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"Exploratory Analysis of pH Reference Materials for Quality Control Acceptance by Chemometrics Algorithms"

European Master in Quality in Analytical Laboratories (EMQAL)

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Declaration of Authorship

I declare that I am the author of this work, which is original. The work cites other authors and works, which are adequately referred in the text and are listed in the bibliography.

(Madonna Atef Boulos Salib)

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Abstrato

O National Institute for Standards and Technology (NIST) tem procedimentos definidos para a aceitação dos seus Materiais de Referência Certificados (MRC) para medição de pH. Estes procedimentos são morosos e destrutivos para a amostra. A sua substituição por métodos quimiométricos recorrendo a técnicas de análise não destrutivas traria grandes benefícios, por um lado em termos da rapidez e simplicidade do procedimento de aceitação de MRC.

Neste trabalho recolheram-se espectros no infravermelho próximo (NIR) e espectros Raman e trataram-se os dados através de análise de componentes principais (PCA), a fim de realizar uma análise qualitativa de três diferentes MRC de pH: carbonato de cálcio (CaCO3), tetraborato de sódio decahidratado (bórax) e hidrogeno ftalato de potássio (KHP).

O método foi testado para distinguir entre MRCs produzidos pelo NIST e outros materiais candidatos a MRC, assim como amostras comerciais de menor pureza que serviram como controlo negativo.

Uma coleção de 87 amostras de CaCO3, 52 amostras de bórax e 63 amostras de KHP foi analisada por espectroscopia NIR e Raman. No caso do CaCO3, o PCA alcançou uma boa discriminação, coerente com o método de referência NIST para aceitação de MRC de pH. No caso do bórax, obteve-se boa discriminação entre as amostras, mas que se revelou inadequada para controle de qualidade dos materiais candidatos. No caso do KHP, a discriminação entre amostras foi insuficiente, mas não exclui a possibilidade de aplicar o método proposto ao controle de qualidade de materiais candidatos visto o método de referência NIST também foi incapaz de distinguir os controlos negativos.

A diferenciação obtida por PCA para o caso do CaCO3 foi explicada por difração de raios-X, tendo-se verificado que os diferentes grupos observados por PCA correspondem a diferentes polimorfos de calcite, aragonite e vaterite.

No caso do bórax, a análise termogravimétrica revelou que o bórax tende a perder parte de sua água de cristalização ao longo do tempo de armazenamento e se transforma lentamente na forma pentahidratada. A análise por PCA diferenciou grupos de acordo com o grau de hidratação das amostras.

As amostras de KHP não foram suficientemente separadas na análise por PCA, com exceção de amostras com maior grau de cristalinidade, o que se explicou devido à presença de água oclusa nos cristais de KHP.

Este estudo prova que o uso de quimiometria e sua capacidade de discriminar entre amostras quimicamente diferentes é potencialmente uma ferramenta poderosa para garantir a identidade e a qualidade de MRC para pH, e para simplificar o procedimento de aceitação de novos MRC.

Abstract

Spectra collected with near infrared (NIR) and Raman spectroscopic methods were used along with unsupervised pattern recognition, namely, principle component analysis (PCA), to conduct a qualitative analysis of three different pH materials: calcium carbonate ($CaCO_3$), sodium tetraborate decahydrate (borax) and potassium hydrogen phthalate (KHP).

The NIR and Raman based fingerprinting with PCA were tested to distinguish between standard reference materials (SRM's) produced by the National Institute of Standards and Technology (NIST), and other potential candidate materials, and commercial samples of lower purity.

A collection of 87 samples of $CaCO_3$, 52 samples of borax and 63 samples of KHP were measured by NIR and Raman spectroscopy. In the case of $CaCO_{3,}$ PCA achieved a good discrimination coherent with the NIST reference method for acceptance of pH SRM's. In the case of borax, good discrimination was obtained between the samples but inadequate for quality control of candidate materials. In the case of KHP, insufficient discrimination was obtained between samples. This does not exclude the possibility of applying the proposed method to the quality control of candidate materials, because the NIST reference method was also unable to distinguish negative controls from SRMs.

The PCA clusters were further explained by X-ray diffraction (XRD) in case of calcium carbonate, which revealed that the PCA discrimination is based on the phase transformation of polymorphs of calcite, aragonite and vaterite.

In the case of borax, thermogravimetric analysis (TGA) revealed that borax is not a stable substance but it tends to lose some of its crystallization water and transforms slowly into the pentahydrate. This is in good agreement with the different groups distinguished on the PCA.

KHP was characterized by the impurities within the samples. The dominant impurity was proven to be the occluded water.

This study proves that the use of chemometrics and its ability to discriminate between chemically different samples could be a powerful tool to assure the identity and quality of the pH buffer materials and to streamline the acceptance procedure of new issues of SRM's.

Contents

Acronyms and Abbreviations

SRM®	Standard Reference Material®
NIST	National Institute of Standards and Technology
NIR	Near infrared spectroscopy
Borax	Sodium Tetraborate Decahydrate
KHP	Potassium Hydrogen Phthalate
PCA	Principal Component Analysis
HCA	Hierarchical Cluster Analysis
XRD	X-ray powder diffraction
SEM	Scanning Electron Microscopy
TGA	Thermogravimetric Analysis
NMI’s	National Metrology Institutes
IUPAC	International Union of Pure and Applied Chemistry
SI units	International System of units
CRM	Certified Reference Material
ORM	Office of Reference Materials
SED	Statistical and Engineering Department
PAT	Process Analytical Technology
PCs	Principal Components
SOM	Self-Organizing Map
SNV	Standard Normal Variate

INMETRO	National Institute of Metrology, Standardization and Industrial Quality
NMR	Nuclear magnetic resonance Spectroscopy

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1. INTRODUCTION

1.1. Objectives

The most frequently measured chemical quantity, often described as a master descriptive variable, is pH. Measurement of pH serves as a key indicator for acid/base equilibria, metal speciation, mineral saturation states and the bioavailability of metals, organic ligands, and proteins. Accurate measurements of pH depend on the availability of pH Certified Materials (CRM's). National metrology institutes such as the National Institute of Standards and Technology (NIST) supply primary pH buffers to ensure the quality of pH measurements worldwide.

The overall control of the quality of NIST pH standard reference materials (SRMs) starts from the candidate pH raw material and continues through the value assignment process which involves the preparation of liquid buffer solutions from solid buffers and an inter-comparison of the new buffer with the former one. The process to examine the quality of candidate materials can be time consuming and non-destructive methods to quickly screen materials is desired. An alternative procedure would involve spectroscopic fingerprinting. Spectroscopic fingerprinting has been widely used as a tool for the quality control of raw materials. However, discussions are still on-going as to whether a single technique provides adequate information to control the quality of pH buffer reference materials. In this study, we applied near infrared spectroscopy (NIR) and Raman spectroscopy in combination with chemometrics approaches to streamline the production process of three pH reference materials: calcium carbonate (used as saturated $Ca(OH)_2$ solution), sodium tetraborate decahydrate (borax) and potassium hydrogen phthalate (KHP). We aimed to reduce the time as well as the procedure needed to accept new candidate pH raw materials. NIR and Raman spectroscopy are known as fast, low cost, non-destructive tools with no or minimal sample preparation; and with the application of chemometrics, we tested the ability to distinguish between different quality levels of raw materials for use as primary pH standards. To accomplish this goal, former batches of pH SRM's were compared against potential candidate materials. Common commercial samples were also tested, functioning as negative control samples.

The traditional method of issuing new pH buffer materials is through wet experiments, which may take up to two weeks such as in the case of calcium carbonate pH buffer material. We believe that the use of an unsupervised classification method for multivariate data treatment such as principal component analysis (PCA) and hierarchical cluster analysis (HCA) would provide a quick and non-destructive qualitative tool to characterize the buffer samples.

It is worth mentioning that the traditional method of characterization is a univariate method using glass electrodes which does not provide as much information as the application of multivariate methods. Therefore, we consider the use of rapid analytical techniques NIR and Raman to examine variance in the buffer materials and supplemented these findings with analysis by techniques such as X-ray powder diffraction (XRD) and thermogravimetric analysis (TGA) for further explanation and interpretation of the results.

1.2. NIST pH SRM program

Measurement and control of pH is required nearly in every laboratory where industrial processes and research work are carried out. It is claimed that commercial pH glass electrodes can measure pH values with a reproducibility of 0.01 and therefore, standard solutions are used to calibrate the electrode reading system. The pH values of the tested samples are compared to those of standard solutions of known pH. National metrology institutes like NIST provide high quality materials which link these pH values to the International System of Units (SI units). Guidelines developed by research and through standard practice at NIST and then agreed upon by the International Union or Pure and Applied Chemistry (IUPAC) outline the qualifications needed to produce a primary pH reference material[1]. These guidelines ensure that pH values assigned to primary pH buffer materials are reproducible and have well defined uncertainties. The pH of six primary standards (potassium hydrogen tartrate, potassium dihydrogen citrate, potassium hydrogen phthalate, potassium dihydrogen phosphate/disodium hydrogen phosphate, sodium tetraborate decahydrate and sodium bicarbonate/sodium carbonate and two secondary (potassium tetroxalate and calcium hydroxide) reference standards are routinely assigned.[2]

1.3. pH concept

The current concept of pH was evolved through several theories. It started by Arrhenius in the last decade of the 19th century; he introduced the theory of electrolytic dissociation where the dissociation constant of water was determined by providing the relationship between hydrogen ions (H^+) and hydroxyl ions (OH^-). This was followed by the establishment of the acidity scale by Friedenthal in 1904 where he examined the color change of indicator dyes in 14 solutions of known hydrogen concentration. Later in 1909, Sørensen, a Danish chemist, introduced the concept of pH with regard to the concentration derived from the ionic product of water with a (zero to 14) scale at 25 ºC. The application of glass electrode (shown in Figure 1) to acidimetric titrations was introduced by Haber and Klemensiewicz in the same year.[3] Later, the concept of pH (Equation 1) was proposed by Sørensen and K. Linderstrøm-Lang as the activity of the hydrogen ions in solution.[3]

$$\mathrm{pH} = -\log a_{\mathrm{H}} = -\log\left(m_{\mathrm{H}}\gamma_{H} / m^{0}\right) \qquad \text{(Equation 1)}$$

where a_H is the relative (molality basis) activity; γ_H is the molal activity coefficient of the hydrogen ion H^+ at the molality $m_{H;}$ and m° is the standard molality (equal to 1 $mol \cdot kg^{-1}$).

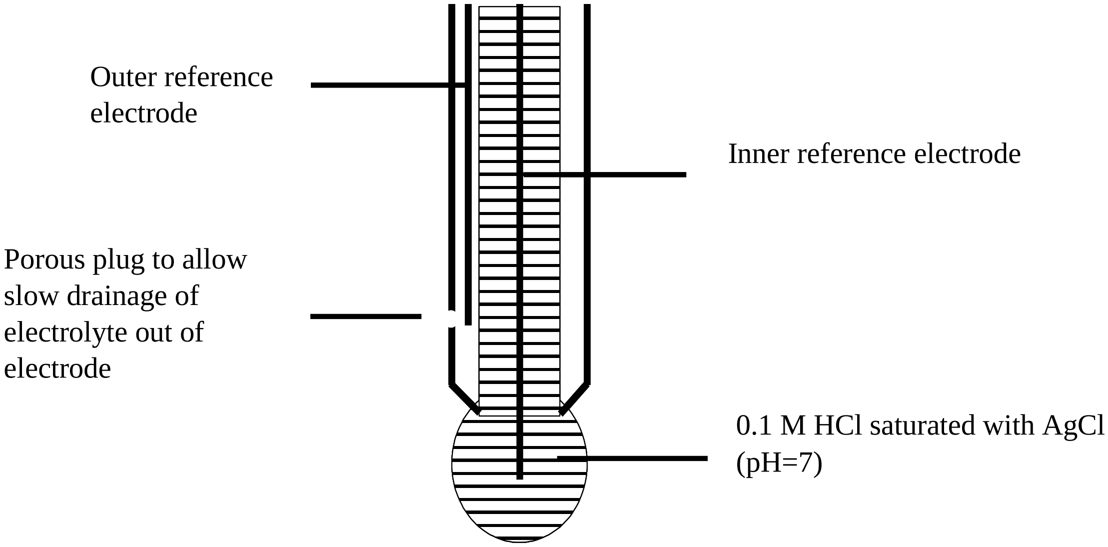


Figure 1 - Structure of the combination pH glass electrode

Values for pH can be expressed as the convention of excess hydrogen ions (proton pressure) or hydrogen ions deficiency (proton deficiency) in water. Measurements of pH can be made using different techniques. Indicator dyes provide a visual way of assessing pH while the hydrogen ion activity is most commonly measured in laboratory settings by glass electrodes using the electrochemical method. However, the concept of hydrogen ion activity is a practical approach and applies to solutions where the ionic strength is sufficiently low in concentration and where the activity coefficient of the hydrogen ion (γ_H+) does not deviate significantly from unity. To overcome this difficulty, Roger G. Bates proposed another more practical definition where the pH is determined relative to that of a standard buffer based on the infinite dilution system from measurements obtained from cells without liquid junction.[4] A buffer is an acid-base system consisting of a mixture of a weak acid/base and its conjugate base/acid. Changes of pH occur when the buffer presented in the solution reacts with the liberated hydrogen or hydroxide ion as expressed in Henderson-Hasselbatch equation (Equations 2):[4]

$$\mathbf{pH = pK_A + \log\frac{[B]}{[A]}} \qquad \textbf{(Equation 2)}$$

where pH is the concentration of $[H^+]$ in mol/L; K_A is the acid ionization constant, and [A] and [B] are the concentrations of the conjugate acid and base, respectively.

Ionic strength (*I*) may affect pH in the following way:[5]

1. Ionic strength is the measure of the total concentration of ions in a solution where the contribution by a given ion is influenced by its charge (Equation 3). An ion with a higher charge gives a larger contribution. This property impacts the dissociation constant and thereby the solubility of salts. High ionic strength reduces the attraction between anions and cations relatively as compared to their attraction in distilled water and therefore promoting the dissociation of salts, increasing their solubility and ultimately affecting the pH of the solution.
2. The activity coefficient depends on the ionic strength as it measures the deviation of behavior from ideality. The activity coefficient ($\gamma_H{}^+$) approaches unity as the ionic strength approaches zero as shown as in equations 3 and 4.

$$\boldsymbol{\mu = \frac{1}{2}\sum c_i z_i^2} \qquad \textbf{(Equation 3)}$$

Where c_i is the concentration of the ith component, z_i is the charge on the ion in question, and

μ is the symbol for the total ionic strength of a solution.

$a_{H^+} = \gamma_{H^+} \cdot [H^+]$ **(Equation 4)**

Several factors can contribute to errors associated with glass electrode pH measurements:[5]

1. The accuracy of pH standards limit sample accuracy by ±0.01 pH units; pH measurement cannot be more accurate than the standards.
2. Junction potential (the porous plug near the bottom of the electrode) contributes uncertainty of at least ~0.01 pH unit due to the change of the junction potential because the ionic composition of the analyte solution within the electrode (~ 3 mol/kg) is different from that of the standard buffer (typically < 0.1 mol/kg); even if the pH of the two solutions is the same, the junction potential will change as shown in Figure 2.
3. Junction potential drift is often caused by two factors. Drift could be initiated if the Ag(s) precipitates due to the presence of a reducing agent in the analyte solution. Junction potential drift could also be an issue when the potassium chloride (KCl) filling solution is diluted causing AgCl to precipitate onto the porous plug. This occurs because most combination electrodes have an Ag|AgCl reference electrode containing saturated KCl solution (around 3 mol kg^{-1}). In both of these cases, the junction potential change will cause a slow drift in the pH reading. This error can be compensated by recalibrating the electrode every two hours.
4. Alkaline or sodium error occurs when $[H^+]$ is much lower than $[Na^+]$ leading to lower measured pH than the actual one.
5. Acid error is evident if the apparent pH is higher than the true one due to the saturation of the glass with H^+ which prevents any further protonation.
6. Equilibrium time impacts a measurement with even in a well-buffered solution taking ~30 s with appropriate stirring to reach equilibrium with an electrode. The time increases for a poorly buffered solution which may require several minutes.
7. Hydration of glass should be maintained by soaking a dry electrode for several hours in order to correctly respond to H^+ in a given medium.
8. Temperature must be stabilized such that the calibration of the pH meter should be carried out at the same temperature at which the measurement will be carried.
9. Cleanliness of the electrode can cause incorrect measurements whereby the reading of an improperly cleaned electrode can drift for hours while it is re-equilibrating with aqueous

solution. Therefore, glass electrodes should be cleaned with a solvent after being exposed to any hydrophobic liquid and then should be well conditioned in aqueous solution.

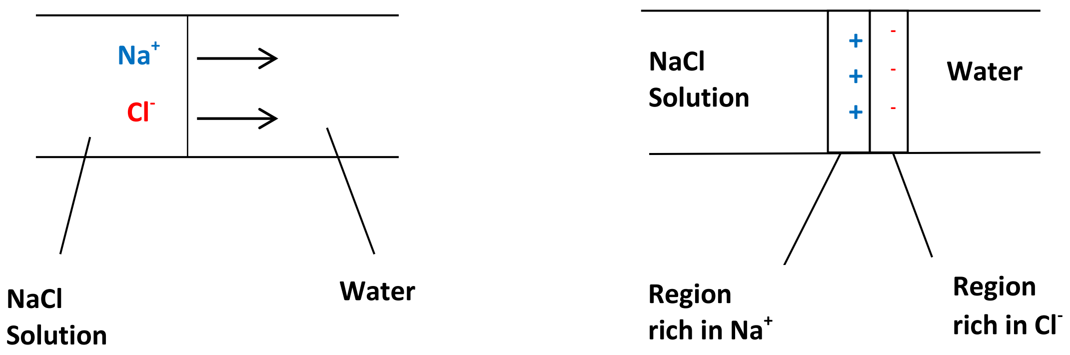


Figure 2 - Development of the junction potential caused by unequal mobility of Na^+ and Cl^-

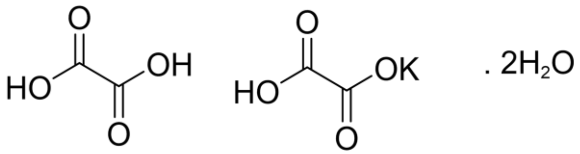
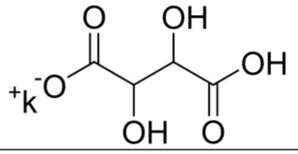
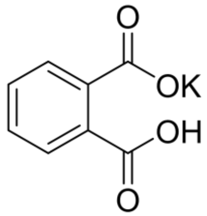
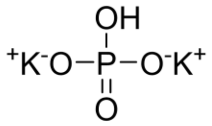
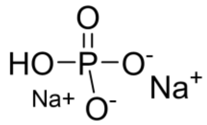
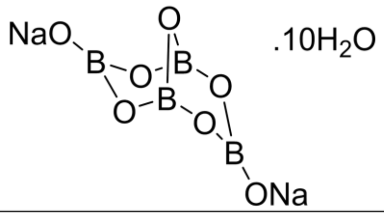
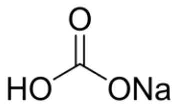
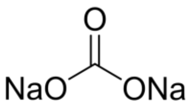
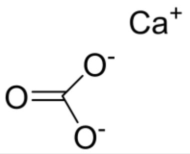
1.4. Certification of NIST pH SRMs

To help control the impact of error associated with glass electrode pH measurements, the proper use of standards to calibrate the electrode is required. NIST along with other National Metrology Institutes (NMI's), issues seven primary pH standards as SRM's as illustrated in Table 1. These SRM's are used by chemical manufacturers to provide traceability of pH measurements for routine analysis.

The primary measurement of pH is carried out with a cell without transference (liquid junction) using the hydrogen gas electrode, known as the Harned cell (see Figure 3). It is used for certifying the seven standard pH reference materials as it has high reproducibility and low uncertainty. The Harned cells fulfill all the essential features to be considered as a primary method of measurement: they function according to a precise measurement equation where all the variables can be experimentally determined in terms of the International System of units (SI). The Harned cell is a cell without liquid junction, defined as Pt | H_2 | buffer S, Cl^- | AgCl Ag, which means there is no separation between reference and sensing electrode. The Harned cell contains standard buffer (S) and potassium and sodium chloride as a source of chloride ions. Both solutions are added in order to use Ag|AgCl reference electrode.[1]

Figure 3 - Harned cell

Table 1 - The seven pH standards as Standard Reference Materials (SRMs) by NIST

pH Standard name	Approximate pH	SRM series	Structure	Year first issued	Average unit sold per year
Potassium Tetroxalate Dihydrate	1.7	189		1964	26
Potassium Hydrogen Tartrate	3.5	188		N/A	6
Potassium Hydrogen Phthalate (KHP)	4.0	185		1967	67
(1) Potassium Dihydrogen Phosphate (2) Disodium Hydrogen Phosphate	2 formulations 6.9 (1:1) 7.4 (1:3.5)	186	 	1965	65
Sodium Tetraborate Decahydrate (Borax)	9.2	187		1970	50
(1) Sodium Bicarbonate (2) Sodium Carbonate	10.0	191	 	1968	34
Calcium Carbonate (used as saturated calcium hydroxide solution)	12.5	2193		N/A	12

According to the NIST definitions of Terms and Modes[6], a Certified Reference Material (CRM) is a reference material, accompanied by a documentation body providing one or more of specified property values with associated uncertainty at a stated level of confidence and traceability using valid procedures while a Standard Reference Material® (SRM®) is a CRM issued by NIST that meets additional specific certification criteria required by NIST. SRMs are supplied with Certificates of Analysis that state the results of their characterizations and provide information about the proper use of the material. Normally, NIST does not produce or manufacture the materials from which SRMs are fabricated. Rather, materials are provided by industry, scientific companies, or groups that meet NIST specifications as shown in Figure 4.

For the pH SRM's, the raw materials are purchased from a chemical manufacturer. After receiving the material, NIST scientists perform acceptance testing then the NIST Office of Reference Materials (ORM) bottles the material for distribution. The bottled material is subsequently tested for homogeneity (heterogeneity) and NIST scientists conduct value assignment on the material with Harned cell measurements. After the value assignment is conducted, the Statistical and Engineering Department (SED) at NIST provides determinations of the overall uncertainty of the values according to the Joint Committee for Guides in Metrology Guide to the Expression of Uncertainty in Measurement.[7] For primary pH SRMs, the following conditions are required for certification:[6]

1. Homogeneity assessment for each pH SRM by inter-comparison of randomly selected aliquots of candidate material(s) normalized to the former issuance of the corresponding pH SRM using a glass electrode.
2. Internal control measures require that if there is a significant difference between the mean pH value of the current candidate material and the certified pH of the preceding SRM issue, the candidate material is rejected.
3. Each pH SRM is certified using Harned cells at each temperature of interest using at least three independently prepared buffer solutions of composite samples of the candidate SRM.
4. The combined uncertainty with the certified value involves the uncertainty in potential Harned cell, standard potential of Ag|AgCl reference electrodes, theoretical uncertainty,

the replication uncertainty for the overall pH value assignment, and the uncertainty from the homogeneity assessment.

