Electrochemical sensor system comprising molecularly imprinted polymer for early warning of urinary tract infections

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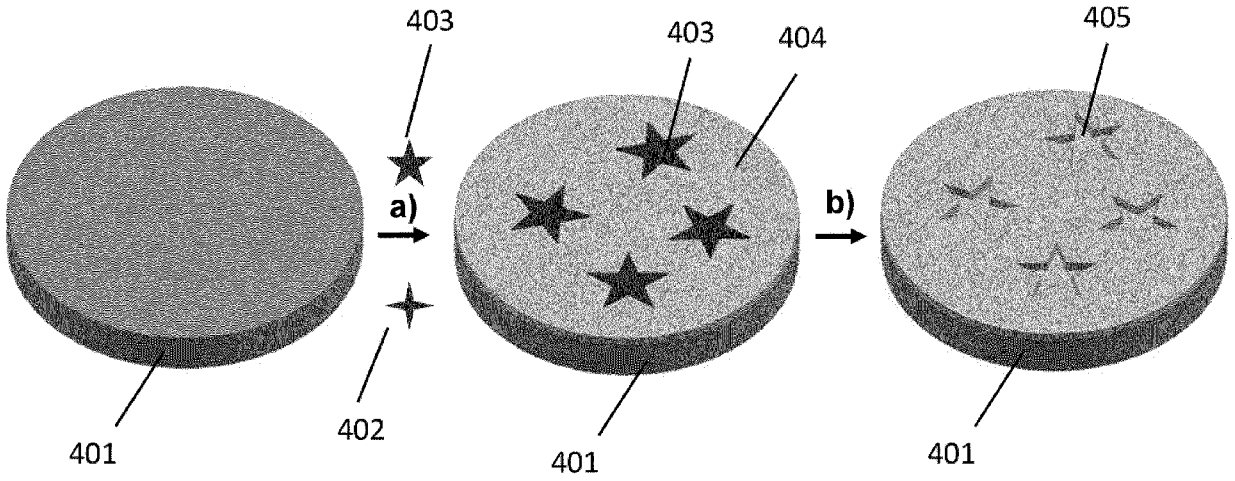

Fig. 4

(57) **Abstract:** A system for specific detection of lactoferrin comprises a molecularly imprinted polymer (404) imprinted with a lactoferrin template (403) may be used in the field of intelligent point-of-care health monitoring systems. More specifically, the system may be used towards for specific detection of lactoferrin in e.g. urine samples for early warnings of urinary tracts infections by integrating the system in an urinary bag, a catheter or an adaptor. Molecularly imprinted polymers (MIPs) specific to lactoferrin may be grafted on a working electrode (401) by electro polymerization. A mixture of a functional monomer (402), an electrolyte, and a lactoferrin template (403) may be deposited on the working electrode (401).

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ELECTROCHEMICAL SENSOR SYSTEM COMPRISING MOLECULARLY IMPRINTED POLYMER FOR EARLY WARNING OF URINARY TRACT INFECTIONS

Technical Field

The disclosure herein relates to the field of intelligent point-of-care health monitoring systems. More specifically, towards a system for specific detection of lactoferrin in e.g. urine samples for early warnings of urinary tracts infections.

Background

Urinary tract infections (UTIs) are among the most common diseases of humans (estimated to be nearly 25% of all the infections), and large numbers of urine specimens are processed daily in most clinical microbiology laboratories. Most screenings of these specimens for microorganisms is a repetitive procedure with a quantitative distinction between positive and negative results. The majority of the test results are negative, leaving positive specimens for further processing. Most such tests are relatively simple and reliable but require an overnight incubation of cultures before results are available. If left undiagnosed and untreated, UTI may spread up to the ureters and the kidneys, and leads to complicated infection which can be accompanied by urinary tract obstruction and renal failure.

For patients such as catheter users, UTIs are assessed as the greatest challenge, and getting a UTI has become a daily concern. In the current paradigm, only symptomatic UTIs draw the attention of doctors, and antibiotic treatment is usually promoted. However, at this stage, patients have already been suffering from symptoms like pelvic pressure, abdominal discomfort, and/or painful urination. In addition, high costed service of medical professionals also poses a significant healthcare burden.

Evidence has shown that a symptomatic UTI is often preceded by an asymptomatic pre-UTI stage, which can be detected by the presence of specific predictive biomarkers.

A promising strategy to improve the management of UTIs is to develop an early warning system that can identify the pre-UTI condition.

Various biosensors such as electrochemical sensors and optical sensors have been demonstrated. Most of these biosensors rely on antibodies to selectively bind to an analyte. Although such system (a bioreceptor system) possess high specificity and affinity, they are expensive and inherently unstable due to the biological origins. Additionally, the high cost and unreliability of such biosensors have prevented patients from using them at a Point-Of-Care (POC) setting. Moreover, the stand¬alone biosensors are not patient specific and cannot provide advice on the risk of a potential UTI.

For the above reasons, there is currently still a need for a rapid report of results leading to diagnosis of UTI, and especially diagnosis of early warnings in connect with a developing UTI.

Summary

Disclosed herein in a **first** aspect is a system for specific detection of lactoferrin, the system comprising an electrochemical sensor, a potentiostat, and a microprocessor, the electrochemical sensor comprising a working electrode, a counter electrode, and a reference electrode, wherein the working electrode comprises a molecularly imprinted polymer imprinted with a lactoferrin template, and wherein the electrochemical sensor is connected to the potentiostat and the potentiostat is connected to the microprocessor.

Lactoferrin, also known as lactotransferrin, is a multifunctional protein of the transferrin family. Lactoferrin is a globular glycoprotein with a molecular mass of about 80 kDa that is widely represented in various secretory fluids, such as milk, saliva, tears, and nasal secretions. Lactoferrin is also present in secondary granules of PMNs and is secreted by some acinar cells. Lactoferrin can be purified from milk or produced recombinant.

Lactoferrin is one of the components of the immune system of the body. Lactoferrin belongs to the innate immune system. Apart from its main biological function, namely binding and transport of iron ions, lactoferrin also has antibacterial, antiviral, antiparasitic, catalytic, anti-cancer, and anti-allergic functions and properties. In particular, lactoferrin provides antibacterial activity to human infants. Lactoferrin interacts with DNA and RNA, polysaccharides, and heparin, and shows some of its biological functions in complexes with these ligands.

The urinary lactoferrin secreted by polymorphonuclear white blood cells (WBC), is an important predictive UTI biomarker. The average concentration of lactoferrin is around 30 ng/ml in healthy urine and 3.3 pg/ml in infected urine.

Measuring the levels of lactoferrin in urine could therefore be a method to indicate the risk of a potential UTI.

Specific detection of lactoferrin is detection of lactoferrin and closely structure related compounds (e.g., similar molecular weight or similar isoelectric point value) such as human serum albumin (HSA), haemoglobin and coagulation factor are not detected with the present system.

A simple electrochemical sensor system based on molecular imprinted polymers may serve as a basis for a cheap and simple sensor for early warning of urinary tract infections. A simple electrochemical sensor may be utilized in a POC setting, making it easy to use by the patient and

cheap to produce. However, if using a simple electrochemical sensor, it is very important to obtain a surface imprinting and a control of the thickness of the polymer layer to increase the reproducibility of the measurements, therefore the imprinting technique to be used for production of such sensor must be able to do such. Furthermore, it is important to use a polymerization technique, which will deposit the molecularly imprinted polymer on the working electrode, such as attached in as a layer onto the electrode.

In contrast to conventional biosensors where antibodies are utilized, the simple electrochemical sensor system employs molecularly imprinted polymers (MIPs) as biomimetic receptors for target recognition. Unlike their biological counterparts they have high robustness and stability under a wide range of conditions, the recognition sites for the specific detection is easy to design, and it is low in production cost.

In biomimetics, living systems are imitated to develop receptors for ions, molecules, and bioparticles. The most pertinent idea is self-organization in analogy to evolution in nature, which created the key-lock principle. Today, modern science has been developing host-guest chemistry, a strategy of supramolecular chemistry for designing interactions of analytes with synthetic receptors. This can be realized, e.g., by self-assembled monolayers (SAMs) or molecular imprinting. The strategies are used for solid phase extraction (SPE), but preferably in developing recognition layers of chemical sensors.

An electrochemical sensor is a device that gives information about a composition by coupling a chemically selective layer/or a recognition element, to an electrochemical transducer. In this way, the chemical energy of the selective interaction between the target species and the sensor is transduced into an analytically useful signal.

A working electrode is an electrode in an electrochemical system on which the reaction of interest is occurring. The working electrode is often used in conjunction with an auxiliary electrode, and a reference electrode in a three electrode system. Depending on whether the reaction on the electrode is a reduction or an oxidation, the working electrode is called cathodic or anodic, respectively. Common working electrodes can consist of materials ranging from inert metals such as gold, silver or platinum, to inert carbon such as glassy carbon, boron doped diamond or pyrolytic carbon, and mercury drop and film electrodes.

