et al. [25], Flottman [27] and Apelblat [26] who obtained respectively 12, 16 and 11.12 g/100 g of water.

Our data, presented in Table 3.2, was measured between 298.2 and 313.2 K. The change of the solubility with temperature can be seen in Figure 3.4. Our data can be compared with the data reported by Apelblat [26] who measured the solubility in a wider temperature range. The solubility reaches 47.5 g/100g of water at 338.15 K, showing that temperature has an important effect on the solubility of oxalic acid.

Table 3.2 Solubility of oxalic acid measured in this work as a function of temperature (standard deviation in parenthesis).

Temperature (K)	Solubility (g oxalic acid/100 g of water)
298.2	11.28 (0.08)
303.2	14.03 (0.14)
308.2	16.89 (0.39)
313.2	21.67 (0.11)

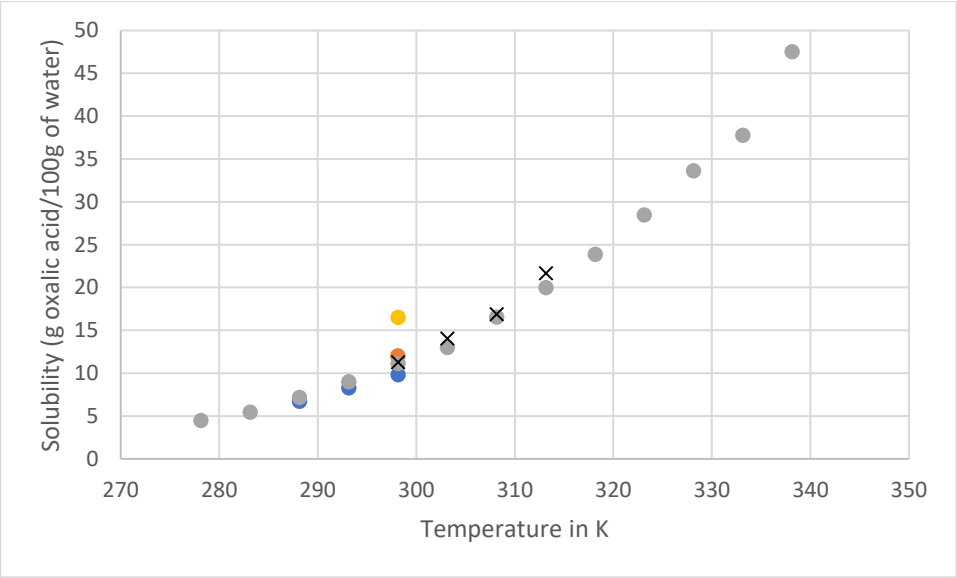

Figure 3.4 Solubility of oxalic acid in water (g/100g of water) as a function of temperature: ● [27], ● [25], ● [28], ● [26], x this work.

3.3.2 Partition coefficients data

3.3.2.1 Partition coefficient of toluene in isooctane/water

The partition coefficient measurements where carried in at least six tubes with the same composition, to assess the precision of the methodology. Considering the low amount of water phase, the very low solubility of toluene in water, and the analytical methods restrictions, a considerable concentration of toluene was dissolved initially in the organic phase (23% w/w).

Table 3.3 presents the average partition coefficient of toluene in isooctane-water system obtained in this work and the corresponding standard deviation.

Table 3.3 Experimental values of log $P_{isooct/w}$ obtained for toluene.

	log $P_{isooct/w}$	
Experiment	**Average value** ± standard deviation	**Individual values**
First round	3.08±0.16	3.13; 3.17; 3.32; 3.00; 2.87; 2.89
Second round	3.24±0,07	3.34; 3.26; 3.18; 3.22; 3.16; 3.30

In preliminary experiments, the mass fractions of toluene in the isooctane phase were determined by UV-Vis spectroscopy which implied a long analytical procedure with several dilutions (dilution factor of 10^3). The partition coefficient obtained was 3.08 with a standard

deviation of 0.16 and the material balance resulted in an average relative error of 24%. To improve the consistency of the material balance results, another method of analysis was adopted for the organic phase measurements. A calibration curve was prepared using a refractometer in an appropriate range of concentrations as described in the previous section. Following this approach, the average value of 3.24 for the partition coefficient was obtained, with 0.07 standard deviation and a material balance with an average error of 3%. This procedure confirms the importance of choosing the adequate analytical methods.