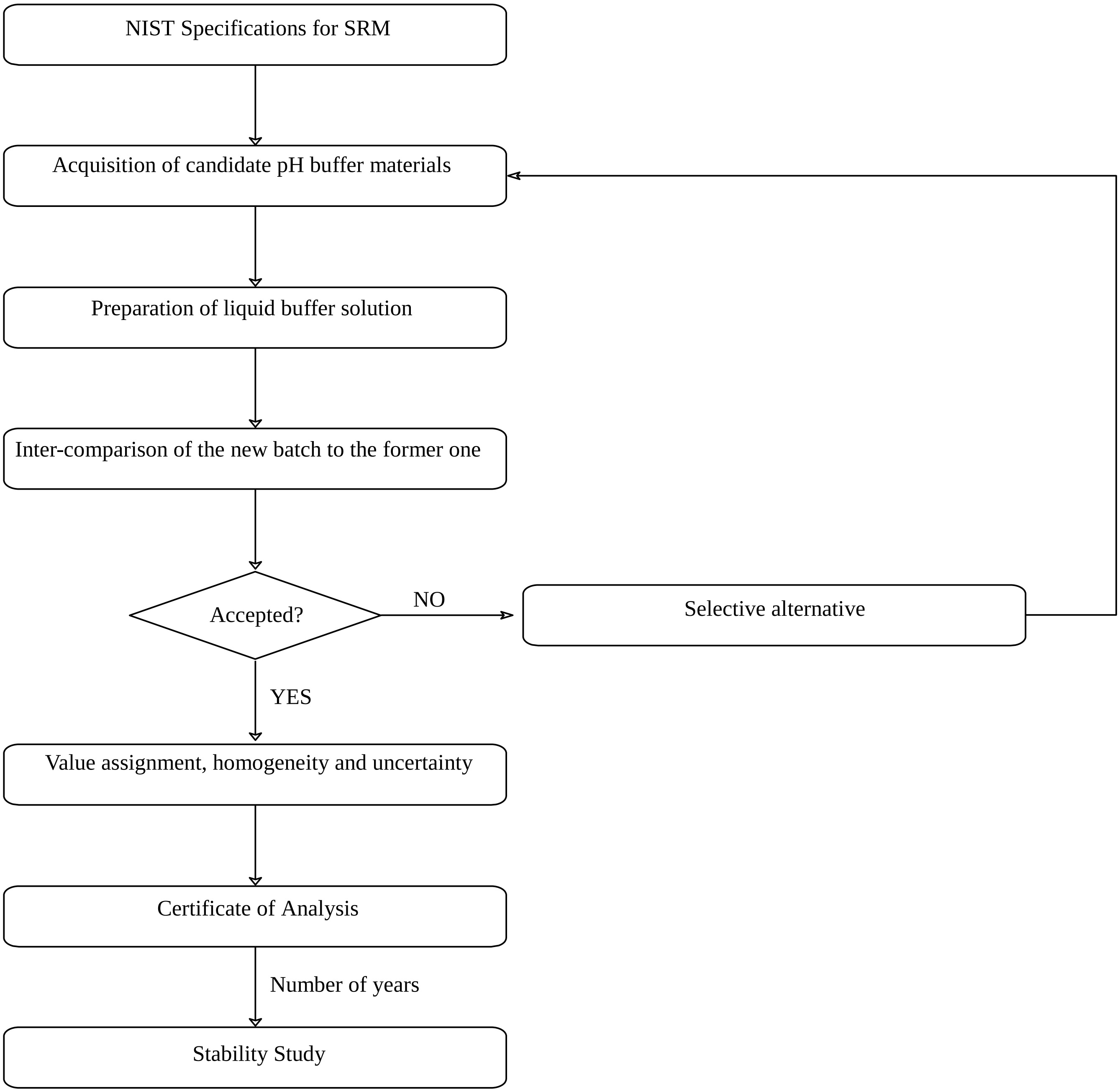


Figure 4 - Schematic Outline of the main steps in producing a pH Standard Reference Material

At NIST, there are several modes to be applied in the value assignment of any SRM for chemical measurement. The choice of mode is based on different factors such as previous experiences and knowledge of the specific matrix, analyte(s) of interest, current measurement capabilities, the quality of the analytical methods results, and the intended use of the material.[6] NIST follows the ISO/Guide 35:2017 Reference materials — Guidance for characterization and assessment of homogeneity and stability for the acceptance testing.[8] For the accepting testing of the raw materials for the primary pH standards, NIST makes liquid buffer solutions from the buffer materials when they arrive from the supplier and each solution is measured using a glass electrode against a former batch of the same material used as a control. This acceptance process may take up to one to two weeks to complete depending on the pH SRM tested. Efforts to decrease the SRM production time are underway and methodologies to streamline the acceptance process will both reduce time and financial investment in the production of a new issue of the pH SRM's. Furthermore, the glass electrode is univariate technique (ΔpH_i) that does not provide an appropriate explanation if the material is deemed unsuitable for use. The currently used electrode method cannot assess the purity of the material or reasons for the variability between batches and/or bottles. This study investigates the use of nondestructive multivariate techniques for use in the acceptance testing of these materials.

1.5. Pharmaceutical case study

The pharmaceutical industry can be used as a solid case study for the streamlining of material acceptance as similar problems are experienced by regulatory bodies for pharmaceutical industry. Many regulatory bodies such as the United Sates Pharmacopeia and the European Pharmacopeia require inspection of every barrel in every shipment of raw materials for the pharmaceutical industry to prevent public health concerns resulting from contaminated or mislabeled materials. Laboratory based analytical techniques such as chromatography, wet chemistry and titrations are traditionally used for pharmaceutical raw material identification or verification of the packaging label. This common quality control practice is time consuming and of high cost, destructive in nature, and labor intensive which is considered challenging to handle an enormous number of analyses.[9] Among the real-world application of this study is the possibility of routine testing of raw materials for acceptance directly in the warehouses. Raw substances for the pharmaceutical industry can be tested for identification and quality conformance in minutes once a chemometrics model for a substance is developed. This procedure is highly convenient to be applied in pharmaceutical industry as an essential part of Process Analytical Technology (PAT).[10] To address this problem, techniques that are non-destructive in nature with minimal manipulation or without sample preparation along with fast acquisition are required.

1.6. Non-destructive techniques

In this study, non-destructive pectroscopic methods such as Near infrared (NIR) spectroscopy and Raman spectroscopy were applied in combination with chemometrics approaches to provide a robust method for characterizing of several of the primary pH SRMs: calcium carbonate ($CaCO_3$), sodium tetraborate decahydrate (borax) and potassium hydrogen phthalate (KHP). Standard Reference Materials (SRMs) quality to streamline the production process along with better understanding of batch to batch variability as shown in Figure 5.

Near infrared (NIR) spectroscopy and Raman spectroscopy are widely used in quality control and assessment of purity in diverse fields such as chemical manufacturing, food production, oil industry and agriculture because they can quickly characterized materials by their spectral properties. They are known as fast, low cost, non-destructive tools with no or minimal sample preparation.[11,12] In near infrared (NIR) spectroscopy, changes in the molecular dipole moment influence the observed spectra and can provide information regarding the chemical and physical properties of the samples. A drawback to NIR is the technique has low sensitivity due to its high sensitivity to water bonds (this can be useful if the highest impurity of a molecule is water), and strong overlap can occur. In these cases, Raman spectroscopy is implemented as a complementary technique to infrared (IR) absorption spectroscopy.[11,12] It is worth mentioning that the development of fiber optics technology and recent improvements made to the instruments have increased the interest and applicability of NIR.[11] Raman spectroscopy is a phenomenon where the laser light is scattered by molecules creating vibrational transitions. The spectrum of scattered light reveals spectral bands which provide information related to the properties of the material, either directly or indirectly.[13,14] The limitation in size and variation of sample database is one of the biggest challenges in applying such techniques and therefore, one goal of this study was to build a library for the qualitative analysis of three pH reference materials (calcium carbonate ($CaCO_3$), sodium tetraborate decahydrate (borax) and potassium hydrogen phthalate (KHP)) by sampling materials from current and past issues of the SRMs and supplementing the dataset with materials from different manufacturers or sources.[15,16] Chemometrics methods simplified the process of data interpretation.

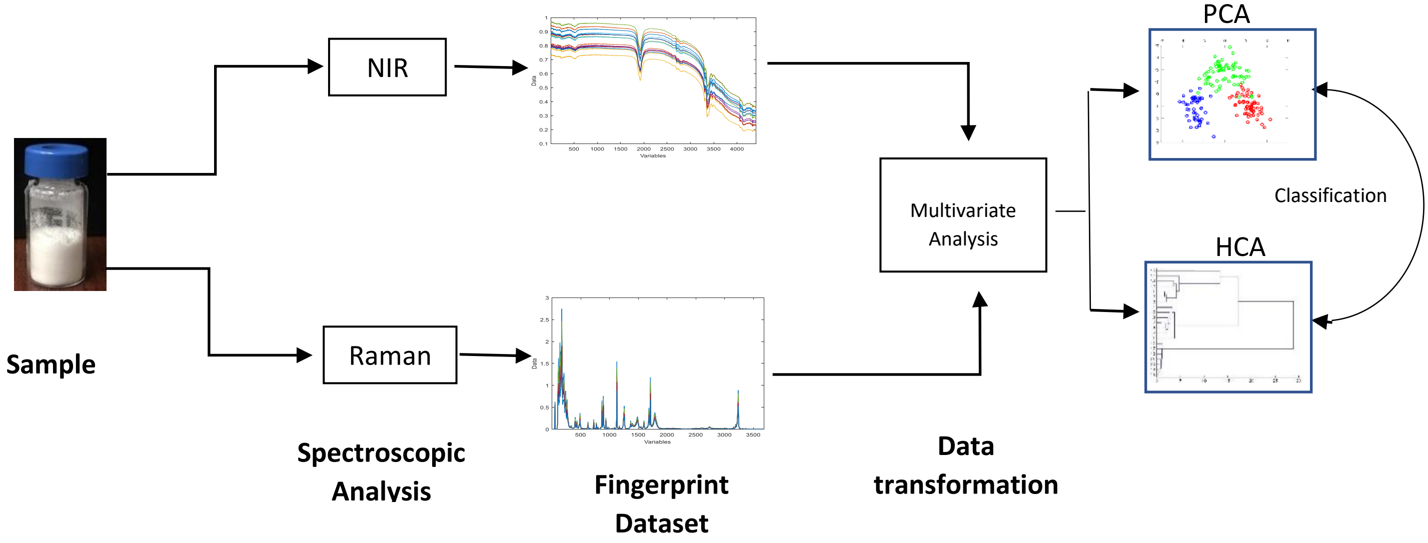


Figure 5 - Workflow of the study design

1.7. Chemometrics

The International Chemometrics Society defines chemometrics as the science of linking measurements made on a chemical system or process to the state of the system through application of mathematical or statistical methods. In other words, chemometrics extract relevant information from complex multivariate data.[14] There are two types of chemometrics classification techniques: the unsupervised method and the supervised method. Concerning qualitative analysis, we use the unsupervised method where samples are classified according to their spectra without any other prior knowledge. In this work, the principal component analysis (PCA) was applied as unsupervised classification method for multivariate data treatment. PCA is a mathematical method that reduces the number of correlated variables of multivariate data into lower dimensional uncorrelated variables expressed as Principal Components (PCs). The analysis of loadings (new variables) provided by PCA contribute to the understanding of the causes of variance, whether they are caused by instrumental, environmental, or sample properties. Loadings are linear combinations of the original variables and can be interpreted like spectra while scores (samples) were plot to confirm statistical differences between different batches (SRM and commercial samples) forming different ellipses or clusters (groups).

On the other hand, the obtained classification was confirmed by the hierarchical cluster analysis (HCA) as this clustering method is characterized by successive divisions of the dataset resulting in a cluster sequence (dendrogram). The main advantage of HCA is the possibility to cluster the samples based on the similarities in terms of the distance and proximity of the samples.[11,14,15]

1.8. Methodology

Multivariate analysis by NIR and Raman spectroscopy was conducted on three pH reference materials: calcium carbonate ($CaCO_3$), sodium tetraborate decahydrate (borax) and potassium hydrogen phthalate (KHP) for chemometrics analysis. The results from the spectroscopic analysis were also compared to those obtained by the reference univariate electrochemical method. Additional investigations were conducted to examine the sources of variation between materials. Information on the samples used for the analysis is summarized in Table 2.

All data was imported to MATLAB R2018a and the principal component analysis (PCA) and the hierarchical cluster analysis (HCA) were performed using the PLS Toolbox.

Furthermore, rapid analytical techniques such as X-ray powder diffraction (XRD) and Thermogravimetric Analysis (TGA) were applied for further explanation and interpretation of the obtained results by chemometrics.

Table 3 - Summary of pH Materials Examined by NIR, Raman, and Supplementary Analysis

Buffer material	**Batch** *(Italic number gives the batch number as plotted in the PCA)*	**Sampling**		
		Number of NIR/Raman Samples	**Reference Method**	**Supplemental Analysis**
$CaCO_3$	SRM 2193b* *(1)*	11 individual bottles	N/A	*One solid composite sample was analyzed by XRD **One solid sample was analyzed by XRD
	SRM 2193a *(2)*	10 individual bottles	A	
	SRM 915b* *(3)*	10 replicates of the same bottle	N/A	
	Baker**	10 replicates of the same bottle	B	
	Sigma**	10 replicates of the same bottle	B	
	Home Science**	10 replicates of the same bottle	B	
	Cuttlefish bone**	5 replicates of the same bottle	B	
	Chalk brand 1	10 replicates of the same bottle (NIR only)	B	
	Chalk brand 2	10 replicates of the same bottle (NIR only)	B	
borax	SRM 187f* *(1)*	One solid composite sample	C	*One solid composite sample was analyzed by TGA **One solid sample was analyzed by TGA
	SRM 187e* *(2)*	10 individual bottles	C	
	Merck**	10 replicates of the same bottle	D	
	Home Science**	10 replicates of the same bottle	D	
	Detergent**	10 replicates of the same bottle	D	
KHP	SRM 185i *(1)*	14 individual bottles	E	Not applicable
	SRM 185e *(2)*	2 bottles (5 replicates per each bottle)		
	SRM 185f *(3)*	2 bottles (5 replicates per each bottle)		
	SRM 185h *(4)*	2 bottles (5 replicates per each bottle)		
	SRM 84L	10 composite solid samples	C	
	Home Science	10 replicates of the same bottle	C	

A: One composite solid sample used as calibrant for the pH combined electrode

B: One solid sample from which one solution was prepared and measured on two different days

C: One composite solid sample from which two solutions were prepared and each was measured in duplicate

D: One solid sample from which two solutions were prepared and each was measured in duplicate

E: One composite solid sample from all SRMs where one part was not ground and from which one solution was prepared and each was measured in duplicate and another part was ground and from which two solutions were prepared and each was measured in duplicate.

2. MATERIALS AND METHODS

2.1. Samples and Reagents

Nine batches of calcium carbonate were examined using NIR Spectroscopy: three issues of calcium carbonate (SRM) 2193 from the National Institute of Standards and Technology (Gaithersburg, MD, USA); five commercial batches: Baker Analyzed Reagent (USA); Sigma chemical Co. (USA); Home Science Tools (USA); Tiza Chalk (USA) and UP&UP Chalk (USA); and 1 natural batch (Cuttlefish bone). Raman Spectroscopy was applied to all the batches except the two chalk batches.

Five batches of sodium tetraborate decahydrate (borax) and six batches of potassium hydrogen phthalate (KHP) were analyzed by NIR and Raman. For the borax, two were issued of the NIST SRM 187 (Gaithersburg, MD, USA), a three were commercial batches: Merck (Germany); Borax Detergent (USA) and Home Science Tools (USA).

For KHP, four batches were pH NIST SRMs (Gaithersburg, MD, USA), one high purity acidimetric NIST SRM 84L (Gaithersburg, MD, USA), and one commercial batch (Home Science Tools, USA).

The water used in the preparation of the buffer solutions was dispensed directly from a deionization-based point-of-use system (Milli-Q system) into the vessel used to prepare the buffer solutions (resistivity > 18 MΩ·cm, conductivity < 0.06 μS/cm). The prepared solutions were protected against evaporation and contamination.

2.2. Measurement of liquid buffers with glass pH electrode (Reference method)

Selected samples were analyzed by the reference method used by NIST to accept candidate pH SRM materials and to determine bottle to bottle heterogeneity of the batch of SRM> Liquid buffers were prepared according to the procedure outlined in the Certificates of Analysis (see section 2.2.1 – 2.2.3).

A Thermo Scientific Orion Micro combination pH electrode was connected to an electrometer (Keithley model 6514) to provide greater sensitivity to pH changes than routine pH meters (Figure 6). Two-point electrode calibrations were performed by bracketing the analysis pH with

SRM buffer solutions of known pH, as shown in Figure 7. The standard buffer solutions were given designated, pH values of pH(S_1) and pH(S_2) and the unknown designated as pH(X) (Table 3). The respective potential differences were measured as $E_V(S_1)$, $E_V(S_2)$, and $E_V(X)$, the pH value of pH(X), was obtained by the following equations:[1]

$$pH(X) = pH(S_1) - [E_V(X) - E_V(S_1)]/k' \qquad \text{(Equation 5)}$$

Where the practical slope factor (k′) is defined as follows

$$k' = [E_V(S_1) - E_V(S_2)]/[pH(S_2) - pH(S_1) \qquad \text{(Equation 6)}$$

Figure 6 - Keithley model 6514 electrometer connected to a Thermo Scientific Orion Micro combination pH electrode

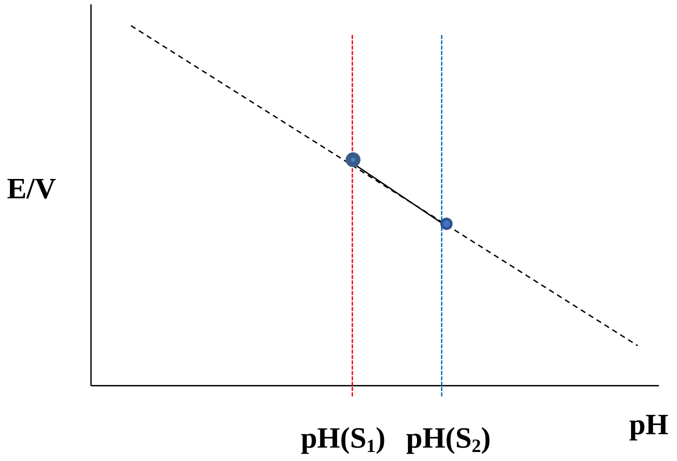


Figure 7 - Two-point calibration or bracketing of a glass electrode

Table 4 - pH values pH(S$_1$) and pH(S$_2$), were used to bracket the unknown pH(X)

pH(X) (unknown)	pH(S$_1$) at 25 °C	pH(S$_2$) at 25 °C
calcium carbonate	sodium tetraborate decahydrate (borax), pH(S$_1$) = 9.195	calcium carbonate, pH(S$_2$) = 12.469
sodium tetraborate decahydrate (borax)	sodium tetraborate decahydrate (borax), pH(S$_1$) = 9.195	calcium carbonate, pH(S$_2$) = 12.469
potassium hydrogen phthalate (KHP)	potassium tetroxalate dihydrate pH(S$_1$) = 1.677	potassium hydrogen phthalate (KHP), pH(S$_2$) = 4.005

Two freshly prepared standard buffers were used to determine the pH electrode response (k′) for each set of sample buffers (Table 3). The pH electrode was kept in the standard buffer solution for 30 minutes prior to the start of the measurements to equilibrate. For each measurement, the sample solution was contained in 2 cm^3 micro-centrifuge tube where the pH electrode was placed and the sample solution was stirred with the electrode for 30 seconds followed by an additional motionless 30 seconds, after which the glass electrode potential was measured.

The calibrant standard reference buffer solution (its pH is close to the sample pH) was measured using the equilibration protocol before and after each sample measurement as a control to account for electrode drift. The other standard buffer solution was measured at the start and end of each set of samples to determine the practical pH electrode response.

All buffer solutions (except calcium carbonate) were prepared gravimetrically by weighing the masses of the salts and adjusting for air buoyancy effects. The quality of prepared buffer solutions was maintained by minimizing atmospheric exposure.

2.2.1. Calcium Carbonate

A saturated calcium hydroxide ($Ca(OH)_2$) solution was prepared from calcium carbonate. About 7.5 g of calcium carbonate was placed into a fused silica (Vycor®) crucible then was slowly heated in a muffle furnace to 950 °C to 1000 °C. The $CaCO_3$ was ignited for one hour at this temperature. Immediately, the obtained product (CaO) was transferred to a desiccator and was allowed to cool. Lumps were removed by gently crushing and 100 mL of carbon dioxide-free water from Milli-Q system was added while stirring to form a calcium hydroxide $Ca(OH)_2$ suspension. This suspension was boiled for 15 minutes and cooled. The final produce was vacuum filtered on a sintered-glass funnel of medium porosity.

The solid $Ca(OH)_2$ was dried in an oven for 2 h at 110 °C and later was crushed by mortar and pestle to a fine powder. Approximately 1 kg carbon dioxide-free water was added to the dried $Ca(OH)_2$ (approximately 5g) into a 1 L plastic bottle. This bottle was shaken for a week about every 2 h and no less than four times per day while being maintained at 25 °C in a thermostated water bath between periods of shaking. The procedure yielded a saturated $Ca(OH)_2$ solution (0.0202 $mol.kg^{-1}$) .

For the analysis, the saturated solution of $Ca(OH)_2$ was filtered immediately before use with a disposable syringe fitted with a 0.45 µm nylon in-line filter.

2.2.2. Sodium Tetraborate Decahydrate (Borax)

Solutions of 0.01 $mol{\cdot}kg^{-1}$ were prepared by weighing approximately 3.7 g of each borax sample by difference to an accuracy of 1 mg, into a clean, dry 1 L polyethylene bottle. The solution was prepared according to the certificate of analysis whereby the weighed mass was multiplied by a conversion factor of 261.841 to give the amount of CO_2-free water needed to make the solution.

The water was weighted to an accuracy of 0.1 g and the solution was then shaken until the solid was totally dissolved. The borax samples were not dried prior to the preparation of the solution.

2.2.3. Potassium Hydrogen Phthalate (KHP)

A solution of the 0.05 mol/kg was prepared by weighing approximately 9.8 g of the material by difference to an accuracy of 1 mg, into a clean, dry 1 L polyethylene bottle. The mass of KHP was multiplied by 97.887, according to the certificate of analysis, to determine the amount of CO_2-free water needed to prepare the solution. The water was weighed to an accuracy of 0.1 g and the bottle was thoroughly shaken until the solid was totally dissolved. The KHP samples were not dried prior to the preparation of the solution.

2.3. NIR and Raman measurement

Acquisition of NIR spectra was done using a Bruker® Vertex 70 FT-IR spectrometer equipped with a Quart-Tungsten Halogen (QTH) lamp and thermo-electrically cooled InGaAS detector as shown in Figure 8. Data were collected and processed using OPUS software. The fiberoptic reflectance was used to record reflectance spectra at 8 cm^{-1} resolution in the spectral range 3500-12500 cm^{-1}. Samples were held in 2 mL vials from Agilent made of Type 1 borosilicate glass because they have invisible reflectance in the NIR region. To collect the background, a piece of Spectralon (polytetrafluoroethylene, PTFE) disc was cut into to fit inside the sample vial. The measurement was carried out by putting the fiberoptic reflectance probe close to the sample. During each measurement, 64 scans were averaged. Three spectra were obtained for each sample. The sample vial was rotated in three different positions with the NIR light being directed through the glass vial where reflected signals being recorded, and the measuring locations distributed as consistently as possible. A white reference standard consisting of Spectralon was used for calibration after every 15 measurements.