A counter electrode, also known as an auxiliary electrode, is an electrode used in a three electrode electrochemical cell for voltammetric analysis or other reactions in which an electric current is expected to flow. The counter electrode is distinct from the reference electrode, which establishes

the electrical potential against which other potentials may be measured, and the working electrode, at which the cell reaction takes place.

A reference electrode is an electrode which has a stable and well-known electrode potential. The high stability of the electrode potential is usually reached by employing a redox system with constant, buffered or saturated, concentrations of each participant of a redox reaction.

Molecular imprinting is a technique to create template-shaped cavities in polymer matrices with memory of the template molecules to be used in molecular recognition. This technique is based on the system used by enzymes for substrate recognition, which is called the "lock and key" model. The active binding site of an enzyme has a unique geometric structure that is particularly suitable for a substrate. A substrate that has a corresponding shape to the site is recognized by selectively binding to the enzyme, while an incorrectly shaped molecule that does not fit the binding site is not recognized. A molecularly imprinted polymer imprinted with a lactoferrin template is a polymer wherein the created template-shaped cavity is created by imprinting the polymer matrix with lactoferrin templates. After removal of the lactoferrin template the polymer matrix is fit to recognise lactoferrin.

The analog part of the system consists of a potentiostat including the working electrode, counter electrode, and reference electrode. The potentiostat sets the voltage between the working electrode (WE) and the reference electrode (RE) in the sample under test, by providing the necessary current through the counter electrode (CE). The usage of the potentiostat is to apply a voltage and to record the current response that is characteristic of the sample being tested.

The microcontroller (i.e. the microprocessor) generates the voltage waveform for the potentiostat to perform the electrochemical measurements and acquires the data. By using a microcontroller low cost, low power consumption, and sufficient computation capability is achieved. The microprocessor may also be used to both generate required voltage signals and communicate with an end user. Using microcontroller for both data acquiring and transmitting makes the system portable, minimizes the size of the device, and reduces the power consumption.

Disclosed herein in a second aspect is a method for producing an electrochemical sensor for specific detection of lactoferrin, wherein the electrochemical sensor comprises a working electrode comprising a molecularly imprinted polymer, and wherein the working electrode is prepared by a method comprising the steps of:

- mixing a composition comprising a functional monomer, an electrolyte, and a lactoferrin template;
- depositing the composition on the working electrode;

- electropolymerization of the composition deposited on the working electrode using cyclic voltammetry, and
- leaching the lactoferrin template.

By using molecularly imprinted polymers (MIPs) as a biomimetic receptor, the biosensor provides high robustness and stability, long shelf life, easy storage, and low production cost.

Electropolymerization is a coating procedure wherein a conducting polymer is formed and deposited from a monomer solution onto a conducting substrate. This may be the method of choice in coating relatively small areas. Care must be taken in choosing the electropolymerization condition, especially the applied potential and current. The applied potential should be high enough to oxidize the monomer and polymerize it, but low enough not to dissolve the metal or induce corrosion.

Cyclic voltammetry is a type of potentiodynamic electrochemical measurement. In a cyclic voltammetry experiment, a working electrode potential is ramped linearly versus time. After the set potential is reached in a cyclic voltammetry experiment, the working electrode's potential is ramped in the opposite direction to return to the initial potential. These cycles of ramps in potential may be repeated as many times as needed.

By the term “leaching the lactoferrin template” is meant the process of extracting the lactoferrin template from a solid, the working electrode, by dissolving it in a liquid. This dissolving process can be assisted/speeded up by e.g. addition of an alkaline substance.

Disclosed herein in a **third** aspect is a system for use in diagnosis of early stages urinary tract infections, wherein the system comprises an electrochemical sensor, a potentiostat, and a microprocessor, the electrochemical sensor comprising a working electrode, a counter electrode, and a reference electrode, wherein the working electrode comprises a molecularly imprinted polymer imprinted with a lactoferrin template, and wherein the electrochemical sensor is connected to the potentiostat and the potentiostat is connected to the microprocessor.

Using a simple and cheap molecular imprinted sensor for detection of lactoferrin may give the patient and earlier indication of urinary tract infections. An earlier warning of UTI may benefit the patient. By earlier indications of UTI, the patient can start on e.g. an antibiotic treatment, which can avoid the patient from suffering from symptoms like pelvic pressure, abdominal discomfort, and/or painful urination. In addition, the high cost for a medical professional may be significantly reduced as less time is spend on each patient and sample.

In addition, when the users get an early warning of a potential UTI, they could initiate steps to avoid it (e.g. increase fluid intake, change catheters more frequently, etc.). As such, early warning could reduce the risk of UTIs, thus reducing the use of antibiotics.

Disclosed herein in a **fourth** aspect is a system for use in diagnosis of Sjogren syndrome, wherein the system comprises an electrochemical sensor, a potentiostat, and a microprocessor, the electrochemical sensor comprising a working electrode, a counter electrode, and a reference electrode, wherein the working electrode comprises a molecularly imprinted polymer imprinted with a lactoferrin template, and wherein the electrochemical sensor is connected to the potentiostat and the potentiostat is connected to the microprocessor.

Sjogren syndrome is a long-term autoimmune disease that affects the body's moisture-producing glands. Primary symptoms are a dry mouth and dry eyes. Other symptoms can include dry skin, vaginal dryness, a chronic cough, numbness in the arms and legs, feeling tired, muscle and joint pains, and thyroid problems. Those affected are at an increased risk (5%) of lymphoma.

Lactoferrin is secreted into tears by the lacrimal gland. Tear lactoferrin level has been reported to be an indicator of lacrimal secretory function. Studies also reported that tear lactoferrin level correlated with the severity of conjunctivocorneal epithelial lesions in patients with primary, secondary, and non-Sjogren syndrome dry eyes. Sjogren syndrome can be diagnosed by analyzing the concentration of lactoferrin in tear fluid, as lactoferrin levels in tear fluid have been shown to decrease in a person diagnosed with Sjdgren syndrome.

Therefore, a system which is used for diagnosis of Sjogren syndrome comprising an electrochemical sensor that measures lactoferrin levels in human tear fluid can enable fast, cost-effective diagnosis of Sjogren syndrome.

Disclosed herein in a **fifth** aspect is a system for use in diagnosis of lactoferrin levels in tears, urine, and/or saliva, wherein the system comprises an electrochemical sensor, a potentiostat, and a microprocessor, the electrochemical sensor comprising a working electrode, a counter electrode, and a reference electrode, wherein the working electrode comprises a molecularly imprinted polymer imprinted with a lactoferrin template, and wherein the electrochemical sensor is connected to the potentiostat and the potentiostat is connected to the microprocessor.

Such a system comprising a sensor also allows for the fast diagnostics of other forms of disease, such as dry eye diseases at the point-of-care settings.

Disclosed herein in a sixth aspect is the use of a system comprising an electrochemical sensor, a potentiostat, and a microprocessor, the electrochemical sensor comprising a working electrode, a counter electrode, and a reference electrode, wherein the working electrode comprises a molecularly imprinted polymer imprinted with a lactoferrin template, and wherein the electrochemical sensor is connected to the potentiostat and the potentiostat is connected to the microprocessor, for specifically detecting lactoferrin in non-medical use, such as detecting lactoferrin in liquid dairy products.

The lactoferrin in milk promotes the development of the immune system in infants. Lactoferrin is also involved in the transport of iron. Lactoferrin is an important additive in food such as infant formula. Therefore, a system comprising the electrochemical sensor for specifically detecting lactoferrin can be used to rapidly and accurately measure lactoferrin in milk/dairy products for quality control.

Disclosed herein in a seventh aspect is the use of a system comprising an electrochemical sensor, a potentiostat, and a microprocessor, the electrochemical sensor comprising a working electrode, a counter electrode, and a reference electrode, wherein the working electrode comprises a molecularly imprinted polymer imprinted with a lactoferrin template, and wherein the electrochemical sensor is connected to the potentiostat and the potentiostat is connected to the microprocessor, for specifically detecting lactoferrin in water samples obtained from a water drilling well.

Detection of lactoferrin in water samples, may be performed on the site of the water drilling, and can hence serve to perform an initial quality control if the water is contaminated with bacteria, and the water drilling can be put in quarantine until further analysis have been performed on the drilling site.

Disclosed herein in an eights aspect is an electrochemical sensor for specific detection of lactoferrin, the electrochemical sensor comprises a working electrode, a counter electrode, and a reference electrode, wherein the working electrode comprises a molecularly imprinted polymer imprinted with a lactoferrin template.

An electrochemical sensor utilizing molecular imprinted polymers may serve as a basis for a cheap and simple sensor for early warning of urinary tract infections. An electrochemical sensor may be utilized in a POC setting, making it easy to use by the patient and cheap to produce.

The molecular imprinted polymer based electrochemical sensor include high selectivity, low cost, simplicity of use for the specific target analysis, short diagnostic time, availability for long-term monitoring, and easy integration in compact analytical devices.

