Project Acronym:	Nutrishield
Grant Agreement number:	818110 (H2020-SFS-2018-IA)
Project Full Title:	Fact-based personalised nutrition for the young

DELIVERABLE

D2.2 – Report on requirements for urine analysis

Dissemination level	PU - Public
Type of Document	Report
Contractual date of delivery	30/04/2019
Deliverable Leader	CSEM
Status & version	Final, V1.0 – 30/04/2019
WP responsible	WP2 (RU)
Keywords:	IR spectroscopy, analytes

Deliverable Leader:	CSEM
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Document History			
Version	**Date**	**Contributor(s)**	**Description**
v0.1	14/03/2019	CSEM	Draft
v1.0	30/04/2019	CSEM	Final complete version
V0.5	03/05/2019	CSEM	Final complete version

Executive Summary

This deliverable summarizes the requirements for urine analysis that were collected together with medical experts and other potential end users. The focus is on parameters that are not or are hardly accessible with current analyser technology.

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Definitions, Acronyms and Abbreviations

Acronym	Title
BMI	Body mass index
CAD	Coronary artery disease
OA	Osteoarthritis
T2D	Type 2 diabetes
PWS	Prader-Willi Syndrome
PYY	peptide YY
GLP-1	Glucagon-Like Peptide 1
CCK	Cholecystokinin
LOD	Limit of detection
PTH	human parathyroid hormone
OSR	Ospedale San Raffaele Srl (Experimental Diabetes Unit-DRI)
HULAFE	Fundacion Para La Investigacion Del Hospital Universitario La Fe De La Comunidad Valenciana (Neonatal Research Unit)
NM-BAPTA	5-nitro-5'-methyl-(1,2-bis(o-aminophenoxy)ethan-N,N,N',N'-tetraacetic acid
QCL	Quantum cascade laser
FTIR	Fourier Transform Infrared Spectroscopy
ATR	Attenuated Total Reflection
PoC	Point-of-Care
EC	Electrochemical
CSEM	Centre Suisse D'Electronique Et De Microtechnique Sa - Recherche Et Development

1. Aim of the obesity study and motivation

Obesity is generally defined as an excess of body fat mass. The prevalence of overweight and obesity is commonly assessed by using body mass index (BMI), defined as the weight in kilograms divided by the square of the height in meters (kg/m^2). A BMI over 25 kg/m^2 is defined as overweight, and a BMI of over 30 kg/m2 as obese [1]. These markers provide common benchmarks for assessment, but the risks of disease in all populations can increase progressively from lower BMI levels.

Recent studies [2] have demonstrated that obesity is a disorder of the energy homeostasis system. The reason why, is related to the interaction between genetic and environmental factors (i.e. metabolic characteristics, physical inactivity, habitual energy intake in relation to expenditure, macronutrient composition of the diet) that affect the energy homeostasis system. The main scientific goal is to understand the obesity disease mechanisms and elucidate obesity pathogenesis in order to better inform public policy. This will help to diminish obesity's public health and economic consequences.

The goal of the Nutrishield project regarding urine analysis is to elucidate innovative roadmaps based on the monitoring of new biomarkers that can be used in the diagnosis of obesity related diseases. The key of success to that is a completely different approach compared to the classical analytical methods and can be summarized in two principal steps. In the first step, the QCL-based sensors will be used for the monitoring of classical biomarkers (i.e. phosphates and creatinine) as an evaluation tool of the renal function. The advantages of using such technology have been described in the paragraph 3.1.3. In a second step, from the metabolomics analysis that is taking place within WP3 an overall fingerprint of biomarkers will be taken into consideration with particular attention to systemic inflammatory markers that are expected to be altered in patients with obesity and diabetes type 2 diseases. This combinatorial analysis study will give the possibility to correlate existing and/or new markers for the prediction of obesity related diseases.

1.1. Health risks and causes

Overweight and obesity cause a large number of health problems and are among the most significant contributors to ill health. The causes of obesity are a complex topic. Although there is a genetic and hormonal behavior on body weight, obesity generally occurs when more calories are assumed by a person than the calories that are burned through exercise and daily activities. This gain in body weight gain (i.e. fat) is affected principally by two factors that are inactivity and unhealthy diet and eating habits. There are also other contributing factors combined to the above mentioned that can cause more obesity to occur:

- Genetic predisposition
- Family lifestyle
- Medical problems
- Certain medications that lead to weight gain
- Social and economic issues
- Age
- Pregnancy
- Lack of sleep
- Quit smoking

Some of the health risks [3] associated with increasing Body Mass Index (BMI) are:

- Metabolic syndrome
- Type 2 diabetes
- Hypertension
- Coronary artery disease (CAD) and stroke
- Respiratory effects
- Cancer
- Osteoarthritis (OA)
- Liver and gallbladder disease

The above mentioned comorbid diseases suggest that obesity increases both morbidity and mortality. For that reason, a correct obesity management is highly recommended in order to diminish severe health consequences.

