

**SPECTROPHOTOMETRIC MEASUREMENTS
AND THEIR UNCERTAINTY IN CHEMICAL
ANALYSIS AND DISSOCIATION CONSTANT
MEASUREMENTS**

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LIST OF ORIGINAL PUBLICATIONS

The thesis consists of four articles listed below and a review. The articles are referred in the text by Roman numerals I–IV. The review summarizes and supplements the articles.

- I. Uncertainty Sources in UV-Vis Spectrophotometric Measurement. Sooväli, L.; Rõõm, E.-I.; Kütt, A.; Kaljurand, I.; Leito, I. *Accred. Qual. Assur.*, **2006**, DOI: 10.1007/s00769-006-0124-x
- II. Uncertainty in Photometric Analysis: A Case Study. Traks, J.; Sooväli, L.; Leito, I. *Accred. Qual. Assur.*, **2005**, *10*, 197-207, DOI: 10.1007/s00769-005-0909-3
- III. Uncertainty Estimation in Measurement of pK_a Values in Nonaqueous Media: A Case Study on Basicity Scale in Acetonitrile Medium. Sooväli, L.; Kaljurand, I.; Kütt, A.; Leito, I. *Anal. Chim. Acta*, **2006**, *566*, 290-303, DOI:10.1016/j.aca.2006.03.020
- IV. Basicity of some P_1 Phosphazenes in Water and in Aqueous Surfactant Solution. Sooväli, L., Rodima, T.; Kaljurand, I.; Kütt, A.; Koppel, I. A., Leito, I. *Organic and Biomolecular Chemistry*, **2006**, DOI: 10.1039/b602797k

Author's contribution

Paper I: Main person responsible for performing the calculations and writing the paper. Performed around half of the experiments.

Paper II: Responsible for performing calculations. Helped to prepare the manuscript.

Paper III: Main person responsible for planning and writing. Performed all calculations.

Paper IV: Main person responsible for planning and writing. Performed all experimental work and calculations.

ABBREVIATIONS

AN	acetonitrile
AN	acceptor number
CG	calibration graph
<i>D</i>	dielectric constant
DBU	2,3,4,6,7,8,9,10-Octahydropyrimidol[1,2- <i>a</i>]azepine (1,8diazabicyclo[5.4.0]undec-7-ene)
dma	N,N-dimethylamino
DMSO	dimethylsulphoxide
<i>DN</i>	donor number
EPA	electron pair acceptor
EPD	electron pair donor
GUM	The Guide to the Expression of Uncertainty in Measurement
HPLC	high performance liquid chromatography
ISO	International Organization for Standardization
MTBD	1,3,4,6,7,8-Hexahydro-1-methyl-2H-pyrimido[1,2- <i>a</i>]pyrimidine (7-Methyl-1,5,7-triazabicyclo[4.4.0]dec-5-ene)
nm	nanometer
OEP	2,3,7,8,12,13,17,18-octaethylporphyrin
PhTMG	N,N,N,N-tertamethyl-N''-phenylguanidine
Pyrr	N-pyrrolidino
R' _n P _n (R'')	phosphazene (iminophosphorane)
SA	standard addition
TBD	1,3,4,6,7,8-hexahydro-2H-pyrimido[1,2- <i>a</i>]pyrimidine (1,5,7-triazabicyclo[4.4.0]dec-5-ene)
TCPP	5,10,15,20-tetrakis(4-chlorophenyl)porphyrin
THF	tetrahydrofuran
TLC	thin layer chromatography
TMP	5,10,15,20-tetramesitylporphyrin
TPP	5,10,15,20-tertaphenylporphyrin
UV	ultraviolet
Vis	visible

INTRODUCTION

Ultraviolet-visible spectrophotometry can be applied to a wide range of measurements – analytical determinations, measurements of equilibrium and rate constants of reactions, studies of solubility, etc. Analytical determinations can be carried out in a wide range of sample matrices, e.g. biochemical, pharmaceutical, clinical and toxicological.^{1,2} This is the reason why spectrophotometry is an instrumental method continuously finding wide application both in routine analysis laboratories and in the field of research. For example, many analytical determinations, including some of the most common (e.g. nitrite³, phosphorus⁴, ammonium⁵) are routinely and in some cases dominantly carried out spectrophotometrically. Spectrophotometry is also extensively used in physico-chemical measurements, in particular determining the acid-base properties of different types of compounds.⁶⁻¹⁰ The instrumentation is relatively inexpensive and very rugged. Although atomic spectrometry methods (for elemental analytes) and chromatographic methods (for organic analytes) are competing with spectrophotometry, it is not to be expected that UV-Vis spectrophotometry will cease to be used in chemical analysis.

After performing the measurements and calculation of the results, the reliability of the latter has to be estimated. Uncertainty estimation of chemical analysis results is becoming more and more of a standard requirement. Results without an uncertainty estimate cannot be considered complete.^{11,12} In the spectrophotometric analysis there are two different groups of uncertainty sources, one of them is denoted as the intrinsic or physical uncertainty sources originating from the spectrophotometer as a measuring device. The uncertainty sources belonging to the other group – the chemical uncertainty sources – originate from the object under study.

Usually it is a common practice that calculating the uncertainty can be far more complicated and time-consuming than the simple calculation of the analysis result.^{11,13}

In the uncertainty estimation clear distinction has to be made between fundamental science measurements and those made at routine analytical laboratories. In a research laboratory it is often possible to devote time for careful study of the uncertainty sources and devise ways for correcting the effects affecting the uncertainty – first of all the systematic effects. This is also the recommended practice in the leading guidance documents.^{12,13} A routine laboratory, however, has usually very limited resources for such studies and most of the systematic effects have to be included in the uncertainty budget.

As stated above, besides simple photometric and spectrophotometric analytical measurements there are some other applications of spectrophotometry – this technique has been used for extensive acidity and basicity measurements in

water,^{7,8,10,IV} AN^{9,14–20} THF^{21–23} and some acid-base data is available for heptane.^{24,25}

The goal of this work was to investigate reliability of spectrophotometric technique, in particular, the sources of uncertainty, provide convenient means for evaluating the sources of uncertainty, to present uncertainty estimation (based on the ISO GUM method) procedures for both a simple photometric analysis and for a complex spectrophotometric scientific experiment and to describe some practical applications of spectrophotometry on examples of determination of pK_a values of organic compounds. It is demonstrated, that the ISO GUM uncertainty estimation method can be applied both to chemical analysis and to pK_a measurement. In the latter case some conceptual difficulties are outlined and the possibilities to overcome these are suggested.

1. CONCEPTS

1.1. The Main Concepts of Uncertainty

1.1.1. The ISO GUM Method

According to the ISO GUM (The Guide to the Expression of Uncertainty in Measurement)¹², the *uncertainty* is a parameter associated with the result of measurement that characterizes the dispersion of the values that could be reasonably be attributed to the measurand.

Usually a measurand Y is not measured directly but is determined from N other quantities X_1, X_2, \dots, X_N through a functional relationship f :

$$Y = f(X_1, X_2, \dots, X_N) \quad (1)$$

The *input quantities* X_1, X_2, \dots, X_N upon which the *output quantity* Y depends may themselves be viewed as measurands and may themselves depend on other quantities.

The uncertainty of the result of a measurement expressed as a standard deviation is called *standard uncertainty*.

The *combined standard uncertainty* $u_c(y)$ is the standard uncertainty of the result of a measurement (output quantity) when that result is obtained from the values of a number of other quantities (input quantities) and it is equal to the positive square root of a sum of squares of standard uncertainties of input quantities.

In the case of uncorrelated input quantities x_i the combined standard uncertainty $u_c(y)$ of a value y is calculated using the following equation:

$$u_c(y) = \sqrt{\left(\frac{\partial y}{\partial x_1} u(x_1)\right)^2 + \left(\frac{\partial y}{\partial x_2} u(x_2)\right)^2 + \dots + \left(\frac{\partial y}{\partial x_i} u(x_i)\right)^2} \quad (2)$$

where $\partial y / \partial x_i$ is the partial differential of y with respect to x_i . This equation can be used if all the input quantities are statistically independent. In some cases there might be many input quantities used for the calculation and some of these may be correlated. According to the ISO GUM method it is possible to find the combined uncertainty $u_c(y)$ of value y and take the correlation into account by using the following equation:

$$u_c(y) = \sqrt{\sum_{i=1}^n \left(\frac{\partial(y)}{\partial x_i} u(x_i)\right)^2 + 2 \cdot \sum_{i=1}^{n-1} \sum_{j=i+1}^n r(x_i, x_j) \cdot \frac{\partial(y)}{\partial x_i} u(x_i) \cdot \frac{\partial(y)}{\partial x_j} u(x_j)} \quad (3)$$

The second term under the square root is introduced for taking into account the correlation between the input quantities x_i and x_j . $r(x_i, x_j)$ is the correlation coefficient of the correlating quantities.

The *expanded uncertainty* U is obtained by multiplying the combined uncertainty $u_c(y)$ by a *coverage factor* k :

$$U = k u_c(y) \quad (4)$$

The result of a measurement is then conveniently expressed as $Y = y \pm U$, which means that the best estimate of the value attributable to the measurand Y is y and that $y - U$ to $y + U$ is an interval that may be expected to encompass a large fraction of the distribution of values that could be reasonably attributed to Y . This interval is also expressed as $y - U \leq Y \leq y + U$.

The coverage factor k is a numerical factor used as a multiplier of the combined standard uncertainty in order to obtain expanded uncertainty. The value of coverage factor is chosen on the basis of the level of confidence required of interval $y - U$ to $y + U$. In general, k will be in the range 2 to 3. In practice, one can usually assume that taking $k = 1$ produces an interval having a level of confidence of approximately 68%, k values 2 and 3 result in the level of confidence of 95% and 99% respectively.

The uncertainty estimation procedure according to the ISO GUM¹² consists of the following steps:

1. Specifying the measurand and definition of the mathematical model;
2. Identification of the sources of uncertainty;
3. Modification of the model (if necessary);
4. Quantification of the measurand and the uncertainty components;
5. And finally calculation of the combined uncertainty.

Before the combined uncertainty is calculated, all uncertainty components are converted to the level of standard uncertainty. In this work we assume that the B-type uncertainties for which no information on distribution function is available and which are expressed with the “ \pm ” sign have rectangular (uniform) distribution.^{12,13} In order to convert them to standard uncertainties they are divided by $\sqrt{3}$.^{12,13}

1.1.2. The Nordtest Method

The Nordtest method²⁶ is another method for uncertainty estimation besides the ISO GUM and is finding increasingly wide application. The ISO GUM method presented in previous section presumes careful investigation of possible uncertainty sources before they are quantified and introduced to the uncertainty budget. The time-consuming in-depth study of the uncertainty sources can be carried out in research laboratories and in laboratories specialized in metrology. The routine analysis laboratories usually are less detailed when inspecting the uncertainty sources.

The Nordtest method can be seen as a borderline case of the ISO GUM method. The uncertainty sources are divided into two major groups: the first

group consists of the uncertainty introduced by the reproducibility within laboratory and the second group consists of the method and laboratory bias estimated by analysis of certified reference materials and/or by the results of interlaboratory comparison measurements. These two groups are then used for uncertainty estimation, the estimation procedure is based on statistical analysis.²⁶

The advantage of the Nordtest method is that less detailed information is needed on the analysis method used if compared to the ISO GUM method and thus the possibility of underestimation of the uncertainty is smaller. On the other hand, the mathematical basis of the Nordtest method is somewhat less rigorous, the use of this method presumes the existence of extensive statistical data and the method provides limited possibility to perform optimization of the analysis procedure based on the data obtained from this method.

1.2. Acid-base Equilibria in Condensed Media

According to the Brønsted-Lowry theory of acid-base interaction the proton transfer from an acid molecule to a solvent molecule with forming a conjugate base of the acid can be expressed as follows:

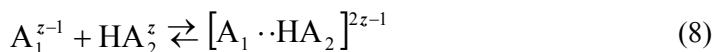


Equation 5 is valid for both neutral ($z = 0$) and cationic ($z = +1$) acids. The dissociation constant K_a of equilibrium in equation 5 expresses as:

$$K_a = \frac{a(\text{HS}^+) \cdot a(\text{A}^{z-1})}{a(\text{HA}^z)}, \quad \text{p}K_a = -\log K_a \quad (6)$$

where a are the activities of the corresponding species. Acid dissociation constant K_a or the corresponding $\text{p}K_a$ value (equation 6) is one of the most important physicochemical characteristics of compounds having acidic or basic properties. Cationic acids are conjugate acids of neutral bases. It is common to express the base strength of a neutral base as the $\text{p}K_a$ value of its cationic conjugate acid (e.g in publication III). Thus “ $\text{p}K_a$ of a base” means in fact the $\text{p}K_a$ value of the conjugate acid (cationic acid, $z = +1$) of the base.

In media of good ability to stabilize and separate ions,²⁷ the situation where the ions are separated to infinity is favored. In media of poor ion separation and stabilization ability these ions tend to aggregate or form conjugate complexes. This separation ability is the dissociating power of the solvent. It is quantitatively described by the dielectric constant D of the solvent. Solvents with large D are called *polar* contrary to the ones with low D which are called apolar or nonpolar. There are two types of important side reactions – homoconjugation (equation 7) and heteroconjugation (equation 8):



It is generally observed that the extent of homoconjugation increases if the number of acidic hydrogen atoms in the molecule increases. Poor steric hindrance of the protonation/deprotonation center is a source of increased homoconjugation.²⁸ The easiest way to decrease the influence of the association process on acid-base equilibria without changing the media, is to use research methods which allow to use very dilute solutions. Ion-pairing is third type of important side reactions, extent of ion-pairing depends on the solvent properties, size of ions and charge distribution in ions, hydrogen bonding and specific solvation possibility. The general trend is that small ions tend to form solvent-separated ion pairs (equation 9) while large ions with delocalized charge tend to form contact ion pairs (equation 10):

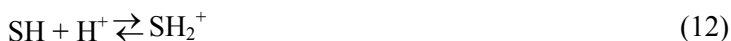


The increase in ion-solvating and ions separating power of the solvent favors formation of the solvent separated ion-pairs. One should also consider the probability of formation of bigger aggregates.

Several medium properties also have an important influence on acid-base reactions in condensed media. The character and extent of the results (solubility, ionization, dissociation, aggregation etc) depends on interactions between solvent and solutes. The important properties from solutes point of view in terms of these interactions are charge and its location, polarity, polarizability and mole ratio (concentration).²⁷

One of the most important properties in acid-base chemistry is the ionizing power of the solvent. It depends mainly on solvents ability to be an electron pair donor (EPD) or electron pair acceptor (EPA). This property is empirically described with donor number (*DN*) and acceptor number (*AN*). Higher values of these mean that solvent has higher ability to ionize neutral ionogen molecules and to stabilize the ions formed.