Disclosed herein in a **ninth** aspect is a catheter comprising an electrochemical sensor for specific detection of lactoferrin, the electrochemical sensor comprising a working electrode, a counter electrode, and a reference electrode, wherein the working electrode comprises a molecularly imprinted polymer imprinted with a lactoferrin template.

By having a catheter comprising a sensor for detection of lactoferrin, a catheter user can be early warned about a urinary tract infection. UTI is very common for catheter. For patients such as catheter users, UTIs are assessed as the greatest challenge, and getting a UTI has become a daily concern. With the early warning system, catheter users can take necessary intervention to prevent the development of UTIs, thereby reducing the clinical consequences as well as patient concerns of UTIs.

A catheter is a thin tube made from medical grade materials serving a broad range of functions. Catheters are medical devices that can be inserted in the body to treat diseases or perform a surgical procedure. By modifying the material or adjusting the way catheters are manufactured, it is possible to tailor catheters for cardiovascular, urological, gastrointestinal, neurovascular, and ophthalmic applications.

Disclosed herein in a **tenth** aspect is a urinary bag comprising an electrochemical sensor for specific detection of lactoferrin, the electrochemical sensor comprising a working electrode, a counter electrode, and a reference electrode, wherein the working electrode comprises a molecularly imprinted polymer imprinted with a lactoferrin template.

By having a urinary bag comprising a sensor for detection of lactoferrin, a catheter user can again be early warned about a urinary tract infection as described above.

A urinary bag collects urine by being attached to the catheter. The attachment of the urinary bag to the catheter may be through one or more connection adaptors.

Connecting an electrochemical sensor directly to a urinary bag via one or more connection adaptors can offer an easy to use system for patients, simplified measurement/sampling procedure, continuous monitoring, and reduced risk for contamination.

Disclosed herein in an **eleventh** aspect is a catheter and urinary bag assembly adaptor comprising an electrochemical sensor for specific detection of lactoferrin, the electrochemical sensor comprising a working electrode, a counter electrode, and a reference electrode, wherein the working electrode comprises a molecularly imprinted polymer imprinted with a lactoferrin template.

By using an assembly adaptor comprising the sensor, the catheter use avoids changing the sensor unit every time the catheter or bag needs to be replaced. Alternative, the sensor can be changed without removing the catheter from the user.

The assembled adaptor and sensor provides a closed system, so that the user does not need to get into contact with the sample, which greatly simplifies the sampling procedures.

Disclosed herein in a **twelfth** aspect is an adaptor comprising an electrochemical sensor for specific detection of lactoferrin, the electrochemical sensor comprising a working electrode, a counter electrode, and a reference electrode, wherein the working electrode comprises a molecularly imprinted polymer imprinted with a lactoferrin template, and wherein the adaptor is suitable for attaching at least the working electrode, the counter electrode, and the reference electrode to a catheter and to a urinary bag such that the working electrode, the counter electrode, and the reference electrode are positioned in-between the catheter and the urinary bag.

An adaptor integrating with an electrochemical sensor allows for monitoring of urine status in real time, simplifying measurement procedures, and reducing introduction of bacteria.

Disclosed herein in a **thirteenth** aspect is a system for specific detection of lactoferrin, the system comprising an electrochemical sensor, a potentiostat, and a microprocessor, the electrochemical sensor comprising a working electrode, a counter electrode, and a reference electrode, wherein the working electrode comprises a molecularly imprinted polymer imprinted with a lactoferrin template, and wherein the electrochemical sensor is connected to the potentiostat and the potentiostat is connected to the microprocessor, wherein the molecularly imprinted polymer is a molecularly imprinted polymer imprinted by electropolymerization.

Disclosed herein in a **fourteenth** aspect is a system for specific detection of lactoferrin, the system comprising an electrochemical sensor, a potentiostat, and a microprocessor, the electrochemical sensor comprising a working electrode, a counter electrode, and a reference electrode, wherein the working electrode comprises a molecularly imprinted polymer imprinted with a lactoferrin template, and wherein the electrochemical sensor is connected to the potentiostat and the potentiostat is connected to the microprocessor, wherein the electrochemical sensor is fabricated using a method

for producing an electrochemical sensor for specific detection of lactoferrin, wherein the working electrode is prepared by a method comprising the steps of:

- mixing a composition comprising a functional monomer, an electrolyte, and a lactoferrin template;
- depositing the composition on the working electrode;
- electropolymerization of the composition deposited on the working electrode using cyclic voltammetry, and
- leaching the lactoferrin template.

Description

The description herein of any aspect or embodiment using terms such as “comprising”, “having,” “including,” or “containing” with reference to an element or elements is intended to provide support for a similar aspect or embodiment of the invention that “consists of, “consists essentially of, or “substantially comprises” that particular element or elements, unless otherwise stated or clearly contradicted by context, e.g. a composition described herein as comprising a particular element should be understood as also describing a composition consisting of that element, unless otherwise stated or clearly contradicted by context. It will be further understood that the terms “comprises," "comprising," "includes" and/or "including," when used in this specification, specify the presence of stated features, integers, steps, operations, elements, and/or components, but do not preclude the presence or addition of one or more other features, integers, steps, operations, elements, components, and/or groups thereof.

As used herein, the singular forms “a,” “an," and “the” are intended to include the plural forms, including “at least one,” unless the content clearly indicates otherwise. “At least one” is not to be construed as limiting “a” or “an.”

The use of any and all examples, or exemplary language (e.g., “such as”) provided herein, is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention unless otherwise claimed. No language in the specification should be construed as indicating any non-claimed element as essential to the practice of the invention.

This invention includes all modifications and equivalents of the subject matter recited in the claims and/or aspects appended hereto as permitted by applicable law.

Unless otherwise defined, all terms used herein (including technical and scientific terms) have the same meaning as commonly understood by those skilled in the art to which this invention pertains. It will be further understood that terms, such as those defined in commonly used dictionaries, should be interpreted as having a meaning that is consistent with their meaning in the context of

the relevant art and will not be interpreted in an idealized or overly formal sense unless expressly so defined in the present specification.

When describing the below embodiments, the present invention envisage all possible combinations and permutations of the below described embodiments with the above disclosed aspects.

In one or more embodiments, the molecularly imprinted polymer is a molecularly imprinted polymer imprinted by electropolymerization.

As disclosed, electropolymerization is a coating procedure wherein a conducting polymer Is formed and deposited from a monomer solution onto a conducting substrate. Electrosynthesis of molecularly imprinted polymers takes place via a process called electropolymerization. Electropolymerization is a deposition procedure in which a conductive polymer layer is formed or coated upon an electrode/supporting substrate material in the presence of the desired template. Such electrochemical coatings are carried out utilizing a typical three-electrode setup, where a working electrode (i.e. where the coating occurs), reference electrode, and a counter electrode are used within a solution containing a monomer, solvent, and a supporting electrolyte; all of which are vital to the surface morphology of the polymeric film.

Electropolymerization, as herein disclosed for fabrication for the electrosynthesis of the molecularly imprinted polymer layers, is a technique were the sweeping of a set range of potentials, via cyclic voltammetry, between the limits of the monomer oxidation and the reduction of the polymerized polymer occurs. Upon control of the voltage range, the molecularly imprinted polymer layer can be fabricated with varying thicknesses and polymer oxidation. This technique also allows the user to control the speed/sweep rate at which the monomer reacts at the electrode surface.

To ensure a specific binding, the thickness and compactness of the molecularly imprinted polymer layer Is very Important. The thickness can be adjusted by the number of cycles during electropolymerization. The more cycles of electropolymerization, the thicker the molecularly imprinted polymer layer will be. The compactness can be tailored by the scan rate of the electropolymerization. If the scanning process (scan rate) is decreased, the rigidity of the molecularly imprinted polymer layer is increased, which may be implying better replica of the protein structure in the molecularly imprinted polymer layer. The scan rate is normally defined in millivolt per second (mV/s).

An alternative and cheap method of creating a molecularly imprinted polymer is be utilizing a chemical method to synthesize the molecularly imprinted polymer. In a chemical method, a bulk polymer is formed by crosslinking a functional monomer (e.g. vinylpyridine) with a template. The

template can then be washed away, leaving cavities for separating out a molecule (the template molecule) in a solution.

Molecularly imprinted chromatography is one of the most traditional applications of molecularly imprinted polymers, especially for liquid chromatography. For this, several synthetic protocols have been developed. Since the first appearance of molecularly imprinted polymers, the most widely-used synthetic protocol has been traditional polymerization. Traditionally, molecular imprints have mostly been made by bulk polymerization. In this process, polymerizable functional monomer (most frequently-methacrylic acid) is prearranged around a template molecule in organic solvent (e.g. chloroform, acetonitrile). The resulting prepolymer complexes are copolymerized with an excess of crosslinker (e.g. ethylene glycol dimethacrylate) in the presence of a free radical initiator under thermal or photochemical conditions. The block of resulting polymer is then ground and sieved by hand in order to produce particles of an appropriate size for experiments. After the removal of the template by extraction, binding sites complementary to the template molecule both in shape and chemical functionality are left within the polymer matrices that allow rebinding of the template with quite specificity.