1.2. Obesity and diabetes type 2

Obesity is linked to medical, psychological, and social conditions. The most devastating of them may be the type 2 diabetes [4]. Type 2 diabetes (T2D) is a complex endocrine and metabolic disorder. The interaction between genetic and environmental factors results in a heterogeneous and progressive disorder with variable degrees of insulin resistance and pancreatic β-cell dysfunction. When β-cells are not able to secrete sufficient insulin to overcome insulin resistance, impaired glucose tolerance progresses to type 2 diabetes [5].

Literature studies [3][4] demonstrated that body mass index (BMI) has a strong relationship to diabetes and insulin resistance. For instance, in obese individuals, the amount of the following substances involved in the development of insulin resistance result increased:

- Non-esterified fatty acids
- Glycerol
- Hormones
- Cytokines
- Pro-inflammatory markers

For that reason, in the frame of the Nutrishield project a complete metabolic fingerprint, focused on the above mentioned markers, is part of the WP3 study.

1.3. Pathophysiology

There are plenty of possible pathophysiological mechanisms involved in the development and maintenance of obesity. Recent findings in the literature [2], [6] give a broad overview on obesity from multiple perspectives, including epidemiological investigation, food addiction, endocrine, and neuroimaging studies on brain circuits associated with eating and obesity. It is worth to elucidate, briefly, the most important pathophysiological mechanisms [6]:

- Binge-eating disorder: is characterized by binge eating without subsequent purging episodes and an association with the development of severe obesity.
- Prader-Willi Syndrome (PWS): is a genetic imprinting disorder that results in hyperphagia and early childhood onset obesity.
- Peripheral hormone participation: leptin, insulin, ghrelin, peptide YY (PYY), Glucagon-Like Peptide 1 (GLP-1)
- Peptides: Cholecystokinin (CCK).

The study of all the above mentioned physiological mechanisms regarding obesity is the demonstration of a better clinical understanding progress related to a high demand for management and intervention.

1.4. Management

The financial impact of treating obesity and obesity-related comorbid diseases is substantial for each society. For that reason, weight loss interventions are important for the prevention and treatment of obesity and prediabetes and can represent a broader social solution. An effective action for obesity treatment has to focus on two main management approaches, the nonpharmacological management, that includes lifestyle changes (i.e. physical activity), diet therapy and behavioral therapy and the pharmacological management including medications, or bariatric surgery [7].

1.5. Childhood obesity

Overweight and obesity in children is epidemic in North America and internationally [8]. The comorbidities that are associated with childhood obesity are generally similar to those found in the adult population. An example of the most frequent comorbidities is summarized below [8]:

- Elevated blood pressure
- Dyslipidemia
- Insulin resistance
- Type 2 diabetes

Despite the cause of obesity in children, it is similar compared to adults. Some emerging [8], [9] data suggests associations between the influence of maternal and fetal factors during intrauterine growth and growth during the first year of life as well. The latter enhances the risk of later development of adult obesity and its related comorbidities.

A research study [8] suggests a multilevel obesity prevention approach that focus on different stages of the child development including very informative guidelines:

1. Perinatal:
   - Good prenatal nutrition
   - Avoid excessive maternal weight gain
   - Control diabetes
   - Help mothers lose weight postpartum
   - Nutrition education.

2. Infancy
   - Encourage increased breast-feeding
   - Delay introduction of solid foods until after 6 months of age,
   - Provide a balanced diet excluding high-calorie snacks
   - Follow weight increase closely.
3. Preschool:
   - Provide early experiences with foods and flavors
   - Help develop healthy food preferences
   - Encourage appropriate parental feeding practices,
   - Monitor rate of weight increases to prevent early adiposity rebound,
   - Provide child and parent nutrition education.
4. Childhood:
   - Monitor weight increase for height (slow down if excessive),
   - Avoid excessive prepubertal adiposity,
   - Nutrition education,
   - Encourage daily physical activity.
5. Adolescence
   - Prevent excess weight increase after growth spurt
   - Maintain healthy nutrition and continue daily physical activity.

2. Requirements for urine analysis

2.1. Medical impact on the markers of choice

This deliverable focuses on the requirements and specifications of the novel monitoring device for urine. A more detailed analysis of urine concerning different biomarkers and metabolic fingerprint will be part of work package 3 “Biomarkers and stress factors analysis & monitoring”.

If a device for the quantification of an analyte is being developed and later on in the process it shows that this analyte is not relevant for the project, it might be possible that the prototype cannot be delivered for the clinical studies in work package 7. Therefore the novel monitoring device for urine analysis within this project will focus on analytes, which we know are already relevant for nutrition and important for the development of infants and children.

2.2. End users feedback

To make it easier for potential end-users to be able to go through the questions faster a questionnaire was set up. After literature research and correspondence with project partners the questionnaire was set up.