Raman spectra were collected with a Bruker® Vertex 70 FT-IR with RAM II module (near-infrared dispersive Raman spectroscopy technique) using a 785-nm (NIR) diode laser for excitation, over a 0– 3500 cm^{-1} spectral range, with a resolution of 4 cm^{-1} (Figure 9). The laser power at the sample was ~500 mW (varied according to sample), and a LN-Ge Diode detector (liquid-nitrogen cooled Ge-diode detector). Samples were held the same 2 mL glass vials used for the NIR measurements. Each sample collected consisted of two spectra at 5 kHz velocity, each spectrum consisting of 32 scans. Naphthalene was used to collect the background prior to the first measurement and after each 20 measurements. For both techniques, samples were subjected to drying and/or grinding conditions according to the certificate of analysis supplied by NIST (Appendix A).

Figure 8 - Bruker® Vertex 70 FT-IR spectrometer and Spectralon (polytetrafluoroethylene, PTFE) fitted inside the HPLC vial

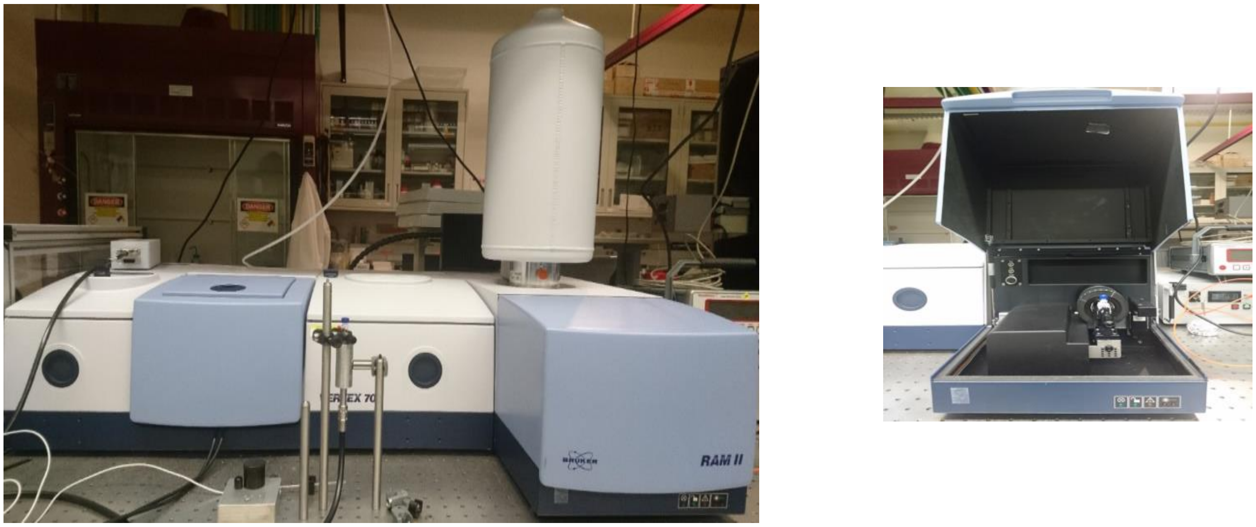


Figure 9 - Bruker® Vertex 70 FT-IR with RAM II module (near-infrared dispersive Raman spectroscopy technique)

2.3.1. Calcium Carbonate

The samples were dried at 200 °C to 210 °C for 4 h and stored in a desiccator over anhydrous magnesium perchlorate before use according to instructions in the certificate of analysis for SRM 915b (See Appendix A). Note that the calcium carbonate for the spectral methods do not require the conversion of $CaCO_3$ to $Ca(OH)_2$.

2.3.2. Sodium Tetraborate Decahydrate (borax)

The samples were used without further preparation as they were not dried in an oven nor stored in a desiccator before use according to the preparation instructions in the certificate of analysis (See Appendix A).

2.3.3. Potassium Hydrogen Phthalate (KHP)

The pH Standards and commercial sample were dried for 2 h at 110 °C and stored in a desiccator over anhydrous magnesium perchlorate before use according to the instructions provided in the certificate of analysis for SRM 185i (See Appendix A).

The acidimetric primary standard (SRM 84L) was ground by hand for a period of 60 s to 90 s in an agate mortar to obtain a fine powder. This preparation step helped to break down larger crystals so that occluded water could be sufficiently released during the drying procedure. The powder was dried at 120 ºC for 2 h and stored over anhydrous magnesium perchlorate in a desiccator.

2.4. X-ray powder diffraction (XRD)

Representative samples for $CaCO_3$ were used as received without any further treatment. Analysis of the samples was carried out by the National Institute of Metrology, Standardization and Industrial Quality in Brazil (INMETRO).

2.5. Thermogravimetric Analysis (TGA)

The thermogravimetric analysis of the sodium tetraborate decahydrate (borax) was performed on an Instrument Specialists Incorporated TGA i1000 to examine weight changes due to the loss of water and other volatiles loosely bound to the samples and to observe sample degradation as function of time and temperature. TGA data was used to predict the decomposition of borate hydrates. The TGA was calibrated using an approximately 10 mg weight whose mass had been verified with a Mettler Toledo XPR2U balance to provide traceability. The oven of the TGA was purged with dry nitrogen gas running at a flow rate of 20 mL/min. The temperature program for the analysis was as follows: the TGA oven was allowed to equilibrate at 25 ^{o}C then the temperature was increased at a heating rate of 10 ^{o}C per minute up to 900 ^{o}C.

Before each sample was measured, a baseline run was performed on an empty platinum pan to correct for apparent fluctuations in mass from buoyancy variations in the sample chamber caused by changes in the temperature of the oven and purge gas. After a smooth baseline was obtained, a sample of the sodium tetraborate decahydrate (borax) was weighed into a platinum crucible (open pan) using an analytical microbalance with a resolution of 0.1 µg. The sample was then run on the TGA using the same temperature program and conditions as the baseline. The baseline was then smoothed and subtracted from the sample runs to give the overall mass versus temperature profile of the sample.

3. DATA ANALYSIS

NIR and Raman provide distinctive and complex fingerprints for each material examined. NIR is associated with the vibration of chemical bonds. Raman is good as a fingerprinting technique because it is dependent on chemical bonds and the local environment of the molecules. Each molecule has a unique spectrum. Therefore, PCA combined with HCA are used as a dimension reduction data analysis tool to explain variation between spectra with as few variables as possible. The data analysis procedure involved two steps. First, a discrimination stage of analysis for the NIR and Raman data using PCA uncovered sources of spectral interferences and differences between NIST SRM's and commercial samples. The cluster analysis on this analysis was carried out with Ward's linkage type combined with the obtained PCs from the PCA analysis.

The second stage consisted assessing some of the differences attributed to properties and characteristics of the materials using either XRD or TGA techniques.

3.1. Data reduction

Each NIR and Raman spectrum is represented by 4407 and 3682 variables, respectively (figures 10 and 11). All spectra were imported to MATLAB R2018a (The MathWorks, Inc.). Firstly, NIR spectra were imported as a function of Reflectance and wavenumber (cm^{-1}). Then, they were converted to Kubelka-Munk (KM) units because the obtained analytical signal is highly complex function that is influenced not only by both physical and chemical properties of the sample but also by the scattering, stray light and inconsistency in the instrument response. Griffiths[17] states in the handbook of near-infrared analysis that the choice of converting the recorded data (spectra) into absorbance values (equation 7) or KM units (equation 8) depends on the type of sample and the spectral region. He also claims that whenever KM units are used, the baseline is irreproducible and the band intensities vary with it. However, Dahm and Dahm[17] do not share the same opinion of Griffiths that log (1/R) plots are more linear in practice than those of KM. The key is the light scatter depends on the physical features related to the particle properties of the sample such as particle size, particle shape and particle size distribution: the more the scatter, the lesser the light will penetrate the sample which impact on the amount of light absorbed by the sample.[17]

$$\mathbf{A = \log \frac{1}{R}} \qquad \textbf{(Equation 7)}$$

Where A is absorbance, R is reflectance

$$\mathbf{\frac{k}{s} = \frac{(1-R)^2}{2R} = f(\boldsymbol{R})} \qquad \textbf{(Equation 8)}$$

f(R) is KM function where R is diffuse reflectance, k is constant coefficient of absorption and s is scattering coefficient.

However, Kubelka-Munk is the most general and widely validated theory. It assumes three easy and clear assumptions:[18]

1- The scattered light is identically distributed;
2- The particles are randomly distributed in the layer and smaller than the layer thickness;
3- The layer is affected by the diffuse reflectance only.

After the conversion of all NIR spectra, averaging of the duplicate measurements of each sample was carried out where X is the data matrix (samples x variables); X1 is calcium carbonate data matrix (87 x 4407), X2 is sodium tetraborate decahydrate (Borax) data matrix (52 x 4407), and X3 is potassium hydrogen phthalate (KHP) data matrix (63x 4407).

Raman spectra were imported to MATLAB R2018a in function of intensity (a.u.) and wavelength known as Raman shift (cm^{-1}) for preliminary spectra assessment and manual removal cosmic ray removal. Each sample was collected in triplicate and the average was calculated where X` is the data matrix (samples x variables); X1` is calcium carbonate data matrix (66 x 3682), X2` is sodium tetraborate decahydrate (Borax) data matrix (52 x 3682), and X3` is potassium hydrogen phthalate (KHP) data matrix (63x 3682). Then, the laser light peak was excluded from the spectra and the regions with no or little information were eliminated as well before any further preprocessing.

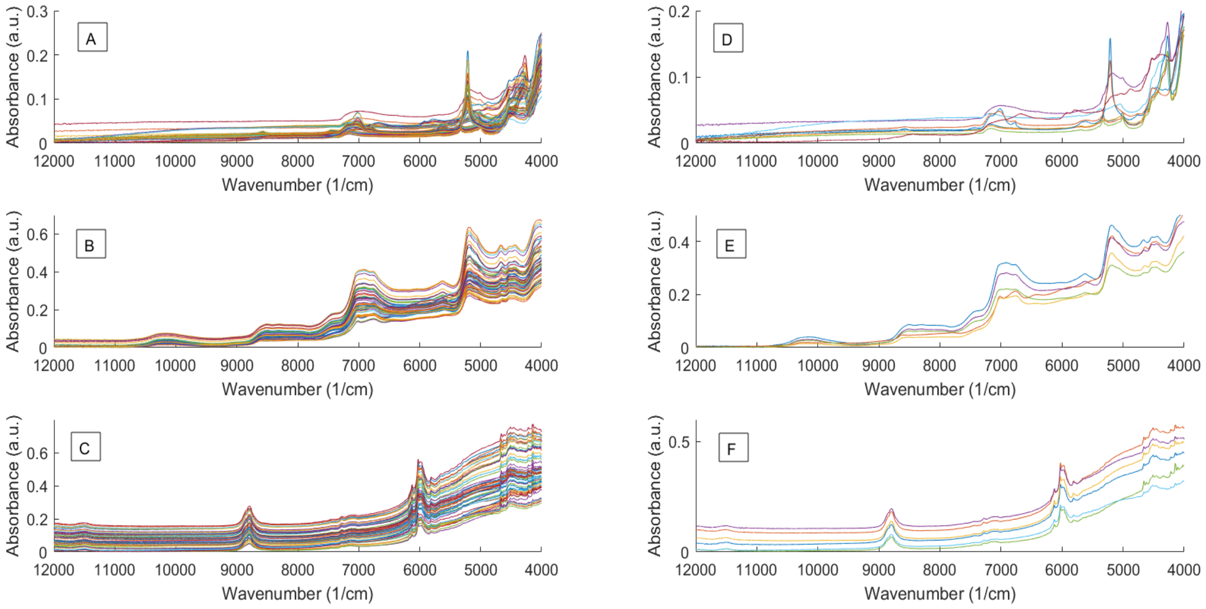


Figure 10 - NIR Raw Spectra. (a) calcium carbonate N=87; (b) borax N=52 and (c) KHP N=63. Subplots (d), (e), and (f) show representative spectra from each batch for $CaCO_3$, borax, and KHP, respectively

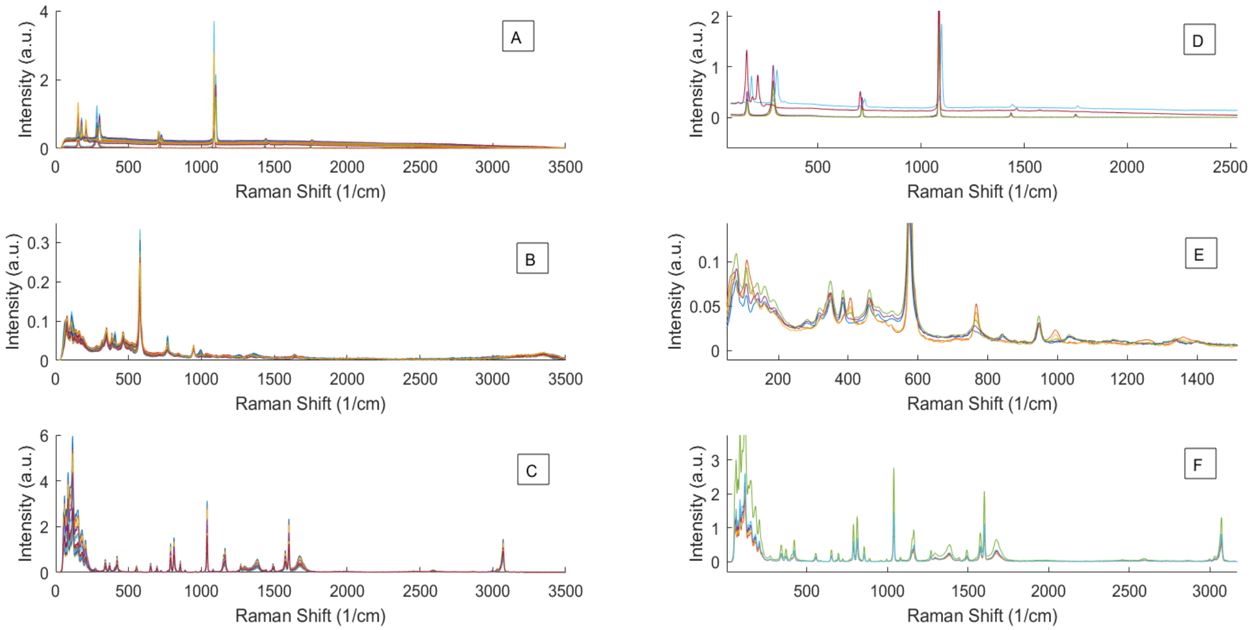


Figure 11 - Raman Raw Spectra. (a) calcium carbonate N=87; (b) borax N=52 and (c) KHP N=63. Subplots (d), (e), and (f) show representative spectra from each batch for $CaCO_3$, borax, and KHP, respectively

3.2. Spectral Pretreatment

Combinations of various data pretreatment methods were investigated for both NIR and Raman spectra. Many spectra pretreatment combinations were investigated to provide a satisfactory level of group separation or discrimination between SRM's and other samples. For NIR spectra, the best results were obtained using first derivative (polynomial order: 2, filter width: 21) followed by Standard Normal Variate (SNV). As shown in Figure 10, the raw spectra of the different materials from each batch displayed broad bands and variability in shape, intensity, and baseline over the whole spectral range. This variability may be caused by uncontrollable physical effects such as non-homogeneous distribution of the particles, changes in refractive index, sample packing/density variability, and sample morphology. In order to minimize or eliminate unwanted variability, spectral pretreatment is necessary. The key is to minimize or eliminate variability unrelated to the key spectral features for classification because some spectral features related to physical properties are significant in differentiating compounds long with chemical differences. Thus, moderate pretreatment is necessary. The key is to minimize or eliminate variability unrelated to the key spectral features for classification thereby differentiating between spectral features related to physical properties which are significant in differentiating compounds from chemical differences. To accomplish this objective, moderate pretreatment was selected. The first derivative was used to obtain more resolvable peaks and to minimize the baseline drift while SNV corrected for both additive and multiplicative effects present in the spectra.[9] The crystalline forms as well as variations of optical path-length and the particle sizes of the sample can affect the NIR spectra. These effects this cannot only be overcome by a well-defined sample preparation procedure but also by applying mathematical pretreatments[11] (Figure 12).

Raman Spectra of each sample was pre-processed before calculating the PCA model. The raw spectra of all compounds and representative spectra from each batch are shown in Figure 11. The first derivative (polynomial order: 2, filter width: 21), normalizing (2-Norm, length = 1) and mean centering were applied to both calcium carbonate and KHP raw Raman spectra while baseline (Automatic Weighted Least Squares, order=2) followed by smoothing (polynomial order: 1, filter width: 15), SNV, and finally mean centering were applied to borax raw Raman spectra.

Mean center is the last preprocessing technique applied in each combination before calculation to reduce data scatter for NIR and Raman data matrix.

Generally, spectral differences are enhanced by the first derivative and the mean centering is applied to track changes in the distribution or to compare the distributions of different types of features as it is the average x and y. This combination of pre-processing diminishes any deviations during measurements such as small temperature variations and other sources of difference impacting the intensity of the peaks.[19] As shown in Figure 12, Raman pre-processed spectral data provides more detailed information about the sample by resolving the previously overlapped bands and by minimizing the baseline drift in the raw spectra.[19]

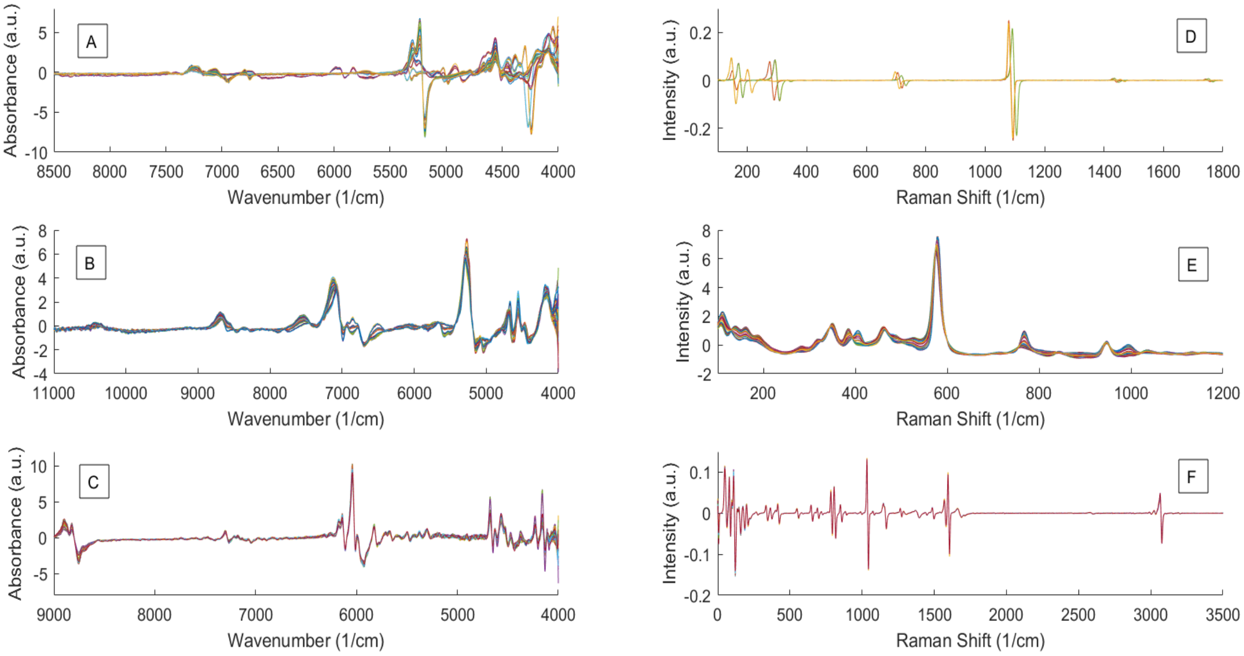


Figure 12 - NIR preprocessed Spectra. (a) calcium carbonate; (b) borax and (c) KHP. Subplots (d), (e), and (f) show Raman preprocessed Spectra for $CaCO_3$, borax, and KHP, respectively

4. RESULTS AND DISCUSSION

4.1. PCA and HCA

4.1.1. Calcium carbonate

The principal component analysis on the calcium carbonate NIR and Raman data matrix are presented in Figure 13. We observe six different ellipses when plotting the first two principal components of NIR data accounting for respectively 72 % and 19% of the total variance. Two overlapped ellipses correspond to NIST SRM's and one of the candidate materials (Sigma). Close to them falls another ellipse that corresponds to the Baker candidate material. Other groups are noticeably distinguishable from the SRM cluster. These outlier materials include the chalk, Home Science batch and the fishbone.

Raman data was not collected for chalk samples because they were dark in color after the drying procedure. The PCA of the $CaCO_3$ Raman samples is differentiated into three distinct groups using PC1 (94%) and PC2 (6%). One group has the SRM batches along with all candidate materials, another corresponds to the natural batch ‘cuttlefish bone’ and the third group includes the commercial batch ‘Home Science’.

Figure 14 shows the loadings plots of PC1 and PC2 for NIR and Raman data. Calcite is characterized by features at 5405, 5000, 4255 and 4000 cm^{-1} in NIR spectra.[20] In our study, all features can be seen in the spectra of our dried SRM samples, Sigma and Baker batches as well.

The interpretation of the loadings plot of Raman spectra provides further interpretation of the variables responsible for the obtained discrimination. The first PC mainly separates according the presence or the absence of calcite; which represent SRM, Baker and Sigma batches. Samples are negatively affected by the first PC due to presence of the calcite peak. The second PC is responsible for the separation according to the dolomite peak.

We plotted representative raw Raman spectra among each batch against those of the SRM and the peaks of calcite, dolomite and aragonite and vaterite were found to be in good agreement with previous literature.[21,22] As shown in Figure 15, both Baker and Sigma are characterized by the presence of calcite peak at 711 cm^{-1} in perfect agreement with the three batches of SRM.

It was proven that Home Science batch is dolomite in nature when we plotted its spectrum against the spectra of SRM batches. Home science is characterized by the presence of dolomite peak at 725 cm^{-1}. According to Kontoyannis[21], aragonite is characterized with a peak at 700 cm^{-1}. The spectrum of the cuttlefish bone indicates the presence of the aragonite peak at 704 cm^{-1}.[22] It is worth mentioning that the absence of vaterite peak at 750cm^{-1} proves that none of our samples is in this phase of crystallization.[21]

One difference to notice is that the NIR method provides more information about the Baker batch as its ellipse in the score plot of NIR shows clear separation away from the ellipse of the SRM’s and the Sigma batch. In contrast, the Baker fingerprint based PCA was not satisfactory in the score plot of Raman, in which the Baker ellipse is overlapping and misclassified along with the SRM’s and Sigma ellipse. This illustrates the fact that NIR technique may be more preferable in the case of calcium carbonate.

The results of the hierarchical cluster analysis are presented in Figure 16 for both NIR and Raman data matrices. The obtained pattern is the same separation obtained by the PCA where the Baker sample stands out as a separate group on the NIR while it shares some similarities with Sigma and SRM’s batches on the Raman data. This confirms that NIR is the most useful technique in the case of calcium carbonate.