For alkane-water systems, there is a clear lack of partition coefficients data. But a comparison can be done with an approximation of log *P* of toluene in other system with an organic phase almost completely immiscible with water such as cyclohexane [13]. According to Abraham's data [12] log $P_{cyc/w}$ is equal to 2.85, calculated using the ratio of molar concentrations, which can be considered in the same range and in good agreement with log $P_{isooct/w}$ obtained from the experimental work. It should also be emphasized that the value obtained here can be quite different from the value obtained under the conditions of low solute concentration. Therefore, further studies should be carried out by increasing the volume of the water phase compared to the volume of the organic phase, to allow the analytical quantification of the aqueous phase.

3.3.2.2 Distribution ratio of benzoic acid in isooctane/water

For this system, an initial solution of benzoic acid in isooctane (BA mass fraction equal to 0.00527) was prepared for the partition experiments. The value of distribution coefficient obtained from this work is log $D_{isooct/w}$ = 0.26 with a standard deviation of 0.035 which apparently disagrees with Abraham's value [12] for the partition coefficient which is -0.71 in cyclohexane-water.

Table 3.4 Experimental values of log $P_{isooct/w}$ obtained for benzoic acid.

log $P_{isooct/w}$	
Average value ± standard deviation	**Individual values**
0.2607±0.03	0.2887/0.2947/0.2462/0.2615/0.2764/0.2656/0.1919

For solvents that are ''almost'' completely immiscible with water, such as alkanes, cyclohexane, hexane, heptane, octane, nonane, decane and most aromatic solvents, as a first approximation the ratio of the solubilities of the solute in the organic phase and in water can

be considered since it will be nearly identical to direct partition [13]. Table 3.5 presents the solubility in weight fraction at 298.15 K of benzoic acid (BA) in different alkanes [29] and their distribution ratios calculated considering the BA solubility in water which is 0.003 in weight fraction at 298.15 K [14].

Table 3.5 Estimated log ($w_{BA,\ in\ alkane}/w_{BA,\ in\ water}$) of BA in several alkanes with water.

	Solubility in weight fraction	log ($w_{BA,\ in\ alkane}/w_{BA,\ in\ water}$)*
Hexane	0.0067	0.35
Heptane	0.0093	0.49
Decane	0.0095	0.50
Cyclohexane	0.007	0.37

*obtained from the ration of weight fractions

We can see that the ratio of solubilities and the partition coefficient available in the literature apparently are not in agreement. It should be noticed that BA is a weak acid that dissociates in water and that is why it is more appropriate to consider a distribution coefficient (D) in this case. Therefore, more experiments should be carried out to obtain the value of the distribution ratio as function of pH and then, estimate the value of log P, using Equation 2.

Chapter 4. Conclusions and future work

Going through the experiments for the implementation of a procedure to measure partition coefficients, one of the most delicate steps was the sampling of the water phase even though we had satisfactory results. An improvement could be achieved by using tubes that can open from below(faucet) which could be interesting an interesting option at least to confirm the current method.

Also, it is highly recommended to avoid big factor dilutions on the volatile compounds (solute or organic solvent), as it can reduce considerably the accuracy of the results. To that problem the refractometer analysis can be considered as a solution since it showed its effectiveness on toluene-isooctane measurements.

For salts and components that react in water, giving rise to different chemical species in solution, the importance of the pH must be considered in these studies since the pH must be known in order to establish then the concentration of each species in solution. In these cases, measurements must be conducted under pH control.

After the partition experiments and going through literature, it is possible to postulate that for partition in organic solvents that are almost totally immiscible with water, the effective partition coefficient and the partition coefficient deduced from solubilities in both phases are approximate values. In this case, parallel analysis of partition coefficient and solubility are very interesting and could give very relevant results.

To continue the assessment of compounds that are important in environmental studies, it is very interesting to consider first a set of experiments to evaluate the time needed to attain equilibria and to change the volumes of each phase.

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Appendix A

Table A.1 Abraham's table of observed and calculated partition coefficient in four systems.