Brønsted acid-base properties of solvent also have influence on the strengths of acids and bases. These properties are the special case of EPD-EPA properties. Solvent's ability to donate (equation 11) or accept (equation 12) a proton characterizes these properties:



A sum of these two equations is quantitatively described by the autoprotolysis constant K_{auto} of the solvent:



$$K_{\text{auto}} = a(\text{S}^-) a(\text{SH}_2^+), \text{p}K_{\text{auto}} = -\log(K_{\text{auto}}) \quad (14)$$

The $\text{p}K_{\text{auto}}$ of the solvent determines how many orders of magnitude can an acidity scale range in the solvent. $\text{p}K_{\text{auto}}$ is one of the most important characteristics of the solvent. If the solvent under consideration has no protons or the reaction described by equation 11 is unfavored, the solvent is called *aprotic*, if there exists the acid dissociation of solvent, the solvent is called *protic*. In *amphiprotic* solvents both the donation (equation 11) and accepting (equation 12) are present. Low acid-base properties make the solvent good *differentiating* solvent. If the solvent is significantly acidic or basic, it is *leveling* solvent for basic and acidic solutes. As the hydrogen bonding plays an important role in the interactions between ions and solvents, the protic solvents tend to solvate anions better and aprotic solvents tend to solvate cations better.

The combination of three solvent properties – ionizing power, dissociative power and the acid-base properties of solute molecules determine the suitability of solvent for acid-base studies for different compounds. It can be concluded that the most suitable solvent for acid-base studies has very low acid-base properties but it should be relatively polar to separate and stabilize ions.

1.3. Spectrophotometry

Ultraviolet-visible (UV-vis) spectrophotometry is primarily a quantitative analytical technique concerned with the absorption of near-ultraviolet (180–390 nm) or visible (390–780 nm) radiation by molecules.¹ These regions of electromagnetic spectrum provide energy that gives rise to electronic transitions in molecules.

The present spectrophotometric technique has developed from colorimetry.²⁹ In colorimetry, the intensity of color was first taken as the measure of concentration, the sample was visually compared with standards, the indicator paper being a typical example. This mainly qualitative technique was then improved by the use of filter-photometers. These measure the attenuation of light in narrow spectral region and compare it with solutions containing only pure solvent. replacement of the filter by a monochromator with a wavelength scanning mechanism then resulted in spectrometry.

The other widely used techniques – infrared spectroscopy, nuclear magnetic resonance spectroscopy and mass spectroscopy are mainly used for the elucidation of structures and identification of compounds. Because of the superimposition of vibrational and rotational transitions the ultraviolet-visible spectrum shows little fine structure. This is the reason why this technique is

seldom used for identification but it is widely used for qualitative analysis. Under certain experimental conditions the amount of radiation absorbed can be directly related to the concentration of the analyte in solution. It can be used for the quantitative determination of both organic and inorganic compounds, the former are determined mainly in the UV range and the latter in the visible range of spectrum. The quantitative analysis can be carried out precisely and reproducibly.

The main areas of application for spectrophotometry are quantitative determinations in food industry, healthcare, pharmacy, etc and as detectors in chromatographic processes (HPLC, TLC). Mixtures as well as pure substances can be studied and the components determined by methods of multicomponent analysis. Modern spectrophotometers operate very rapidly and can be designed using the photodiode arrays. UV-vis spectrophotometry is usable not only for observing stationary systems but also for carrying out repeated determinations very rapidly, sometimes within milliseconds.²⁹

Another widespread application of UV-vis spectrophotometry is the determination of the values of various physico-chemical parameters. A large share of these activities is held by equilibrium constants, in particular acidity constants, pK_a -s.^{6-10,14-25,IV} The majority of spectrophotometric methods for pK_a determination is based on the absorbance measurements of the solutions containing both the acidic form HA^z and the basic form A^{z-1} of the acid investigated. The ratio of concentrations of the acidic and basic forms of the acid and the activity of the hydrogen ion are needed to calculate the pK_a value of the acid. In the case of spectrophotometric method, the ratio of concentrations is found from the absorbance spectra. Therefore the absorbances of the acidic form and neutral form of the acid should be different. If the solution is sufficiently acidic/basic so that the whole acid is in acidic/basic form, then the absorbance spectrum of the acid correspond to the spectra of the respective pure forms. If the pH of the solution favors species both in acidic and basic form, the spectrum is similar to the spectrum of the species prevailing in the solution. The pH of the solution is determined by some other methods, e.g. potentiometrically.

The detailed overview of the theoretical principles of spectrophotometry, instrumentation, special techniques and data treatment can be found elsewhere.^{1,2,29}

2. UNCERTAINTY SOURCES IN UV-VIS SPECTROPHOTOMETRIC MEASUREMENT

2.1. Introduction

A common mistake that is made in uncertainty estimation of chemical measurement results via the ISO GUM¹² approach is that the well-known “classical” (weighing, volumetric operations, etc) uncertainty sources are well accounted for, while the “chemical” sources of uncertainty, such as decomposition of the analyte, adsorption of the analyte, incomplete selectivity, sample preparation, etc. (see reference 13 for a good checklist) are often either underestimated or left out of consideration altogether.

The physical basis of spectrophotometric measurements is very well understood and the sources of uncertainty originating from the instrument itself are very well known and have received ample coverage in analytical chemistry literature.^{2,30} These uncertainty sources are termed as the intrinsic or physical uncertainty sources in publication I.

In spectrophotometric measurement there is another large group of uncertainty sources originating from the object under study (termed as *chemical uncertainty sources* in publication I). These are more difficult to quantify and have received less attention although they often outweigh the intrinsic ones in their contribution to the combined uncertainty of the result. Convenient ways for quantifying such sources of uncertainty seem to be almost missing in the literature.

Below it is demonstrated that the chemical uncertainty sources are often significantly more important than the physical ones. The discussion is meant to be useful from the standpoint of scientific measurements as well as routine analytical determinations as an overview of physical and chemical uncertainty sources encountered in analytical spectrophotometric measurements is given. Besides characterizing the uncertainty sources, on the basis of examples guidance is given on quantitative evaluation of the respective uncertainty components. Overviews of uncertainty sources have been published for e.g. chromatographic methods.³¹ However, to the best of our current knowledge, besides publication I no such publication is available for UV-Vis spectrophotometry. Some uncertainty estimation examples for UV-Vis spectrophotometric measurements have been published, though, both in the literature³²⁻³³ and on dedicated websites.³⁴

Below an overview of the most important uncertainty sources in analytical spectrophotometric measurements is given. With the term *analytical spectrophotometric measurements* the measurements of amount of analyte in an analytical sample using absorption spectrometry in the UV or visible range is referred.

The following discussion focuses on the application of spectrophotometry in analytical determinations and is also relevant for equilibrium constant determinations (e.g. the pK_a values). Absolute measurements of absorbance or absorptivity values are not considered. It is assumed that the spectrophotometer is in good technical condition and the measurements are performed carefully. In addition, it is assumed that in analytical determinations the calibration graph is constructed using the same spectrometer and the same cells that are used for actual determination. Distinction is made between the intrinsic and chemical uncertainty sources, the following discussion focuses on both types of uncertainty sources.

2.2. Physical Uncertainty Sources

2.2.1. Absorbance Repeatability

According to the VIM (International Vocabulary of Basic and General Terms in Metrology)³⁵ *repeatability* is “property of a measuring system to provide closely similar indications for replicated measurements of the same quantity under repeatability conditions”. The most common way of quantitatively expressing repeatability is via the repeatability standard deviation.

Repeatability uncertainty of the spectrophotometer reading in analytical measurements includes the following components: instrumental noise, repeatability of positioning of the cell in the cell compartment, possible temperature fluctuations between the solutions and possible dust particles on the cell windows or in the solution.^{2,36} Experimental determination of repeatability uncertainty should be made using repeated measurements involving taking the cell out of the spectrometer and inserting it again (preferably a spectrum of some other sample should be scanned in between). Repeatability uncertainty should not be confused with the instrumental noise, i.e. the random fluctuations occurring in a signal, which is one of the contributors to the repeatability uncertainty. Uncertainty estimation of an analytical determination involves the overall repeatability of the sample absorbance at a given wavelength, not just the noise only. This is because (1) analytical results are influenced by the whole repeatability not only the noise component and (2) modern spectrophotometers employ quite efficient averaging techniques for noise minimization.

2.2.2. Spectrophotometer drift

According to the VIM³⁵ *drift* is the “change in the indication of a measuring system, generally slow and continuous, related neither to a change in the quantity being measured nor to a change of an influence quantity”. In this work we consider only drift of the absorbance scale. Wavelength drift is of minor importance because most measurements are made at the wavelength of the maximum and the maxima of UV-Vis spectra are broad. There are two types of drift in chemical analysis – the intrinsic or “physical” drift, caused by changes of in the spectrophotometer as a measurement instrument with time and the “chemical” drift, i.e. drift due to changes occurring with time in the solution under study. The former will be discussed in this section, the latter will be discussed below.

The intrinsic drift can also be divided into two different effects:

1. Baseline drift which leads to changes of the intercept of the calibration graph and does not affect the spectrometer response (a translational effect according to reference 37)
2. Drift of the spectrometer response which leads to change in the spectrometer response not affecting the baseline (a rotational effect according to reference 37)

Drift is a systematic effect and hence it should be in principle corrected for rather than taken into uncertainty budget.¹² Drift can be modeled using different functions, including polynomials, exponential functions, etc. However, it is impractical in routine analysis to correct for drift because it is usually small and correcting can be work-intensive. Therefore it is usually included in the uncertainty budget.

There are some important differences between repeatability and drift. Firstly, repeatabilities of absorbance values of different solutions (e.g. calibration standards and samples) are statistically independent and do not depend on the sequence of measuring or the time passed between the measurement of the standards and the samples. As for drift, the opposite is valid – the drift components of uncertainties of the solutions are correlated and dependent on the measuring sequence and time between measurements of standards and samples. Repeatability contribution to uncertainty can be efficiently reduced by repeated measurements whereas the drift contribution cannot be reduced this way. Also, repeatability is fairly constant from day to day, the drift can be very different on different days. And finally, there is no unequivocal way of expressing the drift uncertainty contribution mathematically as it was for repeatability. For the quantification procedure of intrinsic drift (and also repeatability), see publication I.

2.2.3. Non-Linearity of the Spectrophotometer's Absorbance Scale

The instrumental non-linearity of the response is caused by three effects^{2,30}: the stray light, the non-linear character of the detector element used in the spectrophotometer and the finite bandwidth of the radiation passing through the sample. Non-linearity is another systematic effect that in principle should be corrected rather than taken into account in the uncertainty budget. Again, in cases of small deviations it is usually impractical at routine laboratories, thus it should be included in the uncertainty budget. There is an approach based on residual analysis that is applicable to take into account both intrinsic and chemical non-linearity.

The uncertainty contribution to absorbance due to non-linearity is taken as being “not more than” the absolute value of the largest residual. This value is taken as the estimated uncertainty assuming rectangular distribution. The standard uncertainty contribution is found dividing it by square root of three.¹² The approach is illustrated on Figure 1, which presents a case with largely exaggerated non-linearity.

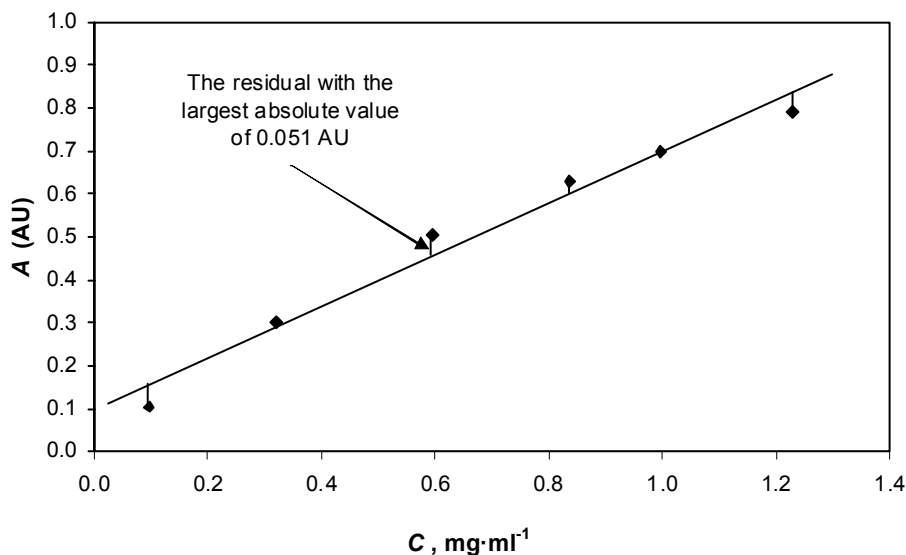


Figure 1. Handling Linearity of Calibration Graph as an Uncertainty Source.^a

^a For the sake of clarity of explanation the non-linearity of the calibration graph in this Figure is greatly exaggerated. In the case of similar highly non-linear response it would certainly be necessary to introduce polynomial or nonlinear calibration.

This approach assumes the uncertainty contribution to be constant throughout the calibration curve (thus somewhat overestimating the uncertainty). This is justified because of the properties of the linear regression model³⁸ – it minimizes the sum of squares of differences of the experimental values from the predicted values – not taking into account in which part of the line the respective data point is located. This constancy has important implications for the uncertainty of the analysis result: it has relatively largest influence in the case of low concentrations. Therefore the overall calibration line in the case of slight non-linearity is well applicable in the case of intermediate concentrations but not in the case of low concentrations. In the latter case a separate calibration should be carried out including only the points from the beginning of the calibration graph.

Other physical sources of uncertainty include the possible partial decomposition of the analyte due to photochemical processes, fluorescence, uncertainty due to rounding of the digital reading and uncertainty due to temperature differences between samples.

2.3. Chemical Uncertainty Sources

The chemical uncertainty sources originate from the system under study rather than from the equipment. Systems under study – normally solutions – can be very different and with different systems different uncertainty sources dominate. Evaluation of the chemical uncertainty contributions is far more difficult than evaluation of the physical sources of uncertainty and has received considerably less attention.

Since most analytes cannot be determined by photometric analysis directly because of absence of strong absorption in suitable wavelength range, photometric analysis is very often carried out using a photometric reagent that under certain conditions forms colored complex with the analyte. This is the “weak link” in photometric analysis. There are some well-known criteria of suitability of a particular reaction for use as photometric complex-forming reaction. Ideally the reaction should have the following properties²: (a) the reaction between the reagent and analyte should be complete and have exact stoichiometry, which is not altered by other species in the solution; (b) the formed complex should be stable; (c) the photometric reagent itself should have zero absorbance at the analytical wavelength; (d) the reagent should not form complexes with other species present in the solution. In many cases the reagents and reactions are wholly adequate, but very often compromises are necessary. In some cases the interfering effects can be minimized by the use of standard addition methods but the most common interference caused by extraneous absorption cannot be overcome this way.