However, the molecular imprinted polymers prepared by traditional method have a number of disadvantages, such as being a time-consuming and complicated preparation process, resulting in less recognition sites inside matrices particles, which in turn provides poor binding capacity and lower binding kinetic of molecularly imprinted polymers for the template molecules. Moreover, the non-regular shape of the particles obtained by bulk polymerization can cause broad asymmetric peaks when using it as stationary phase in LC, CEC or in on-line coupling with LC. Further, the mechanical processing leads to irregular particles with relatively broad size distribution, resulting in packing of irreproducible quality. Additionally, bulk polymerization obtains molecularly imprinted polymers, which requires grinding, require high amount of porogen agent, and the binding sites can be destroyed during the molecularly imprinted polymer elaboration protocol. Overall, this method is not suitable for use in an electrochemical sensor due to at least the above reasons, and due to the fact that this method cannot be used to deposit a molecularly imprinted polymer on a working electrode, which is required for an electrochemical sensor as disclosed herein.
Electropolymerization creates a layer of molecular imprinted polymer on a substrate; while chemical synthesis gives nanoparticles or bulk polymer. In general, chemically synthesized molecularly imprinted polymet cannot be coated on an electrode; in addition, polymers used in chemical synthesis do typically not possess electrochemical properties and can therefore not be applied in an electrochemical sensor setup.

A molecularly imprinted polymer imprinted by electropolymerization may be obtained by using the following steps: Mixing a composition comprising a functional monomer (e.g. pyrrole in the

concentration of 5 mM), an electrolyte (e.g. a PBS buffer at pH 7), and a lactoferrin template (e.g. in the concentration of 5 mg/mL composition); depositing the composition on the working electrode; electropolymerization of the composition deposited on the working electrode using cyclic voltammetry (e.g. a potential scan range between 0 mV and 1200 mV, scan rate at 20 mV/s, and 20 cycles); and leaching the lactoferrin template (e.g. by immersing the electrode in acetic acid solution, 0.3 M, for 15 min). Hereby, a molecularly imprinted polymer layer is obtained on the working electrode with a controlled thickness and compactness, wherein the cavities left in the molecularly imprinted polymer layer is complementary to the template (i.e. lactoferrin), and can therefore be used to recognize lactoferrin, and give a readout when lactoferrin is present in a solution.

In one or more embodiments, the molecularly imprinted polymer is a molecularly imprinted polymer imprinted by electropolymerization.

As disclosed, electropolymerization is a coating procedure wherein a conducting polymer is formed and deposited from a monomer solution onto a conducting substrate.

In one or more embodiments, the thickness of the molecularly imprinted polymer is between 20 and 20 nm.

The thickness is defined as the height of the MIP layer deposited on the electrode. It can be measured by Atomic Force Microscopy (AFM) imaging.

In one or more embodiments, the number of cycles of the electropolymerization is between 10 and 30 cycles, such as between 15 and 25 cycles, such as between 18 and 22 cycles, such as 20 cycles.

The thickness can be adjusted by the number of cycles during electropolymerization. The more cycles of electropolymerization, the thicker the molecularly imprinted polymer layer will be. One cycle is a complete potential scan from the lower potential (e.g. -900 mV) to the higher potential (e.g. 1.200 mV) and back again, or vice versa, from the higher to the lower and back.

In one or more embodiments, the scan rate of the electropolymerization is between 10 and 30 mV/s, such as between 15 and 25 mV/s, such as between 18 and 22 mV/s, such as 20 mV/s.

The compactness can be tailored by the scan rate of the electropolymerization. If the scanning process (scan rate) is decreased, the rigidity of the molecularly imprinted polymer layer is increased, which may be implying better replica of the protein structure in the molecularly imprinted

polymer layer. The scan rate is the amount of potential which is increased/decreased within a predefined period (i.e. the amount of millivolt the potential is changed per second). The compactness can also be called “degree of polymerization”. It equals the rigidity, stiffness, or network density of the polymer.

In one or more embodiments, the molecularly imprinted polymer is imprinted by electropolymerization with a functional monomer, an electrolyte, and a lactoferrin template.

In one or more embodiments, the working electrode comprise a material selected from the group of platinum, gold, titanium, silver, copper, carbon, carbon nanotube, graphite, or combinations hereof.

In one or more embodiments, the working electrode has a form selected from the group of a mesh, a wire, a flag, a sheet, a bar, a three-dimensional sponge, a three-dimensional microneedle array, or combinations hereof.

In one or more embodiments, the counter electrode comprises a material selected from the group of platinum, gold, titanium, silver, copper, carbon, carbon nanotube, graphite, or combinations hereof.

In one or more embodiments, the counter electrode has a form selected from the group of a mesh, a wire, a flag, a sheet, a bar, a three-dimensional sponge, a three-dimensional microneedle array, or combinations hereof.

In one or more embodiments, the reference electrode comprises a material selected from the group of platinum, gold, titanium, silver, copper, carbon, carbon nanotube, graphite, or combinations hereof.

In one or more embodiments, the reference electrode has a form selected from the group of a mesh, a wire, a flag, a sheet, a bar, a three-dimensional sponge, a three-dimensional microneedle array, or combinations hereof.

In one or more embodiments, the reference electrode is selected from the group of a silver/silver chloride electrode, a platinized platinum electrode, or a calomel electrode.

In one or more embodiments, the microprocessor is a digital microprocessor.

In one or more embodiments, the microprocessor is adapted for receiving data from the electrochemical sensor and transmitting data to an end user.

In one or more embodiments, the system further comprises a solution, wherein the electrochemical sensor is immersed in the solution.

In one or more embodiments, the solution is selected from a buffer solution, deionized water, an organic solution, a biological solution, liquid food products, and combinations thereof.

In one or more embodiments, the biological solution is selected from sweat, tears, blood, urine, blood plasma, and combinations thereof.

In one or more embodiments, a limit of detection (LOD) of the electrochemical sensor towards lactoferrin is at least 500 ng/mL, such as at least 250 ng/mL, such as at least 100 ng/mL, such as at least 80 ng/mL, such as at least 70 ng/mL.

The average concentration of lactoferrin was found to be 30.4 ng/ml in health urine and 3,300 ng/ml in infected urines. Thus the electrochemical sensor provides a fast and sensitive way of measuring the level of lactoferrin in urine.

The detection limit, lower limit of detection, or LOD (limit of detection), is the lowest quantity of a substance that can be distinguished from the absence of that substance (a blank value). The limit of detection is estimated from the mean of the blank, the standard deviation of the blank and some confidence factor. A limit of detection of at least 50 ng/mL is able to detect concentrations as low as 50 ng/mL, but is also able to detect higher concentrations such as 100 ng/mL. A limit of detection of at least 15 ng/mL is able to detect concentrations as low as 15 ng/mL, but is also able to detect higher concentrations such as 25 ng/mL. This means that the limit of detection is the lowest concentration the electrochemical sensor can distinguish from a blank sample.

In one or more embodiments, the electrochemical sensor comprises two parts detachably connected, the two parts including:

- a disposable part comprising the working electrode, the counter electrode, and the reference electrode; and
- a reusable part comprising a potentiostat and a microprocessor,

wherein the working electrode, the counter electrode, and the reference electrode are connected to the potentiostat, and wherein the potentiostat is connected to the microprocessor.

The design of disposable and reusable parts minimizes the cost of each test. The user only needs to change the disposable chip for a new test.

By detachably connected is meant that the two parts can be separated/disconnected from each other without breaking parts of the electrochemical sensor.

When an element is referred to as being “connected” to another element, the element is “directly connected” to the other element, or “electrically connected” to the other element with one or more intervening elements interposed therebetween.

In one or more embodiments, the system further comprises a connection adaptor for attaching at least the working electrode, the counter electrode, and the reference electrode to a catheter and to a urinary bag such that the working electrode, the counter electrode, and the reference electrode are positioned in-between the catheter and the urinary bag.

In a system which comprises a connection adaptor, wherein the connection adaptor comprises the working electrode, counter electrode, and reference electrode, the catheter or urinary bag can be changed without requirement of changing the electrodes. Alternatively, the electrodes can be changed without the requirement of changing the urinary bag and/or the catheter.

The adaptors are designed for easy interfacing of the electrochemical sensor with sampling devices such as catheters, so that the sampling method will be non-invasive and convenient for the users.

In one or more embodiments, the system further comprises a catheter.

In one or more embodiments, the system further comprises a urinary bag.

In one or more embodiments, the system further comprises a catheter assembly adaptor.

In one or more embodiments, the system further comprises a urinary bag assembly adaptor.