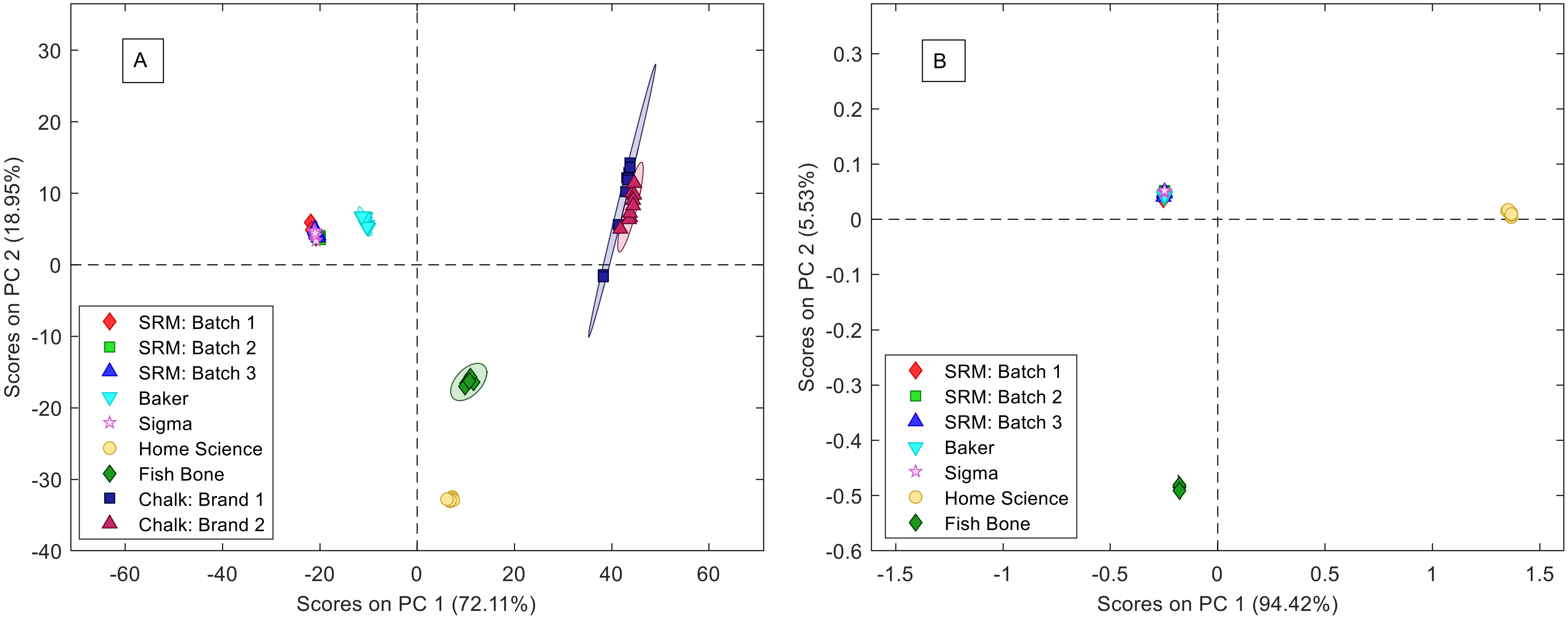


Figure 13 - PCA scores plots of calcium carbonate samples for (a) NIR and (b) Raman

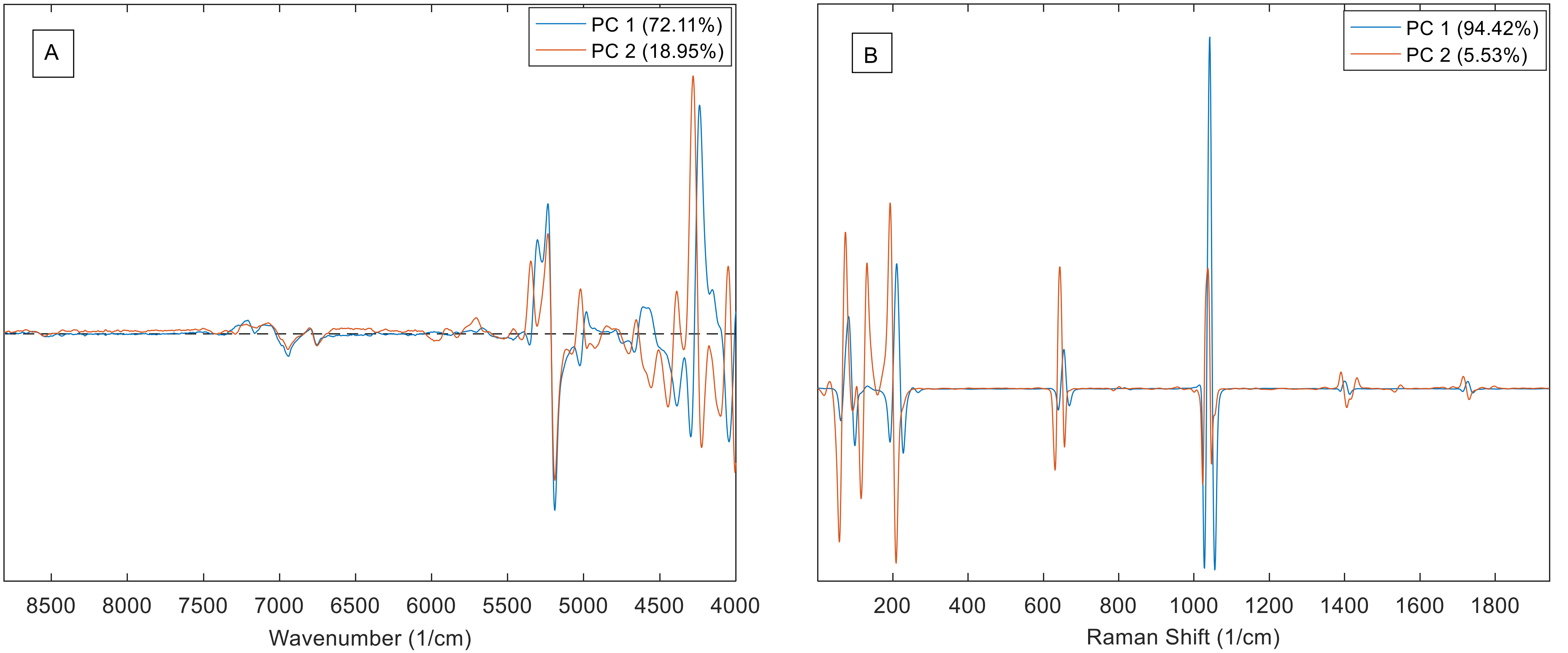


Figure 14 - PCA loadings plots of calcium carbonate samples for (a) NIR and (b) Raman

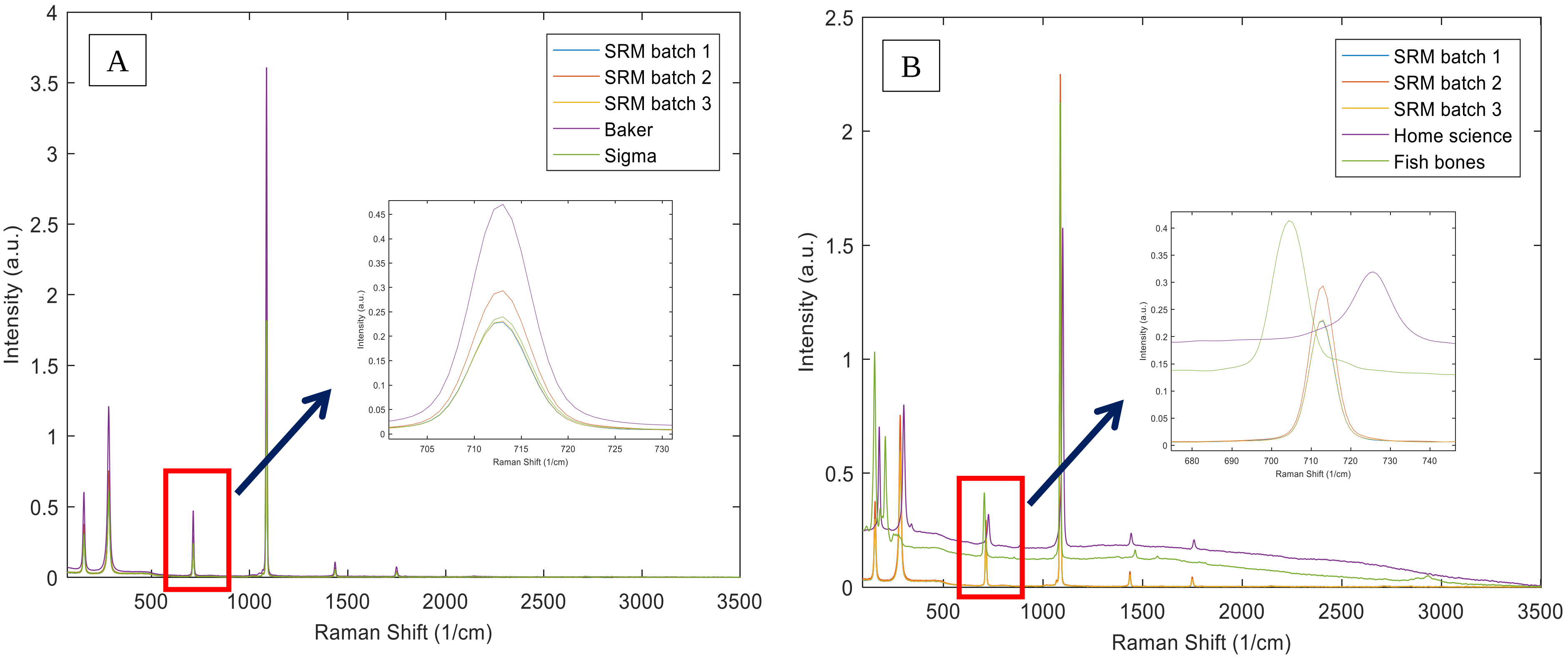


Figure 15 - Raw Raman spectra of SRM batches versus (a) Baker and Sigma and (b) Home Science and Fishbone

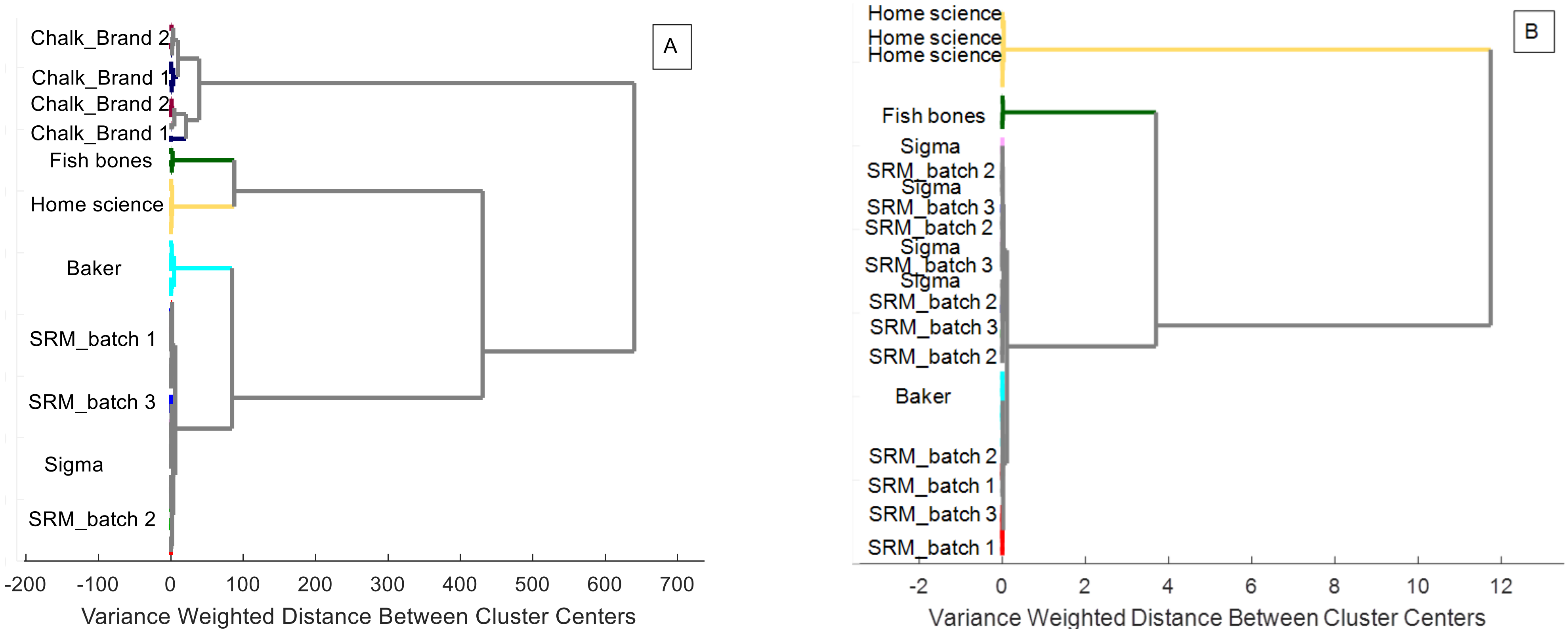


Figure 16 - Dendrogram calculated with the PCA scores of calcium carbonate for (a) NIR and (b) Raman

4.1.2. Sodium Tetraborate Decahydrate (borax)

The PCA scores biplots for NIR and Raman spectra for five different batches are shown in Figure 17. The first two PCs of each method accounted for over 80% of variability, represent an excellent summary variation between data sets. We observe five different ellipses when plotting the first two PCs, which account for respectively 72 % and 20% of the total variance in case of NIR data matrix and 97% and 1% for the Raman data. The ellipses are 95% confidence limits of each batch.

Unexpectedly, the two ellipses representing the SRM’s are not overlapping and show a clear distinction. Since both batches were previously approved by NIST and are already available in the market, we investigated the date of production of each batch to understand more about the stability of the pH reference material, borax. SRM Batch 1, shown as a red ellipse is SRM 187f and was produced in 2016 while the green ellipse is SRM 187e and was produced in 2004.

Further interpretation of the PCA plot shows that the ellipse formed by commercial batch Home Science falls relatively close to that of SRM 187f in NIR and they are overlapping in case of Raman. Checking other clusters, we observe that the blue cluster corresponding to the candidate batch Merck falls near that of SRM 187e in both NIR and Raman data. The Merck batch was acquired in 2010. The negative control batch corresponding to the detergent falls in between both groups of old and newly produced batches.

The visual examination of both SRM batches shows significant differences in the crystals size and texture as shown in Figure 18. SRM 187f is formed of large well-defined crystals while SRM 187e has a fine powdered texture.

According to Naumann R et al.[23], who studied the stability of the pH reference material borax SRM 187c versus a Merck batch, borax is not a stable substance. It is indicated that solid materials are stable as long as they are closed in their original containers, this guarantee a long shelf life as the containers are frequently opened to only withdraw the material. However, borax tends to lose some of its crystallization water, even when stored in its closed original container, to transform from the decahydrate into pentahydrate form which is more stable. Several factors control the rate of transformation of borax such as the storage period of material in the original closed container (age of material), time of atmospheric exposure, relative humidity during subsequent exposures, and temperature.

Further research exploration into the discrimination of the groups based on hydration was conducted by applying TGA to the samples to determine water content.

The dendrograms of NIR and Raman data matrices are shown in Figure 19. The results of the classification are similar to the PCA analysis. All samples are correctly classified in the NIR dendrogram; we can observe five clear groups. Similar batches are close in distance where Merck is adjacent to SRM Batch 2 and Home Science is adjacent to SRM Batch 1. The Detergent falls between and shares some similarities with Home Science as indicated by the PCA as well.

Raman dendrogram shows three well separated groups of which Merck, Detergent and SRM Batch 2 are clustered in one area. Home Science and SRM Batch 1 share several similarities. This discrimination reflects the conclusions obtained by the PCA as all samples were correctly classified.

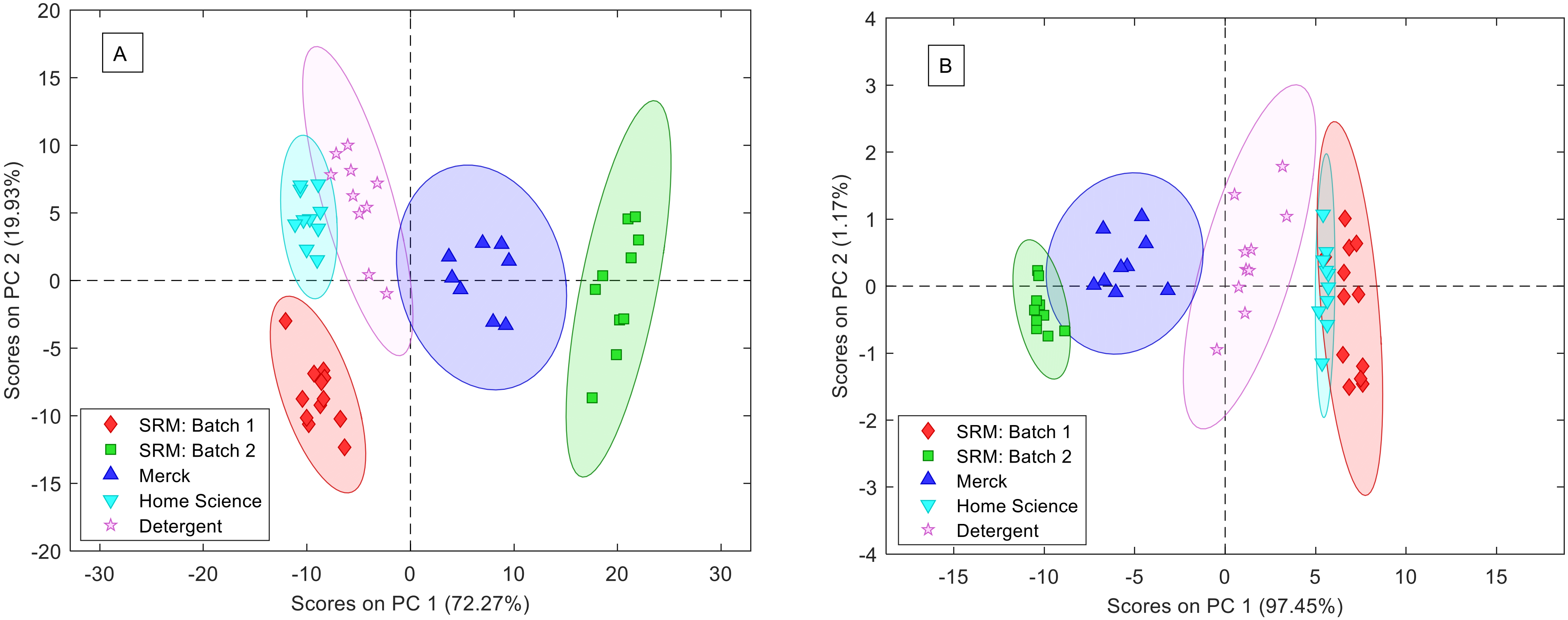


Figure 17 - PCA scores plots of borax samples for (a) NIR and (b) Raman

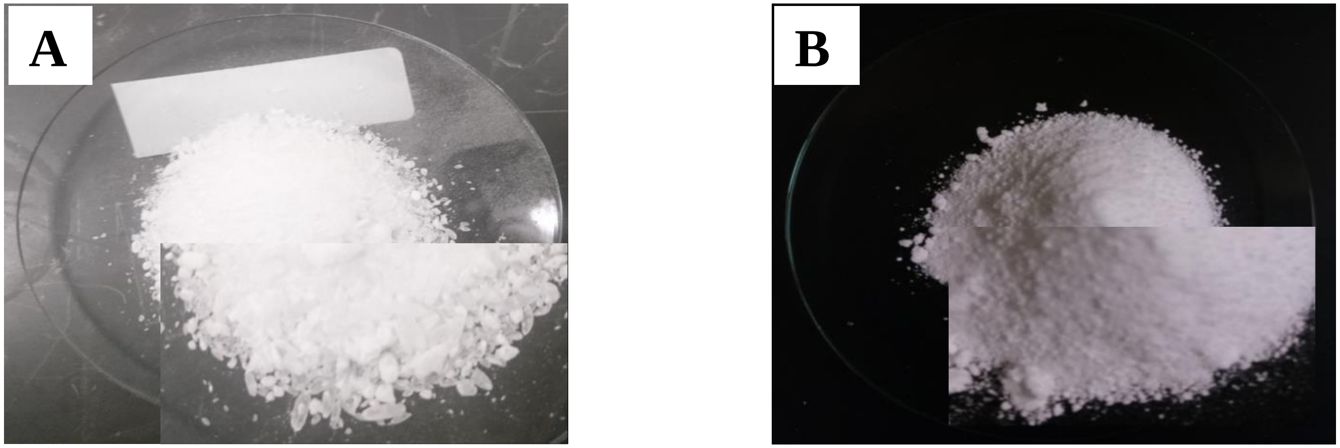


Figure 18 - SRM's Samples. (a) SRM 187f; (b) SRM 187e

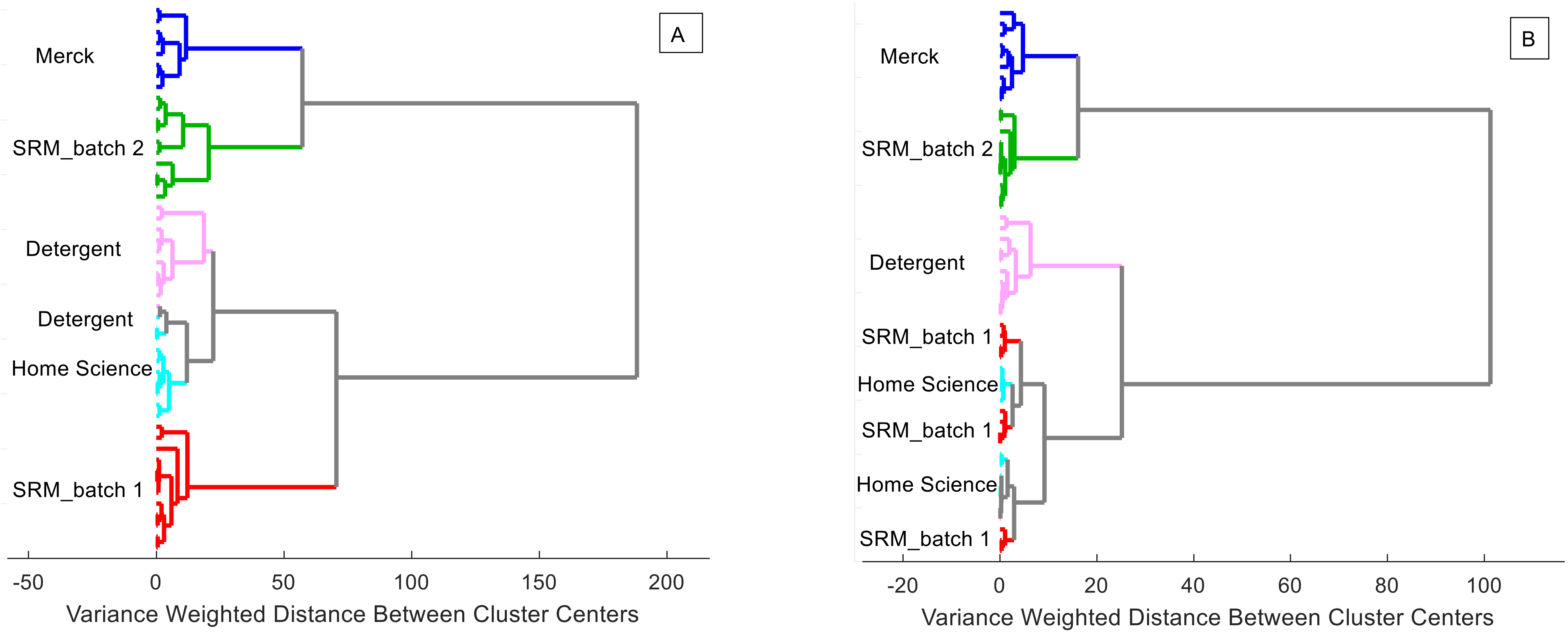


Figure 19 - Dendrogram calculated with the PCA scores of borax for (a) NIR and (b) Raman

4.1.3. Potassium Hydrogen Phthalate (KHP)

Initially, NIR and Raman spectra were collected for three different pH standards, one acidimetric standard and one commercial batch to run the PCA. According to the certificate of analysis of the pH SRM series 185, this standard was prepared to ensure high purity and uniformity, but it is not as a pure substance. On the other hand, the primary acidimetric standard, SRM 84L, consists of a highly purified potassium hydrogen phthalate (KHP) as given in Appendix A. The primary acidimetric standard is intended to be used for acidimetric assays and not as a pH standard. The two SRMs were treated differently as stated in their certificates of analysis, where acidimetric standard should be ground and dried before use while the pH standard should only be dried. The drying step in both cases was carried at the same temperature.