2.3.1. Spectral Interferences

The uncertainty due to spectral interferences refers to the limited selectivity of UV-Vis spectrophotometric measurement caused by broad UV-Vis absorption bands in solution. To overcome this, the common practice is to separate the compounds of interest from the interfering compounds, mask the interfering compounds and/or shift the absorption maximum of the analyte, usually by complex formation, to a longer wavelength – typically in the visible spectral range (approximately 400–700 nm) – where the other components of the sample do not absorb light. Because matrix components can absorb, blank correction is often essential. Worth mentioning are also various multi-wavelength techniques with multi-linear calibration methods³⁹ and standard addition methods.¹¹ Thus there are many possibilities to minimize the interferences – many of them well-known for long time.^{30,40} Insufficient attention has been paid to how to estimate the uncertainty contributions from the (residual) interferences.³⁷

Analysis of real samples generally means analysis of complex mixtures. As in principle every component of the sample affects the result, ideally all these effects should be modeled and taken into account. However since all the components of the sample are not known to us, this approach can only be used in the case of well defined objects (e.g. alloys, pharmaceuticals, chemical formulations) for example in industrial process control using multivariate calibration methods.³⁹ There are usually only one or a few analytes present in the sample to be determined, whereas the other components – the matrix – are of no interest. Although care is taken while working out an analytical method to ensure that the probable constituents of the matrix do not interfere with determination, there always remains some uncertainty regarding the possible interferences that have not been considered during method development and validation or that have contents exceeding the contents assumed during validation. It is usually desirable to correct for extraneous absorbance, the extent of correction depends on the magnitude of this absorbance and on its effect to the measurement result and accuracy. This can, however involve lengthy studies to find out the causes for the interference.

In many standard methods (e.g. references 3 and 5) the interferences have been carefully studied and attached as annexes to the methods. In those annexes it is specified how much will the result of the analysis over- or underestimate the analyte content if a certain amount of an interferent is present in the sample. Usually the effect is not very large and thus can be introduced into the uncertainty budget as follows. First, the maximum possible content of the interferent in the sample has to be estimated by the analyst as m_{\max}^{interf} . Then the estimate of the probable content of the interferent is taken as $m_{\text{probable}}^{\text{interf}} = m_{\max}^{\text{interf}} / 2$ and its uncertainty is estimated as $\pm m_{\max}^{\text{interf}} / 2$. The effects of these amounts on the analysis result are deduced from the annex. As an example, in determination

of nitrite content according to ISO 6777:1984³ the 100 μg amount of Fe^{3+} (one of the important interferents) in the sample causes decrease of the result of measuring nitrite nitrogen content from $m_{\text{N}} = 10.0$ to $m_{\text{N}} = 9.49$ μg , i.e. by -0.51 μg . If the maximum possible content of iron in the sample is estimated as, say, $m_{\text{max}}^{\text{interf}} = 80$ μg , then the $m_{\text{probable}}^{\text{interf}} = 40$ μg . This probable interference is -0.20 μg and thus the result should be corrected by $+0.20$ μg . The uncertainty of this correction is ± 0.20 μg , which assuming rectangular distribution (the safest) leads to standard uncertainty contribution 0.12 μg . This contribution alone accounts for 1.2% of relative standard uncertainty (if $m_{\text{N}} = 10.0$) which is normally above the combined effect of all the intrinsic uncertainty sources of the spectrophotometer. In addition, there can be several interferents, e.g. in reference 3, 27 interfering elements or compounds are listed. In more complex samples the combined uncertainty contribution of all the interferents can be substantial.

The most widespread case of such interference is caused by overlapping of the absorption band of the complex with the “tail” of a shorter wavelength absorption band (see the dash-dot line in Figure 2). A rather useful approach for correcting this interference is presented in Figure 2.

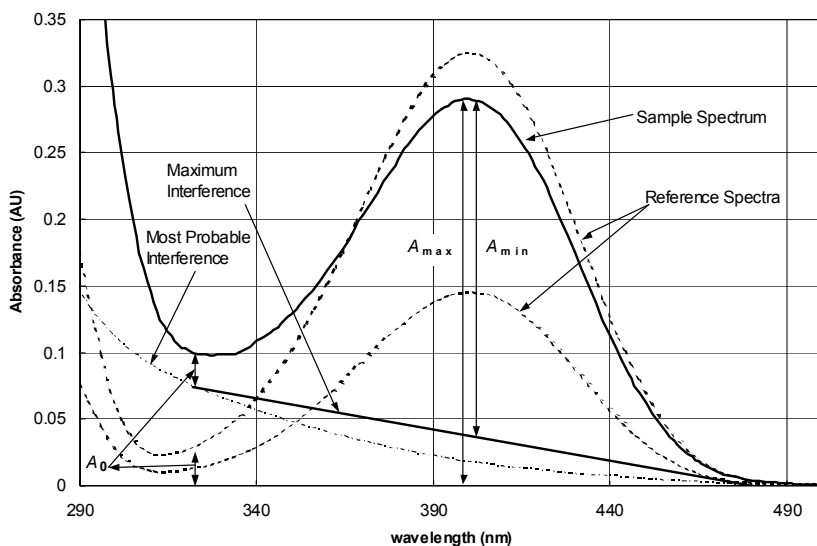


Figure 2. A Possible Way of Correcting for Extraneous Absorbance.^a

^a Solid line – sample spectrum; dotted lines – standard solution spectra; A_0 is the estimated absorbance “offset” of the sample solution at 322 nm in the case of absence of the interference and it is a necessary parameter for drawing the estimated interference lines. The wavelength is chosen to lie between the wavelengths of the minima of the sample spectrum with interference and those of the undisturbed standard solution spectra. The value of A_0 is estimated from the closest standard solution spectrum. The estimated maximum interference of the interfering “tail” are denoted by the solid and dash-dot lines respectively.

Approaches like this one are in principle not new.³¹ What has not been addressed, however, is how to estimate the uncertainty of such corrections. One way to do this is by the following procedure based on two worst-case scenarios. Since the real absorbance of the interference is unknown, the absorbance at the analytical wavelength is corrected in two ways: underestimating the sample absorbance (A_{\min} on Figure 2), by assuming zero absorbance for the “tail”, and overestimating it (A_{\max} on Figure 2), by assuming that the “tail” is linear (the straight line “Maximum interference”, see the footnote of Figure 2 for explanations). The best estimate of the sample solution absorbance value A_{probable} that is used for further calculations and its standard uncertainty $u(A_{\text{probable}})$ are calculated as follows:

$$A_{\text{probable}} = \frac{A_{\min} + A_{\max}}{2} \quad (15)$$

$$u(A_{\text{probable}}) = \frac{A_{\max} - A_{\min}}{2 \cdot \sqrt{3}} \quad (16)$$

This approach fails if the interferent has absorbance maximum near the wavelength of sample absorbance maximum, but the probability for this is low.

Sometimes it is possible to circumvent the whole problem by correcting the result using the procedural blank. Unfortunately this cannot be taken as a general approach, because very often there is no possibility to obtain the procedural blank that contains the interferents in the same quantities as the sample.

2.3.2. Complexing Agent Concentration

The amount of the complexing agent added to the solution is an important factor that can affect the measurement.⁴⁰ The necessary amount of the complexing agent and the extent by which it can be varied is found during procedure development and/or validation. That amount of the complexing agent is usually well above (very often tens or hundreds of times) the stoichiometric amount. Either too high or too low concentration of the complexing agent can adversely affect the results.

2.3.3. Composition Mismatch Between Sample Solution and Calibration Solution

Sometimes there can be a situation where a mismatch between sample solution and calibration solution is observed. This uncertainty source occurs in the case of unstable (e.g. Fe^{2+} , Sn^{2+} , etc.) or ill-defined (oil contamination, dietary fibre content, etc.) analytes. The best way to estimate uncertainty in this case is to estimate the maximum possible effect by monitoring the standard solution by

establishing the maximum time that the solutions can be kept and evaluating the maximum deviation of the results from those obtained with fresh solutions.

2.3.4. Chemical drift

Chemical drift is manifested by changes of absorbance of the solution because of changes taking place in the solution. The changes can be caused by the following: reactions of matrix compounds between themselves (yielding an absorbing species) or with the complex, reactions of oxygen with the analyte or the complex, simple decomposition of the complex. Even in the case of standard methods. Again a good example is the nitrite determination according to ISO 6777:1984.³ This is one of the most common spectrophotometric measurements in routine laboratories. The standard says that after mixing the reagents “The colour develops during 20 minutes and the measurement should be carried out during 2 hours”.

Experience from our laboratory shows that the colour is reasonably stable in simple samples. But even in such samples the decrease of absorbance during two hours occurs and is in the range of 1% of the absorbance value. In the case of absorbance of e.g. 0.4 this makes 0.004 AU.

The situation is still worse in the case on difficult samples like some wastewater samples. The decrease of the absorbance during the same time can be around 10–15% of the absorbance value. 4–6% of this occurs during the first 20 min. The intrinsic drift of the spectrophotometer (or any other uncertainty source considered above) is nowhere near this. Also, most probably this effect operates right from the moment of mixing of the reagents and thus the highest observed absorbance value can also be lowered.

Clearly, in this case the most rigorous approach would be to analyze the water thoroughly and try to separate or mask the interfering compounds. This is, however, work-intensive in most cases and often not practical at routine laboratory level. Therefore this effect has to be included in the uncertainty budget. Besides the matrix composition, aged or impure reagents can cause such drift effects. The possible extent of such effects in the matrices within the scope of the intended use should be investigated during method validation.

To estimate the uncertainty contribution due to chemical drift in this particular case of nitrite, it is suggested to take the highest absorbance value for the calculation and to estimate its uncertainty as \pm the overall absorbance decrease during 2 hours, e.g. $\pm 12\%$. This way also the possible changes that took place already during colour formation will be accounted for. The relative standard uncertainty contribution then will be $\pm 12/\sqrt{3} = 7\%$. This is not an entirely satisfactory approach, but at least the uncertainty estimate of the result will not leave the impression of high precision. The conclusion from the above is that whenever there is a kinetic reaction involved very large uncertainties (usually by far surpassing the other uncertainty components) can be expected.

2.4. Results

Based on the previous sections, we arrive at the following generic uncertainty budget for the absorbance A of a sample solution in spectrophotometric measurement:

$$u_c(A) = \sqrt{u_{\text{rep}}^2(A) + u_{\text{drift}}^2(A) + u_{\text{nonlin}}^2(A) + u_{\text{interf}}^2(A) + u_{\text{mismatch}}^2(A) + u_{\text{chem_drift}}^2(A)} \quad (17)$$

where the uncertainty components are in the following order: repeatability, intrinsic drift, nonlinearity (both intrinsic and chemical), chemical interferences, sample-calibrant mismatch and chemical drift. As a very broad generalization for a “good” case under routine laboratory conditions we can assign the following estimates to these uncertainty components: 0.0001, 0.002, 0.002, 0.003, 0.0 and 0.003 AU (the same order as above, expressed as standard uncertainties, absorbance value $A = 0.40$ AU) (see publication II). The uncertainty contributions of different uncertainty components for this case are presented in Figure 3.

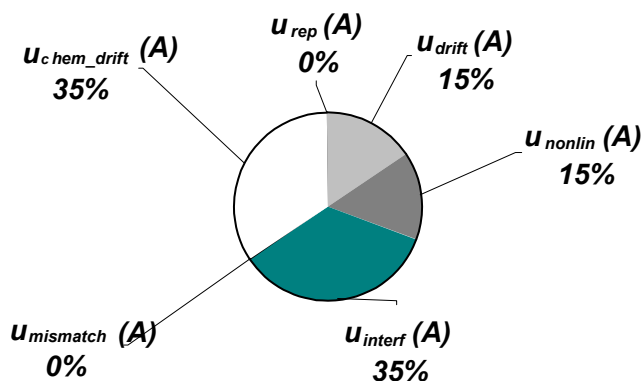


Figure 3. Uncertainty contributions of different uncertainty components of the absorbance A of a sample solution in the “good” case.

These uncertainty components lead to combined standard uncertainty of 0.005 AU, yielding relative combined standard uncertainty 1.2% and expanded uncertainty 2.4% at $k = 2$ level. Thus, even for a well-behaving system the chemical sources of uncertainty dominate, contributing 69% of the overall uncertainty. The intrinsic sources give the remaining 31% (see II for calculation of the percentages).

For comparison an example of a “complicated case” is given. For this case the estimates of uncertainty components given in equation 17 are the following: 0.0001, 0.002, 0.002, 0.0103, 0.0 and 0.028 AU (the same order as above, expressed as standard uncertainties, absorbance value $A = 0.40$ AU). It can be

seen that the numerical values of repeatability, intrinsic drift, non-linearity (both intrinsic and chemical) and sample-calibrant mismatch are the same as were used for the good case. The uncertainty contributions of different uncertainty components in this case are shown in Figure 4. These uncertainty components lead to combined standard uncertainty of 0.03 AU, yielding relative combined standard uncertainty 7.5% and expanded uncertainty 15% at $k = 2$ level. Again, the chemical sources of uncertainty dominate, contributing even 99.1% of the overall uncertainty in this case. The intrinsic sources give the remaining 0.9%.

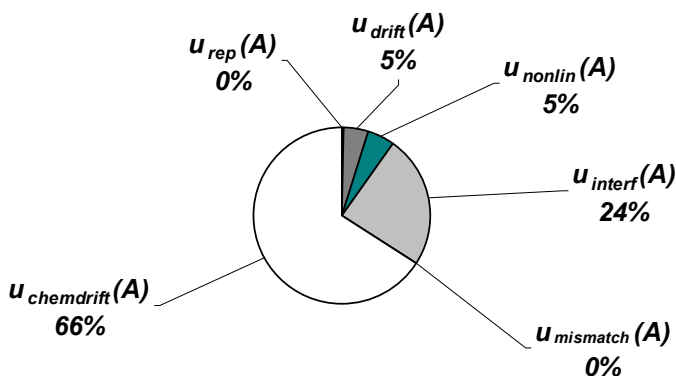


Figure 4. Uncertainty contributions of different uncertainty components of the absorbance A of a sample solution in a “problematic” case.

Skoog, West and Holler in their excellent textbook of instrumental analysis² characterize UV-Vis spectrophotometry as a tool for quantitative analysis by saying the following: “typically, relative uncertainties of 1% to 3% are encountered, although with special precautions errors can be reduced to a few tenths of percent” (ref 2, page 342). Based on the generic example above, we can only agree with this statement. If one’s measurements are concerned with objects that do not undergo decomposition, fading of color, extraneous reactions, etc then the main uncertainty in the measurement arises from the spectrophotometer and can indeed be few tenths of a percent, if special care is taken. It should be noted, however, that in the case of “normal to difficult” samples (e.g. chemical interference, unstable color, etc.) such low uncertainty is not achievable whatever the precautions are. In more common cases the chemical uncertainty sources dominate and relative standard uncertainties of the spectrophotometric measurement between 1% and 3% are realistic. In difficult cases (for example severe chemical drift), however, standard uncertainties in the order of 5–20% are to be expected.

It is important to keep in mind, that all these uncertainty estimates are the uncertainties arising from the spectrophotometric measurement only, not including uncertainty coming from sample preparation (e.g. extraction),

sampling procedures or the calibration procedure, because only the uncertainty sources concerning the absorbance measurement of sample were under consideration. But as will be discussed in Section 3, the calibration does not introduce significantly higher uncertainty. The latter can in the case of low analyte contents or difficult (e.g. heterogeneous) samples contribute more than 90% of the overall uncertainty.⁴¹

3. UNCERTAINTY ESTIMATION IN PHOTOMETRIC ANALYSIS: A CASE STUDY

3.1. Introduction

The discussion in this section focuses on practical applications of the uncertainty sources and their estimation procedures presented in Section 2.