In one or more embodiments, the lactoferrin template is removed by alkaline washing for a predefined period.

Alkaline washing, is washing of at least the working electrode with an alkaline solution, such as an alkaline cleaning agent contain strong bases like sodium hydroxide or potassium hydroxide.

In one or more embodiments, the lactoferrin template is removed by acidic washing for a predefined time period.

In one or more embodiments, the alkaline washing, is washing or immersing in sodium hydroxide or potassium hydroxide solution for a predefined time.

In one or more embodiments, the concentration of the sodium hydroxide or potassium hydroxide solution is between 0.1 M and 0.5 M, such as between 0.1 M and 0.4 M, such as between 0.2 M and 0.4 M, such as 0.3 M.

Acidic washing, is washing of at least the working electrode with an acidic solution, such as an acidic cleaning agent contain strong acids like acidic acid or sulfuric acid.

In one or more embodiments, the acidic washing, is washing or immersing in acetic acid solution for a predefined time.

In one or more embodiments, the predefined time period is between 5 minutes and 30 minutes, such as between 5 minutes and 20 minutes, such as between 10 minutes and 20 minutes, such as 15 minutes

In one or more embodiments, the concentration of the acidic acid solution is between 0.1 M and 0.5 M, such as between 0.1 M and 0.4 M, such as between 0.2 M and 0.4 M, such as 0.3 M.

In one or more embodiments, the electropolymerization of the composition deposited on the working electrode using cyclic voltammetry is adjusted to obtain a predefined thickness of the molecularly imprinted polymer between 20 and 200 nm.

The thickness can be adjusted by the number of cycles during electropolymerization. The more cycles of electropolymerization, the thicker the molecularly imprinted polymer layer will be.

In one or more embodiments, the electropolymerization of the composition deposited on the working electrode using cyclic voltammetry is adjusted to obtain a predefined compactness of the molecularly imprinted polymer.

The compactness can be tailored by the scan rate of the electropolymerization. If the scanning process (scan rate) is decreased, the rigidity of the molecularly imprinted polymer layer is increased.

In one or more embodiments, the cyclic voltammetry is preformed using potential scan range between -900 mV and 1.200 mV (1,2 V) with a scan rate between 30 mV/s and 40 mV/s.

In one or more embodiments, the cyclic voltammetry is preformed using potential scan range between 0 mV and 1.200 mV (1,2 V) with a scan rate between 10 mV/s and 30 mV/s, such as between 15 mV/s and 25 mV/s, such as between 28 mV/s and 22 mV/s, such as 20 mV/s.

In one or more embodiments, the cyclic voltammetry is preformed over at least 4 cycles.

In one or more embodiments, the cyclic voltammetry is preformed over at least 4 cycles, such as at least 6 cycles, such as at least 8 cycles, such as at least 16 cycles, such as 20 cycles.

In one or more embodiments, the functional monomer is selected from pyrrole, 3,4-ethylenedioxythiophene, proDOT-COOH (carboxylic acid functional ethoxythiophene), dopamine, phenol, o-aminophenol, o-phenylenediamine, aniline, scopoletin, pyrrole, aminophenylboronic acid, 2,2'-bithiophene-5-carboxylic acid, or combinations hereof.

In one or more embodiments, the functional monomer is pyrrole.

In one or more embodiments, the functional monomer is in a concentration between 1 mM and 20 mM, such as between 1 mM and 15 mM, such as between 3 mM and 10 mM, such as between 3 mM and 7 mM, such as 5 mM.

In one or more embodiments, the electrolyte is selected from the group of lithium perchlorate, phosphate, sodium chloride, nitric acid, chloric acid, hydrochloric acid, calcium chloride, potassium nitrate, sodium hydroxide, sulfuric acid, sodium acetate, magnesium hydroxide, or combinations hereof.

In one or more embodiments, the liquid dairy products is milk.

When describing the embodiments, the combinations and permutations of all possible embodiments have not been explicitly described. Nevertheless, the mere fact that certain measures are recited in mutually different dependent claims or described in different embodiments does not indicate that a combination of these measures cannot be used to advantage. The present invention envisage all possible combinations and permutations of the described embodiments.

Brief description of the drawings

Figure 1 shows a specific embodiment of the system comprising the electrochemical sensor in an assembly adaptor.

Figure 2 shows a specific embodiment of the system comprising the electrochemical sensor in a catheter.

Figure 3 shows a specific embodiment of the system comprising the electrochemical sensor In a urinary bag.

Figure 4 shows preparation of a molecular imprinted polymer layer on a working electrode by electropolymerization.

Figure 5 shows the electrochemical signals during electropolymerization of an embodiment.

Figure 6 shows a calibration curve of the electrochemical sensor for specific detection of lactoferrin and a potential scan based on increasing concentration of lactoferrin, of an embodiment.

Figure 7 shows a scanning electron microcopy (SEM) image of a sensor electrode after deposition of a molecularly imprinted polymer layer as disclosed herein.

Detailed description of the drawings

Figure 1 shows the system 101 comprises a disposable part 108, and a reusable part 110. The electronic parts 112 and an indication system 105 may be located in the reusable part 110. The core of the disposable part 108 is an electrochemical sensor 112 with a working electrode 107, a reference electrode 109, and a counter electrode 111. An assembly adaptor 104 creates a fluidic channel designed for easy interfacing of the electrochemical sensor 112 with a catheter 102 and urinary bag 106, so that the sampling method will be non-invasive and convenient for the users. The reusable part 110 may additionally comprise a microprocessor for data processing (not shown in the figures), as well as a hardware platform for power supply (not shown in the figures) and a result indicator 113.

Figure 2 shows a second embodiment of the system 201, comprises a disposable part 208, and a reusable part 210. The electronic parts 203 and an indication system 205 may be located in the reusable part 210. The core of the disposable part 208 is an electrochemical sensor 212 with a working electrode 207, a reference electrode 209, and a counter electrode 211. The disposable part 208 is attached to the catheter 202 creating a fluidic channel designed for easy interfacing of the electrochemical sensor 212 with a catheter 202 and urinary bag 206, so that the sampling method will be non-invasive and convenient for the users. The reusable part 210 may additionally comprise a microprocessor for data processing (not shown in the figures), as well as a hardware platform for power supply (not shown In the figures) and a result indicator 213. Alternatively, the disposable part 208 can be connected to a catheter 202 via one or more connection adaptors and

can offer an easy to use system for patients, simplified measurement/sampling procedure, continuous monitoring, and reduced risk for contamination.

Figure 3 shows a third embodiment of the system 301 comprises a disposable part 308, and a reusable part 310. The electronic parts 303 and an indication system 305 may be located in the reusable part 310. The core of the disposable part 308 is an electrochemical sensor 312 with a working electrode 307, a reference electrode 309, and a counter electrode 311 as disclosed herein. The disposable part 308 is attached to the urinary bag 306 creating a fluidic channel designed for easy interfacing of the electrochemical sensor 312 with a catheter 302 and urinary bag 306, so that the sampling method will be non-invasive and convenient for the users. The reusable part 310 may additionally comprise a microprocessor for data processing (not shown in the figures), as well as a hardware platform for power supply (not shown in the figures) and a result indicator 313. Alternatively, the disposable part 308 can be connected to a urinary bag 306 via one or more connection adaptors and can offer an easy to use system for patients, simplified measurement/sampling procedure, continuous monitoring, and reduced risk for contamination.

The sensor chip for the electrochemical sensor may be obtained from a commercial source, e.g. DropSens (Spain). A commercial aviable sensor chip from DropSens comprises three planar electrodes, a working electrode made of gold, a reference electrode made of silver, and a counter electrode made of gold.

Molecularly imprinted polymers (MIPs) specific to lactoferrin may be grafted on a working electrode by electropolymerization. As shown in Figure 4, a mixture of pyrrole (a functional monomer 402), Phosphate-Buffered Saline (PBS) (as an electrolyte), and lactoferrin (a lactoferrin template 403) may be deposited on a working electrode made of gold, a gold electrode 401.

Electropolymerization may be performed using cyclic voltammetry, with potential scan range between 0 mV and 1.200 mV (1,2 V), scan rate at 20 mV/s, and 20 cycles as shown in figure 5. After the molecular imprinted polymer layer 404 is formed, the lactoferrin template 403 is leached by acetic acid washing. The lactoferrin specific cavities 405 left in the molecular imprinted polymer layer 404 are complementary to the lactoferrin template 403, and can therefore be used for selective recognition of lactoferrin.

The constructed working electrode can be tested towards detection of lactoferrin. Different concentrations of lactoferrin are spiked into lactoferrin-free urine samples. When the samples are added to the working electrode of the electrochemical sensor, the functionalized molecular imprinted polymer layer, which may selectively capture the lactoferrin, and the concentration of lactoferrin is determined by voltammetric measurements (voltage vs. current).