The results of the PCA performed on the NIR and Raman data matrix are presented in Figure 20. We observe two different groups when plotting the first two PCs of the NIR data, which account for respectively 65 % and 14% of the total variance. These groups correspond to the separation according to the purity level of the SRMs as well as the commercial batch. The first group involves the pH standards close to the Home Science batch, while the second group corresponds to the acidimetric standard. We observe high intra-variability among the samples of both pH SRMs and Home Science, which are dispersed over a wide area as compared to the ellipse formed by the acidimetric batch. The small cluster by the acidimetric standard proves that the purity and the homogeneity of the acidimetric SRM are high enough to ensure consistency among the samples withdrawn from different bottles.

Although the Home Science batch falls close to the SRM batches on the NIR PCA, the PCA performed on the Raman data matrix shows higher bottle to bottle variability within each batch. The samples are dispersed in one somewhat continuous cluster over PC1 and PC2.

Two different groups are observed when plotting the first three PCs of the Raman data matrix. The three PCs account for respectively 41%, 27% and 17% of the total variance (Figure 20b). The groups distinguish between the less homogenous SRM’s and commercial (Home Science) batch and the “purer” acidimetric SRM.

To understand better, we studied the PCA of the SRM’s samples where we ground two batches of pH SRM versus one batch of acidimetric standard in two different forms. We used a part of the material of the acidimetric standard as-is without any further treatment and we ground the other half. The discrimination that is displayed in Figure 21 shows that the ellipses formed by the ground pH standards are smaller in size as compared to those obtained from not ground samples. Unexpectedly, they look similar to the ground acidimetric samples while the not ground acidimetric standard is characterized by high bottle to bottle variability forming a relatively large ellipse. This indicates that the grinding step is critical as it releases the occluded water within the material which is evaporated later in the drying step.

To test the fact that grinding step is a limiting factor in the discrimination, we studied the acidimetric standard in both forms; ground and not ground. The obtained PCA for both NIR and Raman data matrix is given in Figure 22. Both forms are perfectly separated when plotting the first two PCs of each data matrix, where the ellipse of ground material is relatively small to that of the untreated material. We conclude that the entrapped water within the material is the dominant impurity and the primary factor responsible for the obtained discrimination between the pH and acidimetric standard.

The results of the hierarchical clusters analysis are shown in Figure 23. The dendrograms for both NIR and Raman matrices show no patterns for pH standards while the acidimetric standard, which has a highly purified crystalline form, has a clear distinct separation. The classification is similar to the PCA analysis.

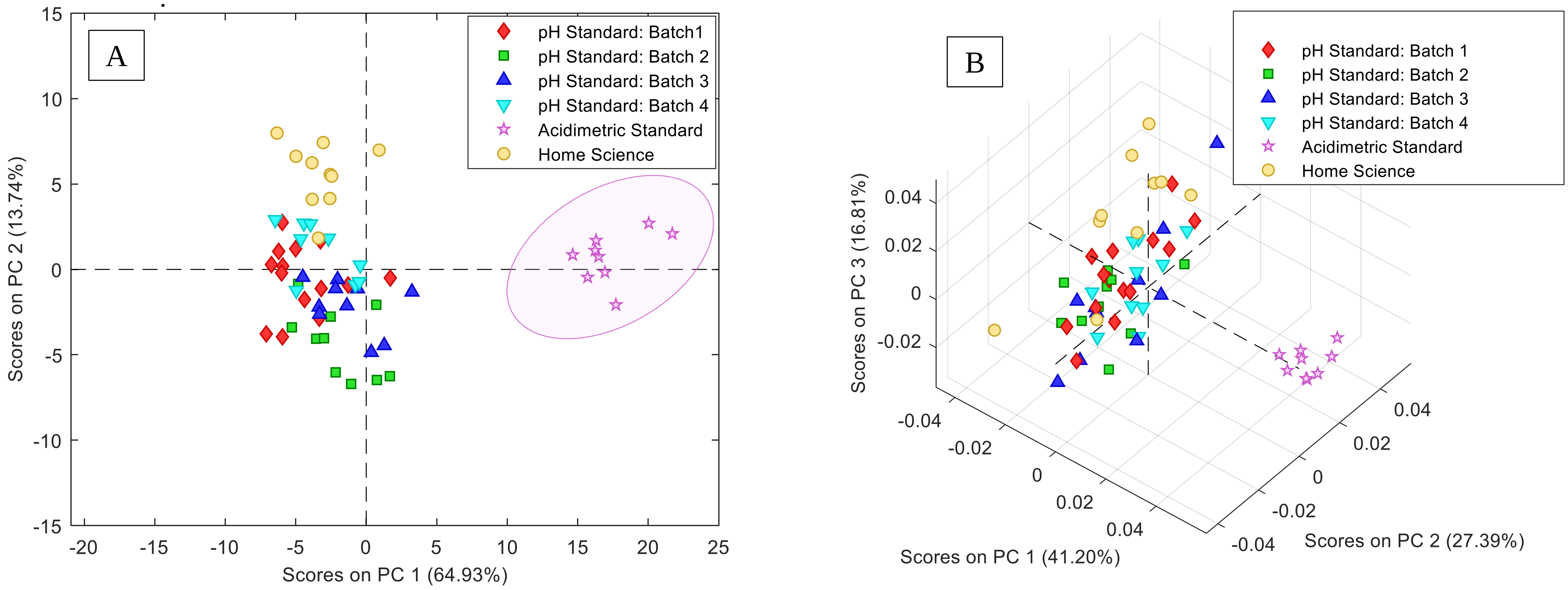


Figure 20 - PCA scores plots of KHP samples for (a) NIR and (b) Raman

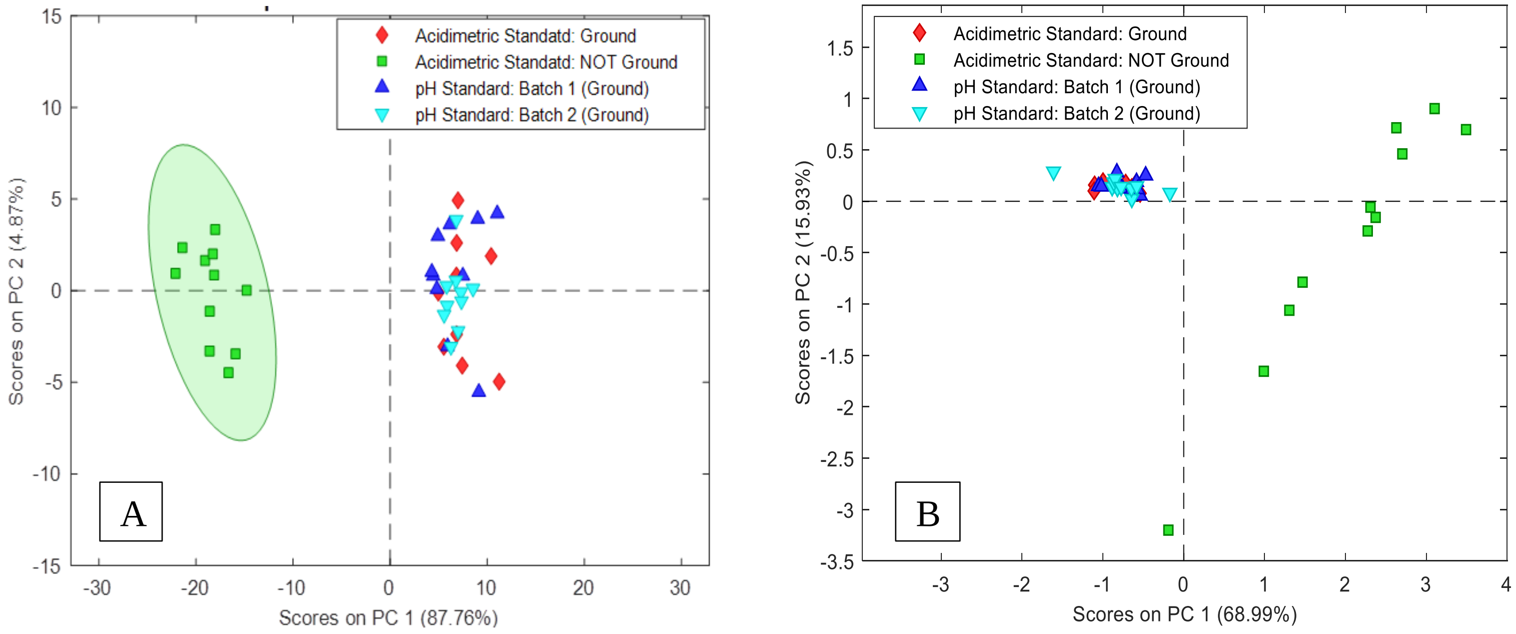


Figure 21 - PCA scores plots of pH standards versus acidimetric standard for (a) NIR and (b) Raman

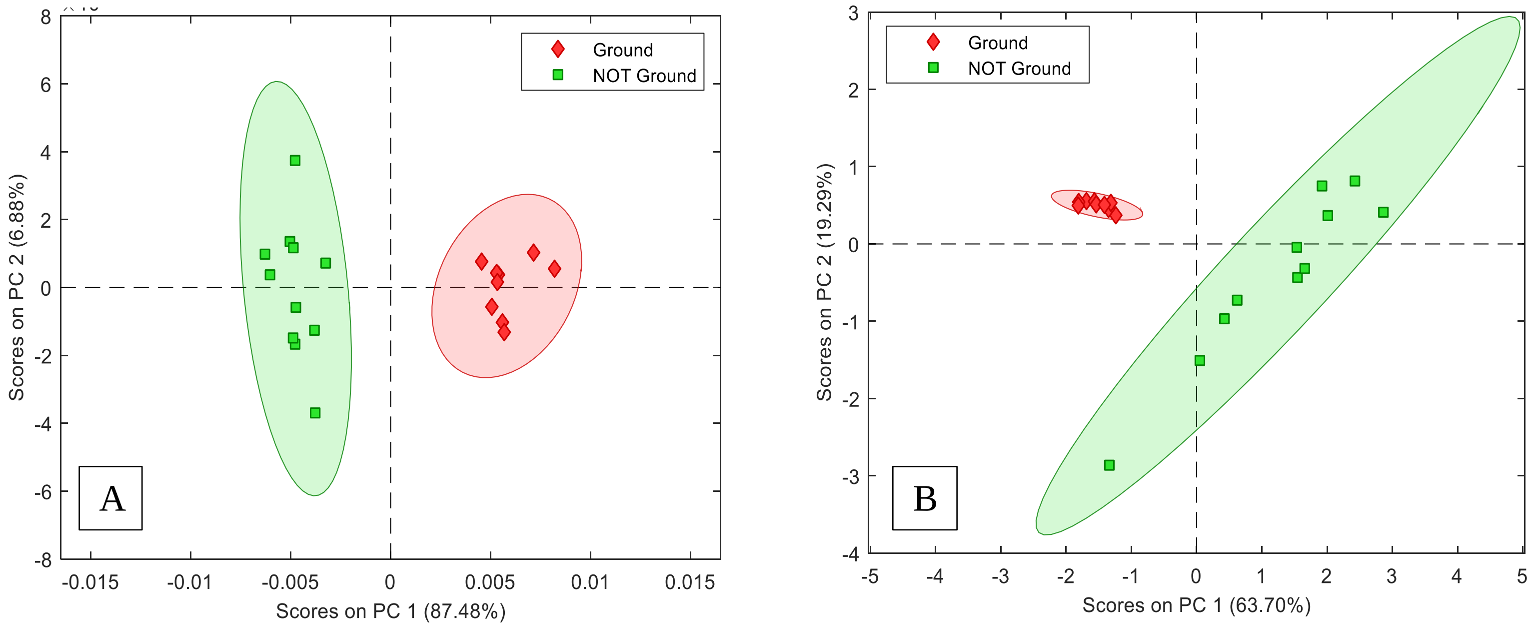


Figure 22 - PCA scores plots of ground / not ground acidimetric standard for (a) NIR and (b) Raman

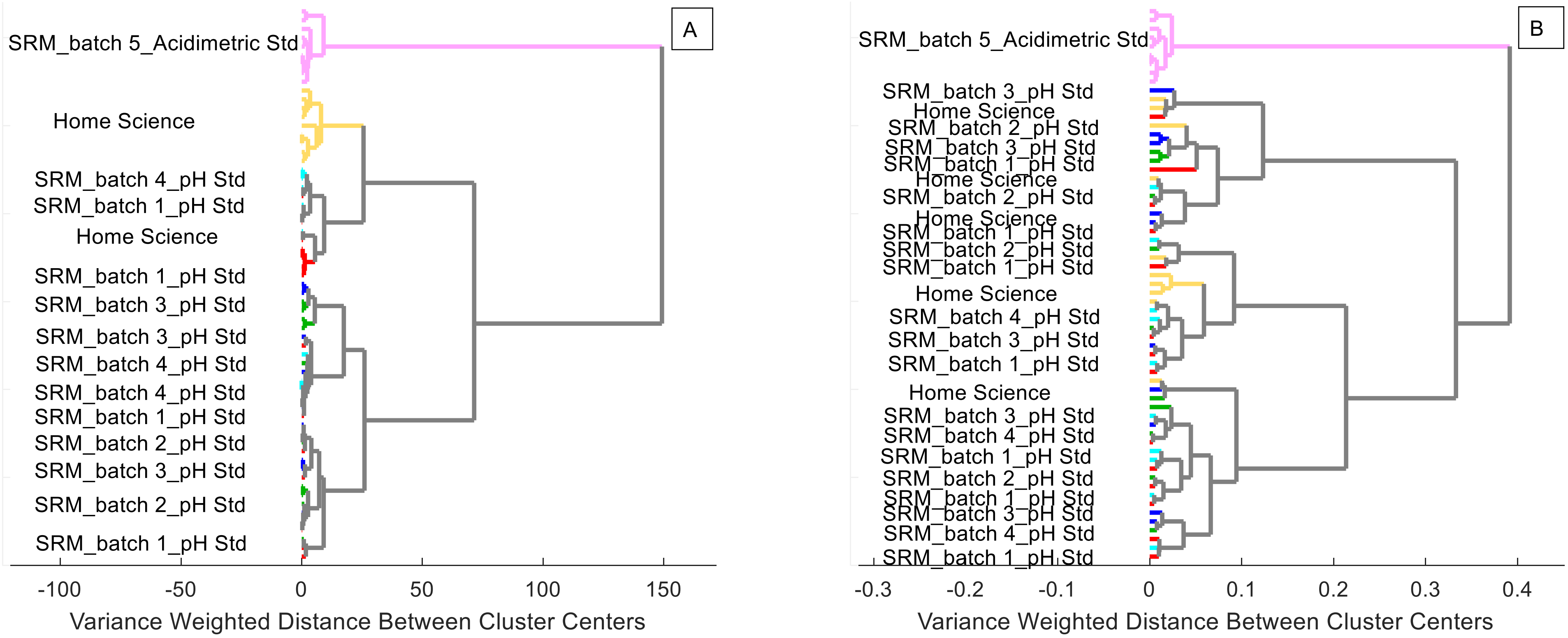


Figure 23 - Dendrogram calculated with the PCA scores of KHP for (a) NIR and (b) Raman

4.2. Reference electrochemical pH method

Each potential reading was recorded to 0.01 mV (corresponding to roughly 0.0002 pH units) and the pH electrode response was measured by the difference in pH (ΔpH) obtained from the mean of the two bracketing of the second standard buffer solutions (calibrant) measured immediately preceding and following the sample.

The change in ΔpH, ΔpH_i, between the standard reference calibrant and each sample was calculated by the following equation:

$$\Delta \mathbf{pH}_i = \left(E_i - \frac{E_A + E_{A`}}{2}\right)/\mathrm{K`} \qquad \textit{(Equation 9)}$$

where EA and EA′ are the potentials recorded for the bracketing calibrant: A (before the sample) and A′ (after the sample). E_i is the potential recorded for the sample and K` is the pH electrode response factor (slope).

4.2.1. Calcium carbonate

The potential readings of pH electrode were determined by using two freshly prepared standard buffers SRM 187f (sodium tetraborate decahydrate) and SRM 2193a (calcium carbonate used as saturated $Ca(OH)_2$ solution). To determine the practical pH electrode response factor (k′) while minimizing any memory effects on the liquid junction potential within the set of calcium hydroxide measurements, the SRM 187f borate buffer was measured at the start and end of each run and the second calibrant and control SRM 2193a was measured before and after each sample measurement as shown in Table 4.

Table 4 shows the calculated ΔpHi for the duplicate samples run on two different days. The value of ΔpHi of each sample for the candidate batches from Baker and Sigma as well as the commercial Home Science batch and Chalk samples functioning as negative control samples is used for acceptance assessment. In the same table, we compared our results to those results of homogeneity assessment for SRM 2193b provided by NIST Statistical Engineering Division analysis.[24] The homogeneity assessment for SRM 2193b was carried out on 12 bottles where the mean and the standard deviation of all samples were given.

Comparing the results of our samples to the mean and the standard deviation given by the SRM

2193b, we will conclude that all our samples are not within the accepted threshold except for the candidate batch of sigma (average ΔpH_i of the two days = 0.0007). Therefore, not all of our samples should be accepted as pH buffer materials.

Both brands of the negative control batches (Chalk) are rejected as their ΔpH_i is too high compared to that of the SRM. Unsurprisingly, the ΔpH_i of the commercial batch Home Science is not within the accepted range as visually appeared to be a lower quality material during the buffer preparation process. The color of the calcium hydroxide $Ca(OH)_2$ suspension was a blueish gray instead of white and the boiling and filtration steps took much longer time relatively to the other batches. This can be explained by the fact that dolomite crystals are less soluble than calcite crystals.

The Baker batch is on the border of the acceptance threshold and it shows inconsistent ΔpH_i from day to day. This means if a larger batch was tested, ΔpH_i may fall within the accepted range as the homogeneity will be increased.

Table 5 - Acceptance testing for calcium carbonate

SRM 187f temp coeff		
t/°C	pH(S)	
15	9.288	
25	9.1950	
dpH/d*T*	-0.0093	pH per °C
19.5	9.24615	

SRM 2193a temp coeff		
t/°C	pH(S)	
15	12.83	
25	12.469	
dpH/d*T*	-0.0361	pH per °C
19.5	12.66755	

ΔpH(X) from Δ*E* data

Run 1 pH(S)			Run 1 *E*/mV			d*E*/dpH	Run 1 T			Sample	ΔpH(X)	ΔmV(X)
9.246	calibration	12.574	-139.44		-336.13	-59.110	22.1		22.1			
	Baker		-336.39	-336.08	-336.40	101.80%	22.1	22.1	22.1	Baker	-0.0053	0.01
	Sigma		-336.34	-336.32	-336.45		22.1	22.1	22.1	Sigma	-0.0013	0.11
	Home Science		-336.15	-335.45	-336.13		22.1	22.1	22.1	Home Science	-0.0117	-0.02
	Chalk (Tiza)		-335.44	-221.85	-335.43		22.1	22.1	22.1	Chalk (Tiza)	-1.9216	-0.01
	Chalk (Up)		-335.42	-283.65	-335.51		22.1	22.1	22.1	Chalk (Up)	-0.8766	0.09
						101.44%						
12.574	calibration check	9.246	-335.48		-139.48	-58.902	22.1		22.1			

Run 2 pH(S)			Run 2 *E*/mV			d*E*/dpH	Run 2 T			Sample	ΔpH(X)	ΔmV(X)
9.246	calibration	12.668	-140.28		-334.24	-56.690	19.5		19.5			
	Baker		-334.36	-335.41	-335.43	97.63%	19.5	19.5	19.5	Baker	0.0087	1.07
	Sigma		-335.29	-335.70	-335.82		19.5	19.5	19.5	Sigma	0.0025	0.53
	Home Science		-335.83	-336.70	-335.25		19.5	19.5	19.5	Home Science	0.0196	-0.58
	Chalk (Tiza)		-335.88	-220.60	-335.13		19.5	19.5	19.5	Chalk (Tiza)	-1.9439	-0.75
	Chalk (Up)		-335.39	-282.28	-335.23		19.5	19.5	19.5	Chalk (Up)	-0.8971	-0.16
						104.47%						
12.668	calibration check	9.428	-335.44		-138.89	-60.663	19.5		19.5			

Table 6 - Results of acceptance testing for our samples of calcium carbonate versus the results of homogeneity assessment for SRM 2193b provided by NIST Statistical Engineering Division analysis

Samples	SRM issue	N	Mean of ΔpH (S)	Standard deviation *s*[ΔpH(S)]	Range
SRM 2193[24]	b	12	-0.0002	0.0011	-
Baker	-	2	0.0017	-	0.014
Sigma	-	2	0.0006	-	0.0038
Home Science	-	2	0.004	-	0.0313
Chalk Brand 1 (Tiza)	-	2	-1.9328	-	0.0223
Chalk Brand 2 (Tiza)	-	2	-0.8869	-	0.0206

4.2.2. Sodium Tetraborate Decahydrate (borax)

The potential readings of pH electrode were determined by using two freshly prepared standard buffers SRM 187f (borax) and SRM 2193b (calcium carbonate used as saturated $Ca(OH)_2$ solution). The SRM 2193a was measured at the start and end of each run and the calibrant SRM 187f borate buffer was measured before and after each sample measurement as shown in Table 6. Two buffer solutions were prepared and each solution was measured in duplicate runs for each batch.

According to the rule of the dilution value of the reference material, the pH values of the prepared solutions using the older issue (SRM 187e) should decrease since the batch was transformed into pentahydrate and lost 5 molecules of water. In contrast to this expectation, the experiments carried out by Naumann R et al. for the re-standardization of new Merck reference material sodium tetraborate decahydrate with respect to an approximately 5 years old NIST SRM 187c, proved that pH value increases with increasing loss of water. They estimated ΔpH to be -0.003 if a linear dependence of pH on concentration is assumed.[23]

In other words, the dehydration process means increasing the concentration of tetraborate if the same amount of material is dissolved and therefore, decreasing the pH value of the prepared solution but it seems that the reaction is more complex since the observed ΔpH = +0.02 with the transformation of decahydrate to pentahydrate. This observed ΔpH is about eight times the predictable change in the contrary direction, which supports the fact that the concentration differences, represented by the decahydrate transformation into pentahydrate, is not the only and the major cause of the observed pH changes.[23]

The average ΔpH of the two buffer solutions of SRM 187e included in this study is 0.0094 with standard deviation 0.0014 (see table 7). Those pH values are after 15 years of the production date (2004) with countless times of exposing the material to the atmosphere while the salt is withdrawn to prepare the solutions. On the other hand, the ΔpH of the freshly produced SRM 187e is 0.0023 with standard deviation 0.0012 according to Pratt who studied the within-laboratory variation of the certified pH value of the NIST pH SRM series.[25]

Comparing the value of the newly produced SRM 187e and that of the same lot after 15 years, we notice that ΔpH increased about +0.0071 which is less than the value stated in the study by

Naumann R et al. (ΔpH = +0.02) while the standard deviation in both cases is nearly the same. This confirms that the transformation of decahydrate into pentahydrate is not the major factor in increasing the ΔpH since of notice that ΔpH started to decrease again after more than 10 years over the storing period stated in the study by Naumann R et al.(5 years).