Numerous papers have been published on the topic of uncertainty estimation of the results of chemical analysis, but the majority of those are rather general. What the laboratories need, however, are real uncertainty estimation procedures/examples applicable (with possible modification) to everyday chemical analysis. A number of such examples are included in the EURACHEM/CITAC uncertainty estimation guide¹³ and some more materials are reported in journal articles⁴²⁻⁴⁷ and also on some measurement uncertainty related websites.³⁴ However, in general the number of publicly available examples is clearly insufficient both in quantity and in versatility. The main goals of the study were to develop two uncertainty estimation procedures based on mathematical models (according to the ISO GUM¹² and the EURACHEM/CITAC guide¹³ for photometric determination of iron in aluminum for both calibration graph (CG) and standard addition (SA) methods, to explore and discuss the influence of various experimental parameters on the uncertainty of the analysis result and to compare the performance of the CG and SA methods. Also two uncertainty estimation examples for the GUM Workbench⁴⁸ software were created and made available.

3.2. The Method

The uncertainty estimation procedure derived is intended for photometric determination of iron in aluminum using sulfosalicylic acid. The analysis can be carried out using either the CG or the SA method. Both these options were treated in terms of uncertainty. See publication II for the details of experimental procedure, specification of the measurement and defining the mathematical model. Also the identification and quantification of the uncertainty components are given there. The uncertainty calculations were carried out using the GUM Workbench software. The detailed description of the software is given elsewhere.⁴⁹

The calculations were performed according to the following equation:

$$u_c(w_{\text{Fe}}) = \sqrt{\sum_i \left(\frac{\partial w_{\text{Fe}}}{\partial x_i} u(x_i) \right)^2} \quad (18)$$

where w_{Fe} is the iron content (%) of the sample. The partial derivatives were calculated numerically in GUM Workbench.

The expanded uncertainty is calculated by multiplying the combined standard uncertainty by coverage factor taking into account the number of effective degrees of freedom. The effective degrees of freedom are calculated according to the approach described in reference 49. It is an extension of the Welch-Satterthwaite method given in ISO GUM.¹² The uncertainty contributions of an input quantity x_j is found according to equation 19 (see reference 12 for background information):

$$\%u(x_j) = \frac{\left(\frac{\partial w_{\text{Fe}}}{\partial x_j} u(x_j) \right)^2}{\sum_i \left(\frac{\partial w_{\text{Fe}}}{\partial x_i} u(x_i) \right)^2} \cdot 100\% \quad (19)$$

Quantification of the uncertainty components with additional explanations and calculating the combined uncertainty is presented in GUM Workbench calculation files in the Electronic Supplementary material of publication II (available at <http://dx.doi.org/10.1007/s00769-005-0909-3>). The calculation files are very convenient in modeling the measurement and tuning the analysis procedure before starting the analysis.

In addition to the data directly corresponding to the above application examples, data are given that correspond to situations when some of the experimental parameters have been changed.

It is useful to point out some features of the GUM Workbench software (see reference 49 for full details). The philosophy of the GUM Workbench software has it that every quantity has only one uncertainty component. At first this may seem an obstacle, because for example the uncertainty of a volume of a pipette usually consists 3 uncertainty components: the repeatability contribution, the uncertainty of the stated volume of the pipette (the calibration uncertainty) and the uncertainty due to incomplete temperature control. There is, however, a very easy way to include all the components, which is also used in calculations performed in this work. For example the volume of the 5 ml pipette V_5 is presented as sum of three quantities: $V_{5\text{cal}}$, $V_{5\text{rep}}$ and $V_{5\text{tem}}$. Their uncertainties are the respective uncertainty components. The value of the volume is contained in $V_{5\text{cal}}$, the values of the two others are set to 0 ml.

Another important and often neglected aspect in uncertainty analysis is correlation.^{49,50} Equation 19 is correct only for uncorrelated input quantities. The GUM Workbench software permits to take correlation of input quantities into account. For this it is necessary to know the correlation coefficients of the pairs of correlated input quantities. In most cases (especially in the case of

routine analysis laboratories) the correlation coefficients are not known. There is another way to take the correlation into account – the solution is to “disassemble” such correlating input quantities in the model into more “fundamental” quantities that are not correlated. A typical example: concentrations of a series of working solutions are correlated because they have been prepared from the same stock solution (the same way, all glassware volumes on the same lab bench correlate due to temperature, slope and intercept of the same regression line correlate, etc.). The way to go in this case is to express the concentrations of the working solutions as interim quantities. In the mathematical model they are calculated using the stock solution concentration and temperature as true input quantities and the working solution concentrations as interim quantities. This approach was also used in this work: all quantities are disassembled down to the level of very simple quantities that are not expected to have any correlation anymore.

3.3. Results and Discussion

As the result of the study two procedures – for CG method and SA method, respectively – of uncertainty estimation of photometric analysis of iron were created. The procedures are based on mathematical models for the measurand, which are built with 64 and 80 input quantities, respectively. The procedures were applied to practical examples of determination of iron in aluminium using the sulphosalicylic acid method. The uncertainty of the result strongly depends on changes in experimental details. These dependencies can be conveniently explored with the aid of the model, the GUM Workbench software was found to be very useful in this respect. It was found that the CG method gives generally results with lower (around 1.3 times) relative uncertainty than the SA method. Taking into account the uncertainties, the methods are comparable as the combined uncertainties overlap.

Below there are some examples given on exploring how modifying various parameters of the model equation influences the uncertainty of the result. The uncertainty budgets for the calibration graph method and the standard addition method containing the uncertainty contributions of the most influential input quantities are represented in Table 1 and Table 2 for the CG and the SA methods, respectively. In the fifth columns of these tables, the data of the iron content measurements carried out practically are presented. The object under study was a reference material (of former Soviet origin) with iron content of 0.31% (the uncertainty of this value is not known). As can be seen from Tables 1 and 2, the iron content of the reference material is well within the uncertainty range of the results obtained with both CG and SA methods.

Table 1. The uncertainty budgets of the calibration graph method under various experimental conditions.

The experimental parameters^a									
m_{Fe}^b	102.8	102.8	102.8	51.4	102.8	205.6	102.8	102.8	102.8
m_{sample}	186.4	279.6	559.2	372.8	372.8	372.8	279.6	279.6	279.6
w_{Fe}	0.3075	0.3078	0.3080	0.3081	0.3079	0.3075	0.1248	0.3083	0.6735
Expanded uncertainties, absolute and relative^a									
U	0.0118	0.0100	0.0098	0.0112	0.0097	0.0104	0.0072	0.0100	0.0216
U_{rel}^e	3.84	3.25	3.18	3.65	3.1	3.38	5.75	3.24	3.21
Effective degrees of freedom	61	43	46	61	41	53	85	43	48
Coverage factor k	2.04	2.06	2.06	2.04	2.06	2.05	2.03	2.06	2.05
Input quantities^c (contributions of various groups of input quantities)^d									
$u(A)$	22.9	21	13.7	13.2	17.9	15.4	19.5	21	12.9
$u(A_1) \dots u(A_5)$	12.6	5	4.9	8.3	3.2	6.6	24.7	5.0	5.3
$u(m_{Fe})$	3.9	5.5	5.7	17.3	5.8	1.3	1.7	5.5	5.6
$U(m_{sample})$	1.2	0.7	0.2	0.3	0.4	0.4	0.2	0.7	0.8
$u(V_{1,st}) \dots u(V_{5,st})$	16.3	7.8	13	13.1	8.3	20.9	34.1	7.8	14
V_5	4.9	6.8	7.1	5.4	7.3	6.3	2.2	6.8	6.9
R	36.2	50.6	52.6	40.1	54.1	46.5	16.1	50.7	51.6
All the rest	1.3	2	2.1	1.4	3.0	2.6	0.7	2.0	2.1

^a All masses are given in milligrams, w_{Fe} and its absolute expanded uncertainty are given in percentages. All expanded uncertainties correspond to 95.45% confidence level (the $k = 2$ level of the Normal distribution). The fifth data column corresponds to the actual example described in text.

^b The input quantity in the left column is given as in text, in the right column as in the GUM Workbench files.

^c Standard uncertainties of the groups of input quantities that have the strongest influence on the final result. In the left column the quantity is given as in text, in the right column the respective quantity is given as in the GUM Workbench files.

^d The uncertainty contributions are given in percentages from the overall uncertainty (uncertainty indexes).

^e The relative combined uncertainty of the iron mass fraction in percent is $\frac{U}{w_{Fe}} \cdot 100\%$.

^f The absorbance values A_1 to A_5 and the sample solution absorbance A for the example discussed in the text are 0.100, 0.170, 0.240, 0.302, 0.370 and 0.223, respectively.

Table 2. The uncertainty budgets of the standard addition method under various experimental conditions.

The experimental parameters^d										
m_{Fe}^b	106.8	106.8	106.8	53.4	106.8	213.6	106.8	106.8	106.8	106.8
m_{sample}	218.2	609.3	872.6	436.3	436.3	436.3	436.3	436.3	436.3	436.3
w_{Fe}	0.3324	0.3093	0.3097	0.3174	0.3174	0.3174	0.1705	0.2479	0.4024	0.4859
Expanded uncertainties, absolute and relative^e										
U	0.0167	0.0116	0.0111	0.0156	0.0125	0.0131	0.0084	0.0111	0.0153	0.0180
U_{rel}^e	5.04	3.76	3.58	4.91	3.94	4.13	4.95	4.47	3.80	3.70
Effective degrees of freedom	46	46	42	29	43	74	45	36	47	46
Coverage factor k	2.06	2.06	2.06	2.09	2.06	2.03	2.06	2.07	2.06	2.06
Input quantities^c (contributions of various groups of input quantities)^d										
$u(A_1) \dots u(A_6)$	50.0	37.1	35.0	51.5	42.7	27.3	51	59.8	37.5	36.9
$u(m_{Fe})$	2.1	3.8	4.2	10.7	3.4	0.8	2.2	2.7	3.7	3.9
$u(m_{sample})$	0.5	0.1	0.0	0.2	0.2	0.2	0.1	0.2	0.2	0.2
$u(V_{1,st}) \dots u(V_{6,st})$	23.1	16.4	13.4	6.8	14	36.9	22.3	7.7	17.1	15.2
R	21.0	37.8	41.7	26.8	34.5	31.2	21.8	26.7	36.9	38.8
All the rest	1.9	3.9	4.9	3.2	3.1	3.6	1.4	2.2	3.6	4.3

^a All masses are given in milligrams, w_{Fe} and its absolute uncertainty are given in percentages. The fifth data column corresponds to the actual example described in text.

^b The input quantity in the left column is given as in text, in the right column as in the GUM Workbench files.

^c Standard uncertainties of the input quantities x_i that have the strongest influence on the final result. In the left column the uncertainty is given as in text, in the right column the respective quantity is given as in the GUM Workbench files.

^d The uncertainty contributions are given in percentages from the overall uncertainty (uncertainty indexes).

^e The relative combined uncertainty of the iron mass fraction in percent is $\frac{U}{w_{Fe}} \cdot 100\%$.

^f The absorbance values A_1 to A_6 are 0.086, 0.161, 0.233, 0.301, 0.369 and 0.434, respectively.

Both calibration graph (CG) and standard addition (SA) method have been extensively used in analytical chemistry.⁵¹ There are some important differences between these two methods:

1. The CG method is an interpolation method while the SA method is an extrapolation method. This difference alone immediately implies that the uncertainty should be higher in the case of the SA method. According to Tables 1 and 2 this is indeed the case.
2. In the case of the CG method the information on sample absorbance is fully contained in the measurement(s) carried out with the sample solution. This is well reflected by the uncertainty contributions, see below. In the case of SA method all the solutions contain the dissolved sample. This way the measurement of the sample is in a way averaged in the case of the SA method. The largest uncertainty contribution is due to the point that has the strongest influence on the line – the one with highest concentration.

For the CG method, the most prominent uncertainty source was the uncertainty of R (the recovery factor which takes the sample preparation procedure into account, see publication II) with 54.1 % of the overall uncertainty. The next contributor to uncertainty was sample absorbance. Three components are included in sample absorbance: repeatability of absorbance, uncertainty due to the drift of the photometer and uncertainty caused by rounding of the digital display. Their contributions were 2.0%, 14.9% and 1.0%, respectively, making their joint contribution 17.9%. The joint contribution of the absorbance measurements of the CG solutions was 3.2%, meaning that from the point of view of photometry the measurement of the sample solution is the single most important issue. In all absorbance measurements drift was the dominating source (although more so with higher absorbance values).

The next important sources of uncertainty were the uncertainty of volume of the sample stock solution transferred by pipetting to the 50 ml flask (7.3%) and the mass measurement of iron taken for the iron stock solution preparation (5.8% of the overall uncertainty). However, no major reduction of uncertainty can be achieved by increasing the mass of iron, as can be seen from Figure 5.

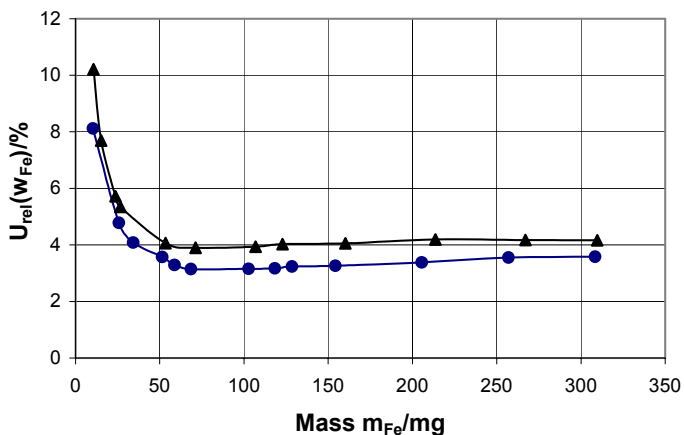


Figure 5. Dependence of the $U_{rel}(w_{Fe})$ on the weight of metallic iron taken for preparation of the iron stock solution. Calibration graph method (*triangles*); Standard addition method (*circles*).

The dependence of the $U_{rel}(w_{Fe})$ on the weight of sample in the case of both methods is presented in Figure 6 (for the case of around 0.3% of iron in the sample). As can be seen from Figure 6 the relative uncertainty has tendency to stabilize at 3% and 4% with both methods.

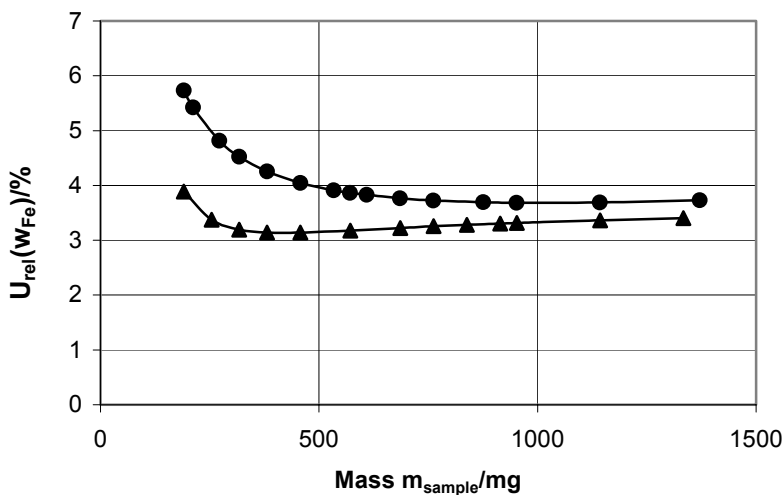


Figure 6. Dependence of the $U_{rel}(w_{Fe})$ on the mass of the sample taken for the analysis. Calibration graph method (*triangles*); Standard addition method (*circles*).