As shown in Figure 6, the limit of detection (LOD) of one embodiment of the biosensor may be determined to be 0.8 nM (corresponding to 65 ng/ml), which is well below the lactoferrin level in infected urines (3300 ng/ml). Hence, the constructed electrochemical sensor may be used to selectively capture the lactoferrin from the complex urine sample. The binding event may be converted into a current signal, which in turn can be converted to a lactoferrin concentration, for early warnings of urinary tract infections.

Figure 7 further shows a scanning electron microcopy image of a sensor electrode after deposition of a molecularly imprinted polymer layer as disclosed herein. Here the working electrode 507, counter electrode 511, and the reference electrode 509 are all seen, and the image shows a zoom in on the working electrode 507 on which the SEM image is shown shows deposition of the molecular imprinted polymer layer as disclosed herein.

When describing the embodiments, the combinations and permutations of all possible embodiments have not been explicitly described. Nevertheless, the mere fact that certain measures are recited in mutually different dependent claims or described in different embodiments does not indicate that a combination of these measures cannot be used to advantage. The present invention envisage all possible combinations and permutations of the described embodiments.

References

101 System
102 Catheter
103 Electronic parts
104 Assembly adaptor
105 Indication system
106 Urinary bag
107 Working electrode
108 Disposable part
109 Reference electrode
110 Reusable part
111 Counter electrode
112 Electrochemical sensor
113 Result indicator
201 System
202 Catheter
203 Electronic parts
205 Indication system
206 Urinary bag

207 Working electrode
208 Disposable part
209 Reference electrode
210 Reusable part
211 Counter electrode
212 Electrochemical sensor
213 Result indicator
301 System
302 Catheter
303 Electronic parts
305 Indication system
306 Urinary bag
307 Working electrode
308 Disposable part
309 Reference electrode
310 Reusable part
311 Counter electrode
312 Electrochemical sensor
313 Result indicator
401 Gold electrode
402 Functional monomer
403 Lactoferrin template
404 Molecularly imprinted polymer layer
405 Lactoferrin specific cavity
507 Working electrode
509 Reference electrode
511 Counter electrode

Claims

1. A system for specific detection of lactoferrin, the system comprising an electrochemical sensor, a potentiostat, and a microprocessor, the electrochemical sensor comprising a working electrode, a counter electrode, and a reference electrode, wherein the working electrode comprises a molecularly imprinted polymer imprinted with a lactoferrin template, and wherein the electrochemical sensor is connected to the potentiostat and the potentiostat is connected to the microprocessor.

2. The system according to claim 1, wherein the molecularly imprinted polymer is a molecularly imprinted polymer imprinted by electropolymerization.

3. The system according to claim 2, wherein a thickness of molecularly imprinted polymer is between 20 and 200 nm.

4. The system according to any of the claims 2-3, wherein a number of cycles of the electropolymerization is between 10 and 30 cycles, such as between 15 and 25 cycles, such as between 18 and 22 cycles, such as 20 cycles.

5. The system according to any of the claims 2-4, wherein a scan rate of the electropolymerization is between 10 and 30 mV/s, such as between 15 and 25 mV/s, such as between 18 and 22 mV/s, such as 20 mV/s.

6. The system according to any of the claims 2-5, wherein the molecularly imprinted polymer is imprinted by electropolymerization with a functional monomer, an electrolyte, and a lactoferrin template.

7. The system according to claim 6, wherein the functional monomer is selected from pyrrole, 3,4-ethylenedioxythiophene, proDOT-COOH (carboxylic acid functional ethoxythiophene), dopamine, phenol, o-aminophenol, o-phenylenediamine, aniline, scopoletin, pyrrole, aminophenylboronic acid, 2,2'-bithiophene-5-carboxylic acid, or combinations hereof.

8. The system according to claim 6, wherein the functional monomer is pyrrole.

9. The system according to any preceding claim, wherein the working electrode comprise a material selected from the group of platinum, gold, titanium, silver, copper, carbon, carbon nanotube, graphite, or combinations hereof.

10. The system according to any preceding claim, wherein the working electrode has a form selected from the group of a mesh, a wire, a flag, a sheet, a bar, a three-dimensional sponge, a three-dimensional microneedle array, or combinations hereof.

11. The system according to any preceding claim, wherein the counter electrode comprises a material selected from the group of platinum, gold, titanium, silver, copper, carbon, carbon nanotube, graphite, or combinations hereof.

12. The system according to any preceding claim, wherein the counter electrode has a form selected from the group of a mesh, a wire, a flag, a sheet, a bar, a three-dimensional sponge, a three-dimensional microneedle array, or combinations hereof.

13. The system according to any preceding claim, wherein the reference electrode comprises a material selected from the group of platinum, gold, titanium, silver, copper, carbon, carbon nanotube, graphite, or combinations hereof.

14. The system according to any preceding claim, wherein the reference electrode has a form selected from the group of a mesh, a wire, a flag, a sheet, a bar, a three-dimensional sponge, a three-dimensional microneedle array, or combinations hereof.

15. The system according to any of the claims 1-13, wherein the reference electrode is selected from the group of a silver/silver chloride electrode, a platinized platinum electrode, or a calomel electrode.

16. The system according to any preceding claim, wherein the microprocessor is a digital microprocessor.

17. The system according to any preceding claim, wherein the microprocessor is adapted for receiving data from the electrochemical sensor and transmitting data to an end user.

18. The system according to any preceding claim further comprising a solution, wherein the electrochemical sensor is immersed in the solution.

19. The system according to claim 18, wherein the solution is selected from a buffer solution, deionized water, an organic solution, a biological solution, liquid food products, and combinations thereof.

20. The system according to claim 19, wherein the biological solution is selected from sweat, tears, blood, urine, blood plasma, and combinations thereof.

21. The system according to any preceding claim, wherein a limit of detection (LOD) of the electrochemical sensor towards lactoferrin is at least 500 ng/mL, such as at least 250 ng/mL, such as at least 100 ng/mL, such as at least 80 ng/mL, such as at least 70 ng/mL.

22. The system according to any preceding claim, wherein the electrochemical sensor comprises two parts detachably connected, the two parts including:

- a disposable part comprising the working electrode, the counter electrode, and the reference electrode; and
- a reusable part comprising a potentiostat and a microprocessor,

wherein the working electrode, the counter electrode, and the reference electrode are connected to the potentiostat, and wherein the potentiostat is connected to the microprocessor.

23. The system according to any preceding claim further comprising a connection adaptor for attaching at least the working electrode, the counter electrode, and the reference electrode to a catheter and to a urinary bag such that the working electrode, the counter electrode, and the reference electrode are positioned in-between the catheter and the urinary bag.

24. The system according to any preceding claim further comprising a catheter.

25. The system according to any preceding claim further comprising a urinary bag.

26. The system according to any preceding claim further comprising a catheter assembly adaptor.

27. The system according to any preceding claim further comprising a urinary bag assembly adaptor.

28. A method for producing an electrochemical sensor for specific detection of lactoferrin, wherein the electrochemical sensor comprises a working electrode comprising a molecularly imprinted polymer, and wherein the working electrode is prepared by a method comprising the steps of:

- mixing a composition comprising a functional monomer, an electrolyte, and a lactoferrin template;
- depositing the composition on the working electrode;

- electropolymerization of the composition deposited on the working electrode using cyclic voltammetry, and
- leaching the lactoferrin template.

29. The method according to claim 28, wherein the lactoferrin template is removed by alkaline washing for a predefined time period.

30. The method according to claim 28, wherein the lactoferrin template is removed by acidic washing for a predefined time period.

31. The method according to claim 30, wherein the acidic washing, is washing or immersing in acetic acid solution for a predefined time.

32. The method according to claim 31, wherein the predefined time period is between 5 minutes and 30 minutes, such as between 5 minutes and 20 minutes, such as between 10 minutes and 20 minutes, such as 15 minutes, and wherein a concentration of the acidic acid solution is between 0.1 M and 0.5 M, such as between 0.1 M and 0.4 M, such as between 0.2 M and 0.4 M, such as 0.3 M.

33. The method according to any of the claims 28-32, wherein the electropolymerization of the composition deposited on the working electrode using cyclic voltammetry is adjusted to obtain a predefined thickness of the molecularly imprinted polymer between 20 and 200 nm.

34. The method according to any of the claims 28-33, wherein the electropolymerization of the composition deposited on the working electrode using cyclic voltammetry is adjusted to obtain a predefined compactness of the molecularly imprinted polymer.

35. The method according to any of the claims 28-34, wherein the cyclic voltammetry is preformed using potential scan range between -900 mV and 1.200 mV (1,2 V) with a scan rate between 30 mV/s and 40 mV/s.

36. The method according to any of the claims 28-34, wherein the cyclic voltammetry is preformed using potential scan range between 0 mV and 1.200 mV (1,2 V) with a scan rate between 10 mV/s and 30 mV/s, such as between 15 mV/s and 25 mV/s, such as between 28 mV/s and 22 mV/s, such as 20 mV/s.

