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PRACTICAL APPLICATIONS OF A NOVEL TIME-RESOLVED LUMINESCENCE BASED METHOD

Joonas Siivonen

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University of Turku

Faculty of Medicine
Cell Biology and Anatomy
Drug Research Doctoral Programme (DRDP)

Supervised by

Professor Pekka Hänninen
Faculty of Medicine
Institute of Biomedicine
Medical Physics and Engineering
University of Turku,
Turku, Finland

PhD Paul Mundil
CTO-emeritus
Aqsens Oy
Espoo, Finland

Reviewed by

Professor Johan Bobacka
Faculty of Science and Engineering
Laboratory of Molecular Science and Engineering
Åbo Akademi University
Turku, Finland

Professor Jouko Korppi-Tommola
Faculty of Mathematics and Science
Department of Chemistry
University of Jyväskylä
Jyväskylä, Finland

Opponent

Professor, Jouko Vepsäläinen
School of Pharmacy, Faculty of Health Sciences
University of Eastern Finland
Kuopio, Finland

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ABSTRACT

In this thesis, a novel method to determine a chemical fingerprint for liquids was developed and it's performance was studied in real world applications.

The fingerprints are generated from interactions between the main components of a sample, a lanthanide-based signal source and carefully chosen chemical modulators. The sample admixed with the signal source is brought to contact with several modulators separately. The modulators interact with the signal source and the sample molecules modifying luminescence efficiency of the signal source, an europium chelate. Recorded luminescence signals under multiple chemical conditions form the digital data base for further statistical analysis to produce the fingerprint of the sample in question.

The method was applied in the following areas: 1) food product and beverage analysis, 2) on-site monitoring of scale inhibitors in oil and gas production processes and 3) rapid tests for oral cancer. The method performance was better than or identical to those of common FT-IR, HPLC and sensory analysis.

The method proved to be extremely versatile, as it can be applied to multiple fields, and novel applications can be easily developed. The technology has been productized, and test kits and instruments have been produced commercially. The fundamental chemistry of the method is robust and well characterized.

KEYWORDS: europium, fingerprinting, lanthanide, multivariate, nonspecific binding, wine, oral cancer. Head and Neck Neoplasms

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TIIVISTELMÄ

Tässä opinnäytetyössä kehitettiin uusi menetelmä nestemäisen aineen kemiallisen sormenjäljen määrittämiseksi, ja sen suorituskykyä tutkittiin käytännön sovelluksissa.

Sormenjäljet luodaan vuorovaikutuksista näytteessä olevien pääkomponenttimolekyylien, lantaanipohjaisen signaalilähteen ja vaihtelevan modulaattorin välillä. Näyte sekoitetaan signaalilähteen kanssa ja tuodaan kosketuksiin useiden ennalta valittujen kemiallisten modulaattorien kanssa yksi kerallaan. Modulaattorit vuorovaikuttavat signaalilähteen ja näytteen molekyylien ja muuttavat signaalilähteen, tässä tapauksessa europium kompleksin luminesenssin voimakkuutta. Mittaamalla näytteen luminesenssisignaalit useissa eri modulaattorien muodostamissa kemiallisissa ympäristöissä on mahdollista luoda luminesenssi intensiteettien muutoksia kuvaava numeerinen tietue, josta tilastollisten menetelmien avulla voidaan muodostaa näytteen sormenjälki.

Menetelmää sovellettiin seuraavilla aloilla: 1) elintarvikkeiden ja juomien analysointi, 2) saostumisenestoaineiden pitoisuuden seurantaan öljy- ja kaasutuotantoprosesseissa ja 3) pikatestinä suusyövälle. Menetelmän suorituskyky oli parempi tai identtinen yleisesti käytettyjen FT-IR, HPLC ja aistinvaraisen analyysin kanssa.

Menetelmä osoittautui erittäin monipuoliseksi, sillä sitä voidaan soveltaa useilla eri aloilla, ja uusia sovelluksia voidaan kehittää empiirisesti. Menetelmän peruskemia sen toiminnan kannalta on hyvin tunnettu ja ymmärretty.

AVAINSANAT: europium, sormenjälki, lantaani, monimuuttuja, epäspesifinen sitoutuminen, viini, suusyöpä. Pää- ja kaula-alueen neoplasma

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List of Original Publications

This dissertation is based on the following original publications, which are referred to in the text by their Roman numerals:

I Hänninen, P.E., Siivonen, J.J., Väisänen, P.I., Tiittanen, S.A., Lehmusto, M.M., Teimonen, T., Törnkvist, N., Sandell, M.A., Knaapila, A.J., Mundill, P. & Härmä, H.J. 2013, "Fuzzy liquid analysis by an array of nonspecifically interacting reagents: The taste of fluorescence", Journal of the American Chemical Society, vol. 135, no. 20, pp. 7422-7425.

II Siivonen, J.J., Väisänen, P.I., Tiittanen, S.A., Lehmusto, M.M., Lehtonen, P., Patrikainen, E., Teimonen, T., Törnkvist, N., Mundill, P., Hänninen, P. & Härmä, H. 2014, "Novel non-specific liquid fingerprint technology for wine analysis: A feasibility study", Australian Journal of Grape and Wine Research, vol. 20, no. 2, pp. 172-177.

III Hänninen, P.E., Siivonen, J.J., Martikkala, E., Desai, D., Teimonen, T., Tiittanen, S.A., Mundill, P., Mukundan, C., Desai, R. & Birur, P. 2016, "A Low Resource Setting Oral Lesion Screening Tool", vol. 1, no. 1, pp. 1-7 Matters.

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1 Introduction

Better analytical methods are in high demand for ensuring better quality products, earlier detection of serious diseases and monitoring authenticity of various raw materials. The more information we can gather, the better outcome we can provide in medical or industrial settings. However, the development of radically different analytical methods over the past years has been rare. To give few examples HPLC was developed in the late 1960s (Ostrowski, 1968), and immunoassays were developed in the late 1950s (Yalow and Berson, 1959). There have been of course notable developments in methodology and instrumentation in the past 50 years. But the fundamentals of assay development have remained quite similar. Typically, the development of novel applications requires thorough understanding of what happens within the measuring system. The approach described in this thesis differs from most analytical methods developed.

The research and development work reported in this thesis describes a novel analytical method and strategy to develop new assays. When performing an assay or developing an application with the method described here, the input and outputs are the most important aspects, rather than what happens within the system. This is an example of black-box philosophy, which is a common topic when discussing artificial intelligence models. A very simplistic but accurate description of the black box system was provided in the late 1950s by W. Ross Ashby (1956) in his book *An introduction to cybernetics*. "The child who tries to open a door has to manipulate the handle (the input) so as to produce the desired movement at the latch (the output); and he has to learn how to control the one by the other without being able to see the internal mechanism that links them. In our daily lives we are confronted at every turn with systems whose internal mechanisms are not fully open to inspection, and which must be treated by methods appropriate to the Black Box. A similar example from the medical field is treating many illnesses even when the treatment is not fully understood at the molecular level. Scientists have still developed safe and effective therapies with decades of research to treat patients (Li et al., 2020).

In the method developed here, a sample is brought into contact with a lanthanide ion signal source and individual cross-reactive modulators in array form, and a luminescent fingerprint is measured. The signal originating from the lanthanide ion,

by default, is highly sensitive to the environment, and modulators are used to change its interaction with the analyte molecules in the sample. Modulated luminescence signals are recorded with a time-gated measurement system, and these detected signals and measurement parameters form a fingerprint for the sample. The measured fingerprint can then be used to train the system (known sample) or be fed into a mathematical model to predict sample properties; alternatively, to determine which group the fingerprint belongs to, it can be compared to stored fingerprints. In most cases, it is impossible to know the interactions that occur inside of the system between the chemical modulator, signal source and analytes.

The suitability of the method for various applications has been demonstrated in the medical setting (detection of oral lesions), in the food and beverage industry (taste, authenticity) and in the oil and gas industry (process monitoring).

1.1 Brief history of Aqsens Oy

Aqsens Oy was set to commercialize the fingerprinting method in 2009 by professor Pekka Hänninen and two start-up experts, Niklas Törnqvist and Timo Teimonen. The aim was to develop a system suitable for performing an analytical test on-site in industrial and medical applications. The system consists of test kits, a suitable reader and other necessary supplies. The author joined Aqsens in the beginning of 2011 as application scientist and has held multiple positions there.

During the early days of the company, it was very difficult to find a focus. We had a technology that could be utilized for almost everything, and selecting the right application for commercialization was difficult. We performed feasibility studies on almost anything we could obtain. The list is long, and a few items we tested were wine, vodkas, food items (such as ketchup, meat and hot sauces), tobacco, drugs (for authenticity) and even contact lens fluids.

The most interesting results from these feasibility studies form the basis for original publications I and II. Most of the approaches were not pursued further due to difficulty in building a business case or finding development partners to partly fund the projects and provide market insight. However, the importance of this period cannot be overestimated. We as a team learned much about the technology, instrumentation and what information is needed to develop a product. In this period, we learned how to develop products as a team. In addition, I went through a personal development journey while leading a product development team.

Kemira Oyj and Aqsens started to collaborate in late 2011(Fagerholm and Teimonen, 2015). We identified some mutually interesting concepts and selected monitoring scale inhibitors in the oil and gas industry as the lead project. The products were ready in early 2014, and they were launched in Aberdeen, Scotland

2014 (Mundill and Rosati, 2014). The reception was overwhelmingly positive; however, the environment we operated would unexpectantly change.

The plummeting of crude oil prices and the downfall of the O&G industry in 2014-15 made it almost impossible to gain any footing. When we introduced our products to the market, more than 440,000 jobs were lost in the upstream sector of the oil and gas industry. More than two out of five individuals lost their job (Wethe, 2017). Therefore, capital expenditure was also low, and every last drop from operational expenditure was squeezed out; only material that was absolutely needed was purchased. The collaboration project ended in 2017 when Kemira Oyj purchased the technology from Aqsens (Salminen and Teimonen, 2017). None of the results related to industrial water analyses have been published in peer-reviewed publications. This thesis provides information derived from public sources, such as conference proceedings and patents for these applications, which are listed in the Other supporting material section.

Aqsens joined a project with the University of Turku focusing on early detection of oral cancer in 2014. We believe that we could develop a suitable low-cost rapid test for the Indian market. Organizing the work in India was an adventure of its own. India was far away from our operation, and traveling there required a considerable amount of time, reducing the time available to work in the laboratory. The collaboration further complicated because exporting biological samples of human origin out of India is difficult (if not impossible). Therefore, we had to set up a laboratory in India. The results of approximately ten trips to India are presented in original publication III. The work to commercialize the tests in medical applications is continuing at Aqsens Health Oy.

2 Review of the Literature

2.1 Food and beverages

The taste of a food or beverage is determined by a vast array of volatile and non-volatile compounds. These compounds interact in multifaceted ways, affecting taste, aroma, and mouthfeel. The concentration and combination of these molecules can significantly alter the sensory experience, making it difficult to predict taste from chemical composition alone. The most difficult factors to measure are taste (or human-observed quality) and authenticity, as they are not determined by a single easily measurable molecule or parameter but by many compounds that determine taste and authenticity in a holistic manner.

Generally, analytical methods developed for food and beverage applications ensure that products are safe (no contaminations), the taste and mouthfeel of products is consistent and the product is authentic (no adulteration). The section 2.1.1 will discuss some of the standard analytical methods used to monitor quality of wine and section 2.1.2 will discuss authenticity monitoring.

2.1.1 Wine quality monitoring

The consumer and retail expectations for quality monitoring have increased. More testing for wine quality is performed than ever. Several key parameters are routinely measured to ensure the quality, stability, and sensory attributes of wine. These parameters include volatile acidity, total acidity, pH, malic acid, dry extracts, and alcohol content. We will focus on these specific parameters as they provide good understanding of wine composition and their results interpretation is routine production.

Volatile acidity (VA) is a critical parameter in winemaking, often considered an indicator of wine spoilage. Elevated levels of volatile acids, particularly above 0.9 g/L, can significantly impair the sensory qualities of wine, imparting an acrid taste and the unpleasant aroma of vinegar(Boulton et al., 2013). While volatile acidity primarily measures the concentration of acetic acid, other acids such as carbonic acid, sulfurous acid, and minor amounts of lactic, formic, butyric, and propionic acids also contribute to the total volatile acidity. The origins of these acids in wine

are diverse. During the desired fermentation process, added yeast converts sugars into ethanol and acetic acid, contributing to the volatile acidity. However, unwanted bacterial growth during the fermentation or aging process can also increase volatile acidity, resulting in spoilage. This bacterial activity is often associated with Acetobacter species, which oxidize ethanol to acetic acid, particularly when exposed to oxygen.

Historically, the measurement of volatile acidity involved steam distillation followed by titration of the distillate with sodium hydroxide, a method prevalent until the mid-1990s (Zoecklein et al., 1995). This traditional approach, while effective, was labor-intensive and time-consuming. In modern winemaking, the determination of volatile acidity has been greatly simplified and refined through the use of Fourier Transform Infrared (FT-IR) spectroscopy. Instruments such as the FOSS Winescan provide rapid, accurate, and non-destructive analysis of volatile acidity, allowing for efficient monitoring and quality control (Warner, 2002).

During the winemaking process, malic acid is converted to lactic acid through malolactic fermentation (MLF). This biochemical transformation is crucial for reducing the high acidity imparted by malic acid, which in excessive concentrations, imparts an unpleasantly sharp, sour taste to the wine (Volschenk et al., 2006). Successful management of malolactic fermentation is essential for producing high-quality wines, as inadequate levels of malic acid can result in a wine with an undesirably flat and insipid taste.

Monitoring malolactic fermentation involves the quantitative determination of malic acid concentration, which can be achieved through several analytical methods. One common approach is the use of enzymatic reactions, typically facilitated by commercial kits designed for use with a UV-spectrophotometer. This method is favored for its specificity and accuracy in measuring malic acid concentrations. High-Performance Liquid Chromatography (HPLC) is another robust technique employed for this purpose, offering high resolution and sensitivity, thus providing detailed profiles of malic acid content in wine samples. Additionally, Fourier Transform Infrared (FT-IR) spectroscopy presents a rapid and non-destructive alternative for assessing malic acid levels, utilizing the characteristic absorption patterns of the compound to quantify its presence in the wine matrix (Iland, 2000).

Total acidity generally refers to the titration of wine (weakly acid solution) with a strong base (NaOH) to a certain end point (typically pH 7.00 or 8.2), but different definitions exist(Darias-Martín et al., 2003; OIV, n.d.). Total acidity in wine cannot be directly linked to its taste or the winemaking process, as various other parameters also influence the wine's flavor profile, and there is no definitive chemical formula for perfect wine. Total acidity can be measured through a simple titration reaction

using a pH meter or bromothymol blue as an indicator, as well as through indirect methods such as Fourier Transform Infrared (FT-IR) spectroscopy.

Although pH is related to total acidity in wine, it does not directly correlate with total acidity or any other single parameter (Beelman and Gallander, 1979). Despite this lack of direct correlation, pH monitoring is important in the winemaking process, particularly in terms of wine aging and spoilage prevention. As a result, pH is routinely measured in winemaking. Even small changes in pH can significantly impact the behavior of wine during aging, influencing its stability, color, and microbial activity(McRae et al., 2013). Therefore, maintaining an optimal pH is considered essential for producing high-quality wine. The determination of pH is a straightforward procedure that can be conducted using various methods. The simplest method involves the use of pH indicator paper, which provides a quick and approximate measurement. For more precise measurements, a pH meter is commonly used, offering accurate and reliable results. Additionally, pH can be assessed indirectly through techniques such as titration FT-IR spectroscopy.

Dry extracts refer to the solid components that remain in wine after the removal of reducing sugars, water, ethanol, and volatile acids. These extracts comprise both organic compounds, which are generated and/or modified during the fermentation process, and inorganic compounds, which originate from the soil where the grapes were cultivated, such as calcium, magnesium, and sodium ions. Wines with high levels of dry extracts are typically perceived as having a heavy or full-bodied taste(Neto et al., 2015).

Measurement of dry extracts can be done with multiple methods. A method involves determining the specific gravity of the alcohol-free wine, that this then corrected for the presence of volatile acids and reducing sugars to get the final result(OIV, n.d.). A second gravimetric method can also be utilized. Wine sample is dried under controlled pressure and temperature on a filter paper to obtain solid residue. To get the final result reducing sugars need to be subtracted(OIV, n.d.). Alternatively, indirect methods such as FT-IR spectroscopy can be employed for a more efficient and non-destructive analysis. The accurate assessment of dry extracts is essential for understanding the wine's mouthfeel and body.

Alcohol content or Ethanol concentration is a critical parameter in winemaking, influencing both the quality and characteristics of the final product. Monitoring ethanol content is essential during the fermentation process, wherein yeast metabolizes grape sugars to produce ethanol and carbon dioxide. This parameter is also subject to regulatory and taxation requirements, necessitating accurate and reliable measurement.

A variety of methods exist for determining ethanol concentration. Official methods typically involve two primary steps: first, the preparation of a distillate,

followed by the determination of ethanol concentration using techniques such as pycnometry, electronic densimetry, hydrostatic balance, hydrometry, and refractometry. While these methods are highly accurate, they are often cumbersome and not suitable for routine analysis due to their complexity and the time required.

To address the need for more efficient and practical solutions, several alternative methods have been developed. HPLC and gas chromatography methods offer precise and rapid quantification of ethanol. Additionally, test devices and indirect Near-infrared and Mid-infrared (NIR/MIR) spectroscopy provide convenient and quick assessments of ethanol content. Spectroscopic methods have demonstrated improved precision compared to traditional techniques, thereby establishing their efficacy for routine monitoring in wine laboratories, in both production and quality control contexts (Wang et al., 2020; Wilkes and Pride, 2022).