The Merck batch which was initially decahydrate in nature and then was converted into pentahydrate since it was produced in 2010 and subsequently exposed to the atmosphere for countless number of times yielded a ΔpH of 0.0178 with a standard deviation of 0.0002. This means that ΔpH was increased and therefore it is confirmed that ΔpH starts to decrease again after a certain number of years obeying the linear dependence of pH on concentration.

The ΔpH of two solutions of Home Science is -0.001 and standard deviation = 0.0005 as given in Table 7. As expected, those values are nearly the same as those obtained from the SRM 187b, 187c and 187e with ΔpH = -0.0003, -0.0014 and -0.0008, respectively, and standard deviation = 0.0008, 0.0021 and 0.0006, respectively.[25] Based on the results and knowledge of the material purchases, the series SRM lot 187 and Home Science are both decahydrate borates.

Although the detergent is pentahydrate borate, its ΔpH was inconsistent among the two preparations (average of ΔpH of the two runs of each preparation were =0.0017 and 0.1436). This was expected as it is a commercial batch and it includes a lot of unknown impurities.

Table 7 - Acceptance testing for borax

SRM 2193b temp coeff		
t/°C	pH(S)	
15	12.83	
25	12.4690	
dpH/dT	-0.0361	pH per °C
19.5	12.66755	

SRM 187f temp coeff		
t/°C	pH(S)	
15	9.288	
25	9.195	
dpH/dT	-0.0093	pH per °C
19.5	9.24615	

ΔpH(X) from ΔE data

Run 1 pH(S)			Run 1 E/mV			dE/dpH	Run 1 T			Sample	ΔpH(X)	ΔmV(X)
12.668	calibration	9.246	-332.05		-140.12	-56.096	19.5		19.5			
	187e (1,1)		-140.23	-140.68	-140.20	96.61%	19.5	19.5	19.5	187e (1,1)	0.0084	-0.03
	187e (1,2)		-140.17	-140.66	-140.19		19.6	19.5	19.6	187e (1,2)	0.0085	0.02
	187e (2,1)		-140.58	-141.08	-140.50		19.6	19.5	19.6	187e (2,1)	0.0096	-0.08
	187e (2,2)		-140.44	-141.23	-140.74		19.6	19.5	19.6	187e (2,2)	0.0114	0.30
	Merck (1,1)		-140.70	-141.84	-140.80		19.6	19.5	19.6	Merck (1,1)	0.0195	0.10
	Merck (1,2)		-140.44	-141.58	-140.93		19.6	19.5	19.6	Merck (1,2)	0.0159	0.49
	Merck (2,1)		-140.67	-141.67	-140.52		19.6	19.5	19.7	Merck (2,1)	0.0191	-0.15
	Merck (2,2)		-140.72	-141.56	-140.51	98.34%	19.6	19.5	19.7	Merck (2,2)	0.0168	-0.21
9.244	calibration check	12.668	-140.33		-335.81	-57.103	19.8		19.8			

Run 2 pH(S)			Run 2 E/mV			dE/dpH	Run 2 T			Sample	ΔpH(X)	ΔmV(X)
12.574	calibration	9.222	-334.46		-141.67	-57.519	22.1		22.1			
	Home Science (1,1)		-141.34	-141.37	-141.39	99.06%	22.1	22.1	22.1	Home Science (1,1)	0.0001	0.05
	HomeScience (1,2)		-141.38	-141.23	-141.405		22.1	22.1	22.1	HomeScience (1,2)	-0.0028	0.03
	Home Science (2,1)		-141.57	-141.26	-141.5		22.1	22.1	22.1	Home Science (2,1)	-0.0048	-0.07
	Home Science (2,2)		-141.82	-141.72	-141.22		22.1	22.1	22.1	Home Science (2,2)	0.0035	-0.60
	Detergent (1,1)		-141.32	-141.68	-141.744		22.1	22.1	22.1	Detergent (1,1)	0.0025	0.42
	Detergent (1,2)		-141.54	-141.65	-141.67		22.1	22.1	22.1	Detergent (1,2)	0.0009	0.13
	Detergent (2,1)		-141.56	-149.90	-141.71		22.1	22.1	22.1	Detergent (2,1)	0.1437	0.15
	Detergent (2,2)		-141.53	-149.79	-141.54	99.52%	22.1	22.1	22.1	Detergent (2,2)	0.1435	0.01
9.246	calibration check	12.574	-141.51		-333.811	-57.791	22.1		22.1			

Table 8 - Results of acceptance testing for our samples of borax versus the results of homogeneity assessment for SRM 187 series provided by Pratt

Samples	SRM issue	n	Mean of ΔpH (S)	Standard deviation $s[\Delta pH(S)]$
SRM 187 (by Pratt[25])	b	11	-0.0003	0.0008
	c	10	-0.0014	0.0021
	d	10	-0.0008	0.0006
	e	11	0.0023	0.0012
SRM 187	e	4	0.0094	0.0014
Merck	-	4	0.0178	0.0002
Home Science	-	4	-0.001	0.0005
Detergent	-	4	0.0727	0.1003

4.2.3. Potassium hydrogen phthalate (KHP)

The potential readings of pH electrode were determined by using two freshly prepared standard buffers SRM 189c (potassium tetroxalate dihydrate) and SRM 185i (potassium hydrogen phthalate). The SRM 189c was measured at the start and end of each run and the calibrant SRM 185i borate buffer was measured before and after each sample measurement as shown in Table 8.

We recorded pH measurements for the acidimetric SRM 84L in both ground and not ground form along with the pH SRM 185i in both forms as well to test the impact of the grinding step. We tested the commercial Home Science batch to compare its ΔpH to that of the pH SRM.

According to Pratt[25], The ΔpH of SRM 185 series d, e, f, g, h and i are respectively equal 0.0014, -0.0025, -0.0007, 0.0007, 0.0018 and -0.0005. The ΔpH values of our samples are within the accepted range as given in Table 9. The absence of grinding step in the case of the acidimetric standard gives respectively average ΔpH = 0.0009 for the two prepared solutions while it gives average ΔpH = 0.0008 in case of the pH SRM. On the other hand, the ground form of pH SRM gives mean ΔpH = 0.0034 for the two prepared solutions.

Home Science batch ΔpH is similar to that of the SRM 84L (not ground form) with ΔpH equal to -0.0002. This can be explained that grinding of the Home Science samples increased the exposed surface area during the drying step and this allows the samples to give a similar ΔpH as the not ground acidimetric SRM.

As given in Table 9, there is no difference when we compare the standard deviation of the not ground acidimetric SRM (0.0016) and the ground pH SRM (0.0015). Those values are within the range of standard deviation for the certified SRM as stated by Pratt.[25]

It is noticeable that the ground pH standard gives a higher ΔpH than the untreated sample of the same standard. This shows the impact of grinding step on releasing the trapped water within the material and therefore on increasing the homogeneity within the same bottle and from bottle to bottle.

Table 9 - Acceptance testing for potassium hydrogen phthalate

SRM 189c temp coeff		
t/°C	pH(S)	
15	1.669	
25	1.6770	
dpH/d*T*	0.0008	pH per °C
19.1	1.67228	

SRM 185i temp coeff		
t/°C	pH(S)	
15	3.999	
25	4.005	
dpH/d*T*	0.0006	pH per °C
19.1	4.00146	

ΔpH(X) from Δ*E* data

Run 1 pH(S)			Run 1 *E*/mV			d*E*/dpH	Run 1 T			Sample	ΔpH(X)	ΔmV(X)
1.672	calibration	4.001	297.31		160.37	-58.796	19		19			
	84L (Sample 1,1 NOT Ground)		160.13	159.87	159.86	101.39%	19.2	19.2	19.2	84L (Sample 1,1 NOT Ground)	0.0022	0.27
	84L (Sample 1,2 NOT Ground)		159.75	159.47	159.43		19.3	19.3	19.3	84L (Sample 1,2 NOT Ground)	0.0020	0.32
	84L (Sample 2,1 NOT Ground)		159.46	159.07	159.14		19.3	19.3	19.3	84L (Sample 2,1 NOT Ground)	0.0039	0.32
	84L (Sample 2,2 NOT Ground)		159.23	158.86	157.96		19.3	19.3	19.3	84L (Sample 2,2 NOT Ground)	-0.0044	1.27
	Home Science (Sample 1,1)		158.77	158.71	158.58		19.3	19.3	19.3	Home Science (Sample 1,1)	-0.0006	0.20
	Home Science (Sample 1,2)		158.72	158.63	158.56	100.88%	19.3	19.3	19.3	Home Science (Sample 1,2)	0.0002	0.16
4.002	calibration check	1.672	158.64		294.91	-58.501	19.30		19.3			

Run 2 pH(S)			Run 2 *E*/mV			d*E*/dpH	Run 2 T			Sample	ΔpH(X)	ΔmV(X)
1.672	calibration	4.002	294.77		158.908	-58.331	19.3		19.3			
	pH Std (Comp Sample 1,1 NOT Ground)		158.65	158.56	158.48	100.59%	19.3	19.3	19.3	pH Std (Comp Sample 1,1 NOT Ground)	0.0001	0.18
	pH Std (Comp Sample 1,2 NOT Ground)		158.66	158.46	158.433		19.3	19.3	19.3	pH Std (Comp Sample 1,2 NOT Ground)	0.0015	0.22
	pH Std (Comp Sample 1,1 Ground)		158.61	158.16	158.64		19.3	19.3	19.3	pH Std (Comp Sample 1,1 Ground)	0.0080	-0.03
	pH Std (Comp Sample 1,2 Ground)		158.63	158.28	158.26		19.3	19.3	19.3	pH Std (Comp Sample 1,2 Ground)	0.0028	0.36
	pH Std (Comp Sample 2,1 Ground)		158.67	158.24	158.364		19.3	19.3	19.3	pH Std (Comp Sample 2,1 Ground)	0.0047	0.31
	pH Std (Comp Sample 2,2 Ground)		158.60	158.45	158.50	101.14%	19.3	19.3	19.3	pH Std (Comp Sample 2,2 Ground)	0.0018	0.10
4.001	calibration check	1.672	158.52		295.115	-58.647	19.30		19.3			

Table 10 - Results of acceptance testing for our samples of KHP versus the results of homogeneity assessment for SRM 185 series provided by Pratt

Samples	SRM issue	N	Mean of ΔpH (S)	standard deviation, *s*[ΔpH(S)]	Range
SRM 185 (by Pratt[25])	d	10	0.0014	0.0008	-
	e	11	-0.0025	0.0011	
	f	11	-0.0007	0.0005	
	g	11	0.0007	0.0017	
	h	11	0.0018	0.0004	
	i	11	-0.0005	0.0003	
SRM 84 (Not ground)	L	4	0.0009	0.0016	-
Home Science	-	2	-0.0002	-	0.0008
pH SRM (Not ground)	Composite sample	2	0.0008	-	0.0013
pH SRM (Ground)	Composite sample	4	0.0043	0.0015	-

4.3. X-ray powder diffraction (XRD) for Calcium Carbonate

The separation by the PCA could be explained by the results obtained from XRD patterns as these groups correspond to the separation according to the crystal phase of calcium carbonate. Vaterite, aragonite and calcite are the three phases of crystallization of anhydrous calcium carbonate. Vaterite and aragonite are precursor phases for calcite as the transformation is thermal dependent taking into consideration that calcite is the most thermodynamically stable form and aragonite is the least stable polymorph.[21,26]

The X-ray spectra of our samples are shown in Figure 24. In the case of SRM 915b and 2193b as well as candidate batches of Sigma and Baker, the calcite phase of $CaCO_3$ perfectly describes these diffraction patterns. The Home Science is structured in the dolomite phase, $CaMg(CO_3)_2$ stoichiometry. Finally, Fish Bone sample is structured in the aragonite phase. Another phase is also present in the Fish Bone sample but we could not assign it to any simple Ca-C-O structure. Most probably this phase is an oxide with a mixture of many elements (Ca, Al, Fe, Ti,…etc.) in its stoichiometry.

According to Ni and Ratner[26], the 2θ peak positions can be used as fingerprint to identify crystal phases where calcite is characterized by a sharp intense peak at 29 which is absent in case of both aragonite and vaterite spectra. Our results are in good agreement with the literature because SRM batches showed the calcite peak as expected. We assumed that the accepted calcium carbonate candidate pH buffer materials are thermo-stable and this is can only be applied to the calcite crystal form. Group 1 is negatively on PC1 and positively on PC2 corresponds to the samples having calcite crystals (SRM, Sigma and Baker batches) while group 2 corresponds to the samples having dolomite crystals (Home Science). According to J. Perić et al.[27] the thermal transformation of vaterite directly and irreversibly into calcite happens upon heating above 730 k (387.778 ^{o}C). We assume this may be what happened to the Fishbone which is natural vaterite, during the sample preparation which includes drying at 200 °C to 210 °C for 4 h. The transformation process into calcite started but since the required temperature was not reached, the vaterite did not reach the stable calcite form. The Fishbone batch is therefore a true negative control in that it contains a complex crystalline and elemental composition with an oxide phase made of a mixture of several elements (Ca, Al, Fe, Ti,…etc.) in its stoichiometry.

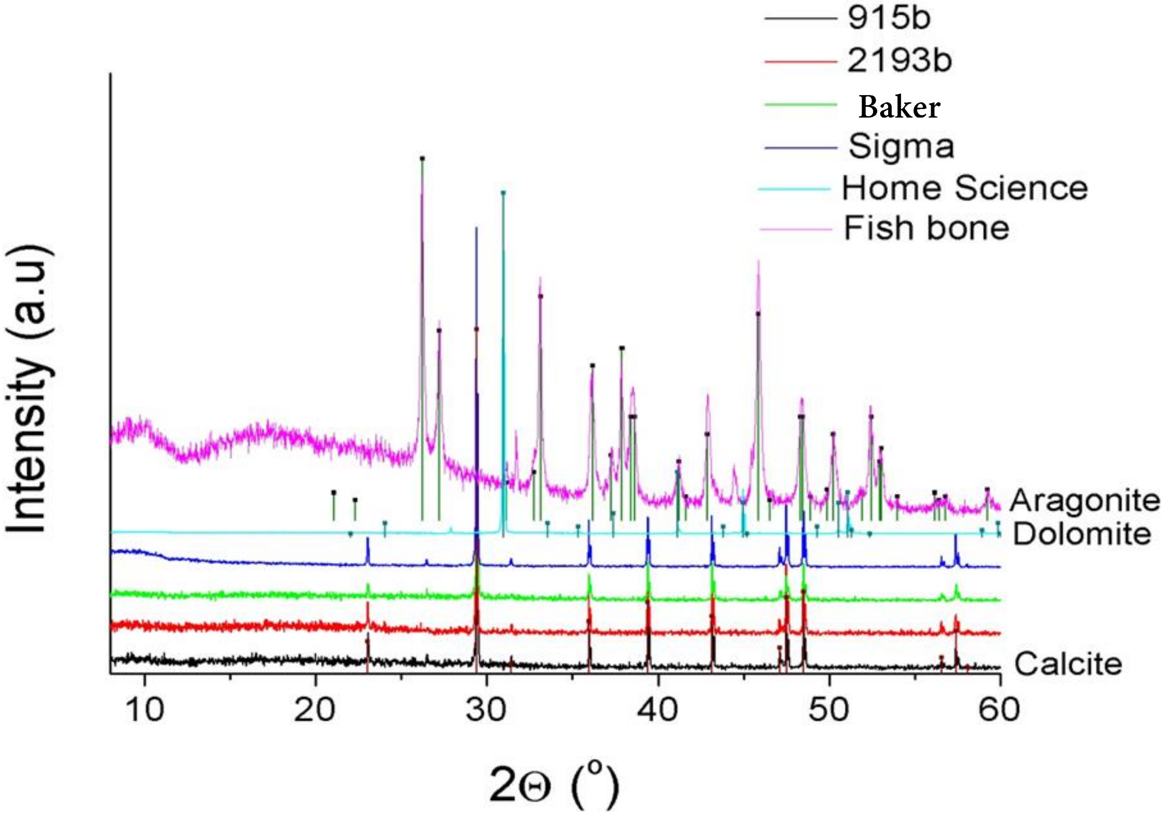


Figure 24 – XRD pattern for calcium carbonate samples

4.4. Thermogravimetric analysis (TGA) of Borax

As it was hypothesized that the separation of borax samples by PCA is due to the different levels of hydration of each sample, TGA was used to study the hydration of sodium tetraborate samples. The moisture loss was determined with the TGA results by measuring the mass loss of the sample in the full TGA curve between 25 ºC and 900 ºC.

The thermal decomposition at high temperature leads to an internal rearrangement forming new crystallization patterns. In both sodium tetraborate decahydrate and pentahydrate forms, two water molecules are involved in the ionic structure of the borate ion as hydroxyl groups. Removal of those water molecules is relatively more difficult whereas the removal of the remaining water molecules which are crystallization water molecules.[28] TGA curves for borax samples are shown in Figure 25. All calculations are detailed in Appendix B.

The dehydration of SRM 187f was investigated by TGA technique. The dehydration occurs in two steps. It was shown that the first curve is related to the removal of the crystallization water in the temperature range between 82.58 ºC and 154.12 ºC followed by the second curve indicating the complete removal of the remaining water at 500 ºC. Ten molecules of water were released from borax SRM 187f.

The TGA result for Home Science batch released approximately nine molecules of water, which is marked by an initial curve of water loss followed by a second curve, that we believe, is related to impurities within the material as we know it is a commercial salt. The last curve indicates the complete loss of water at 500 ºC.

There is an expected difference in the water content of the SRM 187f and Home Science batch versus SRM 187e and Merck batch. The gradual change from the decahydrate form to the pentahydrate form is complete 14 months later to the first exposure of the decahydrate to the atmosphere[23]. That is exactly what happened in the case of SRM 187e as well as Merck batch. Their weight loss decreases with time in accordance with the production year to reach values of 30.93 wt% for SRM 187e (produced in 2004) and 26.37 wt% for Merck (produced in 2010), which is close to the water content of the pentahydrate of 30.92 wt%.

The recently produced and opened samples represented by SRM 187f and Home science consist of nearly 100% sodium tetraborate decahydrate according to the practical water loss of 46.65 wt% and 44.2 wt%, respectively and with a theoretical water content of 47.23 wt% for SRM 187f and of 44.62 wt% for Home Science batch.

On the other hand, SRM 187e and Merck batch consist of nearly 100% sodium tetraborate pentahydrate according to the practical water loss of 30.23 wt% and 27.79 wt%, respectively and with the loss of approximately five or four molecules of water from SRM 187e and Merck, respectively.

The detergent batch lost almost five molecules of water (see appendix B), although it was bought recently but this proves that the decomposition rate of hydrates does not depend only on the frequency with which the material is exposed to the atmosphere but also on the storage time before the opening of the closed container.

The uncertainty of the raw masses was assigned a simple rectangular distribution of 0.0288 mg for the TGA mass loss and 0.0001 mg for the initial masses of the materials measured on the analytical balance.

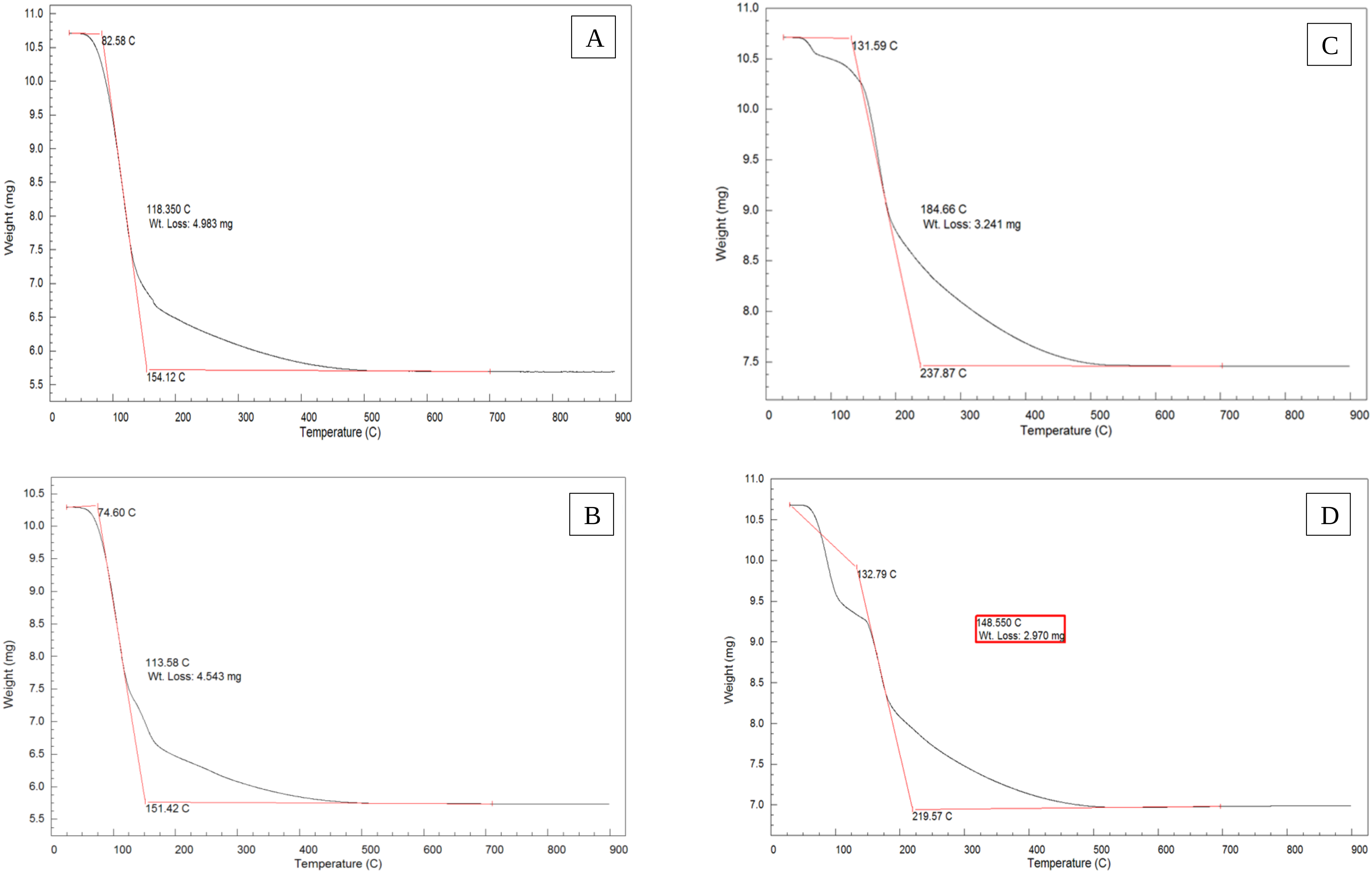


Figure 25– TGA curve for borax samples for (a) SRM 187f; (b) Home Science; (c) SRM 187e and (d) Merck

5. CONCLUSION

This work presents a study of multivariate statistical analysis of NIR and Raman spectra as a powerful tool for quality control of pH raw materials. Comparison of the results of PCA obtained by the NIR and Raman processed spectra to those obtained by the reference electrochemical pH method, demonstrated the ability to distinguish between NIST pH reference materials and other potential candidate materials. In a regulatory setting, having a non-destructive, fast and low cost methodology to ensure the identity of the raw material will help to ensure the quality of the candidate materials and provide evidence that approved candidate materials will supply pH values within the accepted threshold of the SRM's series. The power of these techniques could be used to streamline the production the pH buffer materials.