The questionnaire was divided into the following parts:

1. Introduction
   - 1.1. Mission of Nutrishield
   - 1.2. Motivation
   - 1.3. Concept
   - 1.4. Aim of this questionnaire
2. Interviewee characterization
   - 2.1. Type of industry or organization
   - 2.2. Name and position of the interviewee in the organization
   - 2.3. Interviewee's view on urine and human milk analysis
3. Urine Analysis
   - 3.1. What analytes/analyte groups should be measured with the novel monitoring devices and Why?
   - 3.2. What would be the measurement range of interest of this group of analytes/analyte the interviewee wants to measure and what is the needed accuracy?
   - 3.3. What is the interviewee thinking about the following analytes:

Table 1. Urine analytes suggested by end users.

Analyte	very useful	probably useful	not required	Comment
Ketones				
Protein				
Urea				
metabolic products (e.g. lysine, phenylalanine, histidin, etc.)				
Creatinine				
Glucose				
Phosphate				

3.4. Does the interviewee know other people with interest or expertise in urine analysis?
3.5. Technical aspects like needed LOD, working range, response time, measuring interval.
3.6. Technical restrictions for the instrument?

Very important feedbacks have been collected from external and internal (within the project) medical experts after being asked if phosphates, ketones, protein, urea, creatinine and glucose are of importance to measure.

A summary of their feedback is reported below:

- Calcium and phosphate are already measured. If phosphate is measured, the serum levels have to be known as well, otherwise the measurement is useless. If there is a lack of calcium and PTH (human parathyroid hormone) is high, phosphate is higher as well. This leads to an unreliable measurement.
- There is no need to look at protein, urea or creatinine in clinical routine.
- Glucose is checked in serum levels of preterm infants. However, glucose and ketone in urine is not a state of the art thing.
- Measuring phosphate is a routine analysis in urine of infants, besides calcium, urea, creatinine, potassium and sodium. Of those routine measurements calcium and phosphate are the ones related to nutrition. Human milk is fortified with those molecules if necessary.

Some more specific feedbacks are reported too:

Very useful:

- Albumin (added by OSR)
- Metabolic products (e.g. lysine, phenylalanine, histidine, short chain fatty acids etc.)
- Creatinine
- Glucose

Probably useful:

- Ketone
- Protein
- Phosphate
- NGAL (Neutrophil gelatinase associated lipocalin)
- IgG

2.1.1. Detailed description of the urine sample

Urine is a readily available biofluid that is frequently assessed in biomarker studies focusing on a wide range of different diseases [10]-[12]. Due to its special physiological role, high intra- and inter-individual fluctuations in composition are observed. This demonstrates the sensitivity of the urine to reflect normal physiological changes as well as abnormal pathological changes *in vivo*.

In blood, the homeostasis mechanism is crucial for maintaining the relatively constant physical/chemical properties of the internal environment *in vivo* essential for normal metabolism and various life activities. For maintaining blood homeostasis, a considerable number of molecules are cleared and excreted into urine in order to avoid possible damage or interference to the body. Hence, whereas many biomarkers can be detected only for a fairly short period of time *in vivo* in plasma, their detection is potentially less time-critical in urine.

From a practical perspective, the collection, storage and post-treatment process of urine samples is simpler and cheaper. In comparison to blood or plasma samples, urine is less prone to stability issues related to enzymatic degradation. Therefore, usually urine samples can be directly frozen and stored at -80 ^{0}C after collection without further pretreatment. The non-invasive character of urine samples allows serial determinations and hence offers the possibility of continuously monitoring the patient. In addition, bigger volumes can be obtained even from highly vulnerable patients such as preterm infants, allowing for pre-concentration of the biomarkers of interest before detection thereby directly enhancing the sensitivity of the detection method.

2.1.2. Chemical composition of the urine sample

The major part of urine is water (approximately 91-96%). Total solids in 24h urine samples are on average 59 g. Out of urine dry solids, organic matter makes up between 65% and 85% of, with volatile solids comprising 75–85% of total solids. Urea makes up for more than 50% of the total solids. Elementary analysis showed that on average human urine contains 6.87 g/L of carbon, 8.12 g/L of nitrogen, 8.25 g/L of oxygen, and 1.51 g/L of hydrogen [13]. Furthermore, urine also contains a range of inorganic salts and organic compounds, including proteins, hormones, and metabolites. More detailed information on the urinary metabolome can be found at http://www.urinemetabolome.ca/ [14]. The composition of urine is largely affected by physiologic (e.g. nutrition) and pathologic factors.

2.1.3. Typical concentration ranges

Table 2 shows a summary of biomarkers, detection method and normal concentration ranges as determined routinely in preterm infants.

Table 2. Biomarker, analytical methods and normal concentration ranges determined in preterm infants.

Biomarker	Method	Normal concentration range
Ca	colorimetric determination after reaction with 5-nitro-5'-methyl-(1,2-bis(o-aminophenoxy)ethan-N,N,N',N'-tetraacetic acid (NM-BAPTA)	8.5-10.5 mg dL^{-1}
P	colorimetric determination after reaction of inorganic P with Ammonium molybdate	3.9-6.5 mg dL^{-1}
Urea	2-step kinetic test; reaction with urease and glutamate dehydrogenase	10-50 mg dL^{-1}
Na	ion selective electrode	135-150 mEq L^{-1}
K	ion selective electrode	3.5-5.5 mEq L^{-1}
Creatinine	colorimetric test; reaction with picric acid (Jaffe method)	0.16-0.39 mg dL^{-1}

The urinary biomarkers mentioned in table 2 are routinely used by physicians for medical standard tests in preterm infants.