To summarize there are traditional analytical methods for example electrophoretic, gas and liquid chromatographic methods, enzyme-linked immunological assay (ELISA), polymerase chain reaction (PCR) and microscopic methods have been mostly replaced with spectroscopic methods that are nondestructive and simpler (no sample preparation is necessary, and a high throughput is achieved). This transition has occurred because traditional analytical methods are labor intensive and require specialized knowledge and facilities, making them difficult and expensive to maintain without good laboratory infrastructure. Targeted FTIR or NIR methods combined with data analyses can be used to determine the water, ethanol, glycerol, sugars, polyols, phenols, minerals, organic acids and volatile compounds contained in the wine sample with performance that is equal to traditional analytical methods (Considine and Frankish, 2014; Ranaweera et al., 2021; Rodriguez-Saona and Allendorf, 2011). Table 1 contains comparison of analytical methods for the various parameters and the advantages of modern methods.

Table 1. Comparison of analytical methods for wine.

Parameter	Traditional Methods	Modern Spectroscopic Methods	Advantages of Modern Methods
Volatile Acidity	Steam distillation, titration	FT-IR spectroscopy	Rapid, non-destructive, high throughput
Malic Acid	Enzymatic kits, HPLC	FT-IR spectroscopy	Faster, minimal sample preparation
Total Acidity	Titration (pH meter or indicator)	FT-IR spectroscopy	Automated, less labor-intensive
pH	pH meter, indicator paper	FT-IR spectroscopy	Integrated with other analyses
Dry Extracts	Gravimetric, specific gravity	FT-IR spectroscopy	Non-destructive, efficient
Ethanol Content	Distillation + pycnometry/hydrometry/refractometry	NIR/MIR spectroscopy, HPLC, gas chromatography	Rapid, accurate, suitable for routine use

2.1.2 Wine authenticity monitoring

The ability to monitor authenticity is important, as adulteration leads to large economical gains. For example, wine from Bordeaux, France is more valuable than wine from Chile, and the temptation to fool buyers is high while the likelihood of getting caught is low. The same is true for vintage wines, as a wine from the 1950s can be much more valuable than the same wine from 2018. The options for testing the authenticity of wine or any other food or beverage are limited, as an extremely large databank is needed in which experimental results have been collected for a very long period. Stable isotope ratio analysis (SIRA) and deuterium-nuclear magnetic resonance (2H-NMR) are the most commonly used methods (Christoph, Hermann et al. 2015). The international organization of Vine and Wine (OIV) has published a method OIV-MA-BS-22 Determination by isotope ratio mass spectrometry of the 13C/12C ratio of wine ethanol in spirit drinks of vitivinicultural origin (Type II)(*OIV-MA-BS-22 Determination by isotope ratio mass spectrometry of the 13C/12C ratio of wine ethanol in spirit drinks of vitivinicultural origin (Type II) | OIV*, 2009).This method determines the ratio of carbon isotopes (13C/12C) in ethanol derived from wine. The technique involves combusting the sample and analyzing the resultant gases using mass spectrometry, providing precise isotopic

ratios. The method is relatively complex methods and require extensive laboratory infrastructure and highly trained staff and therefore cannot be performed at wine making sites but only in centralized laboratories

The 13C/12C ratio can be used to determine the origin of alcohol and sugars in wine and spirits. During photosynthesis, plants consume CO_2 through two primary pathways: C3 and Hatch-Slack pathways (C4)(Slack and Hatch, 1967; Stracquadanio et al., 2010). These processes result in different isotope fractionation patterns due to enzymatic preferences and differences in CO_2 isotope diffusion(Farquhar et al., 1982). As a results fermentation products, like sugars and alcohols derived from C4 plants, such as sugar cane and corn contain higher levels of Carbon-13 compared to those from C3 plants. Most plants, including vines and sugar beet, use the C3 pathway. By measuring the Carbon-13 content, it is possible to detect and quantify the presence of C4-derived fermentation products in like musts and wines. These data can be combined with other test results to further increase the confidence of identifying possible alterations(Roßmann et al., 1996).

Recently, nontargeted NIR and MIR methods have been developed to detect the authenticity and origin of wine. Ranaweera et al. used absorbance and transmission excitation-emission matrixes to determine the origin of wine with 100% correct class assignment. Hu et al. (2019) used mid-infrared (MIR) and near-infrared (NIR) spectroscopy to generate country of origin-based fingerprints. Unknown wine samples could then be mapped to the correct origin with a 70-90% recognition rate. These results clearly demonstrate that nonspecific and nontargeted methods have the potential to determine the authenticity and/or origin of a wine.

2.2 Scale inhibitors in oil and gas industry

2.2.1 Introduction

During oil production, more than 95% of the volume extracted at the surface can consist of water, known as produced water (PW). The salts present in PW can precipitate, leading to the blockage of production tubing (Oddo and Tomson, 1994) This phenomenon, termed scaling, can also occur within the geological formation itself, adversely affecting the flow of hydrocarbons before they even reach the production tubing. The measures taken to prevent such blockages are referred to as flow assurance. Scaling can occur in various components of production equipment, including tubing, valves, and water injection facilities or in the formation itself making it unusable. Scale deposition can result in complete flow blockage within a matter of hours, potentially leading to significant economic losses and environmental damage, particularly in the event of it causing oil leaks. Consequently, effective scale

management is crucial for ensuring operational safety and mitigating environmental risks associated with oil and gas production.

The formation of inorganic scale is not limited to the oil and gas industry; it also occurs in various other industrial processes that handle large volumes of water. For example, scale can form in heat exchangers used in sugar production or in the cooling towers of nuclear power plants. These industrial scales can lead to operational inefficiencies and increased maintenance costs, highlighting the importance of effective scale management across different sectors.

2.2.2 Scale formation and prevention

The extent of scaling and the propensity of water to form scales vary significantly depending on the geological characteristics of a location, including the types and concentrations of ions present, as well as the specific production techniques employed, such as water injection, steam injection, and enhanced oil recovery methods. Scaling is frequently triggered by changes in pressure and temperature during the production process or when seawater is injected into the formation, mixing with the formation water. (Hafez et al., 2018; Phakam et al., 2018). The scales can be divided into three categories, low-pH scales, High-pH scales and pH-independent scales based on effect of pH on their formation(Olajire, 2015).

Low-pH scales generally form under acidic conditions and include minerals such as iron sulfides (e.g., FeS)(Kamal et al., 2018). Their formation is commonly linked to the injection of acidic fluids or the natural presence of hydrogen sulfide (H_2S) in oil and gas reservoirs. H_2S, while a natural component of many formations, poses dual challenges: it creates scaling issues and represents a significant health and safety hazard due to its toxicity and corrosive nature. For example, the introduction of acidic corrosion inhibitors to protect production equipment from H_2S can inadvertently create favorable conditions for low-pH scale formation (Okocha and Obeyesekere, 2023). As a result, additional scale control measures are often required to mitigate these risks as part of corrosion control programs and ensure uninterrupted flow in all operating conditions.

High-pH scales predominantly form in alkaline environments, where the solubility of certain ions decreases, leading to precipitation. Notable examples include calcium carbonate ($CaCO_3$) and magnesium hydroxide ($Mg(OH)_2$), which commonly precipitate when pH increases as a result of water mixing or pressure reduction. A significant proportion of the world’s oil fields are in carbonate reservoirs, particularly in regions such as North America and the Middle East(Jiang et al., 2019, p. 1). These reservoirs primarily consist of calcite ($CaCO_3$) and dolostone $CaMg(CO_3)_2$, making these formations inherently rich in carbonate minerals.

pH-Independent Scales: Some scales, such as barium sulfate ($BaSO_4$) and strontium sulfate ($SrSO_4$), are relatively insensitive to pH variations. Their formation is primarily driven by supersaturation resulting from the mixing of incompatible waters. A common example in the O&G industry is when water in a reservoir containing either barium or calcium ions and seawater containing a high concentration of sulfate ions are mixed. This occurs when large amounts of sea water are pumped down to the reservoir to increase the pressure (Puntervold and Austad, 2008). This leads to $CaSO_4$ precipitation (anhydrite, gypsum and bassanite) and/or $BaSO_4$ (barite).

To prevent tube blockage and a subsequent reduction in production rate there are different strategies (1) avoid mixing incompatible waters; (2) dilute the produced water, e.g., in control of halite scale (NaCl); (3) apply pH control, e.g., acid treatment to remove carbonate scale; (4) add a metal chelator to reduce effective concentrations of calcium, barium, or iron; (5) use a water softening agent to remove divalent cations or membrane filtration to reduce sulfate in injection water; and (6) initiate scale formation to reduce the supersaturation(Al Salami and Monem, 2010). Most common method is addition of scale-inhibitors due to the complexity of the other approaches to reach adequate control, but different strategies can be combined.

Scale inhibitors (SIs) are chemical additives designed to prevent the precipitation of scale-forming minerals in oil and gas production systems. They can be broadly categorized into two main groups: polymeric and nonpolymeric inhibitors through chemical structure(Sorbie and Laing, 2004). Polymeric SIs are typically anionic polymers with molecular weights below 5 kDa, such as sulfonated polymeric acids. These are particularly effective in environments where sulfate and carbonate scales are dominant, as they interfere with the nucleation and crystal growth processes. Historically the polymeric scale inhibitors have struggled in high temperature environments (130-200C) and they have been preferred in cooler wells (Wang and Wylde, 2009). Recently polymers with high thermal stability and good scale inhibition properties have been synthesized and this issue has been overcome(Shi et al., 2022). In cooler reservoirs, polymeric inhibitors are often preferred or nonpolymeric because they perform well in preventing sulfate scales under these conditions.

Nonpolymeric SIs, on the other hand, can be inorganic phosphonates, such as diethylenetriamine penta(methylenephosphonic acid) (DTPMP), which is widely used due to their effectiveness in high-temperature and high-pressure conditions. DTPMP exhibits a strong affinity for calcium ions, making it particularly effective in controlling carbonate scales, such as calcium carbonate ($CaCO_3$), even in highly saline brines, such as those commonly found in Middle Eastern reservoirs(Jarrahian et al., 2020). Additionally, its high thermal tolerance (exceeding 150°C) enables its application in deep oil wells and other high-temperature reservoirs. In addition

phosphonate scale inhibitors have been widely used in the oil and gas industry, and extensive data on its compatibility with other production chemicals and equipment is available, demonstrating its high compatibility with a variety of production chemistries. However, a notable downside of phosphonates are their chemical stability, which results in poor biodegradability. This characteristic raises environmental concerns, leading to restrictions on its use in certain regions due to regulatory and sustainability considerations (Mazumder and A, 2020).

The biodegradability and environmental safety of SIs is important, as residual chemicals may be discharged into the environment during production operations. Accumulation to the environment has negative impacts. Modern formulations often aim to balance performance with environmental safety by incorporating biodegradable or less toxic components for example polyaspartate (Ross et al., 1996). Regulations in certain regions especially in the North Sea may restrict the use of non-biodegradable chemicals, further emphasizing the importance of minimizing the usage of SIs and development of greener alternatives (Jordan et al., 2012; Petroleum Safety Authority Norway et al., 2022).

It can be summarized that different types of scale inhibitors perform differently in different conditions, and a suitable product must be selected for each oil production location, as water chemistry and environmental conditions (geology, temperature and pressure) vary. The selection process requires a notable amount of laboratory testing. In addition, there are different computer modelling systems to select the best possible scale treatment option.

2.2.3 Selection of Scale inhibitor

Selecting the appropriate scale inhibitor (SI) and determining the required concentration for a specific site is a complex process that requires careful evaluation of local conditions, water properties, temperatures and environmental requirements(Boak, 2012; Graham et al., 2002). Laboratory testing is crucial for assessing the performance of the scale inhibitors, as conducting tests in the field can present significant logistical, economical and safety challenges. These laboratory tests typically include dynamic scale loop tests, core flood tests, chemical compatibility tests, and thermal stability tests. The primary goal of these tests is to determine which inhibitor is effective, how stable the inhibitor is, the minimum inhibitory concentration (MIC) of the SI, which is the lowest concentration required to effectively prevent scaling under the specific conditions of the site.

A dynamic loop test is performed by circulating test solution through a narrow tubular loop under carefully controlled conditions of temperature, pressure, and flow rate Multiple commercial instruments are available(“F5 Technologie: Dynamic Scale Loop,” n.d.). During the test, a solution that mimics the water composition in

the production system - containing potential scale-forming ions (e.g., calcium, sulfate, or carbonate) is mixed with a scale inhibitor at varying concentrations. As the fluids flow through the system, scaling tendencies are monitored by on-line measuring changes in pressure and flow rate(Al Helal et al., 2019). It is also possible to analyze the type of scale formed by inspecting deposit accumulation on surfaces.

Dynamic loop tests can mimic the shear stress and fluid dynamics encountered in production systems, providing a more accurate assessment of scale inhibitor performance compared to static tests. A static jar test is in practical terms mixing reagents in a test tube and visually observing if scale is formed. Dynamic loop tests are widely used for product screening as they are simplest dynamic test that can be performed. The duration of a single test is under 1h – non-inhibited brine will block the system in less than 5 minutes, poorly inhibited in less than 30 minutes, making it effective to screen through multiple compounds and how changes in conditions affect the results (Borges and Lessa, 2024; Daniels et al., 2014; Kartnaller et al., 2018). It is also highly repeatable as the conditions are fully controlled, the loop can be aggressively cleaned or easily replaced. Therefore, it enables a reliable comparison of different chemicals.

A core flood test is similar to a dynamic loop test but replaces the tubular loop with a core sample of reservoir rock or, in some cases, a synthetic equivalent. In this test, fluids mimicking the actual production waters are injected through the core sample while flow rate, temperature, and pressure are controlled(Baldygin et al., 2014; Graham et al., 2010a). The procedure simulates the interaction between injected fluids, such as water containing scale inhibitors, and the rock matrix. Coreflood testing is a powerful tool for evaluating key factors such as the adsorption and retention of scale inhibitors on the rock surface. This information is critical for understanding whether the inhibitor will remain adhered to the formation or be rapidly produced back(Graham et al., 2012; Mokogwu et al., 2018). Understanding the behavior of SI with the formation rock helps to tailor inhibitor formulations to specific field conditions, as different applications may require varying adsorption and desorption characteristics to ensure effective scaling control. Core flood tests also provide insights into permeability changes and the potential for formation damage caused by scaling or chemical interactions. As SIs might damage reservoir rock causing notable disruption and reducing the production flow.

The challenges of core flood testing are related to the difficulties associated with obtaining and using core samples. Acquiring representative core samples from a reservoir is a not a simple task, as it requires extraction and transport of the sample to a laboratory. The process can be time-consuming and requires significant investments, especially in remote or deep-water oil fields. Given the high cost of obtaining core samples, they are often reused for multiple tests. However, repeated testing can lead to sample degradation, which can cause the results to become

unrepresentative of the true reservoir conditions (Howard and Tx, 2024). Furthermore, because each core sample is unique, it may not fully capture the heterogeneity and complexity of the reservoir, as rock properties can vary significantly across different locations within the formation. This variability increases the risk that core flood results may not accurately represent the actual field conditions(Shell Global Solutions International BV et al., 2021). To mitigate this issue, additional core samples can be incorporated into the testing process, although this increases the overall cost. Therefore, careful planning and experience are required in test design to ensure the results are as reliable and as representative as possible.

In addition to these challenges, the flow dynamics in a typical core flood test rig are relatively simple compared to the complex fluid flow that occurs in actual field conditions. In the field, fluid flow is influenced by factors such as reservoir heterogeneity, varying permeability, and natural fractures, all of which can significantly affect how scale inhibitors interact with the rock matrix. Therefore, while core flood tests provide valuable insights, they may not capture the full complexity of fluid behavior in real-world reservoir environments(Howard and Tx, 2024). Core flood tests are typically limited to a fixed experimental duration, which may not allow for the observation of long-term effects such as inhibitor degradation, accumulation, or scale formation over extended periods. This makes it difficult to predict long-term field performance with complete accuracy.

Overall, it can be stated that core flood testing is a crucial component of the scale prevention strategy selection process. When conducted properly, it provides valuable insights that help mitigate significant economic and environmental risks.

Due to the high cost and time investment associated with these laboratory evaluations, changing an SI after it has been implemented is generally undesirable. Incompatibility or poor performance from an ill-suited inhibitor can lead to operational disruptions, increased scaling risks, and elevated remediation costs. Therefore, thorough pre-deployment testing and careful consideration of reservoir-specific conditions are critical.

2.2.4 Addition of a scale inhibitor

The SIs can be added with two different strategies either by continuous feed to a suitable location in the oil production process upstream of the issue or by a scale squeeze (Slyker, 1969). With the squeeze, a large amount of SIs is deposited near the wellhead and released to the produced water over a long period of time. Initially, a high concentration of the SI is returned, but after some time, the release will stabilize for an extended period(Kan et al., 2020). The effective release period of the squeeze varies from a few months to a few years depending on the outcome and

design; longer release periods are preferred due to the high cost of squeeze jobs but this requires high stability from the SI and good retention of the SI to the geology (Mackay and Jordan, 2003). In some cases, both strategies must be used, as the scaling tendency varies in different parts of the production process, which necessitates the injection of additional SI to certain parts to prevent scaling.

As described in the previous chapter, the SIs must be present in sufficiently high concentrations to effectively prevent scaling. The concentration of SI must be monitored from the produced water because, even with continuous addition, the system is not closed; thus, SIs can escape. Complete blockage of production tubing by scale can occur in less than 24 hours if the scale inhibitor dosage is incorrect. typical MIC is 1-10 ppm, which sets requirements for the analytical methods.