The dependence of the relative uncertainty of the result of photometric determination of iron on the iron content of the sample is presented in Figure 7. The data are given for the case when around 300 mg of sample was taken for the analysis. This amount of sample is suitable for iron contents starting from around 0.3%. If the sample has a lower iron content then the sample size intake should be increased if the uncertainty needs to be lowered.

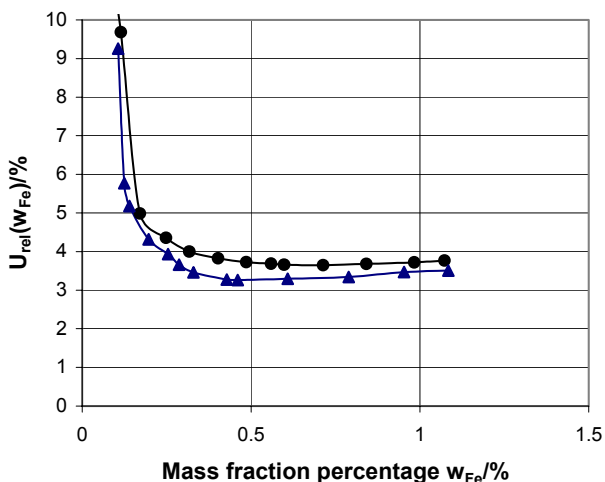


Figure 7. Dependence of the relative uncertainty of the iron content of the sample on the iron content itself. Calibration graph method (*triangles*); Standard addition method (*circles*).

The structure of the uncertainty budget of the SA method has both similarities and differences with that of the CG method. As can be seen from the fifth data column of Table 2, the most prominent uncertainty source is R (33.2 %), as in the case of the CG method. However, $u(R)$ is not dominating as heavily as in the CG method. The next major sources of uncertainty are the absorbance measurements. Their joint contribution for the absorbances A_1 to A_6 (all three components combined) represent 7.6%, 7.2%, 3.8%, 0.2%, 2.7% and 20.8%, respectively, making 42.3 % of the overall uncertainty. The uncertainty contributions are not spread evenly along the line. Instead the last point of the graph alone gives almost as high contribution as the rest taken together. Clearly, several factors are in operation here, including the dependence of the uncertainty of the absorbance value on the value itself (which is increases with increasing absorbance but is not purely proportional), and dependence of the uncertainty on the extent of extrapolation involved. However, the domination of the last point of the line is a common characteristic of the measurements carried out under various conditions. This aspect renders the SA method similar to the CG method: in both cases out of 6 measurements one is clearly dominating in uncertainty contribution. Again drift is the dominating uncertainty component

in the photometric measurement: the drift contributions of the six solutions (in the fifth data column) taken together are 36.4%, leaving only 5.9% to the other two uncertainty sources (repeatability and rounding). The resulting influence on the number of degrees of freedom of absorbances is the same as in the case of the CG method.

Another group of uncertainty sources that is of importance is the uncertainty of volume transferred by pipetting of preparing the solutions for the graph and the mass of the iron taken for standard solution preparation. As in the case of the CG method, the uncertainty increases sharply if the mass is below around 50 mg and remains practically constant at masses over around 100 mg (Figure 5).

The dependence of the results on the mass of the sample and the iron content of the sample is similar to that of the CG method. For the SA method slightly larger samples or iron contents are optimal for achieving low uncertainty than for the CG method.

In the example above not all uncertainty sources were operational that were mentioned in the previous sections. This is normal – calculation of uncertainty should always follow the individuality of the method.

4. UNCERTAINTY ESTIMATION IN MEASUREMENT OF pK_a IN ACETONITRILE MEDIUM

4.1. Introduction

Setting up and expanding acidity and basicity scales by means of pK_a measurements in various non-aqueous media (in particular, in acetonitrile) or gas-phase acidity or basicity measurements has been a core research direction in physical and organic chemistry for decades. Such scales are normally composed of relative acidity or basicity measurements of various acids or bases leading to graphs sometimes called as “ladders” (see Scheme 1), where the compounds are linked by relative measurements (see Schemes 2 and 3 for the structures of the acronyms presented on Scheme 1). The absolute pK_a values can then be found by anchoring the scale to some “anchor” compound(s) with known pK_a value(s). This “ladder” approach was pioneered by the Taft group in the 1970-s⁵² for gas-phase measurements and it has been used extensively since then for the measurements in the gas-phase⁵³⁻⁵⁷ and solution.^{14-22,58}

Recently a large number of pK_a measurements of bases¹⁷ in acetonitrile were merged to form a self-consistent basicity scale¹⁴ containing altogether 89 bases and spanning from 3.8 to 32.0 pK_a units in acetonitrile, that is 28 orders of magnitude. It was also demonstrated that the pK_a values on the scale are consistent with the results of a number of other research groups. However, rigorous uncertainty estimation of the pK_a values on the scale was not done. Instead the whole scale was characterized by the so-called “consistency parameter” s – a standard-deviation-like statistical parameter – which was equal to $s = 0.03$ pK_a units.¹⁷ Although very useful in characterizing the scale as the whole, the meaning of this parameter in terms of uncertainties of individual pK_a values remained unclear.

The measurements of pK_a values in nonaqueous media are notorious for having a large number of uncertainty sources.^{16,59} These include various association side-reactions, difficulties in estimating activity coefficients, trace impurities – most importantly water – in the solvent, impurities in the compounds, etc. Although it is easy to see that these uncertainty sources affect the measurement, their modeling and quantification can be very difficult.

In addition to the technical difficulties in quantifying uncertainty components of the results of the actual measurements there is also the conceptual difficulty of defining the measurand. It can be either the directly measured ΔpK_a value between two bases or the absolute pK_a value of a single base. In the latter case there is the further question, whether the uncertainty of the absolute pK_a value should contain the (quite large) contribution from the uncertainty of the anchor point of the scale or not.

Scheme 1. The pK_a Scale of Bases together with the Standard Uncertainties of the pK_a Values Estimated Using Different Approaches.

Base	Directly measured ΔpK_a	pK_a (AN)	Unc A ^a	Unc B ^b	Unc C ^c	Unc D ^d
1 4-MeO-C ₆ H ₄ P ₂ (dma)	0.51	31.99	0.19	0.22	0.08	0.14
2 PhP ₂ (dma)	-0.80	31.48	0.18	0.21	0.08	0.14
3 2-Cl-C ₆ H ₄ P ₂ (pyrr) ₂ NEt ₂	0.98	31.19	0.18	0.21	0.07	0.14
4 4-CF ₃ -C ₆ H ₄ P ₂ (pyrr)	0.70	30.50	0.17	0.20	0.07	0.14
5 2-Cl-C ₆ H ₄ P ₂ (dma) ₂ NEt ₂	0.32	30.16	0.17	0.20	0.06	0.13
6 2,5-Cl ₂ -C ₆ H ₃ P ₂ (pyrr) ₂ NEt ₂	1.01	29.16	0.16	0.20	0.06	0.13
7 4-CF ₃ -C ₆ H ₄ P ₂ (dma)	1.05	29.10	0.16	0.20	0.06	0.13
8 EtP ₁ (pyrr)	0.64	28.88	0.15	0.19	0.07	0.13
9 t-BuP ₁ (pyrr)	0.61	28.42	0.16	0.20	0.06	0.13
10 4-MeO-C ₆ H ₄ P ₂ (pyrr)	0.86	28.23	0.15	0.19	0.06	0.13
11 PhP ₂ (pyrr)	0.64	27.55	0.15	0.19	0.06	0.13
12 MeP ₁ (dma)	0.69	27.52	0.15	0.19	0.07	0.13
13 HP ₁ (pyrr)	1.21	27.01	0.16	0.20	0.06	0.13
14 t-BuP ₁ (dma)	0.55	26.98	0.15	0.19	0.06	0.13
15 PhP ₂ (dma)	0.46	26.46	0.14	0.18	0.05	0.13
16 TBD	0.61	26.03	0.14	0.18	0.06	0.13
17 HP ₁ (dma)	0.99	25.85	0.15	0.19	0.06	0.13
18 MTBD	0.08	25.49	0.13	0.17	0.05	0.13
19 2-Cl-C ₆ H ₄ P ₂ (pyrr)	1.05	25.42	0.13	0.17	0.05	0.13
20 DBU	0.45	24.34	0.11	0.16	0.05	0.13
21 4-NMe ₂ -C ₆ H ₄ P ₁ (pyrr)	1.20	23.88	0.11	0.16	0.05	0.13
22 4-MeO-C ₆ H ₄ P ₁ (pyrr)	0.77	23.12	0.10	0.16	0.05	0.12
23 PhP ₁ (pyrr)	0.74	22.34	0.10	0.16	0.04	0.12
24 PhP ₁ (dma)	1.09	21.25	0.10	0.15	0.05	0.12
25 4-Br-C ₆ H ₄ P ₁ (pyrr)	0.63	21.19	0.10	0.15	0.05	0.13
26 PhP ₁ (dma) ₂ Me	0.42	21.03	0.10	0.15	0.05	0.12
27 PhTMG	0.44	20.84	0.10	0.15	0.05	0.13
28 1-NaphIP ₁ (pyrr)	0.73	20.61	0.10	0.15	0.04	0.12
29 2-Cl-C ₆ H ₄ P ₁ (pyrr)	0.62	20.17	0.09	0.15	0.04	0.12
30 4-CF ₃ -C ₆ H ₄ P ₁ (pyrr)	0.50	20.16	0.09	0.15	0.05	0.13
31 2-Tol-1-BG	0.6	19.66	0.09	0.15	0.05	0.12
32 pyrrolidine	1.59	19.56	0.10	0.15	0.05	0.12
33 2-Cl-C ₆ H ₄ P ₁ (dma)	0.26	19.07	0.09	0.14	0.04	0.12
34 Et ₃ N	1.69	18.82	0.09	0.15	0.05	0.12
35 Proton Sponge	0.46	18.62	0.10	0.15	0.04	0.12
36 2,6-Cl ₂ -C ₆ H ₃ P ₁ (pyrr)	0.48	18.56	0.09	0.14	0.04	0.12
37 2,5-Cl ₂ -C ₆ H ₃ P ₁ (pyrr)	0.52	18.52	0.09	0.14	0.04	0.12
38 4-NO ₂ -C ₆ H ₄ P ₁ (pyrr)	0.21	18.51	0.09	0.15	0.04	0.12
39 4-Pyrr-Pyridine	0.93	18.33	0.09	0.15	0.04	0.12
40 4-NMe ₂ -Pyridine	0.91	17.95	0.09	0.15	0.04	0.12
41 2-NO ₂ -4-Cl-C ₆ H ₃ P ₁ (pyrr)	0.25	17.68	0.08	0.14	0.04	0.12
42 4-NH ₂ -Pyridine	0.08	17.62	0.08	0.14	0.04	0.12
43 2-NO ₂ -5-Cl-C ₆ H ₃ P ₁ (pyrr)	0.78	17.27	0.07	0.14	0.03	0.12
44 PhCH ₂ NH ₂	1.29	16.91	0.09	0.14	0.04	0.12
45 2-NO ₂ -4-CF ₃ -C ₆ H ₃ P ₁ (pyrr)	1.01	16.54	0.07	0.14	0.04	0.12
46 2-NH ₂ -Acridine	0.85	16.39	0.07	0.14	0.03	0.12
47 2-NH ₂ -1-Me-Benzimidazole	0.57	16.31	0.07	0.14	0.03	0.12
48 2-NH ₂ -Benzimidazole	1.31	16.08	0.08	0.14	0.04	0.12
49 2,3-(NH ₂) ₂ -Pyridine	1.13	15.24	0.07	0.13	0.03	0.12
50 2,4,6-Me ₃ -Pyridine	0.84	14.98	0.06	0.13	0.03	0.12
51 2,4-(NO ₂) ₂ -C ₆ H ₃ P ₁ (pyrr)	0.27	14.88	0.06	0.13	0.03	0.12
52 2,6-(NH ₂) ₂ -Pyridine	0.36	14.77	0.06	0.13	0.03	0.12
53 2-NH ₂ -Pyridine	0.10	14.47	0.06	0.13	0.03	0.12
54 2,6-Cl ₂ -4-NO ₂ -C ₆ H ₃ P ₁ (pyrr)	0.51	14.43	0.07	0.13	0.03	0.12
55 4-MeO-Pyridine	0.22	14.23	0.06	0.13	0.03	0.12
56 3-NH ₂ -Pyridine	0.73	14.17	0.07	0.14	0.03	0.12
57 2,6-Me ₂ -Pyridine	0.74	14.13	0.07	0.14	0.03	0.12
58 2,6-(NO ₂) ₂ -C ₆ H ₃ P ₁ (pyrr)	0.34	14.12	0.08	0.14	0.03	0.12

Scheme 1. Continued.

59	2-Me-Pyridine					13.32	0.06	0.13	0.02	0.12
60	Pyridine					12.53	0.00	0.12	0.00	0.12
61	OEP	-0.66				12.37	0.06	0.13	0.02	0.12
62	4-MeO-Aniline		0.15			11.86	0.04	0.12	0.02	0.12
63	2-methylquinolin-8-amine	0.31		1.01		11.54	0.05	0.13	0.02	0.12
64	N,N-Me ₂ -Aniline	0.07		0.83		11.43	0.07	0.14	0.03	0.12
65	Aniline		0.77		1.15	10.62	0.07	0.14	0.03	0.12
66	2-Me-Aniline					10.50	0.08	0.14	0.03	0.12
67	TPP	0.22				10.41	0.07	0.14	0.03	0.12
68	5-NO ₂ -Benzimidazole			1.58		10.39	0.08	0.14	0.03	0.12
69	TMP	-0.43	0.85			10.15	0.08	0.14	0.04	0.12
70	MePh ₃ P		0.47		0.74	9.96	0.08	0.14	0.04	0.12
71	TCPP	-0.03		1.07		9.94	0.09	0.14	0.04	0.12
72	2-MeO-Pyridine				0.90	9.93	0.07	0.13	0.03	0.12
73	1-Napht-NH ₂	0.16				9.77	0.07	0.13	0.03	0.12
74	3-Cl-Pyridine	0.24	0.38			9.55	0.07	0.14	0.03	0.12
75	4-Br-Aniline	0.11	1.37	0.12	0.33	9.43	0.07	0.14	0.04	0.12
76	2,4-F ₂ -Aniline		1.06			8.39	0.08	0.14	0.03	0.12
77	4-CF ₃ -Aniline	1.57	0.35			8.03	0.08	0.14	0.03	0.12
78	2-Cl-Aniline		0.71	0.35	0.75	7.86	0.09	0.15	0.03	0.12
79	3-NO ₂ -Aniline	0.20			1.77	7.68	0.08	0.14	0.04	0.12
80	4-F-3-NO ₂ -Aniline	0.01		0.04	0.17	7.67	0.08	0.14	0.03	0.12
81	2,6-(MeO) ₂ -Pyridine	0.05			0.07	7.64	0.08	0.14	0.04	0.12
82	Ph ₃ P	0.04	0.88	1.51	0.85	7.61	0.07	0.14	0.03	0.12
83	2-Cl-Pyridine	-1.40		1.43		6.79	0.09	0.14	0.03	0.12
84	4-NO ₂ -Aniline		0.57			6.22	0.08	0.14	0.04	0.12
85	2,5-Cl ₂ -Aniline	0.01		0.25		6.21	0.10	0.15	0.05	0.12
86	Ph ₂ NH	0.25	1.16		1.40	5.97	0.09	0.15	0.04	0.12
87	2,6-Cl ₂ -Aniline	0.91		1.18		5.06	0.09	0.15	0.04	0.12
88	2-NO ₂ -Aniline	-1.30		0.25		4.80	0.09	0.15	0.04	0.12
89	4-Cl-2-NO ₂ -Aniline		0.97			3.80	0.09	0.15	0.05	0.13

^a Uncertainties of pK_a values according to definition (b) calculated using the ISO GUM approach.