The innovation proposed in the Nutrishield project is to be able to measure with the QCL-based sensors (see paragraph 3.1.3) some important routine biomarkers (i.e. creatinine and phosphates) in order to generally evaluate the renal function of the patients.

2.1.4. Environmental conditions for the urine analysis

Urine samples are collected using sterile cotton pads placed in the diaper. Cotton pads are collected with tweezers and urine is expressed from the cotton pads using plastic syringes. Samples are aliquoted and immediately stored at -80 ^{0}C until further processing/measurement without any further preprocessing.

3. State of art of the proposed technology

3.1. Introduction to QCL-based sensing

3.1.1. Specifications

Current sensitivities of QCL-based sensing in aqueous solutions such as urine are estimated to be in the hundreds of mg/L range. The nutrishield sensor for urine will thus focus on two analytes: phosphate and creatinine. These analytes have been selected because i) they are relevant for the Nutrishield project and ii) based on their concentration range it is likely that they can be detected by a QCL-based sensor. Other analytes have also been identified, however, their relevant concentration ranges are prohibitively low to allow for a successful analysis based on direct mid-IR laser spectroscopy.

Developing a mid-IR spectroscopic sensor for quantification of analytes in complex matrices, such as human urine means two important steps have to be taken. First, wavelengths or wavelength ranges that allow to target the analyte(s) have to found. This step can be performed by using conventional mid-IR spectrometers. Second, light sources such as QCLs have to be found, that can be used to miniaturize the sensor.
For selecting the correct wavelength range for an analyte, monovariate or multivariate approaches are available. In a monovariate approach, only a single wavelength or an integrated absorption band is chosen that correlates with the concentration of the analyte and has no matrix interferences. However, for complex analytes, such as urine, often a multivariate approach is better. Here information from many wavelengths are taken into account. A machine learning algorithm is fed with a series of spectra

taken at various analyte and interferent concentrations and tasked to select the contribution of the analyte to the spectrum.
Once a multivariate model for the determination of the analyte has been established, this model can be used to determine the most significant wavelengths. Then QCLs emitting at these wavelengths can be selected. Here a compromise has to be found between the sensitivity of the method and the number of lasers that are required.
Finally, due to the large variation in urine concentration, concentrations in urine are typically either stated as 24 hour amounts or relative to creatinine concentration. Typical concentrations for infant phosphate concentrations lie in the range of 1.2 mol/mol (5th percentile relative to creatinine) to 19.0 mol/mol (95th percentile to creatinine) for the age group of one month to one year old [15]. The maximum typical urinary phosphate concentration decreases with age down to 2.7 mol/mol for the age group of 14-17 year old.
Basic chemical considerations and preliminary experiments demonstrate a strong pH dependence of the phosphate infrared spectrum. This issue can be overcome by ensuring a defined pH or a adding pH variation to the calibration dataset. Furthermore, at high pH phosphate can precipitate as calcium phosphate.

3.1.2. Instrument requirements

The sensor will be designed using a transmission absorption infrared spectroscopy design. Here the sample is placed in a flow cell between two infrared transparent windows. Measurements are performed by recording the transmission through the sample at a series of different wavelengths in the mid-IR spectral range. As the quantum cascade lasers intended for use in the detector emit monochromatic light, a simple detector placed after the sample cell suffices for detection.
Wavelength ranges Preliminary measurements on an FTIR instrument demonstrate that spectral features of phosphate can be found in the range of 900 cm^{-1} to 1200 cm^{-1} (see figure 1). Those of creatinine can be found between between 1000 cm^{-1} and 1400 cm^{-1} (see figure 2). In the same spectral range other major constituents of urine can also be found, such as urea and sulfate. The specific wavelengths needed for sensitive determination of phosphate and creatinine.
Optical materials Typical materials for optical windows for the mid-IR are sapphire, CaF_2, BaF_2, NaCl, KCl, ZnS, ZnSe, diamond, Si and KRS5. For selection of a window material for the nutrishield sensor several requirements have to be met:

1. optical properties
    a. high transmission in the required wavelength range
    b. refractive index similar to the sample to reduce reflections
2. chemical resistance
    a. no solubility in the sample
    b. no reactions with the sample
    c. no corrosion of the optical material at ambient conditions