	LogPoct		**LogP16**		**LogPalk**		**LogPcyc**	
	obs	**calc**	**obs**	**calc**	**obs**	**calc**	**obs**	**calc**
Helium	0.28	0.35	0.28	0.39	0.65	0.58	0.46	0.44
Neon	0.28	0.41	0.38	0.46	0.75	0.65	0.58	0.52
Argon	0.74	0.81	0.78	0.93	1.09	1.1	0.99	1.01
Krypton	0.89	1.03	1	1.18	1.28	1.34	1.24	1.27
Xenon	1.28	1.34	1.35	1.55	1.58	1.7	1.65	1.66
Hydrogen			0.52	0.57	0.78	0.75	0.69	0.63
Methane	1.09	1.04	1.14	1.2	1.37	1.36	1.33	1.29
Ethane	1.81	1.58	1.83	1.82	2.05	1.96	2.06	1.94
Propane	2.36	2.11	2.49	2.44	2.73	2.56	2.79	2.59
n-Butane	2.89	2.65	3.13	3.07	3.38	3.16	3.62	3.25
n-Pentane	1.39	3.19	3.87	3.69	4.06	3.77	4.27	3.9
Cyclohexane	3.44	3.38	3.91	3.87	3.72	3.94	4.15	4.13
Trichloromethane	1.97	2.1	1.69	1.68			1.74	1.83
Trichloroethene	2.42	2.52	2.68	2.58			2.86	2.79
Diethyl ether	0.89	1.08	0.85	0.76			0.66	0.92
Propanone	-0.24	-0.16	-1.09	-1.03	-0.91	-0.92	-0.96	-0.95
Butanone	0.29	0.3	-0.43	-0.37	-0.26	-0.28	-0.25	-0.25
Pentan-2-one	0.91	0.85	0.18	0.27	0.34	0.35	0.44	0.42
Hexan-2-one	1.38	1.38	0.85	0.9	1	0.94	1.12	1.07
Hexan-3-one							1.27	1.1
Heptan-2-one	1.98	1.91	1.53	1.57	1.67	1.54	1.78	1.71
Methyl acetate	0.18	0.25	-0.39	-0.36	-0.2	-0.26	-0.19	-0.26
Ethyl acetate	0.73	0.79	0.15	0.28	0.29	0.36	0.34	0.4
n-Propyl acetate	1.24	1.34	0.77	0.92	0.9	0.99	1.1	1.08
n-Butyl acetate	1.78	1.86	1.41	1.53	1.67	1.57	1.75	1.72
n-Pentyl acetate	2.3	2.39	2	2.15	2.19	2.17	2.39	2.37
Methyl propanoate	0.82	0.82	0.28	0.32	0.5	0.41	0.57	0.46
Methyl pentanoate	1.96	1.88	1.51	1.56			1.81	1.75
Methyl hexanoate	2.42	2.4	2.04	2.16			2.39	2.38
Acetonitrile	-0.34	-0.29	-1.11	-1.23			-1.46	-1.19
Ethylamine	-0.13	-0.39	-1.62	-1.69	-1.78	-1.54	-1.8	-1.61
n-Propylamine	0.48	0.15	-1.08	-1.07	-0.98	-0.95	-0.98	-0.96
n-Butyl amine	0.97	0.67	-0.49	-0.47	-0.57	-0.36	-0.29	-0.33
Diethyl amine	0.58	0.42	-0.6	-0.52	-0.35	-0.41	-0.34	-0.37
Trimethylamine	0.22	0.04	-0.73	-0.61	-0.48	-0.48	-0.44	-0.46
Triethylamine	1.45	1.27	0.72	0.74	0.91	0.81	1.1	0.97
Nitromethane	-0.35	-0.19	-1.06	-1.09	-0.98	-0.97	-0.93	-1.04
1-Nitropropane	0.87	0.84	0.44	0.33	0.45	0.4	0.53	0.44
Acetamide	-1.26	-1.43	-4.68	-4.7			-4.88	-4.77
Propanamide							-4.07	-4.17
N-butylacetamide							-1.9	-2