2.2.5 Scale inhibitor quantitation

There are multiple reasons why scale inhibitors need to be quantified. One must know if the deployment strategy for scale inhibitors is function and the simplest way to do this is to take a sample downstream of the addition point and determine if adequate concentration is present. For continuous injection of SI, the flow rate can be adjusted and for scale squeeze the available lifetime of the treatment can be monitored. In addition to ensuring the concentration is above the minimum limit, overdosing should also be avoided, as it wastes chemicals and money. The available analytical methods depend on the SI's chemical structure. If the molecule contains phosphorus, or particularly designed tags (e.g. fluorescent) those can be quantified directly. Sometimes the chemical itself is detectable e.g. polymeric scale inhibitors can be quantified with HPLC and suitable detectors (UV-VIS). Different methods and approaches have different benefits and downsizes, and these will be discussed in more detail below.

Inductively coupled plasma (ICP) spectroscopy based methods are used for phosphonates and phosphorus-tagged products (Graham et al., 2010b). It is highly effective in detecting and quantifying trace metals and specific elements. For phosphonate-based scale inhibitors, ICP is simply used to quantify the phosphorus content. This method utilizes a plasma source to ionize the sample, and the resulting emissions or ions are analyzed to determine inhibitor concentration when compared to a calibration curve. ICP has sensitivity and ability to detect low concentrations. The high analytical sensitivity makes it possible to incorporate pre-treatments that potentially dilute the sample, increasing the methods tolerance to variations and impurities in the produced water. However, it requires advanced instrumentation, highly trained operators and some level of sample preparation to eliminate interferences. It can also be problematic if multiple phosphorus containing molecules are present in the produced water (e.g. corrosion inhibitors or mixture of scale inhibitors).

One of the industry standards methods used for quantifying polymeric scale inhibitors is the Hyaline method. Hyamine 1622, or other quaternary ammonium salts, reacts with the scale inhibitor in the sample, producing turbidity that correlates with the concentration of the scale inhibitor. The concentration of the sample can be quantified by preparing a calibration series with known concentrations of the scale inhibitor, measuring their absorbance at 500 nm, and comparing the sample's absorbance to the calibration curve. The method is relatively time consuming as samples containing low concentrations (typically below 100 ppm) of active scale inhibitor must be pre-treated with an anion exchange, C18 or similar cartridge to remove any ions that might interfere with the measurement (e.g. chlorine).

The polymeric scale inhibitors can be tagged with fluorescent labels. This enables quantification by measuring prompt fluorescent from the sample and comparing the measured signal to the signals measured from samples containing know amounts of scale inhibitor. In practice all oil field chemical providers have introduced fluorescently tagged polymers to the market (Hills and Chapon, 2005; Moore and Clapp, 2016, 2012; Moriarty et al., 2001, 2001; Morris et al., 2001; Toivonen and Nuutinen, 2014). However, the incorporation of fluorescent tags into scale inhibitors chemical structure creates some challenges. The tagging process can potentially alter the inhibitor's chemical structure, impacting its ability to interact with scale-forming ions and inhibit deposition. For instance, the functional groups responsible for scale inhibition might be sterically hindered or chemically modified by the fluorescent tag, reducing the inhibitor's effectiveness. Therefore, fluorescently tagged inhibitors must be rigorously tested to confirm that their performance remains comparable than their untagged counterparts. The stability of the fluorescent tag is another critical factor. Many operational environments for scale inhibitors, such as high-temperature boilers or extreme pH conditions, can degrade fluorescent molecules, leading to a loss of signal and rendering the tagging ineffective (Vuori et al., 2016). Fluorescent tags have not become popular, most probably due to economic reasons and the cost of fluorescent spectrophotometers.

State-of-the are performance is achieved with High-performance liquid chromatography (HPLC), in particular, is used to separate and quantify individual components within complex mixtures of scale inhibitors with good analytical sensitivity (sub 1 ppm)(Heath et al., 2016). HPLC methods are highly specific and capable of analyzing multi-component formulations, making them valuable for evaluating inhibitor performance under challenging conditions. However, they require advanced instrumentation, longer analysis times, and expertise in method development and operation.

The accurate determination of scale inhibitor (SI) concentrations is essential for maintaining safe and efficient operations in the oil and gas industry. Most analytical techniques used for this purpose, such as those presented above, are standard

procedures widely available in laboratory settings. These techniques are well-suited for controlled environments, where stable conditions and proper equipment maintenance ensure reliable results. The Table 2 contains summary and comparison of various analytical methods discussed.

Table 2. Comparison of Scale inhibitor detection methods and practical considerations.

Method	Advantages	Limitations	Suitability
ICP Spectroscopy	High sensitivity, tolerance to impurities	Complex sample prep, interference issues	Lab-based analysis
Hyamine Method	Cost-effective, simple equipment	Pre-treatment required, time-intensive	Limited field use
Fluorescent Tagging	Rapid analysis, direct detection	Tag stability issues, high cost	Laboratory trials
HPLC	High specificity, handles complex mixtures	Long analysis time, advanced expertise needed	Advanced lab setups

However, their application becomes impractical in remote and harsh environments typical of oil and gas production sites, such as offshore platforms in the North Sea or arid desert regions in the Middle East. At such sites, logistical constraints often necessitate shipping samples to centralized laboratories for analysis. This process introduces significant delays, with the average turnaround time between sample collection and receipt of results being approximately two weeks. In more challenging scenarios, such as when exporting samples involves complex customs procedures or helicopter transport is restricted due to safety regulations, these delays can extend further. Such lengthy intervals between sampling and results present a critical limitation, particularly when operating near the MIC threshold, where real-time decision-making is crucial to prevent scaling.

The delay creates a dilemma for operators when results arrive. If a two-week-old analysis indicates the SI concentration is at the safe limit, the operator must choose between two options: (1) halting production to avoid potential scaling risks, or (2) continuing production while ordering a new test, thereby accepting the associated risks. The oil and gas industry, prioritizing the high costs and operational disruptions caused by scale deposition—such as pipeline cleaning or well redrilling—tends to adopt conservative measures. This often involves performing chemical squeezes more frequently than necessary as a precautionary strategy. While conservative approaches minimize the risk of scaling, they have their own drawbacks. Excessive chemical use leads to increased operational costs and imposes a greater environmental burden, as surplus inhibitors are discharged into the ecosystem. Moreover, unnecessary production halts or interventions result in lost revenue and inefficiencies. These challenges underscore the pressing need for portable, real-time

analytical solutions capable of providing accurate SI concentration data at production sites. Developing such technologies would reduce reliance on off-site laboratories, shorten decision-making timelines, and optimize both economic and environmental outcomes in scale management practices.

2.3 Oral cancer & saliva

Oral cancer represents a significant public health crisis in Southeast Asia, with approximately 400,000 new cases reported annually. Alarmingly, two-thirds of these cases occur in low- and middle-income countries, where access to healthcare and cancer prevention programs is often limited (Vigneswaran and Williams, 2014). India, in particular, bears a substantial burden, with certain regions reporting an oral cancer incidence rate as high as 11.5 per 100,000 males. This alarming rate has been linked to the widespread use of smokeless tobacco products, a deeply ingrained cultural practice in many parts of the region.

Smokeless tobacco products, commonly containing betel quid, areca nut, lime, and other natural ingredients, are highly addictive and carcinogenic. Betel quid, a chewable mixture that often includes areca nut and tobacco, is a major contributor to the high prevalence of oral cancer in Southeast Asia. The International Agency for Research on Cancer (IARC) classifies areca nut and betel quid as Group 1 carcinogens, signifying their definitive role in causing cancer in humans. Chronic use of these products has been strongly associated with the development of oral submucous fibrosis, a precancerous condition that significantly increases the risk of malignant transformation (Asthana et al., 2019).

In addition to their direct carcinogenic effects, smokeless tobacco products exacerbate other risk factors for oral cancer, including poor oral hygiene, malnutrition, and infections such as human papillomavirus (HPV)(Haukioja et al., 2014; Murthy et al., 2017). These compounding factors contribute to the disproportionately high incidence and mortality rates seen in resource-constrained regions. Despite the recognized health risks, the consumption of these products persists, fueled by cultural norms, social acceptance, and aggressive marketing by the tobacco industry.

The majority of cancers are head and neck squamous cell carcinomas (HNSCCs). They develop in the mucous membranes of the mouth, nose, and throat. Generally, these areas receive most of the chemical insults from smokeless tobacco products. The irritated location develops abnormal patches or open sores, and the severity of the damage is difficult to assess visually. Minor and benign abnormalities should be carefully monitored as they can develop into malignancy. Early clinical symptoms (e.g., pain in the mouth, sinus congestion, sore throat, earache, pain when swallowing) are easily confused with other diseases, which delays the correct diagnosis (Rivera, 2015).

Most oral cancers are diagnosed at a late stage, which leads to increased mortality. The earlier an accurate diagnosis is achieved, the better the treatment outcome. The five-year disease-free survival rate is approximately 80-90% if the disease is diagnosed at an early stage (I and II), but the rate is 20% when diagnosed at a late stage (III and IV). In India, approximately 90% of cases are diagnosed at a late stage (Solanki and Gupta, 2017). Even if a patient survives late-stage disease, the treatment often leads to severe disfiguration, and the patient's life after recovery is not full (Day et al., 2003). Early diagnosis is key for patient survival.

There are at least two root causes for late diagnosis. First, no good methods or tests are available for diagnosing oral cancer in point-of-care or assessing the malignancy potential of a lesion. Second, patients are only willing to travel to healthcare facilities when seriously ill, as the socioeconomic issues caused by visits to hospitals are devastating in southeast Asia (ThekkePurakkal et al., 2018). Health workers working in the field do not have the means or skills to convince patients to travel to a more established healthcare facility for accurate diagnosis (Brahmbhatt and Sheth, 2017).

Tests are challenging to develop, as there are currently no bona fide biomarkers for oral cancer (Kaur et al., 2018). The expectation is that saliva should contain biomarkers related to malignancy due to its proximity and direct contact to the lesions. This seems accurate; however, it is almost certain that a high number of biomarkers need to be monitored to obtain acceptable diagnostic sensitivity and specificity (Einhaus et al., 2023).

The gold standard method for diagnosing oral cancer is biopsy and histological staining, which are not available to patients in rural areas. Some rapid tests or test systems have been developed in which the lesions in the oral cavity are stained, the lesions are visually inspected, and the auto luminescence of the lesions is measured. More recently, a method has been suggested that involves analyzing images taken with mobile phones (Davies et al., 2015; Haron et al., 2016; Heidari et al., 2019, 2019). Unfortunately, these approaches have been found to be inaccurate or unsuitable for field conditions and thus have not gained notable popularity.

To support local health workers in their decision making, a rapid, easy-to-use and non-invasive diagnostic or screening test is needed.

2.4 Technology foundations

2.4.1 Time-resolved luminescence labels

Knowledge about lanthanide luminescence dates back to WWII (Weissman, 1942). However, the first applications utilizing lanthanides in solving real world clinical

issues were immunoassays developed in Turku, Finland, in the 1980s (Meurman et al., 1982; Soini and Kojola, 1983).

Lanthanide ions are weakly luminescent and not useful in most applications as labels. Therefore, a lanthanide-containing chelate is used as a signal source. The chelate is formed from a ligand - an organic structure that binds the Ln^{3+} ion and has an antenna to be effectively excited and collect light. The chelates can be divided into two categories based on their formation.

Stable chelates contain Ln ions that are placed there during their synthesis, and the dissociation of the ion generally requires harsh conditions. The chelates can be nonluminescent or intrinsically luminescent. Compared to the original invention, intrinsically luminescent lanthanide chelates were developed much later; however, their assay formats may be simpler, as they do not require the dissociation step (Hemmilä, 1995).

Weak chelates were initially developed to create highly luminescent complexes from Ln ions dissociated from nonluminescent chelates. They are formed in solution and generally contain two to five different molecules that form the luminescent chelate. Weak chelates generally have better quantum yields than that of intrinsically luminescent chelates and are used in applications that require very high sensitivity.

It has long been known that lanthanide chelates are sensitive to the environment in which they are located. This is generally an issue (interference), as these effects disturb the test systems (Bünzli and Piguet, 2005). These interference effects form one of the cornerstones for the fingerprinting method described in this thesis. Modulators can be selected specifically to interfere with a chelate to create the sample's specific fingerprint.

The chelate structure determines many of the optical properties of the chelate, such as the excitation wavelength and the quantum yield (Hemmilä et al., 1997). When a weak chelate is used, the free concentration of the ligand components greatly affects the quantum yield of the chelate. Traditionally, the concentration of the lanthanide ion is determined. However, the same principle can also be used to determine the concentrations of other components (Hemmilä et al., 1984). Therefore, the sample composition can be determined by nonspecific binding or inactivation of chelate components to the sample.

If a sample being fingerprinted contains molecules that may function as part of the weak chelate, their concentration can be estimated based on the signal measured, as this value indicates the number of intact chelates formed. Changes in the excitation spectrum indicate changes in the ligand structure, which can also be used to collect information about the sample. The situation can be reversed as well. The sample might lower the active concentration of a chelate component. For example, this approach is used to detect the scale inhibitor concentration.

Many metal ions (e.g., Mn^{2+}, $Cr^{2/3+}$, Co^{2+}, $Fe^{2+/3+}$ and Cu^{2+}) quench the signal from lanthanide chelates by an ion-pair mechanism, as most chelates are negatively charged (Cano-Raya et al., 2005). Interference can be prevented if the free concentration of the ions is changed, e.g., by using a metal-ion specific chelator, modulator or particles. When a mix of suitable chemicals is used in an array, the ion composition of the environment surrounding the chelate can be estimated. Increasing the concentration of no quenching ions (e.g., Ca^{2+}, Na^{2+}, Ba^{2+}, Mg^{2+}) can also protect the chelate, as the ions compete on the same binding sites with the quenching molecule.

2.4.2 Introduction to TRF instrumentation

In time-resolved (TRF) measurements used, a delay from a few to several hundred microseconds separates the pulsed excitation of the time of the emission detection from the sample. This removes short-lived luminescence and significantly reduces any autoluminescence or background signal and excitation light scattering, allowing sensitive and specific signal detection. An outline of a typical measurement cycle is given in **Figure 1**. In addition, the very large Stokes shift (>200 nm) of lanthanides and their sharp emission peaks (full width at half maximum 10 nm) contribute to the low background signal (Bünzli and Piguet, 2005). These special properties define the rough specification for a TRF-reader.

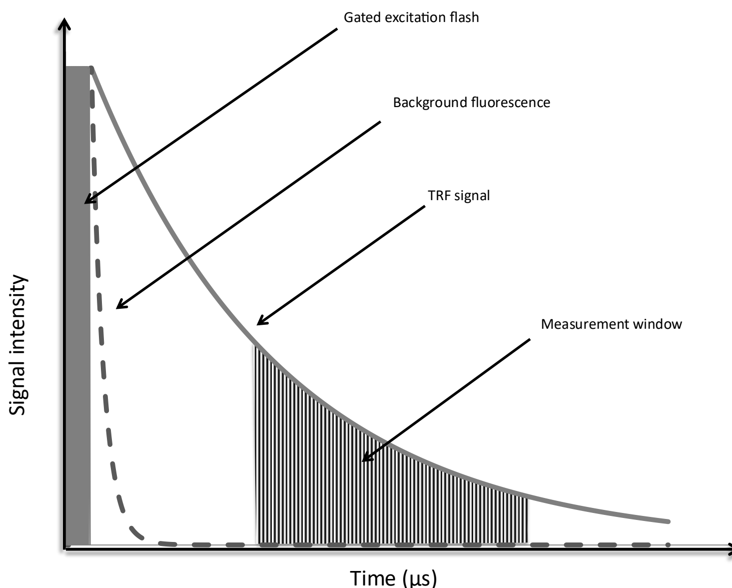

Figure 1. Typical measurement cycle of a time resolved fluorescence reader. There is sharp excitation flash followed by a delay before activating the detector. The dashed line shows how the fluorescence signal drops greatly before the measurement of TRF signal of the lanthanoid is initiated.

A functional reader requires a light source for excitation, a detector to count the photons emitted, optics to control and collect the light travel, filtering for choosing the spectral range to be monitored and electronics to coordinate the actions.

In most commercially available TRF readers, the light source is a xenon flash lamp; however, LEDs currently offer a lower price and more reliable and long lasting option (Moe et al., 2005). The biggest difference is that xenon lamps offer a wide spectrum of excitation light from approximately 200 nm to 800 nm. A high-power LED offers only a narrow bandwidth of approximately 10 nm; thus, if the instrument is not used for a single excitation wavelength, multiple LEDs must be used. In addition, xenon lamps exhibit notable afterglow, whereas most LEDs do not. This makes LEDs suitable for measuring shorter lifetimes and simplify the optical design of the instruments. In addition using LEDs can eliminate the need for excitation filters, which simplifies the instrument design and lowers the cost.

With pulsed illumination the detector needs to be a sensitive photon counting device; generally, only PMTs offer sufficiently high sensitivity, and the detectors are mainly used for luminescence detection in commercial TRF readers. (Kuswandi et al., 2007). Avalanche photodiodes achieve good performance, but their photosensitive surface area is small, and thus they are only useful for microvolume measurements. Silicon PMTs are developing rapidly but have not yet demonstrated sufficiently high sensitivity to be useful in photon counting applications with lanthanides (Hahn et al., 2018). Other electronic components are standard. The timing of the measurments is crucial, but obtaining high-quality components for timing the reader operations is not difficult.

2.4.3 Data analysis methods

Humans are generally unable to handle interactions with more than four variables, but modern analytical methods can generate thousands of variables from a single sample; thus, it is impossible to capture the information from the data without using modern data-analysis technologies (Halford et al., 2005). Multivariate analyses and machine learning have become everyday tools in determining the origin of a valuable food item (Tahir et al., 2022), performing medical diagnostics (Shehab et al., 2022) and controlling the quality of food (Buvé et al., 2022).