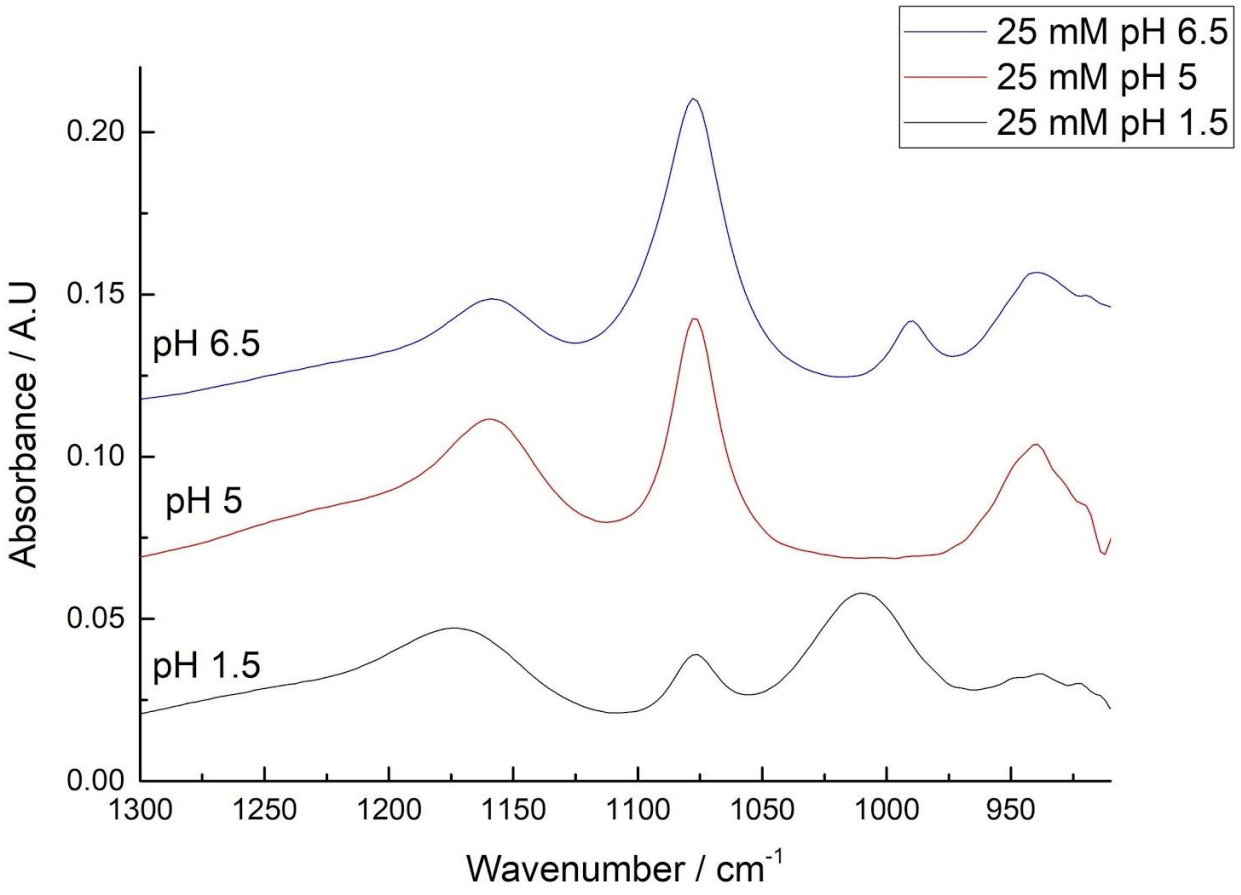

Figure 1. FTIR spectra of phosphate taken at a series of pH values. With different levels protonation the infrared spectrum of the phosphate ion changes.