N.N-Dimethylformamide	-1.01	-1.18	-2.35	-2.51			-2.71	-2.47
Acetic acid	-0.17	-0.18	-3.16	-3.04	-3.06	-2.89	-3.05	-3.08
Propanoic acid	0.33	0.31	-2.45	-2.45	-2.32	-2.32	-2.4	-2.47
Butanoic acid	0.79	0.86	-1.83	-1.8	-1.7	-1.68	-1.76	-1.78
Pentanoic acid	1.39	1.42	-1.14	-1.14	-0.92	-1.05	-1.1	-1.09
Methanol	-0.74	-0.66	-2.77	-2.9	-2.8	-2.72	-2.49	-2.91
Ethanol	-0.3	-0.15	-2.19	-2.1	-2.08	-1.94	-1.89	-2.06
Propan-1-ol	0.25	0.38	-1.53	-1.5	-1.39	-1.34	-1.49	-1.42
Butan-1-ol	0.88	0.91	-0.86	-0.86	-0.82	-0.75	-0.87	-0.77
2-Methylpropan-2-ol	0.76	0.94	-0.89	-0.82	-0.6	-0.7	-0.85	-0.73
Butan-2-ol	0.61	0.69	-1.05	-1.01	-0.8	-0.9	-0.96	-0.92
2-Methypropan-2-ol	0.35	0.6	-1.32	-1.07	-1.18	-0.94	-1.15	-0.96
Pentan-1-ol	1.56	1.45	-0.24	-0.24	-0.28	-0.15	-0.26	-0.13
Pentan-2-ol	1.19	1.22	-0.38	-0.4	-0.27	-0.31	-0.39	-0.28
Hexan-1-ol	2.03	1.98	0.38	0.38	0.48	0.45	0.45	0.53
Hexan-2-ol	1.76	1.75			0.32	0.29	0.23	0.37
Heptan-1-ol	2.72	2.52	1.03	1.01	1.04	1.06	1.12	1.18
2.2.2-Trifluoroethanol	0.41	0.53	-1.93	-1.9			-2.04	-1.95
Hexafluoroethanol	1.66	1.71	-1.37	-1.11			-1.46	-1.19
Methylthiol	0.65	0.69					0.93	0.59
n-Propylthiol	1.81	1.76	1.91	1.69			1.52	1.89
Methyl ethyl sulfide	1.54	1.59					1.73	1.64
Trimethyl phosphate	-0.78	-0.77			-2.2	-2.12	-2.22	-2.08
Triethyl phosphate	0.8	0.68			-0.78	-0.51	-0.14	-0.33
Tri-n-propyl phosphate	1.87	1.95			0.91	0.83	1.18	1.15
Tri-n-butyl phosphate					2.74	2.49	2.9	2.96
Benzene	2.13	2.13	2.15	2.15	2.3	2.21	2.35	2.36
Toluene	2.73	2.66	2.68	2.76	2.85	2.81	2.99	3.01
Chlorobenzene	2.89	2.76	2.84	2.89	2.95	2.93	3.13	3.14
Methyl phenyl ether	2.11	2.19	2.09	2	2.07	2.03	2.19	2.24
Ethyl phenyl ether	2.51	2.66	2.61	2.54			2.77	2.81
Benzaldehyde	1.48	1.47	1.06	0.99	1.05	1.02	1.13	1.21
2-Methylbenzaldehyde	2.09	2.05					1.86	1.92
3-Methylbenzaldehyde							1.8	1.78
4-Methylbenzaldehyde		2.09	2.02		1.6	1.63	1.82	1.87
Acetophenone	1.58	1.69	1.14	1.16	1.11	1.17	1.27	1.4
Ethyl phenyl ketone	2.19	2.18					2.02	2
Benzophenone	3.18	3.24					3.29	3.19
Methyl benzoate	2.12	2.1	1.56	1.72	1.82	1.73	2.08	1.98
Ethylphenyl acetate							2.4	2.42
Benzonitrile	1.56	1.51			0.95	1.07	1.11	1.24
Phenylacetonitrile	1.56	1.6					1.31	1.24
Aniline					-0.05	-0.08	0.05	0.04
o-Toluidine			0.36	0.4	0.52	0.51	0.61	0.69
m-Toluidine					0.45	0.45	0.58	0.62
p-Toluidine					0.39	0.43	0.56	0.61