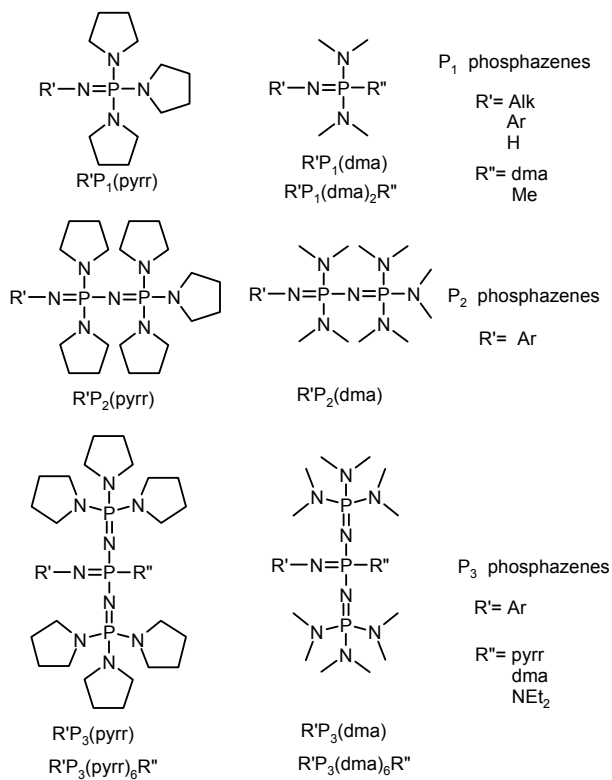
^b Uncertainties of pK_a values according to definition (a) calculated using the ISO GUM approach.

^c Uncertainties of pK_a values according to definition (b) calculated using the statistical approach.

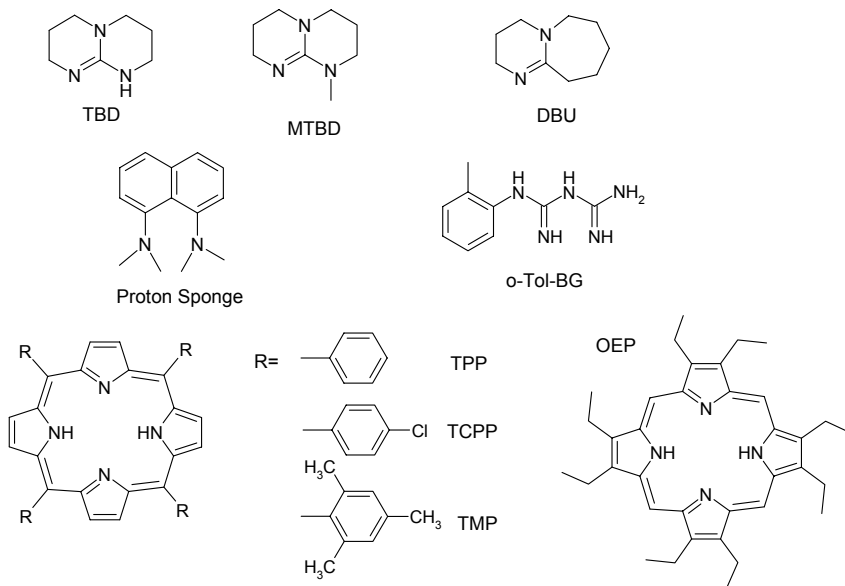
^d Uncertainties of pK_a values according to definition (a) calculated using the statistical approach.

The goal of this investigation (publication III) was to identify the most important uncertainty sources for such pK_a measurements and to attempt to model and quantify them. An uncertainty estimation approach was proposed but also it was demonstrated that there are several problems in uncertainty estimation according to the ISO GUM approach.¹² Also the elaboration of the meaning of the previously used consistency parameter of the scale¹⁷ in terms of the uncertainties of the pK_a values of the bases and to compare these uncertainties with those obtained using the ISO GUM approach. In publication III the uncertainty estimation procedure for the pK_a values on the self consistent basicity scale in acetonitrile is given.

Scheme 2. The structures and definitions of the phosphazene bases presented on Scheme 1.



Scheme 3. The structures and definitions of some other bases presented on Scheme 1.



4.2. The Method

The procedure of uncertainty estimation of the pK_a values of bases on the scale consisted of two steps: the uncertainty of the relative basicity of two bases, the ΔpK_a , had to be estimated first and then based on the uncertainties of the ΔpK_a values on the scale, the uncertainty of the absolute pK_a values was calculated.

The uncertainties of the absolute pK_a values were calculated via two ways – with and without taking the uncertainty of the reference base into account. The absolute pK_a value of a base x on the scale can be expressed as follows:

$$pK_{ax} = pK_a(\text{pyridine}) + \Sigma \Delta pK_a \quad (20)$$

where pK_{ax} is the pK_a value of the base x , $pK_a(\text{pyridine})$ is the pK_a value of pyridine equal according to the best current knowledge to 12.53¹⁷ and $\Sigma \Delta pK_a$ is the sum of differences derived from the ΔpK_a measurements and minimized according to the procedure described in references 17, 16 and 24. The combined uncertainty of the absolute pK_{ax} value should include contributions from both of these summands:

$$u_c(pK_{ax}) = \sqrt{u(pK_a(\text{pyridine}))^2 + u(\Sigma \Delta pK_a)^2} \quad (21)$$

Estimation of the uncertainty of $\Sigma \Delta pK_a$ can be done in quite a rigorous way, by thoroughly analyzing all the uncertainty sources and estimating the magnitude

of their contribution based on the best available information. But there is currently no rigorous approach to estimate the uncertainty of the absolute pK_a value of the reference base pyridine. In a recent work¹⁴ it was corrected from 12.33 to 12.53, so the uncertainty could be estimated as ± 0.2 pK_a units. This will make the uncertainty of the reference pK_a value the dominant uncertainty contribution for the majority of the bases on the scale and will cause quite large uncertainties for the absolute pK_a values of the bases.

The main use of the pK_a values on the scale is to compare the base strengths of different bases. The comparison of absolute pK_a values is much more difficult due to very strong correlation of these values introduced into the uncertainties of the absolute pK_a values by the large uncertainty of the pK_a value of the reference base. When comparing the pK_a values of two bases on the scale, it would be logical to compare their ΔpK_a values relative to the pK_a value of the reference base pyridine, taking the latter as a constant. Obviously these values also will be correlated and that this correlation will have to be taken into account, but this correlation is a lot weaker.

Taking the above mentioned into account, the uncertainty of a pK_a value was defined in two different ways:

1. Definition (a): the uncertainty is the combined uncertainty of the absolute pK_a value taking into account the uncertainties of both summands in equation 21.
2. Definition (b): the uncertainty is the combined uncertainty according to equation 21 but assuming the uncertainty of the pK_a value of pyridine as 0.

The definition (b) is equivalent to the uncertainty of ΔpK_a value of the base under consideration relative to the pK_a value of the reference base pyridine.

The uncertainty according to (a) estimates our ability to obtain absolute basicity data in acetonitrile medium. This uncertainty estimate will be at best tentative because of the impossibility (at this time) to rigorously estimate the uncertainty of the absolute pK_a value of pyridine. The uncertainty according to (b) is a rigorous uncertainty estimate for comparing pK_a values of two bases on the scale.

As for the uncertainty estimation of ΔpK_a value, there was a point which had to be taken into account. Some of the many input quantities in this uncertainty budget are strongly correlated (see a note on correlation in Section 3.2). As the real extent of correlation was not known, two extreme correlation coefficient values were used to take the correlation into account. The uncertainty was calculated using the correlation coefficient $R = 1$ which stands for the case if the change of uncertainty of all the quantities being correlated leads to the entire change in the uncertainty value. The other extreme was the calculation where the change in the uncertainty had no influence on the uncertainties of the quantities being correlated, i.e. the R value 0 was used. The actual extent of

correlation obviously lies somewhere between the two. Thus the uncertainties that were finally ascribed to the pK_a values were found as mean values of the two. The quantities having correlation were the drift component at various wavelengths in the same spectrum and also the drifts in the spectra that are taken within short time intervals are correlated (i. e. the spectra of the fully protonated and fully deprotonated forms among themselves and the spectra of mixtures of forms among themselves) and the systematic components of the chemical uncertainty at different wavelengths in the spectra of the fully protonated or fully deprotonated forms of bases.

As stated above, the complete uncertainty estimation procedure consisted of two subtasks. As there were all in all 180 individual ΔpK_a measurements on the scale and carrying out full uncertainty estimation separately for each of them would have been obviously far too time-consuming, the following approach was used. All the 180 ΔpK_a measurements were divided into two groups according to the reliability of the experimental measurement. In one group there were measurements which are denoted as “normal”, whereas the other group involved “difficult” measurements. Then one “normal” and one “difficult” measurement were chosen as model cases and the uncertainties of these ΔpK_a values were calculated with full rigor using the GUM Workbench software. Then the standard uncertainties obtained were used for all the other measurements in the groups. The absolute pK_a values were found in least squares minimization process. See files in the Electronic Supplementary material of publication III (available at <http://dx.doi.org/10.1016/j.aca.2006.03.020>) for the details of all calculation procedures.

4.3. Results and Discussion

The basicity scale together with the uncertainties of the pK_a values found using different approaches is presented in Scheme 1.

Uncertainties of the ΔpK_a values using the ISO GUM Method. As the different uncertainty sources were analyzed and quantified by this method, it is useful to point out some interesting aspects related to uncertainty calculation of ΔpK_a . As seen from Figure 8 and Figure 9, there are five groups of input quantities that dominate in the uncertainty budget of ΔpK_a values. The other uncertainty components not belonging to these five groups are negligible. Three of the five involve absorbance measurements of the spectra of bases in either fully protonated or fully deprotonated form – the component accounting for the drift of absorbance and both systematic and random chemical uncertainty components of the absorbance. As seen from the mathematical model presented in publication III, the correct measurement of the absorbance of a base in protonated/deprotonated form is very important. The contribution of the systematic uncertainty component in the “difficult” case is larger, being about

double of that in the “normal” case. The random uncertainty component has almost equal contribution in both cases.

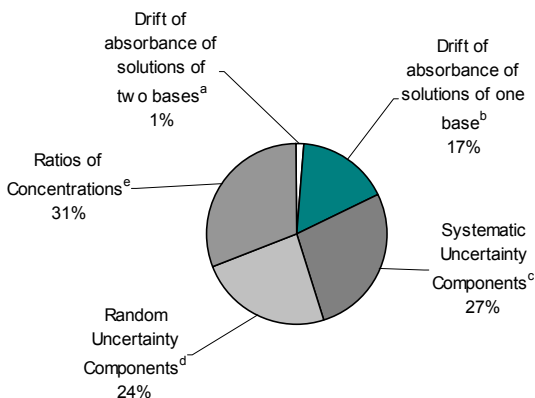


Figure 8. Uncertainty contributions of the most important groups of influencing parameters of ΔpK_a for the average “normal” measurement (correlation coefficient $R=0$).

^a Drift contributions of absorbance values of solutions containing two bases at various degrees of protonation simultaneously in the same solution.

^b Drift contributions of absorbance values of solutions containing pure protonated and deprotonated forms of individual bases.

^c Systematic contributions of absorbance values of solutions containing pure protonated and deprotonated forms of individual bases.

^d Random contributions of absorbance values of solutions containing pure protonated and deprotonated forms of individual bases.

^e Uncertainty components of the concentration ratios of bases.

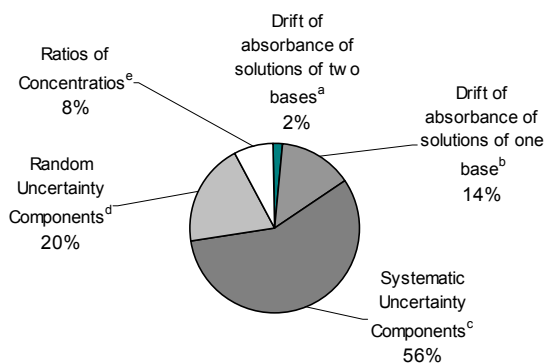


Figure 9. Uncertainty contributions of the most important groups of influencing parameters of ΔpK_a for the average “difficult” measurement (correlation coefficient $R=0$).

^a For the explanation of the uncertainty sources given on this figure, see footnote of Figure 8.

Calculation of uncertainties of the absolute pK_a values. As described above all the ΔpK_a values were divided into two groups according to their reliability. The standard uncertainty values for these two groups were 0.0559 (“normal” case) and 0.0607 (“difficult” case) pK_a units (correlation coefficient $R=1$) or 0.0623 (“normal” case) and 0.0840 (“difficult” case) pK_a units ($R=0$). Then the overall uncertainty for every single ΔpK_a measurement on the scale was calculated using the following procedure. The standard uncertainty value of the ΔpK_a (i.e. 0.0559 and 0.0607 or 0.0623 and 0.0840) was multiplied by a random variable following normal distribution. The central limit theorem was used to get this random variable. Five random variables following rectangular distribution were summarized according to a function having the central value 0 and the standard deviation value 1. The experimentally measured ΔpK_a values to which the product of uncertainty and random variable was added, were used for the minimization process. The sum of squares of differences between directly measured ΔpK_a values and the assigned pK_a values were minimized. As a result of the minimization process new pK_a values slightly different from those given in reference 14 for individual acids were obtained. The minimization process was repeated for 50 times. The anchor compound – pyridine was not involved in the minimization. Then the average and standard deviation for each pK_a was calculated. This calculation procedure was carried out for both cases – i.e $R=1$ and $R=0$, and finally the average value of the standard deviations was found.

Uncertainties of the ΔpK_a values using the Consistency Parameter of the Scale. As was stated in the introduction, the consistency parameter s carries information about the reliability of the pK_a values on the scale. This information is of average nature and is not directly related to any single measurement. Also, several measurements contribute to the ΔpK_a value between any two bases in an averaged way. Therefore the uncertainty of that ΔpK_a value, which contains contributions from the uncertainties of all these measurements, can be estimated using the consistency parameter s . This uncertainty estimation was carried out using the same minimization procedure that was described in the previous section but instead of the uncertainties of ΔpK_a values calculated with GUM Workbench software, the consistency parameter was used.