One of the commonly used tools is principal component analysis. It is used in many multivariate analyses to reduce the dimensionality of data and visualize data. As demonstrated in **Figure 2**, the use of principal component analysis has increased significantly during the past 20 years. The method was developed approximately one hundred years ago (Hotelling, 1933; Pearson, 1901), so the increase in usage is not attributed to its invention. Most likely, software tools become available during this rapid increase that enabled all scientists, not just computer scientists, to explore the

methods (Kuhn, 2008). In addition, principal component analysis is not computationally heavy, and the calculation power of most laptops is adequate to perform the analyses in a reasonable time.

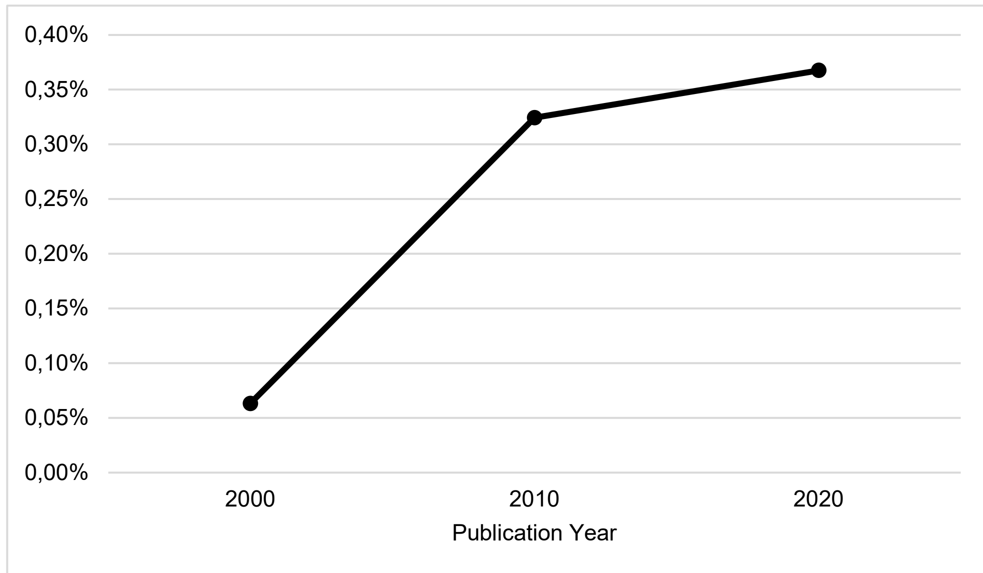

Figure 2. A simple search was performed in PubMed database with term “Principal component analysis” and the number of results is presented as percentage of all publications in PubMed. Clearly, the usage of the method rapidly increased from 2000 to 2010, and since then, the increase has been moderate.

As discussed in the previous sections 2.1 and 2.3, by applying these methods, nontargeted methods can be developed in which the response of the sensors (variables) is not linked directly to the monitored property. An example different from those presented in the previous sections could be the predictions made by Royal Dutch Shell regarding their maintenance needs for production equipment; these predictions were based on information collected from their various IT systems but not on sensors within the machines (Marr, 2015).
The development of any multivariate data analysis or machine learning involves the same steps, which are as follows: 1. Data collection; 2. Data preprocessing; 3. Select/Train Model (calibration); 4. Finetune; and 5. Test (validate) model. Data collection is self-explanatory. The data must be collected from the measuring system to maintain its integrity. Additionally, the data needs to be linked to the experimental methods and data associated with the samples. This all necessitates good laboratory practices and familiarity to any laboratory performing analytical testing.

Data preprocessing can be more complex. In most cases, day-to-day variations must be filtered out; in other words, instrument and method variations need to be calibrated (e.g., temperature effects, light scattering). The methods used are highly dependent on the type of data generated and need to be optimized for each application separately. Some examples of methods that could be used are peak alignment, baseline corrections, Savitzky–Golay (S-G) filtering, Fourier transformation and data normalization (Cozzolino, 2020; Engel et al., 2013; Mehmood et al., 2012; Rinnan et al., 2009).

The most commonly used data analysis methods are artificial neural networks (ANNs), partial least squares (PLS), support vector machines (SVMs) and various clustering algorithms (Lavine and Workman, 2008). Typically, the model must be selected experimentally. Training multiple different types of models and testing the performance are necessary steps to generate the model. Generally, there should not be drastic performance differences between models if their basic assumptions are not broken. If vast differences are observed, there is likely an issue with data quality (Kilkenny and Robinson, 2018). Some of the methods are trained unsupervised, whereas others are supervised. The key difference is that with supervised methods, the generated measurement data are linked to data labels by the operator. In unsupervised methods, the algorithm tries to find patterns within the data without human intervention (Alloghani et al., 2020). For example, PLS is a supervised method, and PCA is an unsupervised method.

When training and validating the model, the data are typically split into two parts. The training set is used to train and fine-tune the algorithm, and the test set or validation set is used to estimate the model performance. The terminology here can be confusing as the training data set is sometimes split into another part to test the performance of the model during finetuning – this might also be called a test set. The independent test/validation data set not used in model training/finetuning produces achieves the most comparable performance numbers for models; therefore, its performance should be used to measure model functionality (Westad and Marini, 2015). In practice, there are almost always limitations due to the difficulty of obtaining proper samples for studies.

The complexity of the methods also raises new ethical questions, as assessing the correctness of the results is no longer possible by visually inspecting the figures or data. As a result, the model performance can be overestimated, and due to positive publication bias, much time may pass before the scientific consensus corrects the results (Dhiman et al., 2023). Importantly, as the data analysis methods are complex, incorrect results can result from a nonoptimal data-analysis protocol without any malicious intent.

Unfortunately, the complexity of the data-analysis methods also enables misconduct. A famous example is Duke University, from which at least 11 papers

were retracted or corrected (approximately 50 required corrections or retraction); in this case, cancer patients received an ineffective treatment, and a scientist was found to be guilty of research misconduct (Potti et al., 2006). Initially, it seemed that data labels were mixed, but later investigations have revealed that the data was willfully manipulated to falsify the results (The Office of Research Integrity, 2015). The best protection against misconduct is full openness; raw data without any analyses or processing should be made available with publications. Furthermore, information needed to reproduce the analyses should be provided.

Modern data-analysis methods can be carefully utilized with high reliability for various tasks in commercial and research applications. However, due to the complexity of these methods, care must be taken when interpreting the results.

3 Aims

The purpose of this study was as follows:

1. Demonstrate the following points.
    1. The developed TRF-based fingerprinting method is suitable for performing analyses with similar or better performance than that of existing analytical methods.
    2. An antibody-free and nonspecific method can tolerate various matrixes and is therefore suitable for a wide range of applications.
2. The method workflow is simple enough to be performed outside of analytical laboratories.
3. The technology is suitable for commercialization and used to solve real world issues.

4 Materials and Methods

4.1 Wine analyses

4.1.1 Red wine samples

Alko Oy (a national alcoholic beverage retailing monopoly in Finland) provided fifteen European red wines for testing. Vintage wines were selected on the basis of their availability (see **Table 3** for details). During their lifetime, the selected wines were stored horizontally in a controlled environment at Alko Inc.

Table 3. The wine samples used in the study.

Alko's identifier	Wine	Vintage	Country of origin
2012-00236-1	Chateau Kirwan	1985	France
2012-00237-1	Chateau Kirwan	1989	France
2012-00238-1	Chateau Kirwan	1993	France
2012-00239-1	Chateau Kirwan	1998	France
2012-00240-1	Travaglini Gattinara	1995	Italy
2012-00241-1	Travaglini Gattinara	1998	Italy
2012-00242-1	Travaglini Gattinara	1999	Italy
2012-00243-1	Travaglini Gattinara	2006	Italy
2012-00249-1	Argiano Brunello di Montalcino	1998	Italy
2012-00250-1	Argiano Brunello di Montalcino	2000	Italy
2012-00251-1	Argiano Brunello di Montalcino	2001	Italy
2012-00252-1	Argiano Brunello di Montalcino	2003	Italy
2012-00253-1	Muga Reserva	2004	Spain
2012-00254-1	Muga Reserva	2005	Spain
2012-00255-1	Muga Reserva	2007	Spain

4.1.2 Modulators for wines

Fourteen different modulators (**Table 4**) were measured under eight different conditions. Modulators were applied on standard microtiter plate plates (Nunc, MaxiSorp C12,437915) using a simple procedure: the modulators were dispensed to each microtiter well in a volume of 40 µl and subsequently dried under airflow for 16 hours.

Table 4. Surface modulators coated on microtiter wells.

Modulator (supplier, #product number)	Concentration
Polyethyleneimine (Nippon Shokubai, Epomin SP-003)	5%
L-arginine (Sigma–Aldrich, #A5006),	1%
L-Cysteine (Sigma–Aldrich, #W326305)	1%
Poly(allylamine hydrochloride) (Sigma–Aldrich, #283215)	0.1%
Poly(sodium 4-styrenesulfonate) (Sigma–Aldrich, #243051)	0.1%
Polyvinylpyrrolidone (Sigma–Aldrich, #PVP10)	0.1%
´Y-globulins from bovine blood (Sigma–Aldrich, #G7516)	0.1%
Gold(III)chloride hydrate (Sigma–Aldrich, #50790)	1 mM
Copper(II)chloride (Sigma-Alrich #222011)	0.1 mM
Iron(III)Chloride (Sigma-Alrich #157740)	10 mM
New Fuchsin (Sigma–Aldrich, #72200)	10 µM
Delfia assay buffer (Perkin-Elmer, #1244)	N/A
Rhodamine 800 (Sigma–Aldrich, #83701)	15 µM
Maxisorb Nunc C12 (Nunc, #437915)	N/A

4.1.3 Sample preparation

4.1.3.1 Buffer solutions

The following buffer solutions were prepared:

Phosphate buffer: pH 7.0, 10.4 g/l Na_2HPO_4 $12H_2O$ 3.3 g/l NaH_2PO_4 $2H_2O$

Carbonate buffer: pH 11.0, 5.3 g/l Na_2CO_3, pH adjusted with HCl

TRIS-HCl buffer: pH 8.0, 6.1 g/l, pH adjusted with HCl.

4.1.3.2 Pretreaments

In a tube, 4600 µl phosphate, carbonate or TRIS-HCl buffer was added to 250 µL of wine sample and combined with 150 µl of 1 µM europium chelate.

In a tube, 250 µl of wine sample was mixed with 25 µl of 30% H_2O_2 (Sigma–Aldrich 316763), 1 M NaOH (Sigma–Aldrich, S5881) or 1 M HCl (Sigma–Aldrich, 71763-11). The mixtures were incubated for 30 minutes, and 4575 µl of phosphate buffer and 150 µl of 1 µM europium chelate were added.

In a tube, 250 µl of wine was mixed with 50 µl of 1 g/l polyethyleneimine (Epomin SP-003, Nippon Shokubai) or with 1 g/l bovine serum albumin (BSA, Sigma–Aldrich A7906) solution. After 30 minutes of incubation, 4550 µl of 50 mM phosphate buffer and 150 µl of 1 µM europium chelate were added.

4.1.4 Analytical method

The prepared samples were added to microtiter wells in duplicate. The microtiter plates were read with a Wallac Victor 1420 HTS (Perkin-Elmer/Wallac Oy, Turku) with standard europium detection settings (emission filter D615 nm, excitation filter D340 nm, delay 400 µs, integration window 400 µs, cycle 1000 µs) after a 20-minute incubation. All measurements were repeated twice for all wines except for Travaglini Gattianara, 2006, which was measured five times.

4.1.5 Reference methods

Reference analyses were performed by the Alcohol Control Laboratory (ACL) of Alko Inc., the official laboratory for alcoholic beverage analyses in Finland.

The wine samples were analyzed using accredited methods for ethanol, total acid, volatile acid, glucose and fructose concentration as well as pH and dry extract using a FOSS WineScan FT120 instrument (Hillerød, Denmark). The samples were diluted 1:20 with distilled water, and their absorbance at 280, 420, 520 and 620 nm was determined using a Thermo Scientific Arena 20XT automated photometric analyzer (Vantaa, Finland).

4.1.6 Data analyses

PCA was carried out with R (R Core Team 2016). Fingerprinting data were mean centered and standardized before the PCA calculations.

4.1.6.1 Aging and prediction of chemical parameters

To assess the wine vintage, the data set was divided into teaching and testing sets. Teaching was performed on the known vintages utilizing the partial least squares regression method of the Molegro Data Modeler (MDM 2009.2.1.0). On the test set (teaching set), the Pearson correlation was 97% (100%), and the Spearman rank correlation was 96% (99,6%).

Chemical parameters (volatile acidity (g/l), total acidity (g/l), dry extract (g/l), color intensity, A450/A520, A280, alcohol by volume (% vol)) were estimated with the REG procedure in SAS stastical analysis software by using the linear regression model with stepwise variable selection. Models were fitted separately for each chemical parameter, and individual variables of the fingerprint data were used as explanatory variables. All the variables (modulators) in the models are significant at the 0.05 level. The analyses for chemical parameters were performed by Numos Oy.

4.2 Foodstuff analyses

4.2.1 Assay preparations

The assay was performed in two formats on 96-well plates (Nunc, Maxisorb C12, 437915) and in a custom-made microfluidic chip (Aqsens Oy Espoo, Finland; IMM, Mainz, Germany; MiniFAB Pty LTD., Melbourne, Australia).

The protocol for coating plates/chips was performed as follows: the coating/modulator agents provided in **Table 5** were pipetted by hand in a volume of 40 µl into each well and subsequently dried at RT under airflow for 16 hours. In the case of the water samples and microchips, the volume used before drying was 2 µl. The modulators were coated in 20 mM NaCl solution excluding the metal ions, which were prepared in water.

Table 5. Modulators and concentrations used to prepare the plates or chips.

Modulator (supplier, #product number)	Concentration
APO 12 (Calbiochem ,#178377)	1% in 20 mM NaCl
Triton X-405 (Sigma–Aldrich, #X405)	0.1-1% in 20 mM NaCl
L-arginine (Sigma–Aldrich, #A5006),	1% in 20 mM NaCl
L-Cysteine (Sigma–Aldrich, #W326305)	0.1-1% in 20 mM NaCl
Poly(allylamine hydrochloride) (Sigma–Aldrich, #283215)	0.1% in 20 mM NaCl
Poly(ethylene glycol) (Sigma–Aldrich, #81255)	0.1-1%
Poly(sodium 4-styrenesulfonate) (Sigma–Aldrich, #243051)	0.1% in 20 mM NaCl
Polyvinylpyrrolidone (Sigma–Aldrich, #PVP10)	0.1% in 20 mM NaCl
Albumine from bovine serum (Sigma–Aldrich, #A7906)	0.1-1 mg/ml in 20 mM NaCl
´Y-globulins from bovine blood (Sigma–Aldrich, #G7516)	0.1-1% in 20 mM NaCl
Gold(III)chloride hydrate (Sigma–Aldrich, #50790)	1-10 mM in H_2O
Silver Nitrate (Sigma–Aldrich, #209139)	1-10 mM in H_2O
Chromium Cloride (Sigma-Alrich, #20050)	0.1-10 mM in H_2O
Copper(II)chloride (Sigma-Alrich #222011)	0.01-1 mM in H_2O
Iron(III)Chloride (Sigma-Alrich #157740)	0.01-10 mM in H_2O
Bromothymol Blue (Sigma–Aldrich, #114413)	10 µM in 20 mM NaCl
Cresol Red, (Sigma–Aldrich, #114472)	10 µM in 20 mM NaCl
Pyridine 2	10 µM in 20 mM NaCl
1,1’-Dipropyl-3,3,3’,3’,tetramethylindocarbocyanine iodede	10 µM in 20 mM NaCl
Leucoberbelin Blue (Sigma–Aldrich, #431299)	10 µM in 20 mM NaCl
New Fuchsin (Sigma–Aldrich, #72200)	10 µM in 20 mM NaCl
Rhodamine 800 (Sigma–Aldrich, #83701)	15 µM
D(+)-Saccharose (Sigma–Aldrich, #84100)	1% in 20 mM NaCl
Maxisorb Nunc C12 (Nunc, #437915)	N/A

4.2.2 Samples

Chokeberry juice samples were prepared from frozen and gently defrosted berries by pressing. Different pure taste molecules simulating sweetness (sucrose), sourness (citric acids) and bitterness (caffeine) were added to pressed juice as described in **Table 6**. The samples were prepared in a laboratory designed for sensory evaluation (ISO 8589) at the Functional Foods Forum, Turku. Prior to analyses, juice samples were diluted 1/20 with respect to water. Two microliters of 1 µM Eu-terpyridine chelate was added per 200 µl of the diluted sample and dispensed onto microtiter plates. The plates were measured after 20 minutes of incubation.

Table 6. The tested chokeberry juices and their taste modifications.

Quality of taste	Taste modification
Natural	No modification, 100% pressed juice from frozen and molten berries
Sweet	Natural + 5% sucrose
BItter	Natural + 0.1% caffeine
Sour	Natural + 0.1% citric acid
SweeT + Sour	Natural + 5% sucrose + 0.05% citric acid
Sweet + Bitter	Natural + 5% sucrose + 0.05% caffeine
Sour + Bitter	Natural + 0.05% citric acid + 0.05% caffeine

The water samples (listed in Table 7) were taken from bottles purchased from local supermarkets and mixed in a volume of 200 µl with 2 µl of 1 µM Eu-Terpyridine (the waters containing CO_2 were opened 24 hours prior to use to evaporate CO_2). This volume was then pipetted into the microchip after 5 minutes of incubation at room temperature. The chips were measured in a custom reader at 2-minute and 8-minute time points. Each time point was considered an individual measurement in the subsequent principal component analysis.

Table 7. Bottled waters and their abbreviations.