2.6-Dimethylaniline					1.21	1.23	1.35	1.47
2-Chloroaniline			1.07	1.05	1.09	1.08	1.17	1.27
3-Chloroaniline			0.64	0.64	0.71	0.67	0.89	0.84
4-Chloroaniline			0.56	0.55	0.58	0.57	0.69	0.74
2-Methoxyaniline			0.33	0.38	0.39	0.4	0.52	0.6
3-Methoxyaniline			-0.33	-0.27	-0.28	-0.25	-0.13	-0.07
4-Methoxyaniline			-0.54	-0.47	-0.54	-0.45	-0.38	-0.26
2-Nitroaniline	1.85	1.85	0.22	0.23	0.2	0.24	0.36	0.42
3-Nitroaniline	1.37	1.54			-0.61	-0.62	-0.42	-0.48
4-Nitroaniline	1.39	1.24			-1.2	-1.15	-1	-1.03
N-Methylaniline					1.04	0.84	1.18	1.04
N.N-Dimethylaniline			2.17	2.24	2.31	2.24	2.47	2.54
1-Naphtylamine					1.15	0.91	1.26	1.26
2-Naphthylamine					1.06	0.9	1.21	1.25
Benzylamine	1.09	0.79			-0.21	-0.36	-0.12	-0.18
Nitrobenzene	1.85	1.84	1.54	1.46	1.45	1.48	1.69	1.68
Benzamide	0.64	0.47			-2.3	-2.34	-1.92	-2.28
Acetanilide	1.16	1.04			-1.85	-1.69	-1.31	-1.59
Benzoic acid	1.87	1.74			-0.71	-0.74	-0.85	-0.7
Phenol	1.46	1.54	-1.08	-0.98	-0.92	-0.9	-0.8	-0.89
o-Cresol	1.98	2.13	-0.09	0	0.08	0.06	-0.04	0.14
m-Cresol	1.98	1.96			-0.22	-0.36	-0.34	-0.3
p-Cresol	1.97	2.07	-0.19	-0.26	-0.3	-0.2	-0.35	-0.13
2.3-Dimethylphenol					0.43	0.42	0.51	0.56
2.4-Dimethylphenol	2.3	2.42			0.36	0.29	0.59	0.43
2.5-Dimethylphenol					0.43	0.37	0.57	0.5
2.6-Dimethylphenol					0.82	0.81	1	0.99
3.4-Dimethylphenol					0.21	0.08	0.25	0.2
3.5-Dimethylphenol					0.26	0.21	0.38	0.33
2-Ethylphenol	2.47	2.44					0.36	0.48
4-Ethylphenol	2.58	2.39			0.24	0.17	0.37	0.29
2.3.5-Trimethylphenol							0.97	0.92
2.4.5-Trimethylphenol							0.94	0.86
3.4.5-Trimethylphenol							0.63	0.61
2-Propylphenol	2.93	2.95					1.18	1.1
3-Propylphenol							0.83	0.89
4-Propylphenol					0.86	0.76	1.03	0.92
4-Butylphenol	3.56	2.87					0.77	0.86
4-Butylphenol	3.56	3.45					1.48	1.57
4-Phenylphenol							0.98	0.95
2-Fluorophenol	1.71	1.88			-0.43	-0.43	-0.3	-0.42
3-Fluorophenol	1.93	1.89					-0.7	-0.74
4-Fluorophenol	1.77	1.69			-0.7	-0.81	-1	-0.83
2-Chlorophenol	2.15	2			0.84	0.61	0.87	0.74
3-Chlorophenol	2.5	2.41			-0.08	-0.18	-0.12	-0.14
4-Chlorophenol	2.4	2.22	-0.76	-0.43	-0.39	-0.38	-0.35	-0.34

2-Bromophenol	2.35	2.28			1.05	0.81	1.16	0.98
3-Bromophenol	2.63	2.57					-0.06	-0.01
4-Bromophenol	2.59	2.42	-0.1	-0.24	-0.08	-0.2	-0.09	-0.12
2-Iodophenol	2.65	2.54			1.01	0.84	1.26	1.08
4-Iodophenol	2.91	2.85			0.35	0.23	0.57	0.39
2-Methoxyphenol	1.32	1.53			0.36	0.22	0.47	0.38
2-Cyanophenol							-1.7	-1.52
4-Cyanophenol	1.6	1.47	-2.04	-2.03			-2.14	-2.01
Indole							0.79	0.82
Pyrazole							-2.91	-2.85
Imidazole							-3.7	-3.69
N-Methylimidazole							-2.16	-1.96