A non-destructive classification analysis for assessing the quality of calcium carbonate samples was carried out by NIR and Raman techniques. However, NIR could detect minor differences which were not rejected by the reference electrochemical method. For instance, Baker samples were overlapped with the SRM's ellipse on the PCA of Raman but they fall outside it on that of NIR. It was shown that the batch is on the margin of the acceptable limit according to the reference method. By applying XRD, it was shown that the difference in the crystal arrangement of the calcium carbonate is responsible for the discrimination of the batches.

NIR and Raman proved to be of similar discriminating power for the sodium tetraborate decahydrate (borax) samples. Five different ellipses representing five different batches were identified when plotting the first two PCs of both data matrices. Further studies on the discrimination of borate samples with the help of TGA represent an efficient technique to distinguish between different samples based on their hydration level.

The separation between the batches of potassium hydrogen phthalate (KHP) was based on the grinding step performed before the collection of the spectra. NIR and Raman techniques are both powerful to provide discrimination wherever water is considered to be the dominant impurity within the samples. Both techniques represent a sensitive and an efficient way to distinguish between the acidimetric standards and the pH standard of potential lesser purity.

It can be concluded that the proposed methodology for analysis of the NIR and Raman spectra implementing chemometrics tools allows a quick discrimination of the samples comparable to the usual reference electrochemical pH method in case of calcium carbonate. This illustrates the fact that non-destructive, fast and low cost techniques such as NIR and Raman techniques combined with chemometrics tools could streamline the acceptance process of calcium carbonate pH reference materials.

This may not be the same in case of borax since two separate ellipses were identified for NIST SRM’s while negative controls could be found in the space between the two accepted SRMs. Hence, it was not possible to identify one clear region for the acceptance of candidate SRM.

NIR and Raman techniques do not provide enough information related to the differences among the KHP batches. This does not eliminate the possibility of applying the proposed method to the quality control of candidate materials; because the NIST reference method was also unable to distinguish negative controls from SRMs. Nuclear magnetic resonance (NMR) spectroscopy may be helpful for further investigations.

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APPENDIX A

National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 2193a

Calcium Carbonate pH Standard
[used as saturated $Ca(OH)_2$ solution]

This Standard Reference Material (SRM) is intended for use in preparing solutions for calibrating electrodes for pH measuring systems at pH values above 11.0. This lot of calcium carbonate ($CaCO_3$) was selected for its low level of alkali metal impurities. However, this SRM is certified **ONLY** as a pH standard, **NOT** as a pure substance. A unit of SRM 2193a consists of 30 g of $CaCO_3$.

Certified Values and Uncertainties: The certified standard pH, pH(S), values provided in Table 1 correspond to $\log(1/a_H)$, where a_H is the conventional activity of the hydrogen (hydronium) ion referred to the standard state ($p° = 1\ \mathrm{atm} = 1.013\,25 \times 10^5\ \mathrm{Pa}$) on the scale of molality. The values were derived from emf measurements of cells without liquid junction by the primary measurement method [1,2]. **NOTE:** These certified values apply **ONLY** to SRM 2193a. Minor variations of pH(S) values (of the order of 0.01 units) may be expected to occur between different lots of this SRM.

The expanded uncertainty in the certified value, U, is calculated as $U = ku_c(y)$, where $u_c(y)$ is the "combined standard uncertainty" calculated according to the ISO/JCGM and NIST Guides [3]. The value of $u_c(y)$ is intended to represent the combined effect of the following uncertainty components associated with the primary measurement method and material homogeneity: curve-fit; standard electrode potentials, E^o; material homogeneity; molality of HCl, b_{HCl}, used for determining E^o; measured cell potentials; correction to the standard pressure for H_2 gas; mean activity coefficient of HCl at b_{HCl}; gas constant; temperature; Faraday constant; the molality of NaCl; and uncertainty [4,5] of the conventional calculation of $\log \gamma_{C1}$ (Bates-Guggenheim convention [6]). The value of $u_c(y)$ has been multiplied by k, obtained by the Student's t-distribution for effective degrees of freedom at the given temperature and a 95 % confidence level. A NIST certified value is a value for which NIST has the highest confidence in its accuracy in that all known or suspected sources of bias have been investigated or taken into account [7]. The measurand is the certified pH, pH(S), values listed in Table 1 as a function of temperature. The certified value is traceable to NIST's primary measurement of pH.

Expiration of Certification: The certification of **SRM 2193a** is valid, within the measurement uncertainties specified, until **01 July 2020**, provided the SRM is handled and stored in accordance with the instructions given in this certificate (see "Instructions for Use"). The certification is nullified if the SRM is damaged, contaminated, or otherwise.

Maintenance of SRM Certification: NIST will monitor this SRM over the period of its certification. If substantive technical changes occur that affect the certification before the expiration of this certificate, NIST will notify the purchaser. Registration (see attached sheet or register online) will facilitate notification.

The experimental work leading to the certification of this material was performed by K.W. Pratt of the NIST Chemical Sciences Division.

Statistical consultation was provided by W.F. Guthrie of the NIST Statistical Engineering Division.

Support aspects involved with the issuance of this SRM were coordinated through the NIST Office of Reference Materials.

Carlos A. Gonzalez, Chief
Chemical Sciences Division

Gaithersburg, MD 20899
Certificate Issue Date: 30 September 2015
Certificate Revision History on Last Page

Robert L. Watters, Jr., Director
Office of Reference Materials

Table l. Certified pH(S) Values and Expanded Uncertainties as a Function of Temperature

Before use for pH calibrations, a freshly filtered, saturated (at 25 °C) solution of $Ca(OH)_2$ must be prepared from SRM 2193a as described in the "Instructions for Use" section of this certificate. The certified pH(S) and U of this solution as a function of temperature are given below.

t/°C	pH(S)	u_c(measurement)[a]	$u_c(y)$[b]	k	U[b]
5	13.232	0.0030	0.0058	2.0	0.011
10	13.026	0.0025	0.0056	2.0	0.011
15	12.830	0.0025	0.0056	2.0	0.011
20	12.645	0.0024	0.0056	2.0	0.011
25	12.469	0.0024	0.0055	2.0	0.011
30	12.303	0.0071	0.0087	2.0	0.017
35	12.145	0.0071	0.0087	2.0	0.017
37	12.084	0.0071	0.0087	2.0	0.017
40	11.995	0.0071	0.0087	2.0	0.017
45	11.853	0.0072	0.0087	2.0	0.017
50	11.717	0.0074	0.0089	2.0	0.017

[a] u_c(measurement) includes components associated with the measurement method and material homogeneity, but does not include the uncertainty of the Bates-Guggenheim convention (0.0050) [4,5].

[b] $u_c(y)$ includes u_c(measurement) combined with u(Bates-Guggenheim convention) = 0.0050 [4,5].

Reference Values: To attain traceability to the NIST reference pH(S) values for SRM 2193a when traceability to the SI is not necessary, the uncertainty of the Bates-Guggenheim convention is excluded from the uncertainty calculation. Each reference value includes the respective pH(S) value in Table 1 and its corresponding expanded uncertainty, U_R:

$$U_R = k_R u_c\,(\text{measurement})$$

where k_R is the coverage factor for U_R. The value of $k_R = 2.0$ at all temperatures. A NIST reference value is a noncertified value that is the best estimate of the true value; however, the values do not meet NIST criteria for certification and are provided with associated uncertainties that may not include all sources of uncertainty [7].

NOTICE AND WARNINGS TO USERS(1)

Source of Material: The calcium carbonate ($CaCO_3$) was obtained from a commercial company. This material conforms to the specifications of the American Chemical Society for "calcium carbonate, low in alkalies" [8].

Storage: SRM 2193a is stable when stored in its original container, with the cap tightly closed, in a dry environment, and under normal laboratory temperatures. The saturated solution of $Ca(OH)_2$, prepared as described below, should be freshly filtered before use in pH calibrations.

INSTRUCTIONS FOR USE

Preparation of Carbon Dioxide-Free Water: Carbon dioxide-free water must be used for making the solutions. This water must be prepared either by (1) boiling a good grade of distilled water (conductivity < 2 µS/cm) for 10 min and guarding it with a soda-lime tube while cooling or (2) dispensing water directly from a deionization-based point-of-use system into the vessel used to prepare the buffer solutions (resistivity > 17 MΩ·cm).

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(1) Certain commercial instruments, materials, or processes are identified in this report to adequately specify the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the instruments, materials, or processes identified are necessarily the best available for the purpose.

Preparation and Use of the Saturated $Ca(OH)_2$ Solution: Put 7.5 g SRM 2193a into a platinum or fused silica (Vycor®) crucible or dish, heat slowly in a muffle furnace to 950 °C to 1000 °C and ignite for 1 h at this temperature. Immediately transfer the product (CaO) to a dessicator and allow to cool. After cooling, gently crush any lumps and add slowly to 100 mL carbon dioxide-free water while stirring. Heat the resulting $Ca(OH)_2$ suspension to boiling for 15 minutes, cool, and filter on a sintered-glass funnel of medium porosity. Dry the resulting solid $Ca(OH)_2$ in an oven for 2 h at 110 °C and crush in a mortar and pestle to a fine powder. Put the obtained $Ca(OH)_2$ (approximately 5 g) into a 1 L plastic bottle, add approximately 1 kg carbon dioxide-free water and shake the bottle periodically (nominally every 2 h, four times per day). Between periods of shaking, maintain the bottle at 25 °C in a thermostated water bath.

To obtain a truly saturated solution ($0.0202\ mol \cdot kg^{-1}$) by this procedure, approximately 1 week is required. However, after 1 day of mixing the excess $Ca(OH)_2$ with water, the pH is lower by only 0.02 pH units than that of the saturated solution. After 2 days, the difference is only 0.01 pH units.

Filter a portion of the saturated $Ca(OH)_2$ solution immediately before use (a syringe feeding a 0.45 µm in-line filter works well). Use the fresh filtrate as the pH standard.

Stability of Prepared Solution: The filtered solution (see above) will develop a $CaCO_3$ film on its surface in a few minutes. Although the pH of this filtered solution changes only slightly in 1 hour, it is preferable to use a fresh filtered solution for each measurement. The stock saturated solution maintains its pH value if stored in the thermostated water bath, provided that excess $Ca(OH)_2$ remains present.

Calibration of pH Electrode-Meter Systems for High-Alkalinity Measurements (pH > 11): For the pH measurement of highly alkaline solutions, a 2-point calibration is suggested. Prepare and use $0.01\ mol \cdot kg^{-1}$ borax (SRM 187d or current renewal) as the first standard and adjust the pH meter accordingly. Then use the freshly filtered, saturated $Ca(OH)_2$ solution prepared from SRM 2193a as the second standard and adjust the temperature compensation to set the pH reading to the certified value.

Notice to User: For pH measurements in highly alkaline solutions using commercial glass-reference electrode systems, larger uncertainties are to be expected. The sources of this uncertainty are: (1) changing liquid junction potential with increasing concentration of the highly mobile OH^- ions; (2) nonideal performance of glass electrodes, including poorer reproducibility, sluggish response, and “sodium error”; and (3) higher sensitivity of pH to temperature changes. An uncertainty of 0.05 pH is not uncommon and is reasonable for pH measurements in highly alkaline solutions.

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Certificate Revision History: 30 September 2015 (Change of expiration date; editorial changes); 13 December 2007 (Update of certification period); 19 June 2003 (original certificate date).

Users of this SRM should ensure that the Certificate of Analysis in their possession is current. This can be accomplished by contacting the SRM Program: telephone (301) 975-2200; fax (301) 948-3730; e-mail srminfo@nist.gov; or via the Internet at http://www.nist.gov/srm.

National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 187f

Sodium Tetraborate Decahydrate (Borax)

pH Standard

This Standard Reference Material (SRM) is intended for use in preparing solutions for calibrating electrodes for pH measuring systems. SRM 187f Sodium Tetraborate Decahydrate ($Na_2B_4O_7 \cdot 10\ H_2O$) was prepared to ensure high purity and uniformity. However, this SRM is certified **ONLY** as a pH standard [pH(S)], not as a pure substance. A unit of SRM 187f consists of 30 g of sodium tetraborate decahydrate.

Certified Values: The certified pH(S) values provided in Table 1 correspond to $\lg(1/a_H)$, where a_H is the conventional activity of the hydrogen (hydronium) ion referred to the standard state ($p = 1$ atm $= 1.013\,25 \times 10^5$ Pa) on the scale of molality. The values were derived from potential measurements of cells without liquid junction by the primary measurement method [1,2]. A NIST certified value is a value for which NIST has the highest confidence in its accuracy in that all known or suspected sources of bias have been investigated or taken into account [3]. The certified pH(S) values and their expanded uncertainties, *U*, are stated in Table 1.

Reference Values: The uncertainty [1,4] of the Bates-Guggenheim convention [5] is excluded from the uncertainty calculation for the reference values provided in Table 2. Reference values are noncertified values that are the best estimate of the true value; however, the values do not meet the NIST criteria for certification and are provided with associated uncertainties that may not include all sources of uncertainty [3].

Traceability: The measurand is the pH of the specified buffer solution. The certified values in Table 1 are metrologically traceable to the International System of Units (SI) of amount-of-substance and mass and to the convention [5] used to define ionic activity, including its uncertainty [1,4]. The reference values in Table 2 are traceable to the SI units for amount-of-substance and mass and to this defining convention [5], taken as an exact value with no uncertainty (the uncertainty of the Bates-Guggenheim convention is excluded from the uncertainty calculation).

Expiration of Certification: The certification of **SRM 187f** is valid, within the measurement uncertainty specified, until **31 August 2021**, provided the SRM is handled and stored in accordance with the instructions given in this certificate (see "Instructions for Handling, Storage and Use"). The certification is nullified if the SRM is damaged, contaminated, or otherwise modified.

Maintenance of SRM Certification: NIST will monitor this SRM over the period of its certification. If substantive technical changes occur that affect the certification before the expiration of this certificate, NIST will notify the purchaser. Registration (see attached sheet or register online) will facilitate notification.

Experimental work leading to the certification of this material was performed by R.A. Easley and J.F. Waters of the NIST Chemical Sciences Division.

Statistical consultation was provided by W.F. Guthrie of the NIST Statistical Engineering Division.

Support aspects involved in the issuance of this SRM were coordinated through the NIST Office of Reference Materials.

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Certificate Issue Date: 29 August 2017

Steven J. Choquette, Director
Office of Reference Materials

INSTRUCTIONS FOR HANDLING, STORAGE AND USE

Storage: SRM 187f is stable when stored in its original container, with the cap tightly closed, in a dry environment, and under normal laboratory temperatures.

Drying Instructions: Use as received. SRM 187f must not be dried in an oven. Do not store in a desiccator before use.

Source Water for Solution Preparation: The water used in the preparation of the SRM 187f buffer solution should be protected from atmospheric carbon dioxide. This water must be prepared either by (1) boiling of distilled water (conductivity < 2 µS/cm) for 10 min and guarding it with a soda-lime tube while cooling or (2) dispensing water directly from a deionization-based point-of-use system into the vessel used to prepare the buffer solutions (resistivity > 17 MΩ·cm, conductivity < 0.06 µS/cm). The prepared solution should be protected against evaporation and contamination.

Preparation of the 0.01 mol/kg Solution: Quantities denoted by m' and associated numerical factors in this paragraph include the effect of air buoyancy, i.e., they correspond to the balance indication in units of mass obtained in the laboratory (the *balance reading*). Weigh by difference approximately 3.7 g of SRM 187f, m'_{187f}, to an accuracy of 1 mg, into a clean, dry 1 L polyethylene bottle. Add a weighed quantity of CO_2-free water, equal to 261.841 multiplied by m'_{187f}, to an accuracy of 0.1 g. Shake until the solid has totally dissolved. Gravimetric preparation in this manner eliminates the need to weigh exactly predetermined masses of solid samples. Proportionately smaller quantities of each SRM may be used in this preparation, provided that m'_{187f} exceeds 0.42 g.

Stability of the Prepared Solution: The solution should be discarded after one month, or sooner if sediment appears or if it has been exposed repeatedly to air containing carbon dioxide. To avoid contamination of the buffer solution with atmospheric carbon dioxide, keep the bottle capped except when removing a portion of the solution.

SOURCE, PREPARATION AND ANALYSIS(1)

Source of Material: The sodium tetraborate decahydrate ($Na_2B_4O_7 \cdot 10\,H_2O$) was obtained from a commercial company. This material conforms to the specifications of the American Chemical Society for primary standard chemicals [6].

Certified Values: The pH(S) and the expanded uncertainty, U, of this solution as a function of temperature are given in Table 1. The expanded uncertainty in the certified value, U, is calculated as $U = ku_c(y)$, where $u_c(y)$ is the “combined standard uncertainty” calculated according to the ISO/JCGM Guide [7]. The value of $u_c(y)$ represents the combined effect of the following uncertainty components: extrapolation to obtain the acidity function, $p(a_H\gamma_{Cl})°$; standard electrode potentials, $E°$; material heterogeneity(2); molality of HCl, b_{HCl}, used for determining $E°$; measured cell potentials; correction to the standard pressure for H_2 gas; mean activity coefficient of HCl at b_{HCl}; gas constant; temperature; Faraday constant; the molality of NaCl; and the uncertainty of the conventional calculation of log γ_{Cl} (Bates-Guggenheim convention [5]). Current expert opinion [1,4] has assessed the uncertainty attributable to the Bates-Guggenheim convention as 0.010 pH (95 % confidence interval). The value of $u_c(y)$ has been multiplied by a coverage factor, k, obtained by the Student’s t-distribution for effective degrees of freedom at the given temperature and a 95 % confidence level. A solution of molality 0.01 mol/kg is recommended for the calibration of pH measuring systems. **NOTE**: These certified values apply ONLY to a 0.01 mol/kg solution prepared from SRM 187f. Minor variations of pH(S) values may be expected to occur between SRM lots.

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(1) Certain commercial equipment, instruments, or materials are identified in this certificate to adequately specify the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

(2) The uncertainty for material heterogeneity includes analysis of the relevant uncertainty arising from the uncertainty in SRM 187e, which was used in the collection of the data for assessing material heterogeneity. The uncertainty from the use of SRM 187e was determined to add only negligible uncertainty to the assessed material heterogeneity value.

Table 1. Certified pH(S) Values and Expanded Uncertainties (95 % Confidence)

Temperature (°C)	pH(S)	Combined Standard Uncertainty, $u_c(y)$	Coverage Factor, k	Expanded Uncertainty, U
5	9.401	0.0052	1.96	0.010
10	9.342	0.0051	1.96	0.010
15	9.288	0.0051	1.96	0.010
20	9.239	0.0051	1.96	0.010
25	9.195	0.0051	1.96	0.010
30	9.155	0.0054	1.96	0.010
35	9.120	0.0053	1.96	0.010
37	9.107	0.0052	1.96	0.010
40	9.088	0.0052	1.96	0.010
45	9.059	0.0052	1.96	0.010
50	9.034	0.0052	1.96	0.010

Reference Values: To attain traceability to the NIST reference pH(S) values for SRM 187f when traceability to the SI units is not necessary, the uncertainty of the Bates-Guggenheim convention is excluded from the uncertainty calculation. The respective pH(S) values in Table 2 are identical to those in Table 1 but are listed to the number of decimal places reported for the expanded uncertainty, U_R:

$$U_R = k_R u_c(\text{measurement}),$$

where k_R is the coverage factor for U_R. The quantities U_R and u_c(measurement) each include all components associated with the measurement method and assessment of material heterogeneity, but **DO NOT** include the uncertainty [1] of the Bates-Guggenheim Convention.

Table 2. Reference pH(S) Values and Expanded Reference Uncertainties (95 % Confidence)

Temperature (°C)	pH(S)	Combined Standard Uncertainty, u_c(measurement)	Reference Coverage Factor, k_R	Expanded Uncertainty, U_R
5	9.4013	0.0013	2.08	0.0028
10	9.3417	0.0012	2.09	0.0024
15	9.2878	0.0010	2.04	0.0021
20	9.2390	0.0010	2.02	0.0019
25	9.1951	0.0010	2.03	0.0020
30	9.1554	0.0019	2.01	0.0038
35	9.1198	0.0017	1.98	0.0033
37	9.1066	0.0016	1.97	0.0031
40	9.0879	0.0015	1.96	0.0029
45	9.0593	0.0015	1.96	0.0029
50	9.0338	0.0014	1.96	0.0028

REFERENCES

[1] Buck, R.P.; et. al.; *Measurement of pH. Definition, Standards, and Procedures*; (IUPAC Recommendation 2002), Pure Appl. Chem, Vol. 74, pp. 2169–2200 (2002); available at http://www.iupac.org/publications/pac/2002/pdf/7411x2169.pdf (accessed Aug 2017).

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[3] May, W.; Parris, R.; Beck II, C.; Fassett, J.; Greenberg, R.; Guenther, F.; Kramer, G.; Wise, S.; Gills, T.; Colbert, J.; Gettings, R.; MacDonald, B.; *Definition of Terms and Modes Used at NIST for Value-Assignment of Reference Materials for Chemical Measurements*; NIST Special Publication 260-136; U.S. Government Printing Office: Washington, DC (20); available at http://www.nist.gov/srm/upload/SP260-136.PDF (accessed Aug 2017).

[4] Baucke, F.G.; *New IUPAC Recommendations on the Measurement of pH – Background and Essentials*; Anal. Bioanal. Chem., Vol. 374, p. 772 (2002).

[5] Bates, R.G.; Guggenheim, E.A.; *A Report on the Standardization of pH and Related Terminology*; Pure Appl. Chem., Vol. 1, pp. 163–168 (1960).

[6] American Chemical Society; *Reagent Chemicals*; 8th ed.; Washington, DC (1993).

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Users of this SRM should ensure that the Certificate of Analysis in their possession is current. This can be accomplished by contacting the SRM Program: telephone (301) 975-2200; fax (301) 948-3730; e-mail srminfo@nist.gov; or via the Internet http://www.nist.gov/srm.

DEPARTMENT OF COMMERCE UNITED STATES OF AMERICA

National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 185i

Potassium Hydrogen Phthalate

pH Standard

This Standard Reference Material (SRM) is intended for use in preparing solutions for calibrating electrodes for pH measuring systems. SRM 185i Potassium Hydrogen Phthalate ($KHC_8H_4O_4$) was prepared to ensure high purity and uniformity. However, this SRM is certified **ONLY** as a pH standard [pH(S)] not as a pure substance. A unit of SRM 185i consists of 60 g of potassium hydrogen phthalate.

Certified Values: The certified pH(S) values provided in Table 1 correspond to $\lg(1/a_\mathrm{H})$, where a_H is the conventional activity of the hydrogen (hydronium) ion referred to the standard state ($p° = 1\ \mathrm{atm} = 1.013\,25 \times 10^5\ \mathrm{Pa}$) on the scale of molality. The values were derived from potential measurements of cells without liquid junction by the primary measurement method [1,2]. A NIST certified value is a value for which NIST has the highest confidence in its accuracy in that all known or suspected sources of bias have been investigated or taken into account [3]. The certified pH(S) values and their expanded uncertainties, U, are stated in Table 1.

Reference Values: The uncertainty [1,4] of the Bates-Guggenheim convention [5] is excluded from the uncertainty calculation for the reference values provided in Table 2. Reference values are noncertified values that are the best estimate of the true value; however, the values do not meet the NIST criteria for certification and are provided with associated uncertainties that may not include all sources of uncertainty [3].