Water	Abbr.	Manufacturing area
BonAqua still	BA	Sweden/Hanvede
Cool-Aqua	CA	Finland/Suomenselkä
Evian	Ev	France/Alps
Hellefors	He	Sweden/Bergslagen
Kevyt olo	Ko	Finland/Lehtimäki
Malavela	Ma	Spain/Girona
Perrier	Pe	France/Vergeze
Pirkka	Pi	Finland/Multiala
Polar springs	PS	Finland/Asikkala
Roberts Coffee still	Ro	EU
Spring Aqua	SA	Finland/Multiala
VeEN Velvet	Ve	Finland/Konisaajo

The cola samples were prepared as follows: Each of the cola samples (5000 µl) was first sonicated for 5 minutes to remove CO_2. Then, 500 µl of pH 11 phosphate buffer and 152 µl of 1 µM Eu-terpyridin were added, and the solutions were dispensed into the well plates. The TRF measurements were performed after 20 minutes of incubation.

The vodka samples were prepared as follows: Vodka samples were taken directly from the bottles, mixed to a final concentration of 5 nM Eu-terpyridin and incubated for 20 minutes prior to measurement. For the reference, ETAX-A (>96% vol) was diluted with water to 40% ethanol concentration (vol) and settled for 24 hours.
The data analysis was performed off-line (Molegro Data Modeler 2.1.0). The principal component analysis for each experiment was calculated separately; thus, the amplitude of the principal component data reflects the amplitude variations in the actual signals, but the PC values between experiments cannot be compared.

4.3 Oral cancer analyses

4.3.1 Sample collection

Each patient rinsed their mouth with 15 ml of 0.9% NaCl solution, which was collected and immediately stored in ice and after being transported to the laboratory in Axxonet, Bangalore at -20°C. The number of patients in this study was 53, which included 40 patients with lesions and 13 healthy patients. The patients included male and female adults between 20 and 60 years old. The collection was performed under the institutional ethical permit of the Biocon Foundation. The institutional ethical

committee consented to the project. (Ethical clearance: KIDS/IEC/11-2014/30). Informed consent was obtained from all the participants.

The patient's status was determined in a visual examination by a clinician and recorded along with personal information and history of tobacco use. The lesions in the tested data set were found in either the buccal or lower labial mucosa, and no distinction in the teaching of the method was made between the positions of the lesions.

4.3.2 Measurement protocol

The measurement protocol was performed as follows:

1. A sample from the freezer was thawed, and Eu-terpyridine chelate (Ramidus Ab, Lund, Sweden) was added to a concentration of 0.76 nM.
2. A 70 µl sample was dispensed in duplicate into a microtiter plate containing the reagents dispensed in water (see the reagent list below).
3. The sample was incubated for 10 min at room temperature.
4. The sample was measured with a TRF plate-reader (Labrox Inc. Turku, Finland) with 340 nm excitation and 615 nm emission filters, in which the delay time was 400 µs and the measurement window was 400 µs Each measurement consisted of 100 cycles (flashes).

The nonspecific chemistries used in the wells were as follows:

- A: o-cresolphtalein complexone (1006 µM final concentration), pH sensitive chemistry.
- B: EGTA (50 µM) + saffrosine (52 µM), protein sensitive chemistry.
- C: EGTA (50 µM) + pyrocathecol violet (101 µM), ion/complex sensitive chemistry.
- D: 3-HPA (50 µM) + bromocresol purple (71 µM), pH & albumin sensitive chemistry.

Since the amount of data was limited to 13 healthy and 40 lesion patients, we decided to generate an artificial training set by utilizing the average signal of each group and chemistry as the starting point. A training set of 300 samples was then generated by adding randomly distributed noise to the average signals. The standard deviation of the noise was the same as that of the real data, and these artificial noise data were evenly distributed around a mean of 0. The test sets were the measured raw data set with the replicates as individual points and with the replicates averaged. The classification methods used from Molegro were K-nearest neighbor (KNN) and

support vector classification (SVC). The program was fine-tuned to find the parameters for optimal training. We also tried splitting the measured data randomly into individual training and testing sets, but due to the low amount of data, the results varied more than with the adopted method depending on the split.

4.4 Industrial water

4.4.1 Assay for scale inhibitors

The assay was performed as previously described (Nuutinen et al., 2018). Artificial brine (600 mM NaCI, 7 mM $MgCl_2*6H_2O$, 15 mM $CaCl_2*2H_2O$, 3 mM KCI and 0.5 mM BaCl) solution was spiked with various amounts of scale inhibitors and was added to a cuvette (Fischer brand Macro cuvette, Polystyrene, FB55143). Thereafter, $EuCI_3$ and HEPES buffer (pH 7.6) were added at 10 pM and 5 mM concentrations, respectively. The time-resolved luminescence signal was measured with a luminescence reader (Aqsens Oy, Turku, Finland) using an excitation wavelength of 395 nm, emission wavelength of 614 nm, delay time of 100 µs and measurement window of 950 µs. A standard curve was prepared, and the concentrations of unknown samples were determined.

The KemConnect scale inhibitor detection protocol was performed according to Johstone (2014).

Before a measurement was taken, the samples needed to be pretreated. If the sample exhibited a scale-inhibitor polymer concentration <100 mg/kg, the samples were gel-filtered. Illustra NAP-25 columns (GE Life Sciences, USA) were equilibrated with 20 ml of brine, 2.0 ml of sample was added, and the sample was eluted with 3.5 ml of brine. With gravity flow, the flow speed was approximately 1.8 ml/min. Sample collection was started when the sample was added to the column so that the sample volume after purification was 5.5 ml. If the sample had >100 mg/kg polymer, the sample was diluted with brine. The following different dilutions were used: 1:150 and 1:1500. A 1:150 dilution was achieved by pipetting 20 µl of sample with 2980 µl of brine. The 1:1500 dilution was performed in two steps. The first dilution was 20 µl of sample with 2980 µl brine followed by 300 µl of the previous dilution with 2700 µl of brine.

The measurements were performed by adding 2.5 ml of the calibrator sample in brine, purified or diluted sample to a cuvette containing the dried reagents. The cuvette was mixed by inverting for 10-15 s, incubated for 10 minutes and mixed again for 10-15 s by inverting. The cuvette was placed in an LED-based TRF reader (Aqsens, Turku) to be measured.

5 Results & Discussion

5.1 Developed fingerprinting method

5.1.1 Introduction

In this thesis, the fingerprinting method originally developed by Professor Pekka Hänninen and his research group at the University of Turku, (Kulpakko et al., 2015; Rozwandowicz-Jansen et al., 2016) was futher developed and refined; thus, the main result is a better understanding of the fingerprinting method.

The developed method generates fingerprints from interactions of the following main components: a sample, a lanthanide-based signal source and a modulator. After the signal source is added to the sample, the mixture is brought into contact separately with several chemicals that interact with the signal source (modulators). The sample strengthens or weakens the interactions between the signal source and a modulator, which is reflected in the measured signal. When a plurality of carefully selected modulators with a very wide range of chemical properties are used, the properties of the sample can be determined after the signals are fed to a trained algorithm. In addition, in many cases, a sample pretreatment is necessary to obtain a reliable result if the sample matrix varies or contains interfering agents. The details of each step are discussed in more detail in the following sections.

The method has been applied in various applications, including water analyses (I), industrial process monitoring (Johnstone et al., 2015, 2014), screening for oral cancer (III), wine analyses (II) and determining the authenticity of foodstuffs (II & III). A few of the interesting results are presented in the following sections – detailed analysis can be found in the publications.

5.1.2 Sample collection

The sample collection devices and method can be selected based on the ease of use and ability to preserve samples. No interference from sample collection devices was observed during the studies presented in I-III.

However, regardless of the analysis method, some samples easily spoil during storage before the analyses. For example, water samples that precipitate need to be

preserved by acids because reactions can occur, such as the oxidation of iron; in addition, sample matrixes containing enzymes (e.g., saliva, oral rinse) or bacteria (bioprocesses) that degrade (bio)markers need to be frozen as quickly as possible after collection. These are standard practices for all analytical methods, so no special protocols are needed; however, the information needs to be considered when planning sample collection protocols.

Based on the results and methods presented in I-III and in other supporting material, the sample collection is simple and does not differ from any other analytical method. In other words, no special requirements are needed for sample collection or storage with the developed method. All techniques used, such as techniques involving the collection of water and foodstuff samples, are expected to be compatible with the developed method.

5.1.3 Pretreatment

5.1.3.1 Introduction

Pretreatment here is defined widely as any sample processing that occurs prior to introducing the modulators and/or signal source. The aim of the pretreatments is to improve the method performance (repeatability and analytical sensitivity and specificity). Through its chemical nature, the pretreatments fixes the pH and ion strength, removes intense color, prevents bubbles/foam from forming and removes any solids. In all cases reported in publications I to III, some level of pretreatment was needed except for drinking water and strong alcohol samples, which were analyzed directly.

Pretreatments are needed either to remove disturbing factors, such as intense color and particles, and/or to ensure that the measurement conditions are repeatable or to change the chemical composition of the samples, generating a more pronounced sample variation. This makes it possible to collect more information with the array of modulators. Notably, all the pretreatments used were simple and cost effective.

The pretreatment can involve a simple addition of salts or buffering reagents, multistep purification with chromatography columns or something in between. The pretreatments make it possible to work with the most challenging samples, such as oral rinses (viscosity, clarity), water produced in the oil and gas industry (high composition variability; sand and oil contaminations) and wine (intense color), while achieving good analytical performance. The following sections summarize the pretreatments that are useful for particular cases as well as detail experimental examples.

5.1.3.2 Dilution in a buffer solution

Dilution is by far the most commonly used method for sample pretreatment. It is cost effective, simple, and repeatable. Diluting samples in a buffer solution effectively removes matrix variance and intense colors, lowers viscosity, and adjusts analyte concentrations and the solution pH. The fingerprints are formed mostly by weak chemical interactions and ionic interactions between the molecules contained in the sample, the modulator and the signal source. Therefore, maintaining a constant pH and ionic strength is important for obtaining repeatable and reliable results.

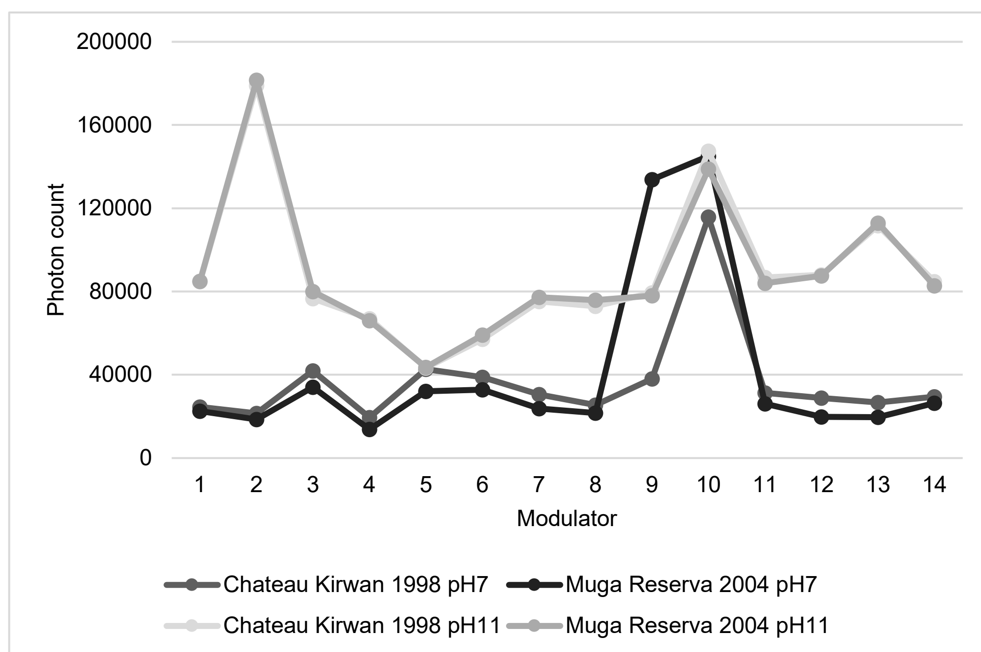

Figure 3. Example fingerprints of two wines from original Publication II. The modulators are ordered as in **Table 4**. The results demonstrate that the pH used in the assay can have substantial effects on the performance of the method. When the pH is 11.0, the wine samples are inseparable; however, when the pH is 7.0, the samples clearly differ.

Changing the pH can have a profound effect on interacting molecules, and changes in the interaction pattern can be very noticeable. In **Figure 3,** raw results from the original publication II are presented; they clearly demonstrate the need to optimize the dilution buffer for each application. To further elaborate on **Figure 3,** modulator 9, which produces the clearest separation at pH 7.0, is poly(sodium 4-styrenesulfonate), a strongly negatively charged polymer. One would expect the interaction with negatively charged modulator to diminish as the sample becomes increasingly negatively charged. As the separation or prediction of a parameter is

driven by a modulator with a negative charge to lower the pH, which might improve the performance, the performance should deteriorate when the pH is increased, as observed in this example.

Extreme pH can also be used to remove disturbing molecules. For example, in original publication I experiments, the pH of carbonated drink samples was increased (to approximately pH 11.0) before analysis to release the CO_2 outside of the measuring system, as gas bubbles negatively affect the optical measurement.

In summary, a suitable dilution factor and pH need to be experimentally derived for each application. Based on the original publications, the optimal dilution factors are from 1 to 20. The pH varies, but the best analytical performance is typically obtained at approximately neutral pH; however, a pH range from 3 to 11 has been used.

5.1.3.3 Oxidation

Optical measurements are disturbed by intensely colored samples. For example, red wine contains organic structures that are responsible for the intense color. The hypothesis was that adding H_2O_2 would reduce the color intensity and reduce the interference.

The results presented in original publication II demonstrate that H_2O_2 treatment caused an unexpected effect. The mathematical models predicting the optical properties of wine (absorbance at 420 nm, 520 nm and 280 nm and color intensity) all use data derived from H_2O_2-treated samples. Of the other predicted parameters, only one used data derived from this pretreatment. One hypothesis was that the H_2O_2 treatment reduces the color intensity to a level at which differences are more reliably detected.

Based on the results, H_2O_2 treatment shows the potential to be used with intensely colored samples, but more research is needed to identify the mechanism and usefulness.

5.1.3.4 Masking

Iron is relatively commonly found in produced water. It binds with high affinity to many scale inhibitors and disturbs their detection. Adding a high affinity iron chelator was a good solution and masked any effects of iron to scale inhibitor detection (Siivonen et al., 2018). Based on the results presented, the performance was completely lost in the presence of iron (recovery <10%) but was recovered using the chelator (recovery >90%). The chelator that was preferred due to its best stability and affinity was deferoxamine mesylate salt, which is an organic metal chelator of

bacterial origin and is used, for example, in the treatment of iron poisoning (Keberle, 1964).

Another type of masking that was useful was separating two scale inhibitors. Phosphonate scale inhibitors (such as DTMP) exhibit low affinity toward many alkaline earth metal ions (e.g., calcium), whereas polymeric scale inhibitors show higher affinity toward these ions. The addition of high amounts of calcium was successfully used to develop a specific method for detecting phosphonates (Siivonen et al., 2016).

Based on these results, masking is a good option for pretreatment when the interferent is known. The masking agent must be rationally selected, and based on the results, masking can be a very successful strategy to overcome challenging interferences.

5.1.3.5 Column pretreatment/filtration

Using gravity flow chromatography columns offers an efficient way of removing disturbing small molecules, performing a buffer exchange, and adjusting salt concentrations in a few steps. Gel-filtration columns were utilized with phosphonate (Siivonen et al., 2016) and polymeric detection techniques (Siivonen et al., 2018).

Based on the research, gel filtration can be a useful pretreatment technique when a simple workflow is needed, when diluting the sample in buffer is insufficient to manage matrix variations or when the necessary dilution factors are not acceptable based on the analytical sensitivity required for the assay.

5.1.4 Signal source

In all the presented tests in the original publications I to III, the signal is ultimately derived from europium ions; in theory, other lanthanide ions can also be used, but to simplify the signal detection and achieve simpler instrument handling, only europium ions were used in practice. Europium ions can be added in multiple formats as part of a stable chelate, as a weak chelate or as free ions. The signal source can be added into the sample as a concentrated solution (Original publications I-III), and the sample can be added to a tube or similar device that contains a fixed amount of signal source (Johnstone et al., 2014) or dried with the modulator (unpublished). The typical concentration ranges for different types of europium-based signal sources and further experimental examples are given in Table 6.

A stable chelate is a chemical entity that is generally used to label antibodies in traditional bioassays; it contains tightly bound europium ions and is intrinsically fluorescent. An example structure of a stable chelate is given in **Figure 4**. The

chelates are known to be sensitive to the environment, and this sensitivity is then enhanced with the modulators and assay conditions.

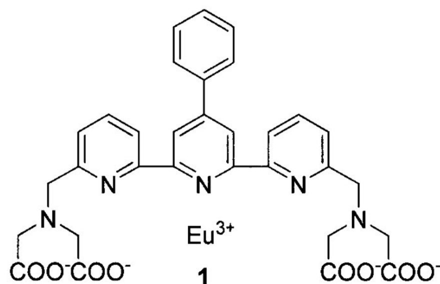

Figure 4. Stable terpyridine based Eu-chelate. A chelate with this structure was used in the original publications I-III.

Unstable chelates are formed in solution from europium ions, and this type of chemistry is used, for example, in the DELFIA system from PerkinElmer (Hemmilä, I., Holttinen et al. 1987). The chelate formation and quantum yield are sensitive to the environment, and as with stable chelates, this formation can be controlled with the assay conditions (for example, pH, ionic strength, detergents).