Appendix B. Calibration curves

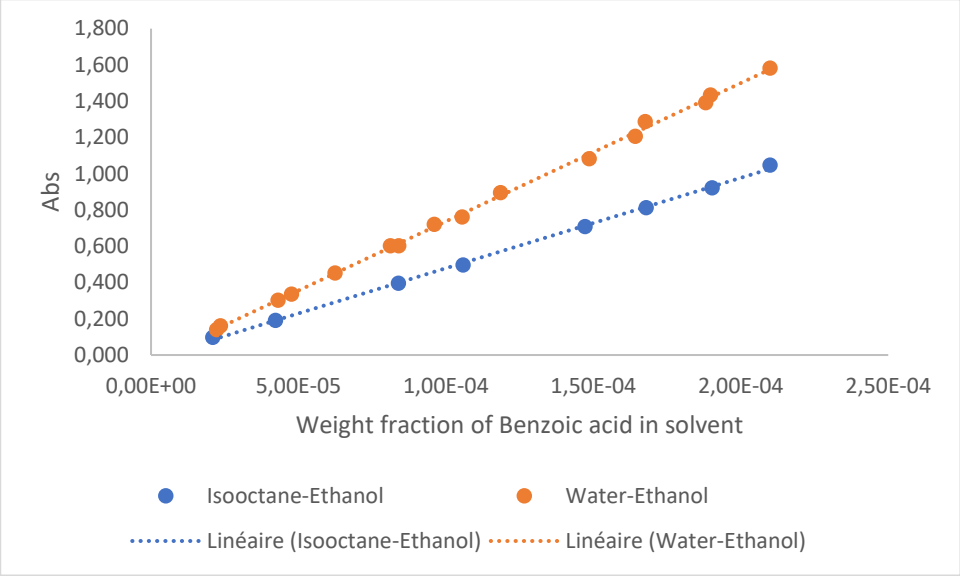

Figure B.1 Calibration curves of benzoic acid in water and isooctane.

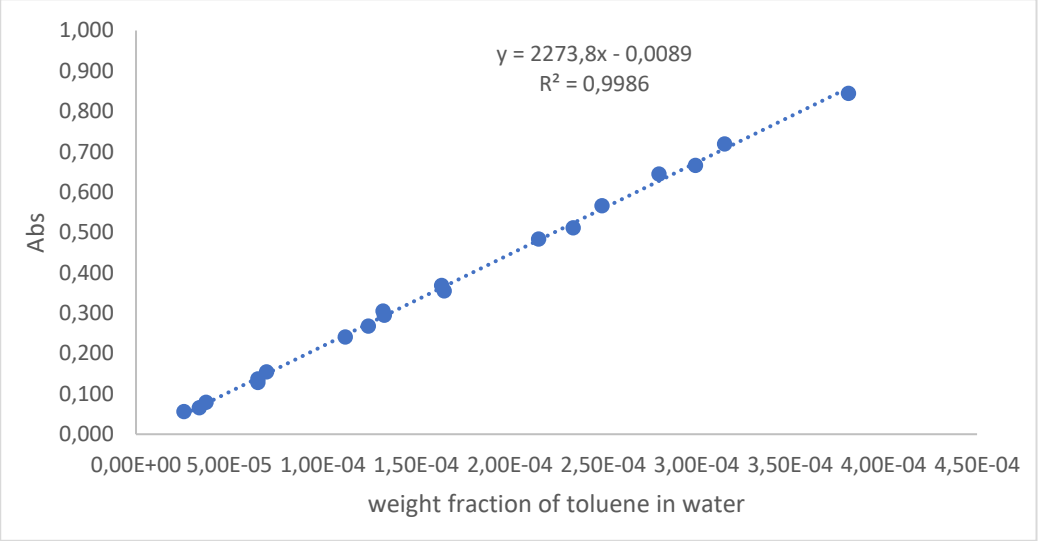

Figure B.2 Calibration curve of toluene in water.

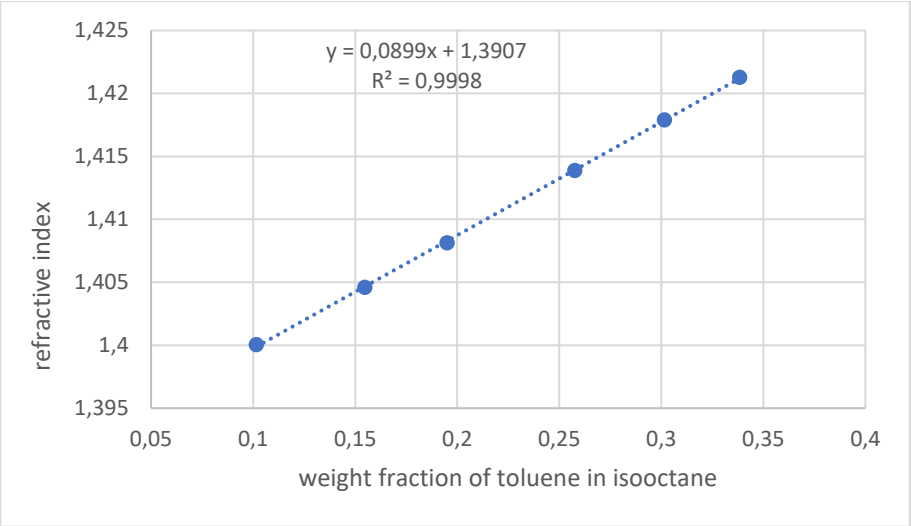

Figure B.3 Calibration curve of toluene in isooctane.