37. The method according to any of the claims 28-35, wherein the cyclic voltammetry is preformed over at least 4 cycles, such as at least 6 cycles, such as at least 8 cycles, such as at least 16 cycles, such as 20 cycles.

38. The method according to any of the claims 28-37, wherein the functional monomer is selected from pyrrole, 3,4-ethylenedioxythiophene, proDOT-COOH (carboxylic acid functional ethoxythiophene), dopamine, phenol, o-aminophenol, o-phenylenediamine, aniline, scopoletin, pyrrole, aminophenylboronic acid, 2,2'-bithiophene-5-carboxylic acid, or combinations hereof.

39. The method according to claim 38, wherein the functional monomer is pyrrole.

40. The method according to any of the claims 28-39, wherein the functional monomer is in a concentration between 1 mM and 20 mM, such as between 1 mM and 15 mM, such as between 3 mM and 10 mM, such as between 3 mM and 7 mM, such as 5 mM.

41. The method according to any of the claims 28-40, wherein the electrolyte is selected from the group of lithium perchlorate, phosphate, sodium chloride, nitric acid, chloric acid, hydrochloric acid, calcium chloride, potassium nitrate, sodium hydroxide, sulfuric acid, sodium acetate, magnesium hydroxide, or combinations hereof.

42. The method according to any of the claims 28-41, wherein the working electrode comprise a material selected from the group of platinum, gold, titanium, silver, copper, carbon, carbon nanotube, graphite, or combinations hereof.

43. The method according to any of the claims 28-42, wherein the working electrode has a form selected from the group of a mesh, a wire, a flag, a sheet, a bar, a three-dimensional sponge, a three-dimensional microneedle array, or combinations hereof.

44. A system for specific detection of lactoferrin, the system comprising an electrochemical sensor, a potentiostat, and a microprocessor, the electrochemical sensor comprising a working electrode, a counter electrode, and a reference electrode, wherein the working electrode comprises a molecularly imprinted polymer imprinted with a lactoferrin template, and wherein the electrochemical sensor is connected to the potentiostat and the potentiostat is connected to the microprocessor, wherein the molecularly imprinted polymer is a molecularly imprinted polymer imprinted by electropolymerization.

45. A system for specific detection of lactoferrin, the system comprising an electrochemical sensor, a potentiostat, and a microprocessor, the electrochemical sensor comprising a working electrode, a counter electrode, and a reference electrode, wherein the working electrode comprises a molecularly imprinted polymer imprinted with a lactoferrin template, and wherein the electrochemical sensor is connected to the potentiostat and the potentiostat is connected to the microprocessor, wherein the electrochemical sensor is fabricated using the method according to any of claims 28-43.

46. A system for use in diagnosis of early stages urinary tract infections, wherein the system comprises an electrochemical sensor, a potentiostat, and a microprocessor, the electrochemical sensor comprising a working electrode, a counter electrode, and a reference electrode, wherein the working electrode comprises a molecularly imprinted polymer imprinted with a lactoferrin template, and wherein the electrochemical sensor is connected to the potentiostat and the potentiostat is connected to the microprocessor.

47. A system for use in diagnosis of Sjfigren syndrome, wherein the system comprises an electrochemical sensor, a potentiostat, and a microprocessor, the electrochemical sensor comprising a working electrode, a counter electrode, and a reference electrode, wherein the working electrode comprises a molecularly imprinted polymer imprinted with a lactoferrin template, and wherein the electrochemical sensor is connected to the potentiostat and the potentiostat is connected to the microprocessor.

48. A system for use in diagnosis of lactoferrin levels in tears, urine, and/or saliva, wherein the system comprises an electrochemical sensor, a potentiostat, and a microprocessor, the electrochemical sensor comprising a working electrode, a counter electrode, and a reference electrode, wherein the working electrode comprises a molecularly imprinted polymer imprinted with a lactoferrin template, and wherein the electrochemical sensor is connected to the potentiostat and the potentiostat is connected to the microprocessor.

49. Use of a system comprising an electrochemical sensor, a potentiostat, and a microprocessor, the electrochemical sensor comprising a working electrode, a counter electrode, and a reference electrode, wherein the working electrode comprises a molecularly imprinted polymer imprinted with a lactoferrin template, and wherein the electrochemical sensor is connected to the potentiostat and the potentiostat is connected to the microprocessor, for specifically detecting lactoferrin in non-medical use, such as detecting lactoferrin in liquid dairy products.

50. Use of a system according to claim 49, wherein the liquid dairy products is milk.

51. Use of a system comprising an electrochemical sensor, a potentiostat, and a microprocessor, the electrochemical sensor comprising a working electrode, a counter electrode, and a reference electrode, wherein the working electrode comprises a molecularly imprinted polymer imprinted with a lactoferrin template, and wherein the electrochemical sensor is connected to the potentiostat and the potentiostat is connected to the microprocessor, for specifically detecting lactoferrin in water samples obtained from a water drilling well.

52. An electrochemical sensor for specific detection of lactoferrin, the electrochemical sensor comprises a working electrode, a counter electrode, and a reference electrode, wherein the working electrode comprises a molecularly imprinted polymer imprinted with a lactoferrin template.

53. A catheter comprising an electrochemical sensor for specific detection of lactoferrin, the electrochemical sensor comprising a working electrode, a counter electrode, and a reference electrode, wherein the working electrode comprises a molecularly imprinted polymer imprinted with a lactoferrin template.

54. A urinary bag comprising an electrochemical sensor for specific detection of lactoferrin, the electrochemical sensor comprising a working electrode, a counter electrode, and a reference electrode, wherein the working electrode comprises a molecularly imprinted polymer imprinted with a lactoferrin template.

55. A catheter and urinary bag assembly adaptor comprising an electrochemical sensor for specific detection of lactoferrin, the electrochemical sensor comprising a working electrode, a counter electrode, and a reference electrode, wherein the working electrode comprises a molecularly imprinted polymer imprinted with a lactoferrin template.

56. An adaptor comprising an electrochemical sensor for specific detection of lactoferrin, the electrochemical sensor comprising a working electrode, a counter electrode, and a reference electrode, wherein the working electrode comprises a molecularly imprinted polymer imprinted with a lactoferrin template, and wherein the adaptor is suitable for attaching at least the working electrode, the counter electrode, and the reference electrode to a catheter and to a urinary bag such that the working electrode, the counter electrode, and the reference electrode are positioned in-between the catheter and the urinary bag.