The following can be observed from the results:

1. The uncertainty of ΔpK_a of a base is the larger the farther away the base is removed from pyridine on the scale. The uncertainty of the ΔpK_a of the first and last base on the scale is around two times larger than the uncertainty of the ΔpK_a value of adjacent bases (see Figure 3 in publication III).
2. When comparing the four different uncertainties given in Scheme 1 it can be seen that if the uncertainty of the pK_a value of the “anchor compound” – pyridine – is taken as $\pm 0.2 pK_a$ units – definition (a) – then the additional uncertainty obtained by the minimization procedure is quite small. This is expected because the uncertainty of the pK_a value of pyridine is

the dominant component in the uncertainty calculation and thus pushing down the effect of the uncertainty obtained by the minimization procedure.

It is of interest to compare the ISO GUM uncertainty estimates and those obtained with the statistical approach. As can be seen from Scheme 1, the latter are around two times smaller. This is not unexpected and this may have the following reason.

The ISO GUM uncertainty of the ΔpK_a between the base x and pyridine includes all effects that are possible to take into account, both random and systematic with respect to different ΔpK_a measurements (i.e. measurements of the same base against different reference bases). This leads to some overestimation of the uncertainty of a ΔpK_a because all but two such systematic effects should cancel out when summing up the uncertainty contributions, but this is impossible to do in the framework of the calculation procedure used. The ones that should not cancel out are those at the beginning and the end of the ΔpK_a “chain”.

The basis for the statistical approach is the consistency parameter s . It can be regarded as the part of uncertainty composed only of the random components. It does not take into account any systematic affects (also not those at the beginning and the end of the “chain”) leading thus to some underestimation of uncertainty.

It can be concluded that the best uncertainty estimate of the ΔpK_a value between base x and pyridine – uncertainty of pK_a value according to definition (b) – lies between the values (is “bracketed” by the values) given in columns A and C of Scheme 1. As for the definition (a), the best uncertainty estimate of the pK_a value lies between the values given in columns B and D of Scheme 1.

Although it is not possible to give one “definite” pK_a uncertainty estimate for every single base on the scale as the result of the uncertainty estimation procedure discussed, but it is still possible to make some suggestions for estimating uncertainties of pK_a values on the scale. The uncertainty estimates given in column A be used as the reliability indicators of the pK_a values on the scale if comparing them with values that are related to the same scale (definition (b)) and uncertainty estimates given in column B be used if comparing the pK_a values with those that are external to this scale (definition (a)). These columns contain the ISO GUM uncertainty values, which are a bit overestimated but they are based on a scientifically more justified approach than the uncertainty values found using statistical approach. Also, the used statistical approach is wholly based on within-laboratory data, thus leaving any possible laboratory bias out of consideration. This is quite a serious issue and it has been shown recently that the laboratory bias effects on pK_a values can be very large.⁶⁰ The ISO GUM uncertainty values take these laboratory bias effects into account at least to some extent, because all uncertainty sources – also those that are of systematic nature – are considered.

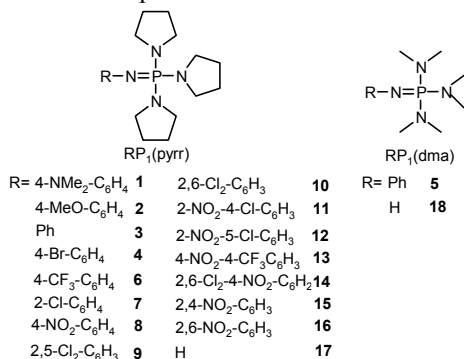
It is obvious that the uncertainty estimation of pK_a values in nonaqueous media is complicated and the procedures described here are not yet ideal. There are several sources of uncertainty which are currently quantified only as rough approximations. The detailed quantification of some of these sources would definitely involve an extremely large experimental effort and it is doubtful whether the value of the obtained additional information would justify this.

5. BASICITY OF SOME P₁ PHOSPHAZENES IN WATER AND IN AQUEOUS SURFACTANT SOLUTION

5.1. Introduction

In the course of research aimed at design and basicity measurements of strongly basic phosphazene bases their basicity has been earlier investigated in acetonitrile^{17,14,18,19,20} (AN), in tetrahydrofuran²¹⁻²³ (THF) and in the gas phase.^{22,23,61} Still, to the best of current knowledge, no basicity data of phosphazenes in the most common solvent – water – are available. This is probably due to the low solubility of most of the alkylated phosphazene bases used in practice and also due to the high basicity of many of them. Thus the aim of this work was to fill this gap by measuring the p*K*_a values of a series P₁ phosphazenes in water (see Scheme 4 for compound definitions, numbering and acronyms) and to compare the basicities of phosphazenes in water and in other media.

Scheme 4. Structures of the Phosphazene Bases Studied in this Work.



The solubility in water of all the phosphazene bases studied in this work is limited. With some bases it was possible to make aqueous solutions of sufficient concentration. With others the measurements were conducted in dilute solutions of nonionic surfactant Tween 20 to improve the solubility.

Quite some studies of acidic and basic properties of molecules in surfactant solutions have been carried out.⁶²⁻⁶⁶ A study of spectral and acid-base properties was carried out with some solvatochromic acid-base indicators in self-assembled surfactant aggregates.⁶² Another work carried out with acid-base indicators focused on tuning the p*K*_a values with changing the properties of medium by adding different surfactants.⁶³ In reference 64 behavior of 2-aminofluorene has been studied as a function of concentration of different surfactants of the Tween family at fixed pH value and as a function of pH at

given Tween concentration. It was observed that the higher the concentration of Tween and also the larger the molecules of Tween used, the bigger were the shifts in the pK_a values obtained. It has also been reported, that the presence of surfactants leads to the change in the transition interval of acid-base indicators thymol blue and bromthymol blue.⁶⁵ The pK_a values of ascorbic and maleic acid were determined potentiometrically, spectrophotometrically and conductometrically in cationic, anionic and nonionic surfactants and it was concluded that the acid-base properties of these acids are dependant on the type and concentration of surfactants used.⁶⁶

The data available from the literature thus suggest that surfactants often shift the pK_a values of acidic or basic species when compared to the pK_a values in pure water. In this work the shifts observed were very small as verified by the measurements with a control set of bases that had satisfactory solubility. This might be explained by the fact that the concentration of surfactant solution used in this work was 1–2 orders of magnitude lower than that used in other reports.^{62–66}

5.2. The Method

The pK_a values of the phosphazene bases were measured using a combined method of UV-Vis spectrophotometry and potentiometry. For the calculation of pK_a values from the absorbance data, analytical wavelengths were picked for the compounds corresponding to the maximum difference in absorbances between the neutral and protonated form. Two calculation methods were used. Detailed description of the experimental setup and calculation methods can be found in publication IV.

5.3. Results and Discussion

The pK_a values of phosphazenes and some other bases are presented in Table 2. Altogether 23 basicity measurements in water or 0.1% Tween 20 solution were carried out for 16 phosphazenes previously measured in acetonitrile and tetrahydrofuran.

Due to the limited solubility pK_a values of the compounds **7** and **9–16** presented in Table 2 were determined in 0.1% solution of nonionic surfactant Tween 20. As there are hydrophobic interactions between the phosphazene molecules (and possibly also the phosphazenium cation) and the surfactant molecules the pK_a values obtained this way do not necessarily coincide with those obtained in pure water as solvent. Also, the pH electrode system (especially the reference electrode) may behave differently in 0.1% Tween 20 solution than in aqueous buffers where it was calibrated. For assessing the possible effect of the surfactant on the pK_a values, those phosphazene bases that

were sufficiently soluble in pure water (**1–6**, and **8**) were determined in both media. As it can be seen from Table 2 the differences between the values are with both signs. The average difference is -0.022 pK_a units and the standard deviation of the differences is 0.055 pK_a units. This means that there is no well-defined systematic effect. The maximum difference was observed with **3** and was 0.08 pK_a units.

Table 2. The pK_a values of some phosphazenes in aqueous solution (this work, if not indicated otherwise), in acetonitrile (AN), in tetrahydrofuran (THF) and their gas-phase basicities (GB).

Compound	Base	pK_a				
		H ₂ O	0.1% Tween 20	AN ^a	THF ^b	GB (kcal/mol)
1	4-NMe ₂ -C ₆ H ₄ P ₁ (pyrr)	12.00	12.07	23.88	17.1	
2	4-MeO-C ₆ H ₄ P ₁ (pyrr)	11.94	12.00	23.12	16.6	255.2 ^c
3	PhP ₁ (pyrr)	11.52	11.60	22.34	15.9	252.0 ^c
4	4-Br-C ₆ H ₄ P ₁ (pyrr)	11.23	11.27	21.19	15.3	
5	PhP ₁ (dma)	10.64	10.60	21.25	15.3	
6	4-CF ₃ -C ₆ H ₄ P ₁ (pyrr)	10.65	10.59	20.16	14.6	
7	2-Cl-C ₆ H ₄ P ₁ (pyrr)		9.98	20.17	13.2	251.1 ^c
8	4-NO ₂ -C ₆ H ₄ P ₁ (pyrr)	9.22	9.24	18.51		
9	2,5-Cl ₂ -C ₆ H ₃ P ₁ (pyrr)		9.21	18.52	11.9	248.4 ^c
10	2,6-Cl ₂ -C ₆ H ₃ P ₁ (pyrr)		9.00	18.56	11.8	
11	2-NO ₂ -4-Cl-C ₆ H ₃ P ₁ (pyrr)		8.37	17.68	10.8	
12	2-NO ₂ -5-Cl-C ₆ H ₃ P ₁ (pyrr)		8.33	17.27	10.1	
13	2-NO ₂ -4-CF ₃ -C ₆ H ₃ P ₁ (pyrr)		8.14	16.54	9.6	
14	2,6-Cl ₂ -4-NO ₂ -C ₆ H ₂ P ₁ (pyrr)		7.50	14.43	7.8	
15	2,4-NO ₂ -C ₆ H ₃ P ₁ (pyrr)		7.34	14.88	8.0	
16	2,6-NO ₂ -C ₆ H ₃ P ₁ (pyrr)		6.82	14.12	7.5	
17	HP ₁ (pyrr)	13.93 ^d		27.01	20.8	255.1 ^c
18	HP ₁ (dma)	13.32 ^d		25.85	19.7	249.6 ^c
Reference Compounds						
19	TMG	13.6 ^f		23.3 ^g	15.3	234.8 ^h
20	PhTMG	11.77	11.76	20.84	14.0	240.4 ⁱ
21	Pyrrolidine	11.27 ^f		19.56	13.5	218.8 ⁱ
22	Et ₃ N	10.7 ^j		18.82	12.5	227.0 ⁱ
23	4-NMe ₂ -Pyridine	9.66	9.65	17.95	11.2	232.1 ⁱ

^aReference 14. ^bReferences 21 and 22. ^cReference 22. ^dEstimated from the pK_a values determined in AN and THF. ^eReference 67. ^fReference 7. ^gReference 68. ^hReference 69. ⁱReference 70. ^jReference 10.

Similar measurements in both media were also made with some other bases: **20** and **23**. The agreement between the value obtained in water and the value obtained in 0.1% Tween 20 solution is good: the difference is 0.01 pK_a units for

both **20** and **23**. These measurements validate the approach for pK_a determination of bases not readily soluble in water. As the average deviation given above was small, it is clear that there is no need to introduce any correction factors and thus it is possible to handle the obtained pK_a values as estimates of the aqueous pK_a values. The combined uncertainty of the pK_a values determined directly in aqueous medium was estimated as $\pm 0.15 pK_a$ units ($k = 2$). In the case of pK_a determination in Tween solution the effect of the surfactant on the pK_a values was estimated as $\pm 0.1 pK_a$ units ($k = 2$). Combining these values according to the uncertainty propagation rules gives the combined uncertainty $\pm 0.18 pK_a$ units ($k = 2$) for the values determined in the surfactant solution. It is also necessary to mention that the uncertainties of the pK_a differences of the bases are smaller than this value due to the strong correlation of the values determined using the same method.

These experimental observations of small influence of the surfactant on the pK_a values of bases are also supported by the general knowledge that the pK_a values of bases (that is pK_a values of their cationic conjugate acids) are by far less sensitive to medium than the pK_a values of neutral acids. This is not unexpected since dissociation of a cationic acid involves only rearrangement of the cationic charge between different species in solution while dissociation of a neutral acid involves generation and separation of two charged species. As an example, comparison of pK_a values of a wide selection of different bases in water and DMSO from reference 71 yields the following correlation:

$$pK_a(\text{H}_2\text{O}) = -0.317 + 1.023pK_a(\text{DMSO}) \quad (22)$$

$s(\text{intercept}) = 0.93$, $s(\text{slope}) = 0.11$, $n = 31$, $r^2 = 0.764$, $S = 1.55$.

One sees that not only the sensitivity but also the absolute pK_a values are similar in these rather different solvents. Another very relevant example is determination of pK_a values in solutions of quaternary ammonium salts.⁷² It has been demonstrated that upon moving from water to 7.75 molal solution of tetrabutyl ammonium bromide the pK_a value of various amines changes maximum only by 1.21 pK_a units. At the same time 7.75 molal tetrabutylammonium bromide solution contains 71 % of the salt, being thus a medium very different from water and by far less water-like than the very dilute surfactant solution used in this work. Tetrabutylammonium bromide is itself a rather powerful surfactant providing thus a relevant comparison for this work.

As seen from Table 2, the pK_a of substituted $\text{PhP}_1(\text{pyrr})$ phosphazenes varies from 6.82 (2,6-dinitro-) to 12.00 (4-(dimethyl)amino-). The whole span is thus 5.18 pK_a units. The comparison with the AN and THF as well as with the gas phase shows, that as expected, the differentiating ability of water is the lowest among all the media considered. The same span in AN and in THF is nearly two times wider: 9.8 and 9.6 pK_a units, respectively. Correlation of the phosphazene pK_a values (compounds **1–16**) in water and AN results in the following equation:

$$pK_a(\text{H}_2\text{O}) = -0.920 + 0.550pK_a(\text{AN}) \quad (23)$$

$$s(\text{intercept}) = 0.48, s(\text{slope}) = 0.025, n = 16, r^2 = 0.972, S = 0.29$$

Correlation of the phosphazene pK_a values in water and THF yields the following correlation line:

$$pK_a(\text{H}_2\text{O}) = 3.06 + 0.522pK_a(\text{THF}) \quad (24)$$

$$s(\text{intercept}) = 0.22, s(\text{slope}) = 0.017, n = 16, r^2 = 0.986, S = 0.22$$

As the amount of gas-phase data for the phosphazenes studied is limited, no meaningful correlation between the aqueous pK_a values and the gas-phase acidities can be obtained so far. In order to compare the basicity of different types of $P_1(\text{pyrr})$ and $P_1(\text{dma})$ phosphazenes, it would be interesting to know also the aqueous pK_a values of the parent compounds $HP_1(\text{pyrr})$ and $HP_1(\text{dma})$. As these compounds are obviously too basic for direct measurement in aqueous solution and do not have chromophores for the spectrophotometric method, we took advantage of the correlations given above. Using these correlations of the phosphazene pK_a values in water and AN (equation 23) or THF (equation 24), it is possible to predict the pK_a values of $HP_1(\text{pyrr})$ and $HP_1(\text{dma})$ in aqueous solution. Both correlations resulted in very similar pK_a values for both $HP_1(\text{pyrr})$ and $HP_1(\text{dma})$. For $HP_1(\text{pyrr})$, these pK_a values are 13.95 (correlation with AN) and 13.91 (correlation with THF), for $HP_1(\text{dma})$ these values were 13.31 and 13.33, respectively. Thus the estimates of the pK_a values of $HP_1(\text{pyrr})$ and $HP_1(\text{dma})$ can be obtained as follows: 13.9 and 13.3. These estimates are somewhat crude but allow to draw qualitative conclusions.