If free ions are used as signal sources, it is also possible to use some of the components contained in the sample as part of the chelate – changes in the availability are then reflected in the measured signal (quantum yield). Good examples are phosphonates, such as DTPMP; metal chelators, such as EDTA; and negatively charged polymers, such hydrolyzed polyacrylamide (Nuutinen et al., 2018; Siivonen et al., 2018, 2016).

Table 8. Different types of signal sources that can be used with the fingerprinting method.

Type	Typical concentration (as Eu-ion concentration)	Experimental example	Reference
Stable chelate	pM to nM	Determining the volatile acidity in wine	Original publications I-III.
Free ions	µM to mM	Scale inhibitor concentration detection in various matrixes	Nuutinen et al., 2018; Siivonen et al., 2016b, 2016a

5.1.5 Common characteristics of modulators

Modulator selection is the most important part when developing an assay for a new application. Rational approaches can be used if the sample composition and the changes of interest are well known. However, the biggest benefit of the developed

method is that the changes in a sample can be tracked without knowing the changes; specifically, the modulator–signal source interactions can be nonspecific/nontargeted. This means that modulators can be randomly screened from a library of molecules for a particular application.

To provide some guidance on the selection of modulators and help future modulator library development, the modulators used in original publications I to III were analyzed, and their key features were summarized. The modulators used can be divided into seven groups based on their structure and function. The groups are amino acids, metal chelators, polymers, dyes, metal ions and carbohydrates. The distribution of the modulators used in the original publications to these groups is presented in Figure 5. It seems that very different types of molecules can function as modulators, and no specific structure or property is needed.

However, most of the modulators (68%) used exhibited optical properties. The definition optical property was that water solutions of the modulator are intensely colored, and/or the structure contains conjugated double bonds or aromatic structures. Based on the high proportion of optically active molecules used in the present study, some level of optical activity from the compounds is a beneficial property for a modulator. Modulators may influence luminescence of the lanthanoid via several mechanisms, like by 1) absorbing excitation light and becoming an excited emitting species 2) experiencing in energy transfer from the source molecule, which would reduce the quantum yield 3) absorbing in the emission region of the lanthanoid

If the mechanism is known, it can be used to optimize the instrument parameters to achieve the most sensitive detection.

The molecular weight of nonpolymeric modulators is typically 150-500 g/mol, and for polymeric and protein-based modulators, the molecular weight varies from 300 to 80 000 g/mol. Based on the high variability of the molecular weight, it seems that it is not an important parameter when selecting a modulator.

It seems that the chemical nature of the modulators can be extremely variable. This might also highlight the differences between the applications reported in the original publications I-III. It is beneficial if the modulator molecule shows a specific activity to a targeted sample property and improves its optical detection.

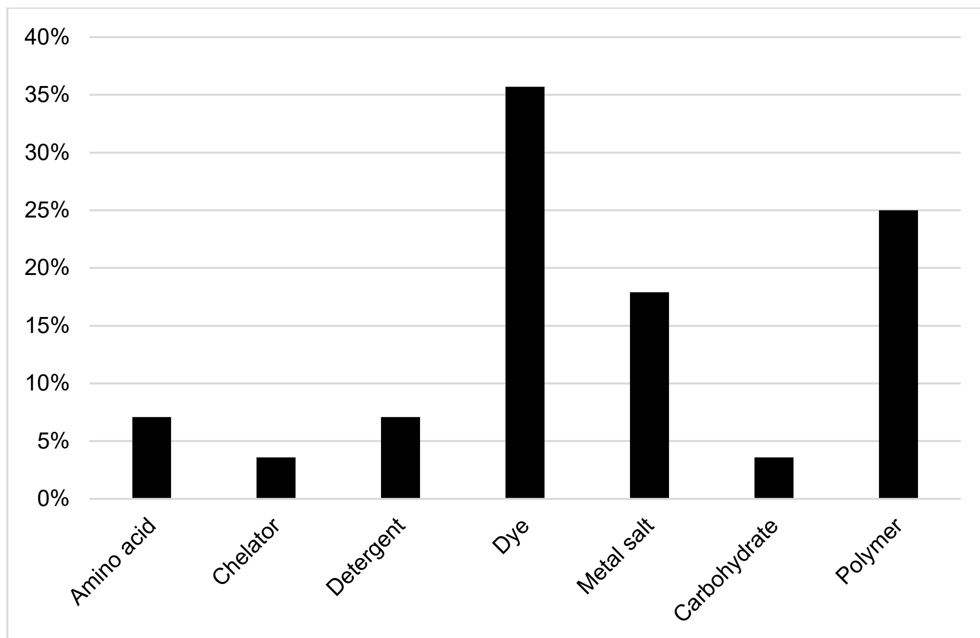

Figure 5. Distribution of the modulators used in the original publications into different functional/chemical groups. The definitions of the groups are self-explanatory except for dyes. Dye group contains molecules that are used as tissue stains, indicators in titrations or as fluorescent labels or probes. Other groups are defined as normally performed in biochemistry. It can be concluded that the chemical nature of the modulators is highly varied.

5.1.6 Data analyses

Each application requires tailored calibration and application-specific data analyses. At minimum, a calibration curve needs to be drawn (only one or two modulators are needed), or in more complex cases, a training set with known samples is needed to build a model to give the results. For the cases where a good sample set is available, the methods and workflow described in section 2.4.3 can be followed.

The model parameter optimization and selection have some effect on the performance, but the quality of the data is of the highest importance. Better raw data leads to better results, and changing the prediction algorithms does not radically change the performance of the assay as long as their basic assumptions are not broken. As an example, oral lesion screening data were analyzed with two algorithms, and as shown in **Table 9,** the performance is highly similar independent of the algorithm used. This demonstrates the high quality of the underlying data.

Table 9. Two different prediction algorithms were used to predict the presence of oral lesions. The algorithms perform similarly, although the models are drastically different mathematically. The table is modified from original publication III.

Algorithm	Sensitivity (%)	Specificity (%)
KNN (k=2)	89	88
SVC	90	88

The data set generated during application development is difficult to analyze due to the large number of variables (a signal read from modulator-signal source-pretreatment combinations). In this stage, 10-100x measured variables are compared to the sample count. For feasibility studies that involve a limited number of samples, a good overview of the performance can be obtained by dimension reduction and visualization, as in Original Publication I. Dimension reduction can be performed by using PCA and then visualizing the data using a few of the components. Visual inspection gives an idea of the performance, but no actual performance numbers (such as sensitivity/specificity) can be calculated. In addition, PCA calculations can be utilized to identify the modulator-signal source-pretreatment combinations that give the desired response. This information can be used to reduce the number of measured variables in further experiments.

5.1.7 Summary

To obtain reliable results, it was confirmed that the following steps are needed in all the assays: 1. Sample collection, 2. Pretreatment, 3. Addition of signal source, 4. Addition of a modulator, 5. Measurement and 6. Data analysis. In some cases, steps 3 and 4 can occur simultaneously in a single addition step.

It can also be concluded that when developing a novel application, the signal source, pretreatment, modulators, and data-analysis methods need to be experimentally selected for the application to obtain the best results. The selection process for the assay components can be based on careful selection, random high throughput screening or a combination of both approaches. It is feasible to carefully select the assay components when the sample composition changes being detected are well known, such as when the concentration of a critical compound is monitored in an industrial process. The chemical properties and reactivity of the compound can be assessed, and most suitable modulators can be selected to give the best possible conditions for the measurements.This will reduce the time used for the screening.

A key benefit is that the developed method can be tailored to applications in which the changes in the sample composition are unknown, such as when no biomarker is available for a disease, or when parameters that predict the success of

a production process are unknown. In these cases, however, contrived samples cannot be used for method development or for quality control. Instead, samples from the actual process are needed. This is generally not a challenge with industrial processes and foodstuffs but can be challenging in medical applications.

5.2 Performance testing in applications

5.2.1 Detection of scale inhibitors

In the O&G industry, untagged scale-inhibitor polymers are favored over tagged and more easily detectable products due to cost and environmental reasons. The tags include phosphorus for ICP (Inductively Coupled Plasma) and fluorescent labels for spectroscopic determination. The most commonly used method for untagged polymer detection is size exclusion chromatography, such as HPLC. HPLC-based methods have demonstrated good performance but highly trained personnel are needed to operate the instruments (Thompson, Kotlar et al. 2008). O&G production sites are generally located in difficult-to-reach locations; thus, shipping samples to a central laboratory is a slow process and finding technicians is difficult. Hyamine methods are also used to some extent, but they are generally unreliable due to interference from salts in the sample (Boak and Sorbie, 2010).

Here, the aim was to develop a reliable method for determining scale-inhibitor performance. To study the method performance in a very realistic setting and to remove any risk of sample spoiling during transport, a core flood experiment was performed in which samples were analyzed with three different methods. The results are presented in **Figure 6**. All three methods produce very similar return curves, indicating that the methods perform equally. The method calibrations are not linked, so a level difference is expected.

In addition, based on the recoveries and standard deviations reported, decision making in the sub 10 ppm level with high certainty may be achievable with the developed method; the standard deviation determined at the 1 ppm level is 0.2 ppm and at 10 ppm 0.6 ppm in produced water (Johnstone et al., 2014). This performance is equal to those reported for ICP- and hyamine-based methods (Boak and Sorbie, 2010).

It can be concluded that the developed method achieves a performance adequate for routine use in monitoring scale inhibitor concentrations and does not require the addition of tags to the scale inhibitor. The instrumentation is simple, and the protocol has been simplified so that minimal training is needed to reliably perform the assay (Johnstone et al., 2014). Thus, the assay is an excellent tool to monitor scale inhibitor concentrations and efficiently manage scale prevention programs. The method was

commercialized, reagent kits were produced on a large scale, and the product has been successfully used in remote locations.

This type of method enables analytical procedures to be transferred from a central laboratory next to an oil well. Reducing the time to results increases operational reliability and safety, which enables notable economic gains. If we assume that a squeeze can provide effective scale protection for 6 months and with on-site analytics, the effective period can be extended by 2 weeks on average. The increased production only would be worth approximately 1.3 M€ (~50€/barrel).

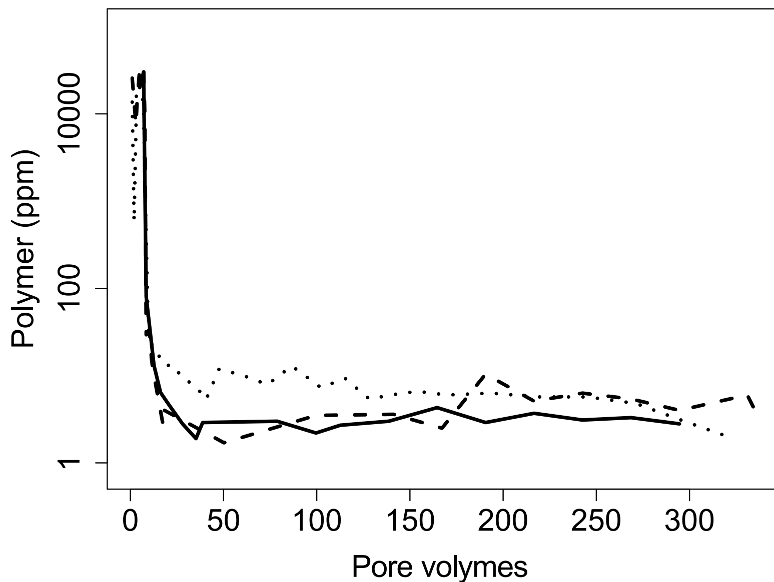

Figure 6. Core flood experiment with untagged sulfonated scale inhibitor polymer. The concentration of SI polymer was monitored by HPLC (solid line), Hyamine (dotted line) and with fingerprinting system (dashed line). Reproduced from Johnstone et al., 2014

5.2.2 Sensory evaluation

The relatively simple experiment presented in **Figure 7** demonstrates that when the taste of fruit juices is modified artificially, the changes can be monitored with the fingerprinting system. In addition, the location in the XY plane is indicative of taste modification. For example, we can see that sweet juices are in the top-left corner and sour & bitter in the bottom right corner. This demonstrates the versatility and capability of the nonspecific detection system.

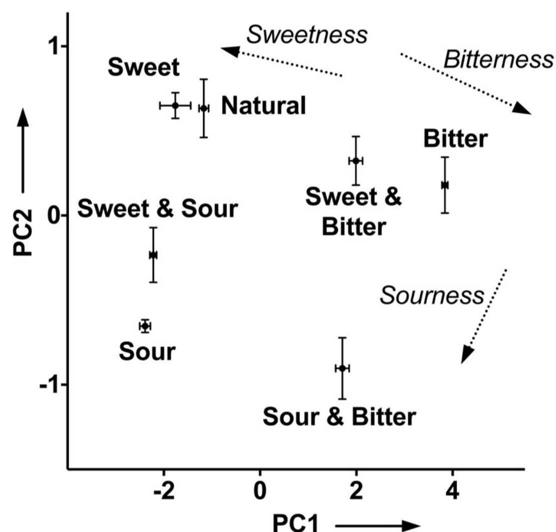

Figure 7. The juices were modified artificially for sweetness, bitterness, and sourness. The dotted arrow approximates the directions of the effects on the PC1/PC2 plot of the artificial taste modifier. Reproduced from original publication I.

The gold standard for the quality control of taste is sensory analysis. It is relatively easy for humans to determine if they like something, whether two items are identical or if one item is better than another when compared simultaneously. This is because humans can create a holistic view from the food sample and easily capture the complexity of taste. The comparison requires notably more training and experience if the comparison is made against historical tasting or some general standards (Ares and Varela, 2017). As a result, companies are very dependent on a small number of trained key employees. The facilities needed for consumer testing are also expensive, and recruiting volunteers can be challenging. There is a clear need for methods that can be trained to identify the complex changes that occur in various food items to identify authenticity and taste.

Based on the results presented in Original publication II, there is a high likelihood that the fingerprinting system could be further developed to an E-nose/tongue application, in which it could be used to support professional tasting panels or tasters that are commonplace in the food and beverage industry to monitor the quality of foodstuffs.

5.2.3 Wine analyses

FT-IR spectroscopy is a widely used method for various industrial applications and is routinely used in thousands of laboratories. Therefore, it is a good benchmark for

assessing the performance of the developed fingerprinting method (Basalekou, Kallithraka et al. 2019). The developed fingerprinting method was compared to a set of FTIR-based methods used by the Alcohol Control Laboratory (ACL) of Alko Oy. A subset of the results of the comparison presented in **Table 10** and the full results can be found in original publication II**.**

The measured results in **Table 10** are typical for red wine; the sample is representative of wines in general. The assay repeatability and measured values were equal to the data measured at the ACL, excluding the alcohol content. The accuracy does not meet requirements for routine analysis, as the tax classification rules are strict and sub +/- 0.1%. However, the accuracy reached with other parameters is adequate for routine quality control of wines.

We can therefore conclude that the fingerprint method performance is comparable to that of well-established FT-IR methods. A wine analyzer could be obtained by further developing the modulator array and data analyses. In addition, in the original publication, the II fingerprinting method has shown potential to determine the origin of wine (authenticity) and aging monitoring.

The method is very promising, but further development and testing with larger data sets is necessary before the method can be used routinely or commercialized.

Table 10. Repeatability of the fingerprint and the reference FT-IR methods for the 2006 Travaglini Gattinara red wine. The accuracy was calculated from five independent repeats for the fingerprint method. Reproduced from original publication II.

	FT-IR		**Fingerprinting**	
	Measured	Accuracy	Measured	Accuracy
Volatile acidity (g/L)	0.91	±0.02	0.95	±0.06
Total acidity (g/L)	5.7	±0.03	5.7	±0.16
Dry extract (g/L)	27.9	±1.40	28.2	±0.83
Alcohol content (% v/v)	13.5	±0.03	13.5	±0.36

5.2.4 Oral cancer

Oral rinse samples were tested to detect the presence of a lesion in original publication III. Oral rinse samples were used as it is a noninvasive method for sample collection and preferred by patients and caretakers. In previous studies, collecting saliva was difficult (the volumes were low and patients experienced discomfort).

The results are presented in Two different prediction algorithms were used to predict the presence of oral lesions. The algorithms perform similarly, although the models are drastically different mathematically. The table is modified from original publication III. **Table 9**. The diagnostic sensitivity and specificity are acceptable;

they are close to 90% regardless of the classification method. In addition, averaging or alternatively using the replicates as individual measurements did not have a significant effect on the results. This indicates that the measurement-to-measurement repeatability was good enough for training the model and obtaining reliable results. Therefore, to summarize, the results indicate that the nonspecific/nontargeted fingerprinting method can be used in differentiating oral rinse samples by using multivariate data-analysis tools. The key limitation of this study is that the sample set did not contain malignant lesions. The presence of lesions can be confirmed by visually inspecting the oral cavity, but more information is needed to select the correct treatment or follow-up. A larger study with reliable information from lesion classification would be needed to develop and validate a method for screening and correctly classifying lesions.

There have been multiple promising studies in which multiple biomarkers have been used in combination to predict the presence of cancerous lesions. Yu et al. (2016) identified a seven-protein panel using an LC–MS-MS/MS-based proteomics approach and reached sensitivity (87.5%) and specificity (80.5%) for detecting oral squamous cell carcinoma. There are also similar approaches based on DNA methylation (Mori et al., 2022) and miRNA expression (Galindo Torres et al., 2023), in which very similar performances have been reported. However, the methods are complex and require complex instrumentation; thus, they are not suited for a low resource setting.

Despite the limitations, the method has shown potential to screen for oral lesions. Compared to the gold-standard method (biopsy), the method is noninvasive. The fingerprinting method is noninvasive, is suited for low-resource settings and eliminates the need for patients to travel to central hospitals or clinics equipped for performing biopsies or complex assays.