Traceability: The measurand is the pH of the specified buffer solution. The certified values in Table 1 are metrologically traceable to the International System of Units (SI) of amount-of-substance and mass and to the definition of single ion activity. The reference values in Table 2 are traceable to the SI units of amount-of-substance and mass and to the convention [5] used to define the single ion activity.

Expiration of Certification: The certification of **SRM 185i** is valid, within the measurement uncertainty specified, until **15 July 2023**, provided the SRM is handled and stored in accordance with the instructions given in this certificate (see “Instructions for Handling, Storage and Use”). The certification is nullified if the SRM is damaged, contaminated, or otherwise modified.

Maintenance of SRM Certification: NIST will monitor this SRM over the period of its certification. If substantive technical changes occur that affect the certification before the expiration of this certificate, NIST will notify the purchaser. Registration (see attached sheet) will facilitate notification.

The experimental work leading to the certification of this material was performed by J.F. Waters and K.W. Pratt of the NIST Chemical Sciences Division.

Statistical consultation was provided by W.F. Guthrie of the NIST Statistical Engineering Division.

Support aspects involved in the issuance of this SRM were coordinated through the NIST Office of Reference Materials.

Carlos A. Gonzalez, Chief
Chemical Sciences Division

Gaithersburg, MD 20899
Certificate Issue Date: 08 August 2013

Robert L. Watters, Jr., Director
Office of Reference Materials

INSTRUCTIONS FOR HANDLING, STORAGE AND USE

Storage: SRM 185i is stable when stored in its original container, with the cap tightly closed, in a dry environment, and under normal laboratory temperatures.

Drying Instructions: SRM 185i should be dried for 2 h at 110 °C and stored in a desiccator over anhydrous $Mg(ClO_4)_2$ before use.

Source Water for Solution Preparation: The water used in the preparation of the SRM 185i buffer solution need not be protected from atmospheric carbon dioxide, and elaborate precautions for the exclusion of air from the solution are not necessary. Distilled water with an electrolytic conductivity not greater than 2 µS/cm or water directly obtained from a point-of-use, deionization-based system, of electrolytic conductivity less than 1 µS/cm, may be used, without boiling. The solution should, however, be protected against evaporation and contamination by mold spores.

Preparation of the 0.05 mol/kg Solution: Quantities denoted by m_W and associated numerical factors in this paragraph include the effect of air buoyancy, i.e., they correspond to the balance indication in units of mass obtained in the laboratory (the *balance reading*). Weigh by difference approximately 9.8 g of SRM 185i, $m_{W,185i}$, to an accuracy of 1 mg, into a clean, dry, 1 L polyethylene bottle. Add a quantity of water, equal to 97.887 multiplied by $m_{W,185i}$, to an accuracy of 0.1 g. Shake thoroughly until the solid has totally dissolved. Gravimetric preparation in this manner eliminates the need to weigh exactly predetermined masses of solid samples. Proportionately smaller quantities of each SRM may be used in this preparation, provided that $m_{W,185i}$ exceeds 4.0 g.

Stability of Prepared Solution: Solutions should be discarded after one month or sooner if mold or sediment appears.

PREPARATION(1)

Source of Material: The potassium hydrogen phthalate ($KHC_8H_4O_4$) was obtained from a commercial company. This material conforms to the specifications of the American Chemical Society for primary standard chemicals [6].

Certified Values: The pH(S) and the expanded uncertainty, U, of this solution as a function of temperature are given in Table 1. The uncertainty in the certified value, U, is calculated as $U = ku_c(y)$, where $u_c(y)$ is the "combined standard uncertainty" calculated according to the ISO/JCGM Guide [7]. The value of $u_c(y)$ represents the combined effect of the following uncertainty components associated with the primary measurement method and material homogeneity: extrapolation to obtain the acidity function, $p(a_H\gamma_{Cl})°$; standard electrode potentials, $E°$; material homogeneity; molality of HCl, b_{HCl}, used for determining $E°$; measured cell potentials; correction to the standard pressure for H_2 gas; mean activity coefficient of HCl at b_{HCl}; gas constant; temperature; Faraday constant; the molality of NaCl; and the uncertainty of the conventional calculation of log γ_{Cl} (Bates-Guggenheim convention [5]). Current expert opinion [1,4] has assessed the uncertainty attributable to the Bates-Guggenheim convention as 0.010 pH (95 % confidence interval). The value of $u_c(y)$ has been multiplied by a coverage factor, k, obtained by the Student's *t*-distribution for effective degrees of freedom at the given temperature and a 95 % confidence level. A solution of molality 0.05 mol/kg is recommended for the calibration of pH measuring systems.

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(1) Certain commercial equipment, instruments, or materials are identified in this certificate to adequately specify the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

Table 1. Certified pH(S) Values and Expanded Uncertainties (95 % Confidence)

Temperature (°C)	pH(S)	Combined Uncertainty, $u_c(y)$	Coverage Factor, k	Uncertainty, U
5	4.003	0.0050	1.96	0.010
10	3.999	0.0050	1.96	0.010
15	3.999	0.0050	1.96	0.010
20	4.001	0.0050	1.96	0.010
25	4.005	0.0050	1.96	0.010
30	4.012	0.0051	1.96	0.010
35	4.021	0.0051	1.96	0.010
37	4.025	0.0051	1.96	0.010
40	4.031	0.0051	1.96	0.010
45	4.044	0.0051	1.96	0.010
50	4.058	0.0051	1.96	0.010

Reference Values: To attain traceability to the NIST reference pH(S) values for SRM 185i when traceability to the SI units is not necessary, the uncertainty of the Bates-Guggenheim convention is excluded from the uncertainty calculation. The respective pH(S) values in Table 2 are identical to those in Table 1 but are listed to the number of decimal places reported for the expanded uncertainty, U_R:

$$U_R = k_R u_c(\text{measurement}),$$

where k_R is the coverage factor for U_R. The quantities U_R and u_c(measurement) each include all components associated with the measurement method and assessment of material homogeneity, but **DO NOT** include the uncertainty of the Bates-Guggenheim Convention.

Table 2. Reference pH(S) Values and Expanded Reference Uncertainties (95 % Confidence)

Temperature (°C)	pH(S)	Combined Uncertainty, u_c(measurement)	Reference Coverage Factor, k_R	Reference Uncertainty, U_R
5	4.0025	0.0005	2.04	0.0011
10	3.9993	0.0004	1.99	0.0008
15	3.9988	0.0004	2.00	0.0008
20	4.0009	0.0004	1.99	0.0008
25	4.0053	0.0004	2.05	0.0007
30	4.0120	0.0010	1.96	0.0020
35	4.0207	0.0010	1.96	0.0020
37	4.0247	0.0011	1.96	0.0021
40	4.0313	0.0011	1.96	0.0022
45	4.0438	0.0011	1.96	0.0021
50	4.0579	0.0011	1.96	0.0022

REFERENCES

[1] Buck, R.P.; et. al.; *Measurement of pH. Definition, Standards, and Procedures*; (IUPAC Recommendation 2002), Pure Appl. Chem, Vol. 74, p. 2169–2200 (2002); available at http://www.iupac.org/publications/pac/2002/pdf/7411x2169.pdf (accessed Aug 2013).

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[6] American Chemical Society; *Reagent Chemicals*; 8th Ed.; Washington, DC (1993).

[7] JCGM 100:2008; *Guide to the Expression of Uncertainty in Measurement*; (GUM 1995 with Minor Corrections), International Bureau of Weights and Measures, Joint Committee for Guides in Metrology, Sèvres Cedex, France (2008); available at http://www.bipm.org/utils/common/documents/jcgm/JCGM_100_2008_E.pdf (accessed Aug 2013); see also Taylor, B.N.; Kuyatt, C.E.; *Guidelines for Evaluating and Expressing the Uncertainty of NIST Measurement Results*; NIST Technical Note 1297, U.S. Government Printing Office: Washington, DC (1994); available at http://www.nist.gov/pml/pubs/tn1297/index.cfm (accessed Aug 2013).

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National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 84L

Potassium Hydrogen Phthalate

$KHC_8H_4O_4$
Acidimetric Primary Standard

This Standard Reference Material (SRM) is intended for use as an acidimetric primary standard. It consists of highly purified potassium hydrogen phthalate (KHP), $KHC_8H_4O_4$. A unit of SRM 84L is supplied as crystalline material in a 60 g unit.

Certified Value: The certified value listed in Table 1 is the mass fraction of total acid (replaceable H^+) expressed as KHP. A NIST certified value is a value for which NIST has the highest confidence in its accuracy in that all known or suspected sources of bias have been investigated or taken into account [1].

Table 1. Certified Value (a) for SRM 84L Potassium Hydrogen Phthalate

99.9934 % ± 0.0076 %

(a) The certified value is expressed as the value ± its expanded uncertainty, U. The expanded uncertainty is calculated as $U = ku_c$, where k is the coverage factor and u_c is the combined standard uncertainty calculated according to the ISO Guide [2]. The value of u_c represents the combined uncertainty in the certified value, at the level of one standard deviation, and includes the replication uncertainty of the 30 titrations of the SRM and all sources of uncertainty inherent to the coulometric method. The value of k controls the approximate level of confidence associated with U. For this SRM, $k = 2.04$. This value corresponds to a level of confidence of approximately 95 %. The value of k is obtained from the Student's t-distribution with effective degrees of freedom, $\nu_{\mathrm{eff}} = 31$.

Information Value: The theoretical total organic carbon (TOC) content is 47.05 %, based on the 2005 Atomic Weights [3]. This TOC value is a noncertified value with no reported uncertainty, as there is insufficient information to assess the uncertainty. An information value is considered to be a value that will be of interest to the SRM user, but insufficient information is available to assess the uncertainty associated with the value or only a limited number of analyses were performed [1].

Expiration of Certification: The certification of **SRM 84L** is valid, within the measurement uncertainties specified, until **01 April 2024**, provided the SRM is handled in accordance with instructions given in this certificate (see "Instructions for Use"). This certification is nullified if the SRM is damaged, contaminated, or otherwise modified.

Maintenance of SRM Certification: NIST will monitor this SRM over the period of its certification. If substantive technical changes occur that affect the certification before the expiration of this certificate, NIST will notify the purchaser. Registration (see attached sheet) will facilitate this notification.

The overall direction and coordination of technical measurements leading to certification was provided by T.W. Vetter of the NIST Analytical Chemistry Division.

Coulometric analyses were performed by K.W. Pratt of the NIST Analytical Chemistry Division.

Stephen A. Wise, Chief
Analytical Chemistry Division

Robert L. Watters, Jr., Chief
Measurement Services Division

Gaithersburg, MD 20899
Certificate Issue Date: 01 February 2010

Statistical consultation was provided by W.F. Guthrie of the NIST Statistical Engineering Division.

Support aspects involved in the issuance of this SRM were coordinated through the NIST Measurement Services Division.

INSTRUCTIONS FOR USE

Stability and Storage: This SRM should be stored in its original bottle at room temperature. It must be tightly recapped after use and protected from moisture, ammonia, and light.

Homogeneity: Tests indicate that this SRM is homogeneous within the uncertainty limits for test portions with a mass greater than 300 mg. Test portions with a mass less than 300 mg are not recommended, to avoid possible inhomogeneity.

Drying Instructions: As issued, SRM 84L contains some entrapped (occluded) water that is released by a combination of grinding and drying. The following procedure was used in the certification of this material and must be followed to attain the certified value. Grind a test portion to a fine, flour-like powder, taking special care to fragment the larger crystals. Dry the powder at 120 ºC for 2 h and store over anhydrous magnesium perchlorate in a desiccator.

The SRM 84L material was ground by hand for a period of 60 s to 90 s in an agate mortar for this certification. Other methods that do not introduce contaminants may also yield a suitable powder.

Analyses of dried, unground test portions of SRM 84L yielded non-certified values from 99.94 % to 99.99 %, owing to variations in the frequency or size of inclusions of water in the KHP crystals in the given test portion. Such inclusions are well known for KHP [4,5] and a mass fraction of occluded water in the range of 0.01 % to 0.15 % was present in previous issues of this SRM [4].

Intended Use: This SRM is certified for acidimetric assay **ONLY** and is not intended for use as a pH standard. The current issue of SRM 185 Potassium Hydrogen Phthalate, pH Standard, is certified for pH.

SOURCE, PREPARATION, AND ANALYSIS[1]

Source of Material: The material used for this SRM was obtained from a commercial supplier. The material was examined for compliance with the specification for reagent grade KHP as specified by the American Chemical Society [6]. The material was found to meet or exceed these specifications in all respects.

Assay Technique: The certified value is based on the results of coulometric assays of ground and dried material (see Drying Instructions). The assay value for this material was obtained by automated coulometric titration [7] to the inflection point (pH ≈ 8.4) of weighed test portions of KHP. The certified value represents the result of 30 titrations of test portions taken from 16 bottles selected by stratified random sampling from the entire lot of SRM 84L. The value of the Faraday constant used in this work was 96 485.3399 C/mol [8]. The 2005 values for the atomic weights [3] were used.

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[1] Certain commercial equipment, instruments or materials are identified in this certificate to adequately specify the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

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[1] May, W.; Parris, R.; Beck II, C.; Fassett, J.; Greenberg, R.; Guenther, F.; Kramer, G.; Wise, S.; Gills, T.; Colbert, J.; Gettings, R.; MacDonald, B.; *Definition of Terms and Modes Used at NIST for Value-Assignment of Reference Materials for Chemical Measurements*; NIST Special Publication 260-136 (2000); available at http://ts.nist.gov/MeasurementServices/ReferenceMaterials/PUBLICATIONS.cfm.

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[8] Mohr, P.J.; Taylor, B.N.; Newell, D.B.; *CODATA Recommended Values of the Fundamental Physical Constants: 2006*; Rev. Mod. Phys., Vol. 80(2), pp. 633-730 (2008); available at http://physics.nist.gov/cgi-bin/cuu/Value?f|search_for=faraday.

Users of this SRM should ensure that the certificate in their possession is current. This can be accomplished by contacting the SRM Program at: telephone (301) 975-2200; fax (301) 926-4751; e-mail srminfo@nist.gov; or via the Internet at http://www.nist.gov/srm.

APPENDIX B

1. Practical and theoretical mass loss for SRM 187f

% mass loss (practically) = $\Delta m/m_o$ = (4.983/10.6819) x 100 = 46.65 %

% mass loss (theoretically) = ($10.H_2O$) / (m_{borax} + $10.H_2O$) = (10 x 18.015) / [201.217+ (10 x 180.15)] x 100 = 47.23 %

Balance uncertainty = $\mu_{balance}$ = 0.0001 mg

Graph scale uncertainty (half of one dimension) = m = 0.05 mg

μ (rectangular) = 0.05/√3 = 0.0288 mg

$\mu_{\Delta m} = \sqrt{(\mu_m^2 + \mu_m^2)} = \sqrt{[(0.05/\sqrt{3})^2 + (0.05/\sqrt{3})^2]} = 0.0408$ mg

$(\mu\ \% / \text{mass loss}\ \%) = \sqrt{[(\mu_{\Delta m} / \Delta m)^2 + (\mu_{mo} / m_o)^2]} = \sqrt{[(0.0408/4.983)^2 + (0.0001/10.6819)^2]} =$ 0.00818 x 100 = 0.8187 %

μ % = $\mu_{\text{mass loss}}$ = 0.00818 x mass loss % = 0.00818 x 46.65 = 0.3815 %

C.I = 46.65 ± 0.3815 ($Z_{95\%}$ = 1.96 ~ 2)

(46.6 ± 0.8) % for 95 % confidence

N of H_2O molecules = (m_f H_2O x m_{borax}) X [m_{H2O} (1 x m_f H_2O)] = 9.77 molecules.

2. Practical and theoretical mass loss for Home Science

% mass loss (practically) = $\Delta m/m_o$ = (4.543/10.2895) x 100 = 44.15 %

% mass loss (theoretically) = ($9.H_2O$) / (m_{borax} + $9.H_2O$) = (9 x 18.015) / [201.217+ (9 x 18.015)] x 100= 44.62 %

Balance uncertainty = $\mu_{balance}$ = 0.0001 mg

Graph scale uncertainty (half of one dimension) = m = 0.05 mg

μ (rectangular) = 0.05/√3 = 0.0288 mg

$\mu_{\Delta m} = \sqrt{(\mu_m^2 + \mu_m^2)} = \sqrt{[(0.05/\sqrt{3})^2 + (0.05/\sqrt{3})^2]} = 0.0408$ mg (μ % / mass loss %) = $\sqrt{}$ [($\mu_{\Delta m}$ / Δm)^2 + (μ_{mo} / m_o)^2] = $\sqrt{}$ [(0.0408/4.543)^2 + (0.0001/10.2895)^2] = 0.00895 x 100 = 0.89588 %

μ % = $\mu_{\text{mass loss}}$ = 0.00895 x mass loss % = 0.00895 x 44.15 = 0.3951 %

C.I = 44.15 ± 0.3951 ($Z_{95\%}$ = 1.96 ~ 2)

(44.15 ± 0.8) % for 95 % confidence

N of H_2O molecules = (m_f H_2O x m_{borax}) X [m_{H2O} (1 x m_f H_2O)] = 8.8 molecules.

3. Practical and theoretical mass loss for SRM 187e

% mass loss (practically) = Δm/mo = (3.241/10.7216) x 100 = 30.23 %

% mass loss (theoretically) = (5.H_2O) / (m_{borax} + 5.H_2O) = (5 x 18.015) / [201.217+ [(5 x 18.015)] x 100= 30.93 %

Balance uncertainty = μ balance = 0.0001 mg

Graph scale uncertainty (half of one dimension) = m = 0.05 mg

μ (rectangular) = 0.05/√3 = 0.0288 mg

μΔm = $\sqrt{}$(μ_m^2 + μ_m^2) = $\sqrt{}$ [(0.05/√3)2 + (0.05/√3)2] = 0.0408 mg

(μ % / mass loss %) = $\sqrt{}$ [(μΔm / Δm)^2 + (μ_{mo} / m_o)^2] = $\sqrt{}$ [(0.0408/3.241)^2 + (0.0001/10.7216)^2] = 0.012588 x 100 = 1.2588 %

μ % = μ mass loss = 0.012588 x mass loss % = 0.012588 x 30.23 = 0.3805 %

C.I = 30.23 ± 0.3805 (Z95% = 1.96 ~ 2)

(30.23 ± 0.8) % for 95 % confidence

N of H_2O molecules = (mf H2O x m_{borax}) X [mH_2O (1 x mf H_2O)] = 4.8 molecules.

4. Practical and theoretical mass loss for Merck

% mass loss (practically) = $\Delta m/m_o$ = (2.970/10.6874) x 100 = 27.79 %

% mass loss (theoretically) = ($4.H_2O$) / (m_{borax} + $4.H_2O$) = (4 x 18.015) / [201.217+ [(4 x 18.015)] x 100= 26.37 %

Balance uncertainty = µ balance = 0.0001 mg

Graph scale uncertainty (half of one dimension) = m = 0.05 mg

µ (rectangular) = 0.05/√3 = 0.0288 mg

$\mu_{\Delta m} = \sqrt{(\mu_m^2 + \mu_m^2)} = \sqrt{[(0.05/\sqrt{3})^2 + (0.05/\sqrt{3})^2]}$ = 0.0408 mg

(µ % / mass loss %) = $\sqrt{[(\mu_{\Delta m} / \Delta m)^2 + (\mu_{mo} / mo)^2]} = \sqrt{[(0.0408/2.970)^2 + (0.0001/10.6874)^2]}$ = 0.0001877 x 100 = 0.01877 %

µ % = µ mass loss = 0.0001877 x mass loss % = 0.012588 x 27.79 = 0.3498 %

C.I = 27.79 ± 0.3498 (Z95% = 1.96 ~ 2)

(27.79 ± 0.7) % for 95 % confidence

N of H_2O molecules = (mf H2O x m_{borax}) X [mH_2O (1 x mf H_2O)] = 4.3 molecules.

5. Practical and theoretical mass loss for Detergent

% mass loss (practically) = $\Delta m/m_o$ = (2.975/10.0666) x 100 = 29.55 %

% mass loss (theoretically) = (5.H2O) / (mborax + 5.H2O) = (5 x 18.015) / [201.217+ [(5 x 18.015)] x 100= 30.92 %

Balance uncertainty = µ balance = 0.0001 mg

Graph scale uncertainty (half of one dimension) = m = 0.05 mg

µ (rectangular) = 0.05/√3 = 0.0288 mg

$\mu\Delta m = \sqrt{(\mu m^2 + \mu m^2)} = \sqrt{[(0.05/\sqrt{3})^2 + (0.05/\sqrt{3})^2]}$ = 0.0408 mg

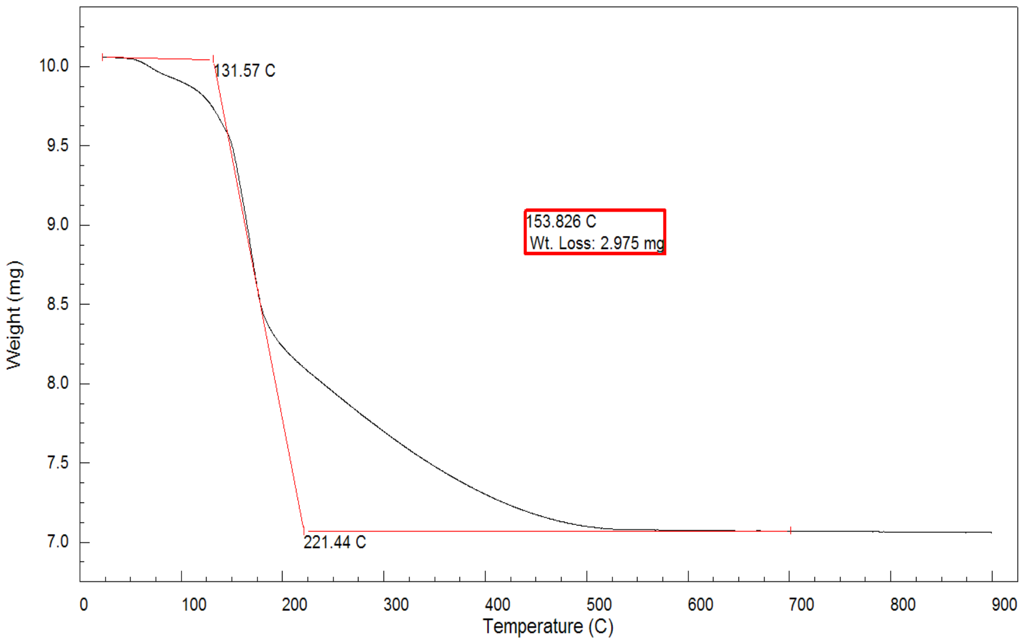
$(\mu\,\% / \text{mass loss}\,\%) = \surd\,[(\mu_{\Delta m} / \Delta m)^2 + (\mu_{mo} / m_o)^2] = \surd\,[(0.0408/2.970)^2 + (0.0001/10.6874)^2] =$ 0.01368 x 100 = 1.368 %

$\mu\,\% = \mu$ mass loss = 0.01368 x mass loss % = 0.01368 x 29.55 = 0.4042 %

C.I = 29.55 ± 0.4042 (Z95% = 1.96 ~ 2)

(29.55 ± 0.8) % for 95 % confidence

N of H_2O molecules = (mf H2O x m_{borax}) X [mH_2O (1 x mf H_2O)] = 4.6 molecules.



TGA curve for Detergent