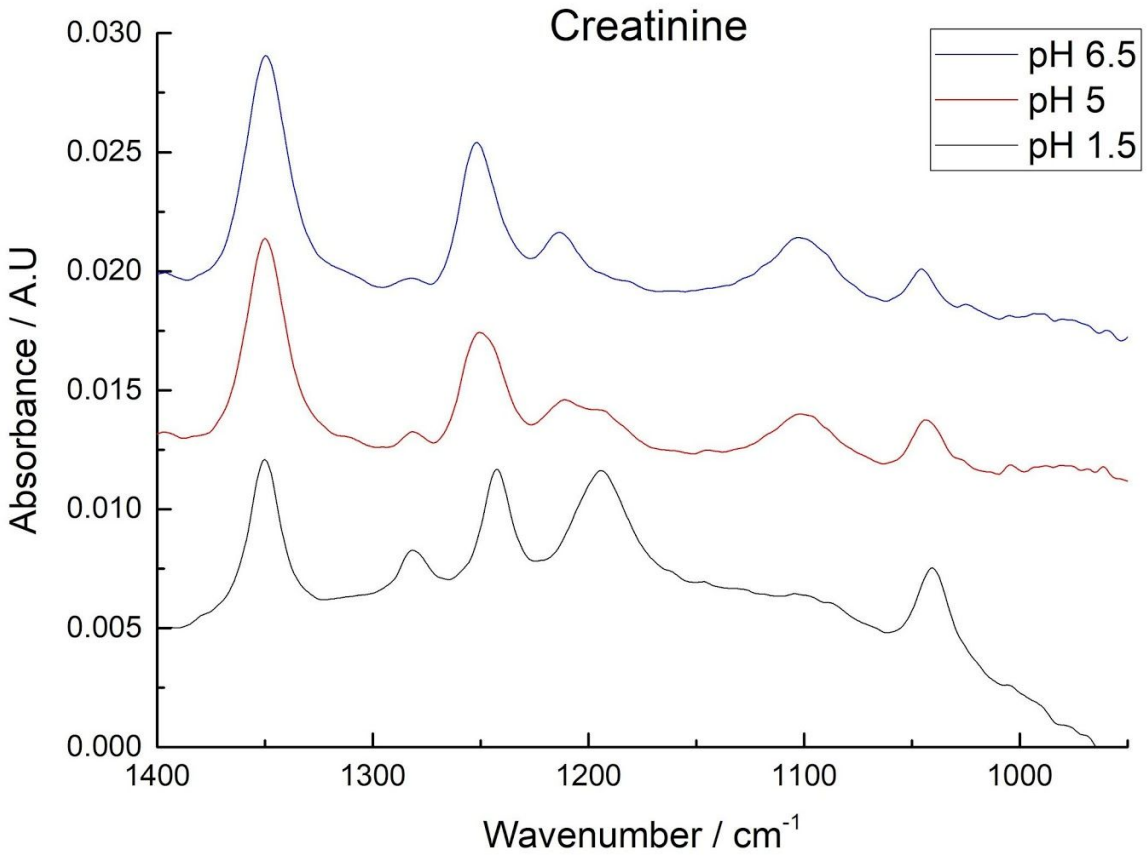

Figure 2. FTIR spectra of creatinine taken at a series of pH values. The mid-IR spectrum of creatinine shows some dependence on pH but the changes are less pronounced than those of phosphate.

The wavelength range needed for the sensor precludes the use of sapphire. ZnSe, KRS-5, ZnS, diamond and Si all have high refractive indices (n>2), significantly larger than the refractive index of the sample (n=1.3 - 1.4). NaCl and KCl have high solubility in water and corrode in humid air. Finally, BaF_2 has the necessary refractive index and transmission as well as the resistance to humidity. However, in presence of sulphate ions BaF_2 is dissolved and $BaSO_4$ is precipitated. The precipitate has a strong absorption in the range needed for the nutrishield sensor (see figure 3). Hence direct contact between the liquid and the sample has to be avoided. Here thin layer of polymer, e.g. PE on the sample side of the flow cell. Alternatives are furthermore CaF_2 being less water soluble than BaF_2 but with a reduced transmission range and diamond. Especially concerning the latter a small size is required due to cost issues. A wedged window or antireflection coating will be required in case window materials with refractive index > 2 are used to avoid fringes in the transmission configuration.