6 Summary/Conclusions

The developed method achieved a performance equal to that of FT-IR-based methods in wine quality control; in scale inhibitor concentration determination, the method performance was equal to that of HPLC and hyamine methods in difficult matrixes. In medical applications, the presence of oral lesions was predicted from an oral rinse sample with high sensitivity and specificity.

The fingerprinting method has also been produced, and test kits and instruments have been produced commercially for monitoring scale inhibitors. This demonstrates that basic chemical interactions relevant for the applicability of the method are well known implying that the method is suitable for commercial applications.

In conclusion, the method is versatile, robust, and easy to use. In addition, there is great potential to develop novel applications with the method. All the aims for the study have been successfully completed.

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Joonas Siivonen

References

Al Helal, A., Soames, A., Iglauer, S., Gubner, R., Barifcani, A., 2019. Evaluating chemical-scale-inhibitor performance in external magnetic fields using a dynamic scale loop. Journal of Petroleum Science and Engineering 179, 1063–1077. https://doi.org/10.1016/j.petrol.2019.04.093

Al Salami, A.R., Monem, A.A., 2010. Downhole and Topside Scale Challenge Removal, Prevention and Inhibition Strategy. Presented at the Abu Dhabi International Petroleum Exhibition and Conference, OnePetro. https://doi.org/10.2118/137906-MS

Alloghani, M., Al-Jumeily, D., Mustafina, J., Hussain, A., Aljaaf, A.J., 2020. A Systematic Review on Supervised and Unsupervised Machine Learning Algorithms for Data Science, in: Berry, M.W., Mohamed, A., Yap, B.W. (Eds.), Supervised and Unsupervised Learning for Data Science, Unsupervised and Semi-Supervised Learning. Springer International Publishing, Cham, pp. 3–21. https://doi.org/10.1007/978-3-030-22475-2_1

Ares, G., Varela, P., 2017. Trained vs. consumer panels for analytical testing: Fueling a long lasting debate in the field. Food Quality and Preference 61, 79–86. https://doi.org/10.1016/j.foodqual.2016.10.006

Ashby Ross, W., 1956. An Introduction to Cybernetics. Chapman and Hall.

Asthana, S., Labani, S., Kailash, U., Sinha, D.N., Mehrotra, R., 2019. Association of Smokeless Tobacco Use and Oral Cancer: A Systematic Global Review and Meta-Analysis. Nicotine Tob. Res. 21, 1162–1171. https://doi.org/10.1093/ntr/nty074

Baldygin, A., Nobes, D.S., Mitra, S.K., 2014. New Laboratory Core Flooding Experimental System. Ind. Eng. Chem. Res. 53, 13497–13505. https://doi.org/10.1021/ie501866e

Beelman, R., Gallander, J., 1979. Wine Deacidification, in: Advances in Food Research. Academic Press, pp. 1–44.

Boak, L.S., 2012. Factors that impact scale inhibitor mechanisms. Heriot-Watt University, Institute of Petroleum Engineering.

Boak, L.S., Sorbie, K.S., 2010. New Developments on the Analysis of Scale Inhibitors. Society of Petroleum Engineers. https://doi.org/10.2118/130401-MS

Borges, E., Lessa, J., 2024. Scale inhibitor qualification: evaluation for treatment in Brazilian pre-salt fields. Braz. J. Chem. Eng. 41, 713–720. https://doi.org/10.1007/s43153-023-00385-0

Boulton, R.B., Singleton, V.L., Bisson, L.F., Kunkee, R.E., 2013. Principles and Practices of Winemaking. Springer Science & Business Media.

Brahmbhatt, M., Sheth, J., 2017. Focused Group Discussion of urban ASHA workers regarding their workrelated issues. Indian Journal of Community Health 29, 187–190.

Bünzli, J.-C.G., Piguet, C., 2005. Taking advantage of luminescent lanthanide ions. Chem. Soc. Rev. 34, 1048–1077. https://doi.org/10.1039/B406082M

Buvé, C., Saeys, W., Rasmussen, M.A., Neckebroeck, B., Hendrickx, M., Grauwet, T., Van Loey, A., 2022. Application of multivariate data analysis for food quality investigations: An example-based review. Food Research International 151, 110878. https://doi.org/10.1016/j.foodres.2021.110878

Cano-Raya, C., Ramos, M.D.F., Vallvey, L.F.C., Wolfbeis, O.S., Schäferling, M., 2005. Fluorescence Quenching of the Europium Tetracycline Hydrogen Peroxide Complex by Copper (II) and other Metal Ions. Appl Spectrosc 59, 1209–1216. https://doi.org/10.1366/000370205774430945

Considine, J.A., Frankish, E., 2014. Chapter 9 - Principles of Analysis, in: Considine, J.A., Frankish, E. (Eds.), A Complete Guide to Quality in Small-Scale Wine Making. Academic Press, San Diego, pp. 127–136.

Cozzolino, D., 2020. The Sample, the Spectra and the Maths—The Critical Pillars in the Development of Robust and Sound Applications of Vibrational Spectroscopy. Molecules 25, 3674. https://doi.org/10.3390/molecules25163674

Daniels, J.K., Littlehales, I., Lau, L., Linares-Samaniego, S., 2014. Laboratory Methods for Scale Inhibitor Selection for HP/HT Fields. Presented at the SPE International Oilfield Scale Conference and Exhibition, OnePetro. https://doi.org/10.2118/SPE-169774-MS

Darias-Martín, J., Socas-Hernández, A., Díaz-Romero, C., Díaz-Díaz, E., 2003. Comparative study of methods for determination of titrable acidity in wine. Journal of Food Composition and Analysis 16, 555–562. https://doi.org/10.1016/S0889-1575(03)00032-2

Davies, K., Connolly, J.M., Dockery, P., Wheatley, A.M., Olivo, M., Keogh, I., 2015. Point of care optical diagnostic technologies for the detection of oral and oropharyngeal squamous cell carcinoma. The Surgeon 13, 321–329. https://doi.org/10.1016/j.surge.2015.06.004

Day, T.A., Davis, B.K., Gillespie, M.B., Joe, J.K., Kibbey, M., Martin-Harris, B., Neville, B., Reed, S.G., Richardson, M.S., Rosenzweig, S., Sharma, A.K., Smith, M.M., Stewart, S., Stuart, R.K., 2003. Oral cancer treatment. Curr. Treat. Options in Oncol. 4, 27. https://doi.org/10.1007/s11864-003-0029-4

Dhiman, P., Ma, J., Andaur Navarro, C.L., Speich, B., Bullock, G., Damen, J.A., Hooft, L., Kirtley, S., Riley, R.D., Van Calster, B., Moons, K.G., Collins, G.S., 2023. Overinterpretation of findings in machine learning prediction model studies in oncology: a systematic review. Journal of Clinical Epidemiology 157, 20–133. https://doi.org/10.1016/j.jclinepi.2023.03.012

Einhaus, J., Han, X., Feyaerts, D., Sunwoo, J., Gaudilliere, B., Ahmad, S.H., Aghaeepour, N., Bruckman, K., Ojcius, D., Schürch, C.M., Gaudilliere, D.K., 2023. Towards multiomic analysis of oral mucosal pathologies. Semin Immunopathol 45, 111–123. https://doi.org/10.1007/s00281-022-00982-0

Engel, J., Gerretzen, J., Szymańska, E., Jansen, J.J., Downey, G., Blanchet, L., Buydens, L.M.C., 2013. Breaking with trends in pre-processing? TrAC Trends in Analytical Chemistry 50, 96–106. https://doi.org/10.1016/j.trac.2013.04.015

F5 Technologie: Dynamic Scale Loop [WWW Document], n.d. URL https://www.f5-tech.de/en/products/dynamic_scale_loop.php?gad_source=1&gclid=CjwKCAiA9vS6BhA9EiwAJpnXwyumfqfJP4s9lAPuuRDcoh2O6ZwMJy37kQXDpMpuFSHwMpjFx8DLmRoC1t8QAvD_BwE (accessed 12.14.24).

Fagerholm, H., Teimonen, T., 2015. Kemira ja teknologia start-up Aqsens yhdistävät voimansa uudenlaisessa yhteistyössä - Kemira.com.

Farquhar, G.D., O'Leary, M.H., Berry, J.A., 1982. On the Relationship Between Carbon Isotope Discrimination and the Intercellular Carbon Dioxide Concentration in Leaves. Functional Plant Biol. 9, 121–137. https://doi.org/10.1071/pp9820121

Galindo Torres, B.P., García Girón, C., Alcaraz Ortega, R., Saiz López, P., Adiego Leza, M.I., Grijalba Uche, M.V., 2023. Knowledge and expectations about miRNAs as biomarkers in head and neck squamous cell cancers. American Journal of Otolaryngology 44, 103771. https://doi.org/10.1016/j.amjoto.2022.103771

Graham, G., Stalker, R., Hoeth, L.W., Kidd, S.L., Lagarde, F., Orski, K., 2012. Effective Laboratory Simulation of Scale Inhibitor Squeeze Treatments -Core to Field II. Presented at the SPE International Conference on Oilfield Scale, OnePetro. https://doi.org/10.2118/157105-MS

Graham, G.M., Collins, I.R., Stalker, R., Littlehales, I.J., 2002. The Importance of Appropriate Laboratory Procedures for the Determination of Scale Inhibitor Performance. Presented at the International Symposium on Oilfield Scale, OnePetro. https://doi.org/10.2118/74679-MS

Graham, G.M., Kidd, S., Fitzpatrick, R., Wright, R., 2010a. Appropriate Simulation of Field Treatments Using Core Flood Data. Presented at the SPE International Conference on Oilfield Scale, OnePetro. https://doi.org/10.2118/131131-MS

Graham, G.M., Munro, I., Harvison, N., Marshall, K., Kyle, M., 2010b. Improved Scale Inhibitor Assay for Sulphonated Polymers in Oilfield Brines. Presented at the SPE International Conference on Oilfield Scale, OnePetro. https://doi.org/10.2118/131100-MS

Hafez, O.M., Shoeib, M.A., El-Khateeb, M.A., Abdel-Shafy, H.I., Youssef, A.O., 2018. Removal of scale forming species from cooling tower blowdown water by electrocoagulation using different electrodes. Chemical Engineering Research and Design 136, 347–357. https://doi.org/10.1016/j.cherd.2018.05.043

Hahn, A., Dettlaff, A., Fink, D., Mazin, D., Mirzoyan, R., Teshima, M., 2018. Development of three silicon photomultiplier detector modules for the MAGIC telescopes for a performance comparison to PMTs. Nuclear Instruments and Methods in Physics Research Section A: Accelerators, Spectrometers, Detectors and Associated Equipment 912, 259–263. https://doi.org/10.1016/j.nima.2017.11.071

Halford, G.S., Baker, R., McCredden, J.E., Bain, J.D., 2005. How Many Variables Can Humans Process? Psychol Sci 16, 70–76. https://doi.org/10.1111/j.0956-7976.2005.00782.x

Haron, N., Zain, R.B., Nabillah, W.M., Saleh, A., Kallarakkal, T.G., Ramanathan, A., Sinon, S.H.M., Razak, I.A., Cheong, S.C., 2016. Mobile Phone Imaging in Low Resource Settings for Early Detection of Oral Cancer and Concordance with Clinical Oral Examination. Telemedicine and e-Health 23, 192–199. https://doi.org/10.1089/tmj.2016.0128

Haukioja, A., Asunta, M., Söderling, E., Syrjänen, S., 2014. Persistent oral human papillomavirus infection is associated with smoking and elevated salivary immunoglobulin G concentration. J. Clin. Virol. 61, 101–106. https://doi.org/10.1016/j.jcv.2014.06.012

Heath, S., Ross, G., Graham, G., MacKinnon, K., 2016. The Importance of Scale Inhibitor Analysis in Scale Management - A State of the Art Overview to Provide Cost Effective Scale Control from Simple to Complex Production Scenarios. Presented at the SPE International Oilfield Scale Conference and Exhibition, OnePetro. https://doi.org/10.2118/179900-MS

Heidari, A.E., Sunny, S.P., James, B.L., Lam, T., Tran, A.V., Yu, J., Ramanjinappa, R.D., K, U., Birur, P., Suresh, A., Kuriakose, M.A., Chen, Z., Wilder-Smith, P., 2019. Optical Coherence Tomography as an Oral Cancer Screening Adjunct in a Low Resource Settings. JSTQE 25, 1–8. https://doi.org/10.1109/JSTQE.2018.2869643

Hemmilä, I., 1995. Luminescent lanthanide chelates—a way to more sensitive diagnostic methods. Journal of Alloys and Compounds 225, 480–485. https://doi.org/10.1016/0925-8388(94)07069-5

Hemmilä, I., Dakubu, S., Mukkala, V.-M., Siitari, H., Lövgren, T., 1984. Europium as a label in time-resolved immunofluorometric assays. Analytical Biochemistry 137, 335–343. https://doi.org/10.1016/0003-2697(84)90095-2

Hemmilä, I., Mukkala, V.-M., Takalo, H., 1997. Development of luminescent lanthanide chelate labels for diagnostic assays. Journal of Alloys and Compounds 249, 158–162. https://doi.org/10.1016/S0925-8388(96)02834-4

Hills, E., Chapon, P., 2005. Tagged scale inhibiting polymers, compositions comprising the same, and method for preventing or controlling scale formation. WO2005000747A2. Paten Publication

Hotelling, H., 1933. Analysis of a complex of statistical variables into principal components. Journal of Educational Psychology 24, 417–441. https://doi.org/10.1037/h0071325

Howard, S., Tx, M., 2024. Long-term Coreflood Testing with Biopolymers—A Laboratory Investigation Showing How Return Permeability Improves From 0 to 100 Percent by Getting a Critical Parameter Right. Presented at the SPE International Conference and Exhibition on Formation Damage Control, OnePetro. https://doi.org/10.2118/217909-MS

Hu, X.-Z., Liu, S.-Q., Li, X.-H., Wang, C.-X., Ni, X.-L., Liu, X., Wang, Y., Liu, Y., Xu, C.-H., 2019. Geographical origin traceability of Cabernet Sauvignon wines based on Infrared fingerprint

technology combined with chemometrics. Sci Rep 9, 8256. https://doi.org/10.1038/s41598-019-44521-8

Iland, E., Sitters, Markides, Bruer, 2000. Techniques For Chemical Analysis and Quality Monitoring During Winemaking. Patrick Iland Wine Promotions.

Jarrahian, K., Singleton, M., Boak, L., Sorbie, K.S., 2020. Surface Chemistry of Phosphonate Scale Inhibitor Retention Mechanisms in Carbonate Reservoirs. Crystal Growth & Design 20, 5356–5372. https://doi.org/10.1021/acs.cgd.0c00570

Jiang, T., Sun, H., Deng, X., 2019. Chapter 1 - Typical characteristics of fractured vuggy carbonate gas reservoirs, in: Jiang, T., Sun, H., Deng, X. (Eds.), Dynamic Description Technology of Fractured Vuggy Carbonate Gas Reservoirs. Gulf Professional Publishing, pp. 1–29. https://doi.org/10.1016/B978-0-12-818324-3.00001-2

Johnstone, J., Peltokoski, B., Toivonen, S., Griffin, R., Hurd, M., O'Brien, A., Fournier, F., Siivonen, J., Väisänen, P., Tittanen, S., Lehmusto, M., Mäkinen, P., Wahrman, J., Mundill, P., 2015. Application of a Novel Method for Real-Time Monitoring of Scale Control Products at the Site of Use. Presented at the SPE International Symposium on Oilfield Chemistry, OnePetro. https://doi.org/10.2118/173779-MS

Johnstone, J., Toivonen, S., Griffin, R., O'Brien, A., Mundill, P., Siivonen, J., Väisänen, P., Tiittanen, S., Lehmusto, M., Härmä, H., Hänninen, P., Teimonen, T., 2014. Novel Method for Real-Time Monitoring of Scale Control Products at the Site of Use. Presented at the SPE International Oilfield Scale Conference and Exhibition, OnePetro. https://doi.org/10.2118/SPE-169797-MS

Jordan, M.M., Sorhaug, E., Marlow, D., 2012. Red vs. Green Scale Inhibitors for Extending Squeeze life—A Case Study From the North Sea, Norwegian Sector—Part II. SPE Production & Operations 27, 404–413. https://doi.org/10.2118/140752-PA

Kamal, M.S., Hussein, I., Mahmoud, M., Sultan, A.S., Saad, M.A.S., 2018. Oilfield scale formation and chemical removal: A review. Journal of Petroleum Science and Engineering 171, 127–139. https://doi.org/10.1016/j.petrol.2018.07.037

Kan, A.T., Dai, Z., Tomson, M.B., 2020. The state of the art in scale inhibitor squeeze treatment. Pet. Sci. 17, 1579–1601. https://doi.org/10.1007/s12182-020-00497-z

Kartnaller, V., Venâncio, F., F. do Rosário, F., Cajaiba, J., 2018. Application of Multiple Regression and Design of Experiments for Modelling the Effect of Monoethylene Glycol in the Calcium Carbonate Scaling Process. Molecules 23, 860. https://doi.org/10.3390/molecules23040860

Kaur, J., Jacobs, R., Huang, Y., Salvo, N., Politis, C., 2018. Salivary biomarkers for oral cancer and pre-cancer screening: a review. Clin Oral Invest 22, 633–640. https://doi.org/10.1007/s00784-018-2337-x

Keberle, H., 1964. The Biochemistry of Desferrioxamine and its Relation to Iron Metabolism. Annals of the New York Academy of Sciences 119, 758–768. https://doi.org/10.1111/j.1749-6632.1965.tb54077.x

Kilkenny, M.F., Robinson, K.M., 2018. Data quality: "Garbage in – garbage out." HIM J 47, 103–105. https://doi.org/10.1177/1833358318774357

Kuhn, M., 2008. Building Predictive Models in R Using the caret Package. Journal of Statistical Software 28, 1–26. https://doi.org/10.18637/jss.v028.i05

Kulpakko, J., Kopra, K., Hänninen, P., 2015. Time-resolved fluorescence-based assay for rapid detection of Escherichia coli. Anal. Biochem. 470, 1–6. https://doi.org/10.1016/j.ab.2014.09.002

Kuswandi, B., Huskens, J., Verboom, W., 2007. Optical sensing systems for microfluidic devices: A review. Analytica Chimica Acta 601, 141–155. https://doi.org/10.1016/j.aca.2007.08.046

Lavine, B., Workman, J., 2008. Chemometrics. Anal. Chem. 80, 4519–4531. https://doi.org/10.1021/ac800728t

Li, M., Yao, X., Sun, L., Zhao, L., Xu, W., Zhao, H., Zhao, F., Zou, X., Cheng, Z., Li, B., Yang, W., Cui, R., 2020. Effects of Electroconvulsive Therapy on Depression and Its Potential Mechanism. Front Psychol 11, 80. https://doi.org/10.3389/fpsyg.2020.00080

Mackay, E.J., Jordan, M.M., 2003. SQUEEZE Modelling: Treatment Design and Case Histories. Presented at the SPE European Formation Damage Conference, Society of Petroleum Engineers. https://doi.org/10.2118/82227-MS

Marr, B., 2015. Big Data In Big Oil: How Shell Uses Analytics To Drive Business Success. Forbes.