It is of interest to compare the pK_a values of the pyrrolidinyl- and dimethylaminophosphazenes **3** and **5**. The pyrrolidinyl phosphazene is stronger in all media, the differences being 0.88, 1.09 and 0.60 pK_a units in water, AN and THF respectively. Using the correlations above, the basicity differences can be normalized to a common medium (water in this case) by multiplying them with the slopes of the respective correlation lines. The following basicity differences are then found for water, AN and THF: 0.9, 0.6, and 0.3 respectively. The different differentiating ability of the solvents is taken into account by the normalization procedure. Thus, if all other effects were absent then the differences would be equal. The main additional effect that should be considered is the increase of level of steric hindrance when moving from dimethylamino-phosphazene to the more bulky pyrrolidino-phosphazene. This increase in steric hindrance reduces the possibility of solvation of the protonation centre of the protonated phosphazene molecule. The pattern of change of the normalized differences supports this qualitative interpretation: it is easy to see that the larger is the solvent molecule the smaller is the normalized difference decreasing from 0.9 for the smallest solvent – H_2O – to 0.3 for the bulkiest solvent under consideration – THF.

Another interesting point would be the comparison of pK_a values of phosphazenes with other types of bases (see Figure 10).

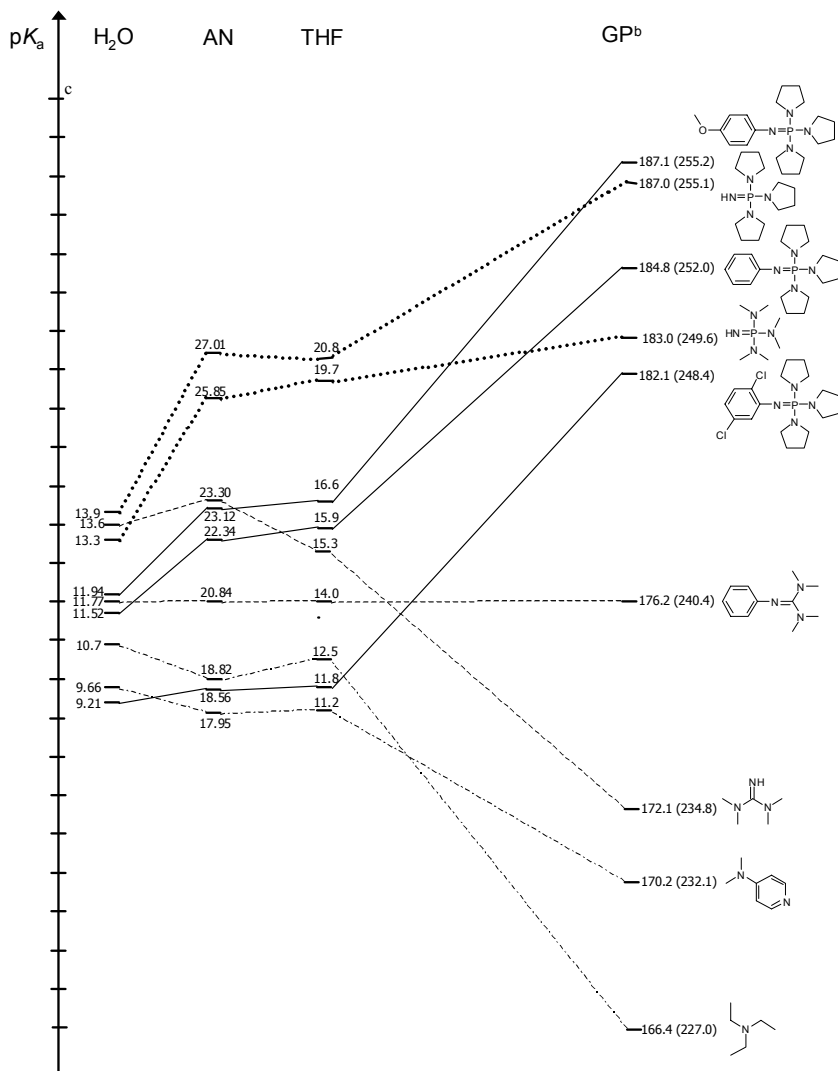


Figure 10. Interrelations Between Basicities of Bases Belonging to Different Families in Different Media.^a

^a The absolute pK_a values in different media are not directly comparable, so PhTMG has been used as an arbitrary reference compound for placement of the scales on the figure.

^b The pK_a values for the gas phase (GP) are found according to the following equation $pK_a(GP) = GB \cdot 2.30 / RT = GB / 1.364$. The gas-phase basicities (in kcal/mol) are given in brackets.

^c The pK_a arrow is divided into pK_a units but as the pK_a values in different media cannot be compared directly, the numbers were not added to the division markers.

If the pK_a values of the reference compounds (**19–23**) in water are examined (see Table 2 and Figure 10), it can be pointed out that guanidines (**19**, **20**) are more basic than the phosphazenes under study. As for amines (**21**, **22**) and pyridine (**23**), the basicity of these compounds in water is “bracketed” between the pK_a values of phosphazenes. The situation is similar in AN and THF, but on an average the pK_a values of phosphazenes are slightly higher relative to the reference compounds. In the gas phase the situation is completely different from that in the condensed media: it is possible to clearly distinguish between two groups – phosphazenes and reference compounds. Phosphazenes are in the gas phase obviously more basic than guanidines, amines and pyridines in the reference group.

Another interesting point is to compare the basicities of the substituted $\text{PhP}_1(\text{pyrr})$ phosphazenes studied in this work with corresponding anilines. pK_a values of substituted anilines in water correlate well with the pK_a values of the respective substituted $\text{PhP}_1(\text{pyrr})$ phosphazenes in water:

$$pK_a(\text{Phosphazene}) = 8.98 + 0.495pK_a(\text{Aniline}) \quad (25)$$

$$s(\text{intercept}) = 0.11, s(\text{slope}) = 0.03, n = 12, r^2 = 0.960, S = 0.35$$

As seen, the basicity of $\text{PhP}_1(\text{pyrr})$ phosphazenes is around two times less sensitive towards the substitution in the aromatic ring than the basicity of anilines. The corresponding correlation can be seen in Figure 11.

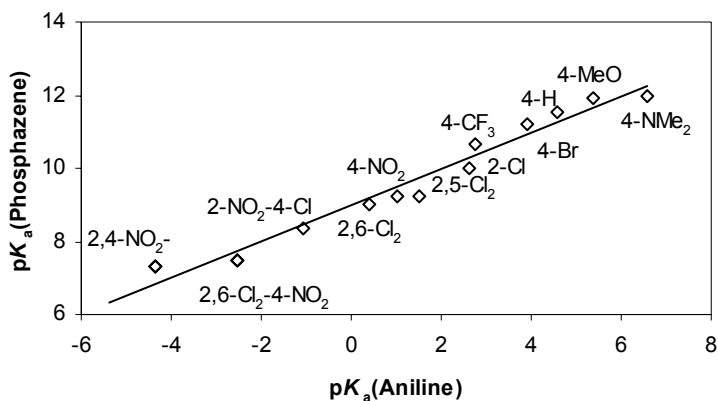


Figure 11. Correlation of pK_a values in water of phenyl-substituted anilines and $\text{PhP}_1(\text{pyrr})$ phosphazenes. The pK_a values of anilines are from references 7, 14,73.

The effect of the substitution of the imino-hydrogen by phenyl ring in tetramethylguanidine and in $\text{P}_1(\text{pyrr})$ and $\text{P}_1(\text{dma})$ phosphazenes is also an aspect to be considered when comparing the basicities of phosphazenes with other types of compounds. In all condensed media the basicity decreasing effect is larger in the phosphazenes (compare **3** and **17**; **5** and **18**) than in tetramethylguanidine, the effects are following: 2.3 (in water), 4.67 (in AN) and 4.8

(in THF) pK_a units for **17** and **3**; 2.64, 4.6 and 4.4 pK_a units for **18** and **5**; 1.83, 2.46 and 1.3 for **19** and **20** (the same order of solvents). The effects of substitution of the imino-hydrogen in guanidines has been thoroughly discussed⁶⁹ and the comparison of substituted imino-hydrogen in guanidines with phosphazenes is given in reference 74, which also describes the basicity decrease. In the gas phase the substitution of the imino H in tetramethylguanidine by a phenyl ring leads to basicity increase, in the phosphazenes to a small basicity decrease. These data for three different media allow us to generalize the earlier suggestion about the nature of the P=N double bond in phosphazenes. In reference 17 it was proposed that this bond in phenyl P₁ pyrrolidino phosphazenes and in phenyl P₁ dimethylamino phosphazenes has a certain contribution of the ylidic (zwitterionic) structure. The basicity decreasing effects of the compounds studied in this work lead to the same conclusion.

6. SUMMARY

The present work focused on UV-vis spectrophotometric technique from the standpoint of reliability (publications I–III) and applications (publication IV).

In the first part of this work (Section 2) the uncertainty sources of spectrophotometric measurements were discussed. The effect of two different groups of uncertainty sources – the intrinsic or the physical uncertainty sources and the chemical uncertainty sources was considered. As the result it was concluded that the chemical uncertainty sources dominate over the physical ones even in chemically well-behaving systems.

The second part of the work (Section 3) focused on practical applications of the uncertainty sources and their application procedures. Two uncertainty estimation procedures based on mathematical models for photometric determination of iron in aluminum for both calibration graph and standard addition methods were developed.

The third part (Section 4) described uncertainty estimation of complicated scientific spectrophotometric experiments. Two different uncertainty estimation approaches were presented and applied to the pK_a values of the compounds on a previously established self-consistent spectrophotometric basicity scale in acetonitrile. The uncertainty of these pK_a values was estimated by using two different approaches – the ISO GUM approach and the statistical approach. The uncertainties including the uncertainty of the pK_a value of the reference base – pyridine – were in the range of 0.12–0.22 (ISO GUM) and 0.12–0.14 (statistical) pK_a units. When the uncertainty of the reference pK_a value was excluded, the uncertainty was in the ranges of 0.04–0.19 and 0.02–0.08 pK_a units. It was demonstrated that the ISO GUM approach can be used for uncertainty estimation of such measurements although some of the uncertainty sources can be very difficult to take into account. It was also demonstrated that definition of the measurand is of utmost importance in such uncertainty studies.

The Section 5 was devoted to pK_a measurements of some phosphazene bases in water and in aqueous surfactant solution. The pK_a values in water and in dilute surfactant solution for 15 ring-substituted phenyl P_1 pyrrolidino phosphazenes $\text{PhN}=\text{P}(\text{NC}_4\text{H}_8)_3$ and the phenyl P_1 dimethylamino phosphazene $\text{PhN}=\text{P}(\text{NMe}_2)_3$ were reported. The pK_a values of the studied phosphazenes in aqueous medium vary from 6.82 (2,6-dinitro-) to 12.00 (4-dimethylamino-). The basicity span is 5.18 pK_a units.

Besides routine analysis laboratories, these results may be helpful for fundamental researchers hopefully to encourage them to perform in-depth estimation of all uncertainty components in their experiments. As it was shown, a thorough uncertainty estimation can be carried out even in the case of complex fundamental research.

7. KOKKUVÕTE

Spektrofotomeetriselised mõõtmised ja nende määramatus keemilisel analüüsil ja dissotsiatsioonikonstantide määramisel

Keemiliste analüüside usaldusväärsuse hindamine on muutumas järjest enam standardnõudeks, analüüsi tulemust mõõtemääramatuse hinnanguta ei saa pidada täielikuks. Käesolevas väitekirjas uuriti mõõtamääramatuse ja usaldusväärsuse seisukohalt ühte rutiinanalüüsi- ja ka teaduslaborites laialt kasutatavat meetodikat – UV-vis spektrofotomeetriat.

Töö esimeses osas keskenduti puhtalt spektrofotomeetrisest mõõtmisest (ehk siis proovilahuse neeldumise mõõtmisest) tulenevatele määramatuse allikatele. Vastavad määramatuse allikad jagati kahte gruppi: spektrofotomeetrist kui mõõtevahendist tulenevad ja keemilised ehk siis mõõdetavast objektist tulenevad määramatuse allikad. Selgus, et kaugelt olulisema tähtsusega on keemilised määramatuse allikad, mis keerukate mõõtmiste korral võivad moodustada isegi kuni ca 99% neeldumise mõõtmisega seotud määramatusest.

Järgnevalt vaadeldi määramatuse allikaid ja mõõtemääramatuse põhjalikumalt hindamist keemilise analüüsi korral. Loodi kaks matemaatilistel mudelitel põhinevat mõõtemääramatuse hindamise meetodikat raua määramiseks sulfosalitsüülhappega alumiiniumsulamites nii kaliibrimisgraafiku meetodi kui ka lisamismeetodi jaoks.

Töö neljas osa käsitles mõõtemääramatuse hindamist keeruka teadusliku eksperimendi puhul. Leiti mõõtemääramatus varasemalt atseetonitriilis koostatud kooskõlalise aluselise skaala aluste pK_a väärtustele. Mõõtemääramatuse hindamisel kasutati kahte erinevat meetodit – ISO GUM meetodit ja statistilist meetodit. Lõpptulemus, ehk siis aluste pK_a väärtuste mõõtemääramatus, esitati kahel viisi – ühel juhul arvestati ka skaala ankrupunkti, püridiini, pK_a väärtuse määramatust. Koos püridiini pK_a väärtuse määramatusega jäid uuritud pK_a väärtuste määramatused vahemikku 0.12–0.22 (ISO GUM meetod) ja 0.12–0.14 (statistiline meetod) pK_a ühikut. Püridiini pK_a väärtuse määramatuse mitteamistamisega olid tulemused vastavalt järgmised: 0.04–0.19 ja 0.02–0.08 pK_a ühikut. Leiti, et ISO GUM meetod on rakendatav taoliste mõõtmiste mõõtemääramatuse hindamiseks, kuigi mõningaid määramatuse allikaid on üsnagi raske kvantitatiivselt arvesse võtta. Ka leiti, et mõõdetava suuruse täpne defineerimine on väga olulise tähtsusega sedatüüpi määramatuse uuringute korral.

Lisaks spektrofotomeetriselise meetodi usaldusväärsuse hindamisele teostati ka mõned pK_a väärtuste määramised spektrofotomeetriselisel meetodil. Töö neljandas osas määrati 15 fenüülasedandatud P_1 pürrolidino fosfaseeni $\text{PhN}=\text{P}(\text{NC}_4\text{H}_8)_3$ ja fenüül- P_1 dimetüülamino fosfaseeni pK_a väärtused vees ning lahjas pindaktiivse aine lahuses. Uuritud fosfaseenide vesilahuse pK_a väärtused varieeruvad vahemikus 6.82 (2,6-dinitro-) kuni 12.00 (4-dimetüülamino-) pK_a ühikut.

Käesoleva töö tulemused näitavad, et lisaks rutiinanalüüsi tulemuste mõõtemääramatuse hindamisele on võimalik tulemuste usaldusväärsust edukalt kontrollida ka keeruka ja põhjaliku teaduseksperimendi korral.

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