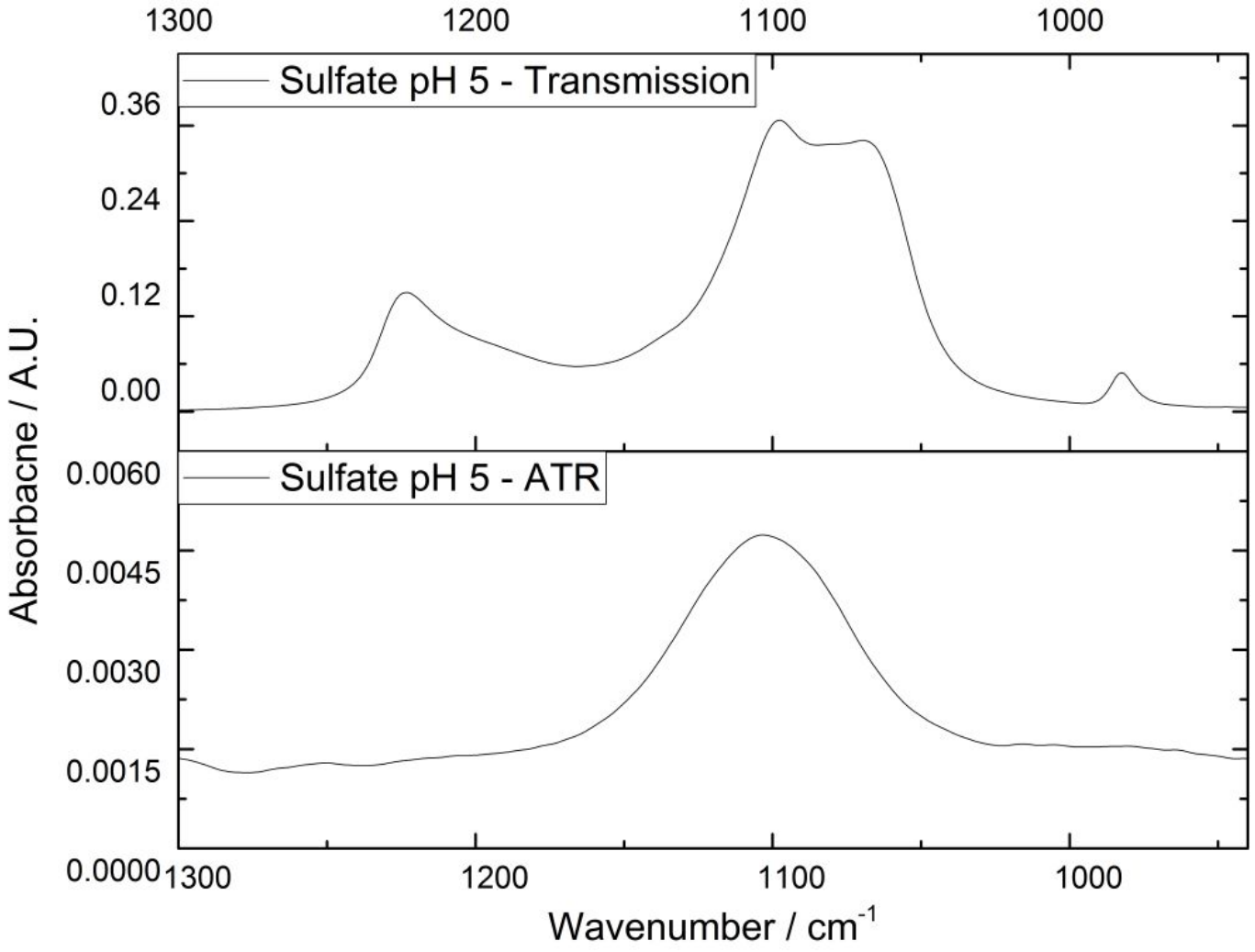

Figure 3. FTIR-ATR spectra of sulfate. The mid-IR spectrum of sulphate taken in a cell with BaF_2 and on a diamond attenuated total reflection (ATR) optical element. The BaF_2 windows react to $BaSO_4$.

In-line sample preconcentration: In the research to be conducted mesoporous silica with pore sizes of approximately 4 to 13 nm will be applied to the window material of the flow-cells used by the spin-coating procedure. The thickness of these layers will be a few hundreds of nanometers. The surface chemistry of the pores can be adjusted to the needs of analysis. Immobilizing anionic change moieties, such as quaternary ammonium ions, will allow to preconcentrate anions including phosphate from the sample to be analysed. In this regard a compromise for the applied thickness of the mesoporous material needs to be made. Larger thickness will increase the achievable analyte preconcentration, however, it will also cause a reduced transmission due to absorption of mid-IR radiation by the mesoporous material itself. It is expected that sensitivity can be increased by one order of magnitude. Similarly, application of hydrophobic moieties on the pores surface, such as C18, will allow to preconcentrate hydrophobic material from the sample. Likewise, contribution of the matrix molecules not targeted by the analysis will be reduced in the overall spectrum, thus simplifying quantitative analysis. In any case the selectivity of the possible recognition mechanism will not provide high

specificities toward a single target analyte in the presence of representatives of the same substance class. Therefore it is expected that also concerning this approach chemometric data analysis will be required. The applicability of this approach will be evaluated in more detail during Nutrishield.

3.1.3. Advantages compared to standard analytical techniques

Conventionally, phosphate is measured via reaction with ammonium molybdate leading to a phosphomolybdate complex that is then quantified using photometry. In PoC applications creatinine is determined using dipstick test, which are based on enzymatic reactions and either colorimetric or electrochemical detection principles. The most common colorimetric assay for creatinine is based on the Jaffe reaction. However, this reaction suffers from non-specificity and spectral interferences [15]. For both analytes, reagents and consumables are needed. In contrast, the nutrishield urine sensor can be designed to be reagent free if a calibration for a range of pH values is performed. Furthermore, the sensor will allow parallel determination of two analytes in a single measurement. Mid-IR spectroscopy is a generic measurement principle that allows to further extend the nutrishield sensor to additional analytes for parallel determination by adding additional wavelengths/QCLs. Finally, by being able to sense creatinine, the nutrishield sensor can provided absolute as well as creatinine referenced concentrations.

3.1.4. Sample preparation

No sample preparation is needed for the nutrishield sensor if a calibration at multiple pH values is performed. If such a calibration is not performed, the sample has to be held at a defined pH during measurement. This is achieved by adding a small aliquot of either a buffer or a strong acid, such as HCl before the measurement. If creatinine referenced measurements are determined, the precise volume of buffer added is not important, as phosphate and creatinine are determined in parallel from the same solution.

3.2. Electrochemical sensors

3.2.1. Introduction

Electrochemical (EC) sensors have proved to be specific, selective and easy to use in the determination of metabolites in diagnostics. This is also the reason why, EC sensors is the most growing class of bio/chemical sensors used in clinical diagnostics. In principle, a bio/chemical sensor is able to give a response (signal) that is directly related to the quantity of a specific bio/chemical species.