Mazumder, J., A, M., 2020. A Review of Green Scale Inhibitors: Process, Types, Mechanism and Properties. Coatings 10, 928. https://doi.org/10.3390/coatings10100928

McRae, J.M., Kassara, S., Kennedy, J.A., Waters, E.J., Smith, P.A., 2013. Effect of Wine pH and Bottle Closure on Tannins. J. Agric. Food Chem. 61, 11618–11627. https://doi.org/10.1021/jf403704f

Mehmood, T., Liland, K.H., Snipen, L., Sæbø, S., 2012. A review of variable selection methods in Partial Least Squares Regression. Chemometrics and Intelligent Laboratory Systems 118, 62–69. https://doi.org/10.1016/j.chemolab.2012.07.010

Meurman, O.H., Hemmilä, I.A., Lövgren, T.N., Halonen, P.E., 1982. Time-resolved fluoroimmunoassay: a new test for rubella antibodies. J. Clin. Microbiol. 16, 920–925.

Moe, A.E., Marx, S., Banani, N., Liu, M., Marquardt, B., Wilson, D.M., 2005. Improvements in LED-based fluorescence analysis systems. Sensors and Actuators B: Chemical 111–112, 230–241. https://doi.org/10.1016/j.snb.2005.01.057

Mokogwu, I., Wilson, S., Graham, G.M., 2018. Formation Damage Versus Chemical Performance - Important Aspects for Upscaling Scale Inhibitor Squeeze Core Floods for Carbonate Systems. Presented at the SPE International Conference and Exhibition on Formation Damage Control, OnePetro. https://doi.org/10.2118/189560-MS

Moore, L., Clapp, L., 2016. Tagged scale inhibitor compositions and methods of inhibiting scale. US9309137B2. Patent Publication

Moore, L., Clapp, L., 2012. Tagged scale inhibitor compositions and methods of inhibiting scale. US20120032093A1.Patent Publication

Mori, K., Hamada, T., Beppu, M., Tsuchihashi, H., Goto, Y., Kume, K., Hijioka, H., Nishi, K., Mishima, Y., Sugiura, T., 2022. Detecting Early-Stage Oral Cancer from Clinically Diagnosed Oral Potentially Malignant Disorders by DNA Methylation Profile. Cancers 14, 2646. https://doi.org/10.3390/cancers14112646

Moriarty, B.E., Wei, M., Hoots, J.E., Workman, D.P., Rasimas, J.P., 2001. Fluorescent monomers and polymers containing same for use in industrial water systems. US6312644B1. Patent Publication

Morris, J.D., Moriarty, B.E., Wei, M., Murray, P.G., Reddinger, J.L., 2001. Fluorescent monomers and tagged treatment polymers containing same for use in industrial water systems. WO2001081654A1. Patent Publication

Mundill, P., Rosati, L., 2014. Kemira to launch novel scale inhibition monitoring technology at the SPE International Oilfield Scale Conference - Kemira.com.

Murthy, V., Calcuttawala, A., Chadha, K., d'Cruz, A., Krishnamurthy, A., Mallick, I., Nair, S., Teni, T., Pawar, S., Talapatra, K., Patil, A., Bhatt, A., Chatterjee, S., Swain, M., Narayanan, P., Ghadyalpatil, N., Singhal, M., Kuriakose, M., Prabhash, K., Agarwal, J., Parikh, P., 2017. Human papillomavirus in head and neck cancer in India: Current status and consensus recommendations. South.Asian J.Cancer. 6, 93–98. https://doi.org/10.4103/sajc.sajc_96_17

Neto, F.S., Castilhos, M.B. de, Telis, V.R., Telis-Romero, J., 2015. Effect of ethanol, dry extract and reducing sugars on density and viscosity of Brazilian red wines. Journal of the Science of Food and Agriculture 95, 1421–1427. https://doi.org/10.1002/jsfa.6835

Nuutinen, V., Toivonen, S., Johnstone, J., Härmä, H., Lehmusto, M., Tiittanen, S., Väisänen, P., Siivonen, J., Mundill, P., 2018. Method for Analysing a Sample Comprising at Least a First and a Second Scale Inhibitor. WO/2015/075309. Patent Publication

Oddo, J.E., Tomson, M.B., 1994. Why Scale Forms in the Oil Field and Methods To Predict It. SPE Production & Facilities 9, 47–54. https://doi.org/10.2118/21710-PA

OIV, n.d. Total Acidity (Type-I) | OIV, OIV-MA-AS313-01.

OIV, n.d. Total dry matter (densimétrie) (Type-IV) | OIV [WWW Document]. URL https://www.oiv.int/standards/compendium-of-international-methods-of-wine-and-must-

analysis/annex-a-methods-of-analysis-of-wines-and-musts/section-2-physical-analysis/total-dry-matter-%28densimetrie%29-%28type-iv%29 (accessed 5.19.24b).

OIV, n.d. Total dry matter (gravimétrie) (Type-I) | OIV [WWW Document]. URL https://www.oiv.int/standards/compendium-of-international-methods-of-wine-and-must-analysis/annex-a-methods-of-analysis-of-wines-and-musts/section-2-physical-analysis/total-dry-matter-%28gravimetrie%29-%28type-i%29 (accessed 5.19.24c).

OIV-MA-BS-22 Determination by isotope ratio mass spectrometry of the 13C/12C ratio of wine ethanol in spirit drinks of vitivinicultural origin (Type II) | OIV, 2009.

Okocha, C., Obeyesekere, N., 2023. Novel Combination Iron Sulfide-Corrosion Inhibitor Development, Testing and Field Assessment for an Integrated Scale Management System. Presented at the ADIPEC, OnePetro. https://doi.org/10.2118/216830-MS

Olajire, A.A., 2015. A review of oilfield scale management technology for oil and gas production. Journal of Petroleum Science and Engineering 135, 723–737. https://doi.org/10.1016/j.petrol.2015.09.011

Ostrowski, W., 1968. Michael S. Tswett--inventor of column chromatography. (On the occasion of 65th anniversary of his lecture on the column chromatography technique). Folia Biol. (Krakow) 16, 429–448.

Pearson, K., 1901. LIII. On lines and planes of closest fit to systems of points in space. The London, Edinburgh, and Dublin Philosophical Magazine and Journal of Science 2, 559–572. https://doi.org/10.1080/14786440109462720

Petroleum Safety Authority Norway, Norwegian Directorate of Health, Norwegian Environment Agency, Norwegian Food Safety Authority, Norwegian Radiation and Nuclear Safety Authorit, 2022. Regulations relating to conducting petroleum activities.

Phakam, B., Moghaddam, L., Wellard, M., Doherty, W.O.S., 2018. The effect of cleaning agents on the structural features of heat exchanger deposits from sugar factories. Journal of Food Engineering 226, 65–72. https://doi.org/10.1016/j.jfoodeng.2018.01.014

Potti, A., Dressman, H.K., Bild, A., Riedel, R.F., Chan, G., Sayer, R., Cragun, J., Cottrill, H., Kelley, M.J., Petersen, R., Harpole, D., Marks, J., Berchuck, A., Ginsburg, G.S., Febbo, P., Lancaster, J., Nevins, J.R., 2006. Genomic signatures to guide the use of chemotherapeutics. Nature Medicine 12, 1294–1300. https://doi.org/10.1038/nm1491

Puntervold, T., Austad, T., 2008. Injection of seawater and mixtures with produced water into North Sea chalk formation: Impact of fluid–rock interactions on wettability and scale formation. Journal of Petroleum Science and Engineering 63, 23–33. https://doi.org/10.1016/j.petrol.2008.07.010

Ranaweera, R.K.R., Capone, D.L., Bastian, S.E.P., Cozzolino, D., Jeffery, D.W., 2021. A Review of Wine Authentication Using Spectroscopic Approaches in Combination with Chemometrics. Molecules 26, 4334. https://doi.org/10.3390/molecules26144334

Rinnan, Å., Berg, F. van den, Engelsen, S.B., 2009. Review of the most common pre-processing techniques for near-infrared spectra. TrAC Trends in Analytical Chemistry 28, 1201–1222. https://doi.org/10.1016/j.trac.2009.07.007

Rivera, C., 2015. Essentials of oral cancer. International journal of clinical and experimental pathology 8, 11884.

Rodriguez-Saona, L.E., Allendorf, M.E., 2011. Use of FTIR for Rapid Authentication and Detection of Adulteration of Food. Annual Review of Food Science and Technology 2, 467–483. https://doi.org/10.1146/annurev-food-022510-133750

Ross, R.J., Low, K., Shannon, J.E., 1996. Polyaspartate Scale Inhibitors-Biodegradable Alternatives to Polyacrylates. Presented at the Corrosion 1996, OnePetro, Denver, Colorado.

Roßmann, A., Schmidt, H.-L., Reniero, F., Versini, G., Moussa, I., Merle, M.H., 1996. Stable carbon isotope content in ethanol of EC data bank wines from Italy, France and Germany. Z Lebensm Unters Forch 203, 293–301. https://doi.org/10.1007/BF01192881

Rozwandowicz-Jansen, A., Kulpakko, J., Hänninen, P., 2016. Novel liquid fingerprinting technology as a tool for detection of bacteria in milk, in: New Biotechnology. p. S174. https://doi.org/10.1016/j.nbt.2016.06.1321

Salminen, A., Teimonen, T., 2017. Kemira acquires liquid fingerprinting technology from Finnish start-up Aqsens - Kemira.com.

Shehab, M., Abualigah, L., Shambour, Q., Abu-Hashem, M.A., Shambour, M.K.Y., Alsalibi, A.I., Gandomi, A.H., 2022. Machine learning in medical applications: A review of state-of-the-art methods. Computers in Biology and Medicine 145, 105458. https://doi.org/10.1016/j.compbiomed.2022.105458

Shell Global Solutions International BV, Berg, S., Unsal, E., 2021. The certainty in uncertainty: Quantifying coreflood data errors. STXD 2021. https://doi.org/10.52196/208316.21.02

Shi, Y., Li, Zhi, Li, Zishuo, Chen, S., Yang, X., Duan, L., Cai, J., 2022. Synthesis and evaluation of scale inhibitor with high-temperature resistance and low corrosion capability for geothermal exploitation. Journal of Petroleum Science and Engineering 218, 110976. https://doi.org/10.1016/j.petrol.2022.110976

Siivonen, J., Mäkinen, P., Tiittanen, S., Wahrman, J., Lehmusto, M., 2018. Saostumanestoaineanalyysi. FI 127572 B. Patent Publication

Siivonen, J., Tiittanen, S., Lehmusto, M., 2016. A Method to Measure Phosphonates. FI 125976 B. Patent Publication

Slack, C.R., Hatch, M.D., 1967. Comparative studies on the activity of carboxylases and other enzymes in relation to the new pathway of photosynthetic carbon dioxide fixation in tropical grasses. Biochem J 103, 660–665.

Slyker, J., 1969. Squeeze treatment of producing oil wells. US3483925A. Patent Publication

Soini, E., Kojola, H., 1983. Time-Resolved Fluorometer for Lanthanide Chelates - A New Generation of Nonisotopic Immunoassays. CLIN.CHEM 29, 65–68.

Solanki, J., Gupta, S., 2017. Prevalence of quid-induced lichenoid reactions among western Indian population. J.Exp.Ther.Oncol. 11, 63–66.

Sorbie, K.S., Laing, N., 2004. How Scale Inhibitors Work: Mechanisms of Selected Barium Sulphate Scale inhibitors Across a Wide Temperature Range. Presented at the SPE International Symposium on Oilfield Scale, OnePetro. https://doi.org/10.2118/87470-MS

Stracquadanio, G., Umeton, R., Papini, A., Liò, P., Nicosia, G., 2010. Analysis and Optimization of C3 Photosynthetic Carbon Metabolism, in: 2010 IEEE International Conference on BioInformatics and BioEngineering. Presented at the 2010 IEEE International Conference on BioInformatics and BioEngineering, pp. 44–51. https://doi.org/10.1109/BIBE.2010.17

Tahir, H.E., Arslan, M., Komla Mahunu, G., Adam Mariod, A., B.H. Hashim, S., Xiaobo, Z., Jiyong, S., El-Seedi, H.R., Musa, T.H., 2022. The use of analytical techniques coupled with chemometrics for tracing the geographical origin of oils: A systematic review (2013–2020). Food Chemistry 366, 130633. https://doi.org/10.1016/j.foodchem.2021.130633

The Office of Research Integrity, 2015. Case Summary: Potti, Anil

ThekkePurakkal, A.S., Naimi, A.I., Madathil, S.A., Kumamangalam Puthiyannal, S.H., Netuveli, G., Sacker, A., Schlecht, N.F., Nicolau, B., 2018. Differential impact of socioeconomic position across life on oral cancer risk in Kerala, India: An investigation of life-course models under a time-varying framework. Community Dentistry and Oral Epidemiology 46, 592–600. https://doi.org/10.1111/cdoe.12404

Toivonen, S., Nuutinen, V., 2014. Tagged scale inhibiting polymer compositions and methods of inhibiting scale formation. WO2014009445A1. Patent Publication

Vigneswaran, N., Williams, M.D., 2014. Epidemiological Trends in Head and Neck Cancer and Aids in Diagnosis. Oral Maxillofac Surg Clin North Am 26, 123–141. https://doi.org/10.1016/j.coms.2014.01.001

Volschenk, H., Van Vuuren, H.J.J., Viljoen-Bloom, M., 2006. Malic acid in wine : origin, function and metabolism during vinification. South African Journal of Enology and Viticulture 27, 123–134.

Vuori, V., Nuutinen, V., Honkanen, T., Griffin, R., Johnstone, J., Peltokoski, B., Lehtonen, A., Suominen, H., Toivonen, S., 2016. Accurate Detection of Tagged Polymeric Scale Inhibitors in Oilfield Produced Water Samples. Presented at the SPE International Oilfield Scale Conference and Exhibition 2016, Society of Petroleum Engineers, Aberdeen, Scotland, UK. https://doi.org/10.2118/179908-MS

Wang, M.-L., Choong, Y.-M., Su, N.-W., Lee, M.-S., 2020. A rapid method for determination of ethanol in alcoholic beverages using capillary gas chromatography. Journal of Food and Drug Analysis 11, 133–140. https://doi.org/10.38212/2224-6614.2710

Wang, S., Wylde, J., 2009. Scale Inhibitor Selection For Deepwater High Temperature Applications. Presented at the CORROSION 2009, OnePetro.

Warner, M., 2002. WineScans: Testing at a New Level. WineBusiness Monthly - Winemaking.

Weissman, S.I., 1942. Intramolecular Energy Transfer The Fluorescence of Complexes of Europium. The Journal of Chemical Physics 10, 214–217. https://doi.org/10.1063/1.1723709

Westad, F., Marini, F., 2015. Validation of chemometric models – A tutorial. Analytica Chimica Acta 893, 14–24. https://doi.org/10.1016/j.aca.2015.06.056

Wethe, D., 2017. Oil industry growing again after losing half-million jobs. World Oil.

Wilkes, E., Pride, R., 2022. Comparison of common methods for measuring alcohol in wine. AWRI Technical Review 2022, 7–10.

Yalow, R.S., Berson, S.A., 1959. Assay of Plasma Insulin in Human Subjects by Immunological Methods. Nature 184, 1648–1649. https://doi.org/10.1038/1841648b0

Yu, J.-S., Chen, Y.-T., Chiang, W.-F., Hsiao, Y.-C., Chu, L.J., See, L.-C., Wu, C.-S., Tu, H.-T., Chen, H.-W., Chen, C.-C., Liao, W.-C., Chang, Y.-T., Wu, C.-C., Lin, C.-Y., Liu, S.-Y., Chiou, S.-T., Chia, S.-L., Chang, K.-P., Chien, C.-Y., Chang, S.-W., Chang, C.-J., Young, J.D., Pao, C.C., Chang, Y.-S., Hartwell, L.H., 2016. Saliva protein biomarkers to detect oral squamous cell carcinoma in a high-risk population in Taiwan. Proceedings of the National Academy of Sciences 113, 11549–11554. https://doi.org/10.1073/pnas.1612368113

Zoecklein, B.W., Fugelsang, K.C., Gump, B.H., Nury, F.S., 1995. Volatile Acidity, in: Wine Analysis and Production. Springer US, Boston, MA, pp. 192–198. https://doi.org/10.1007/978-1-4757-6978-4_11

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