Regarding the development of novel sensory platforms based on EC sensors, CSEM's vision has been focused on simplicity, robustness, versatility and low-cost production. These very important criteria are completely covered by a synergic action of non-invasive sampling and mass production of robust and reproducible sensors integrated in a disposable technology in which CSEM has a high expertise.

The bio/chemical sensors mainly consist of two essential units: a transducer able to transform the biochemical response into a detectable signal (i.e. voltage, current) using a modern instrumentation, and a selective biochemically modified layer, which isolates the response of the analyte of interest from its natural environment (i.e. biological fluids).

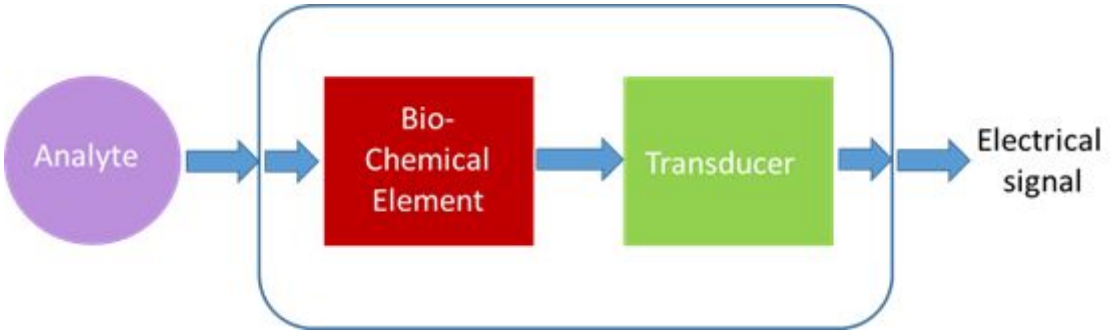

Figure 4. Electrochemical bio-chemical sensor components.

3.2.2. Advantages compared to standard analytical techniques

The advantage of using electrochemical biosensors can be summarized as follows:

- The sample can be processed immediately after collection
- It does not require complex separation steps and

- The response time is in few minutes or seconds.
- Specificity of the immobilized bio/chemical layer
- Selectivity of the electrochemical transducer, which makes the sensor suitable for measurements in biological fluids.

An additional advantage of electrochemical detection regards the simplicity of the instrumentation that lead to low electrical power requirements. The latter is a crucial factor especially for in-field use.

CSEM has developed a powerful know-how based on the use of thin layer technology. The advantages of using this technology regard the implementation of low-cost and versatile materials; screen-printed carbon electrodes are an example and perfectly compatible for electrochemical detection due to:

- Low cost production,
- Disposability and versatility
- Flexibility in design
- Customized chemical modification

Figure 5. Example of CSEM screen printed sensors

3.2.3. Specifications

At CSEM a plethora of amperometric (i.e. glucose, uric acid) biosensors and potentiometric (i.e. pH, sodium, potassium and magnesium) sensors based on screen-printing technology have been successfully developed and tested for the monitoring of the abovementioned clinically relevant biomarkers within body fluids such as urine, saliva and sweat. In table 3 a list of disposable EC sensors with specifications is reported. The sensors mentioned below could be considered a good alternative to the standard methods and for this purpose are proposed in the present deliverable.

Table 3. List of CSEM disposable EC sensors.

Analyte	Concentration range available, and calibration medium
pH	3-11, phosphate/citrate buffer saline
glucose	(0.05 - 5) mM, in 0.05 M phosphate buffer + 0.1 M KCl pH 7.4
Uric acid	(0.03 - 0.5) mM, in PBS
K^+, Na^+	typical range 10^{-1} M to 10^{-5} M in aqueous solutions

3.2.4. Instrument requirements and sample preparation

All the electrochemical measurements using the CSEM fabricated EC sensors have been performed using a battery powered Palmsens 4 Potentiostat/Galvanostat instrument supplied by Palmsens BV (https://www.palmsens.com/product/palmsens4/)

Figure 6. Instrumentation used for the tests.

Preliminary experiments performed in urine samples using pH, potassium and sodium sensors demonstrated that no sample treatment is required. As for the enzymatic sensors (i.e. glucose and uric acid) a slight sample preparation (i.e. dilution and filtration) is highly recommended in order to obtain accurate measurements.

CSEM has also developed a customized sensor system for saliva monitoring that includes sample collection, preparation and analysis modules.

Figure 7. Schematic representation of CSEM activity for a complete sample analysis. Example of saliva specimen processing and steps: A) Collection, B1-B2) Pre-treatment of the sample and delivery to the sensing cartridge, C) Tailored electrochemical read out able to transform the chemical signal in electrical and wireless data transmission to user-friendly interfaces.

A similar model of analysis can be adopted for urine monitoring if needed. Such model is going to be a laboratory-based tool of analysis complementary to the Nutrishield prototype. The goal to be reached is that the sensitivity of CSEM sensors has to be comparable and/or equal, if not higher, to the standard methods (see table 2) used by HULAFE for the analysis of the same biomarkers. In this case CSEM sensors could be used for the urine measurements according to the HULAFE protocols.

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