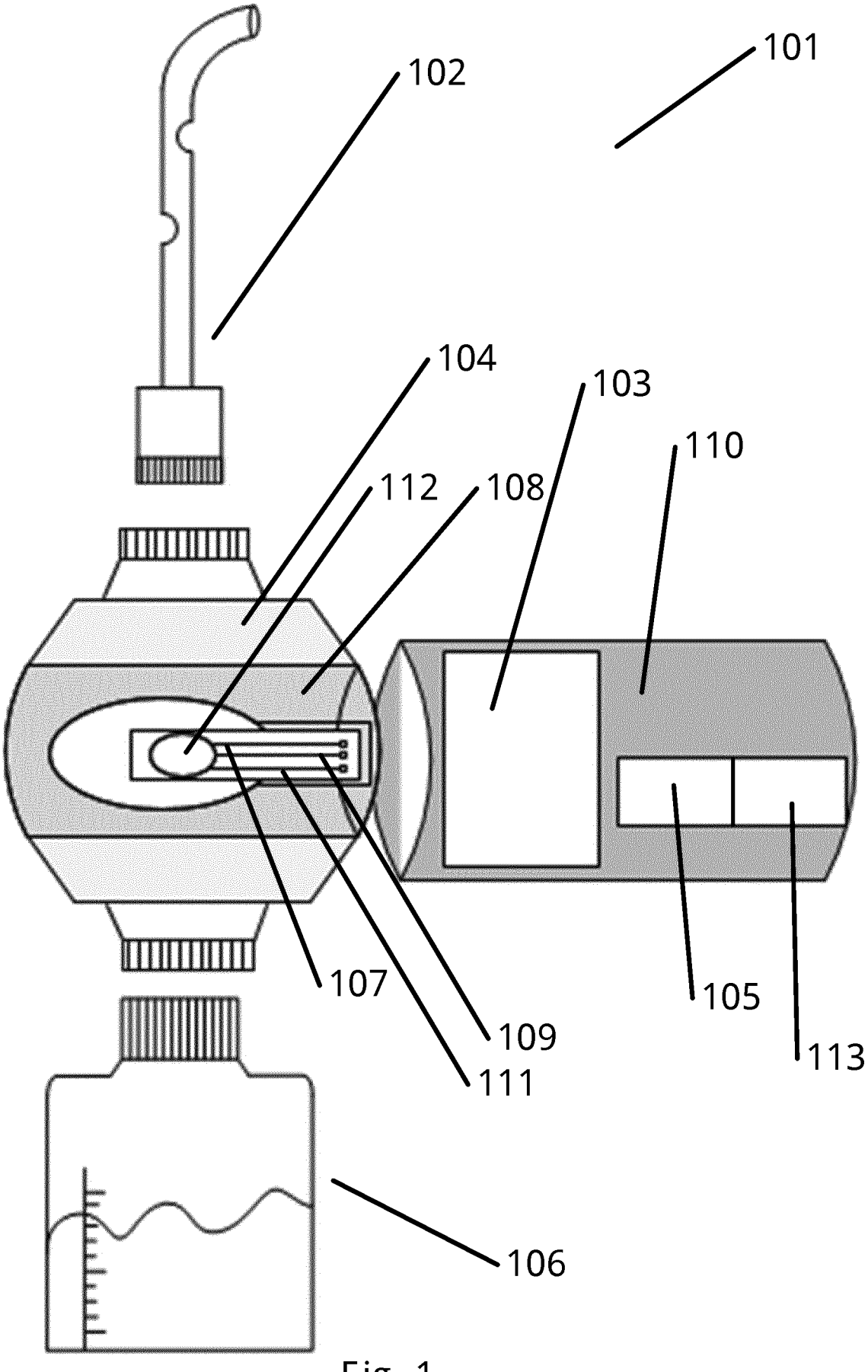

Fig. 1

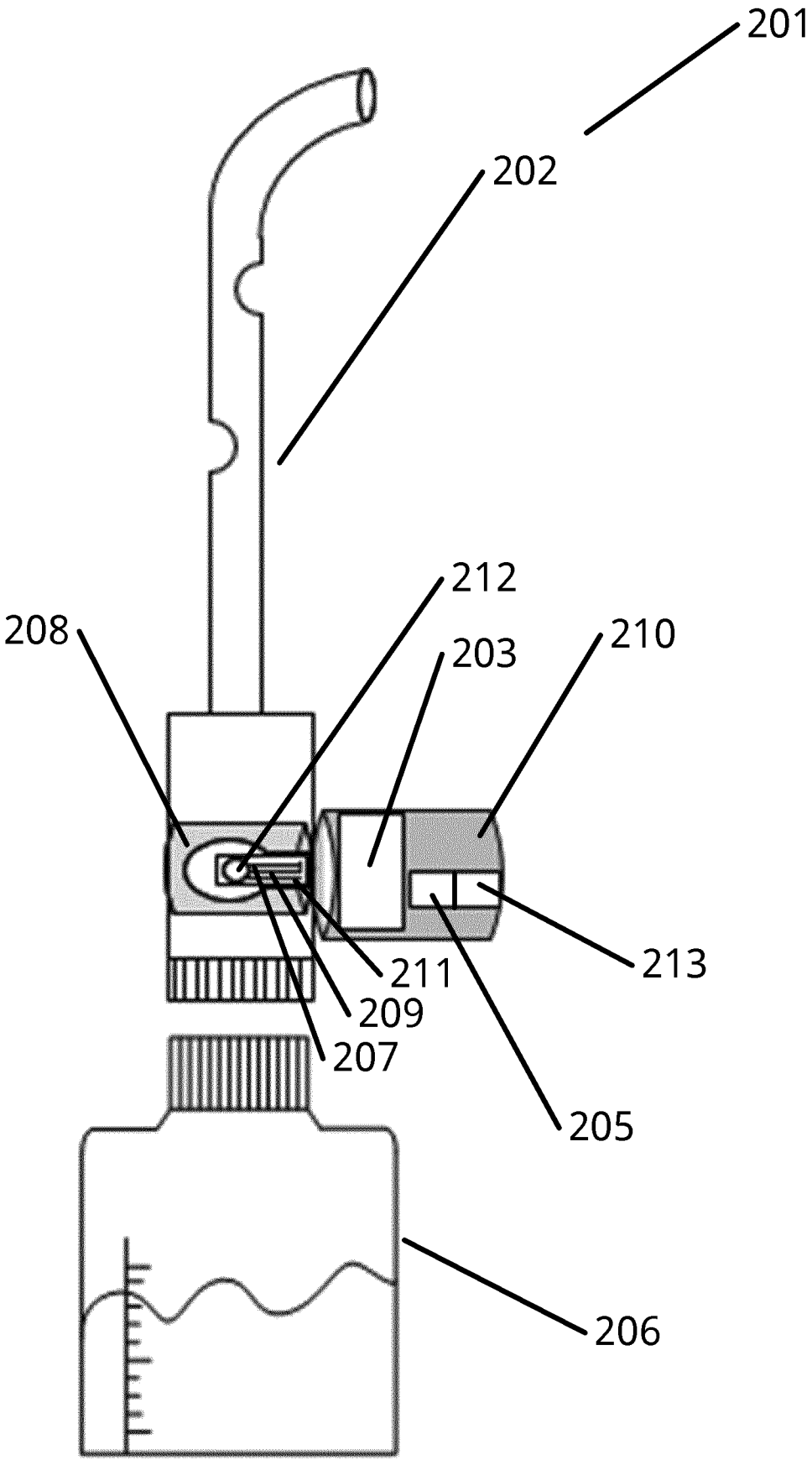

Fig. 2

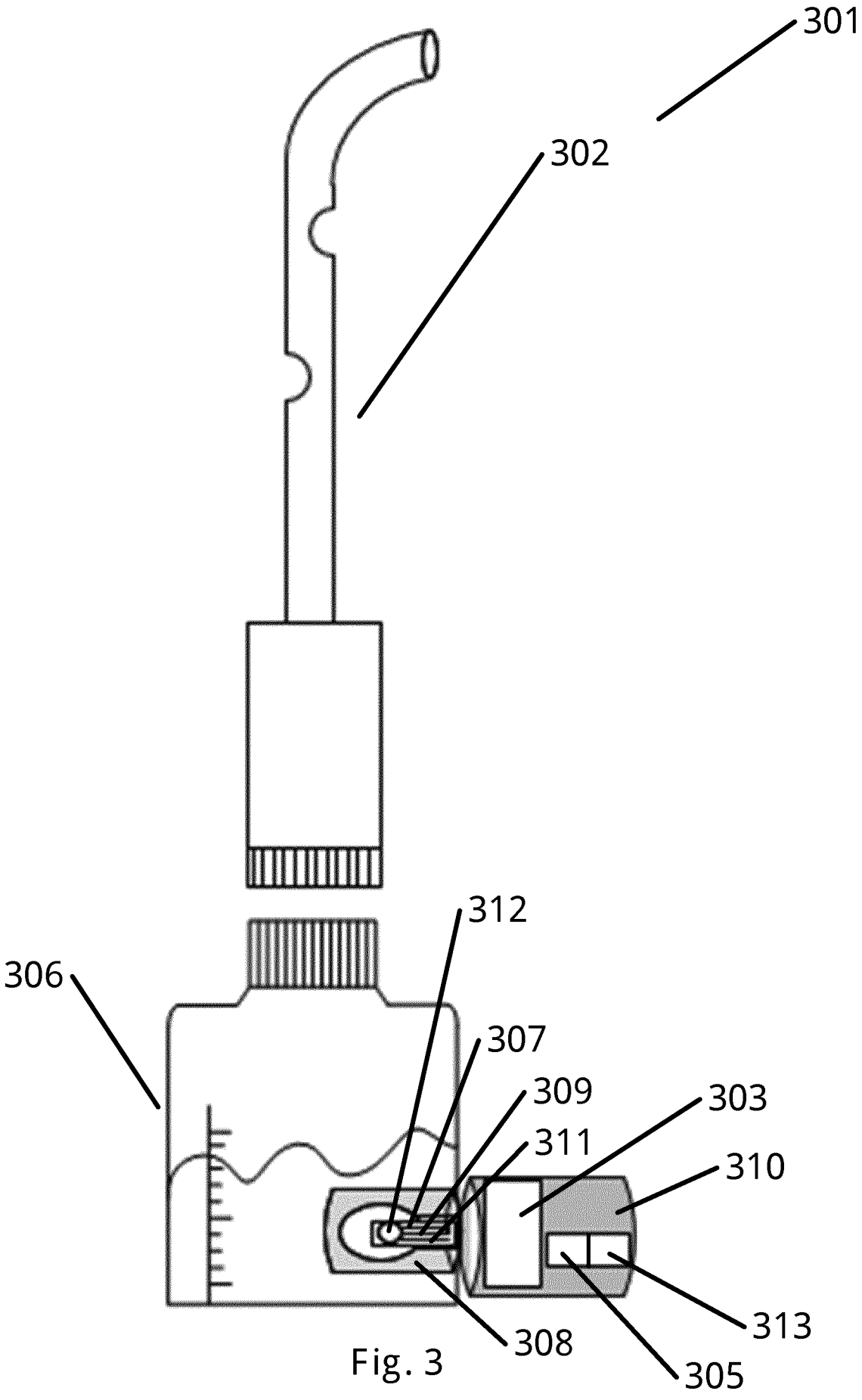

Fig. 3

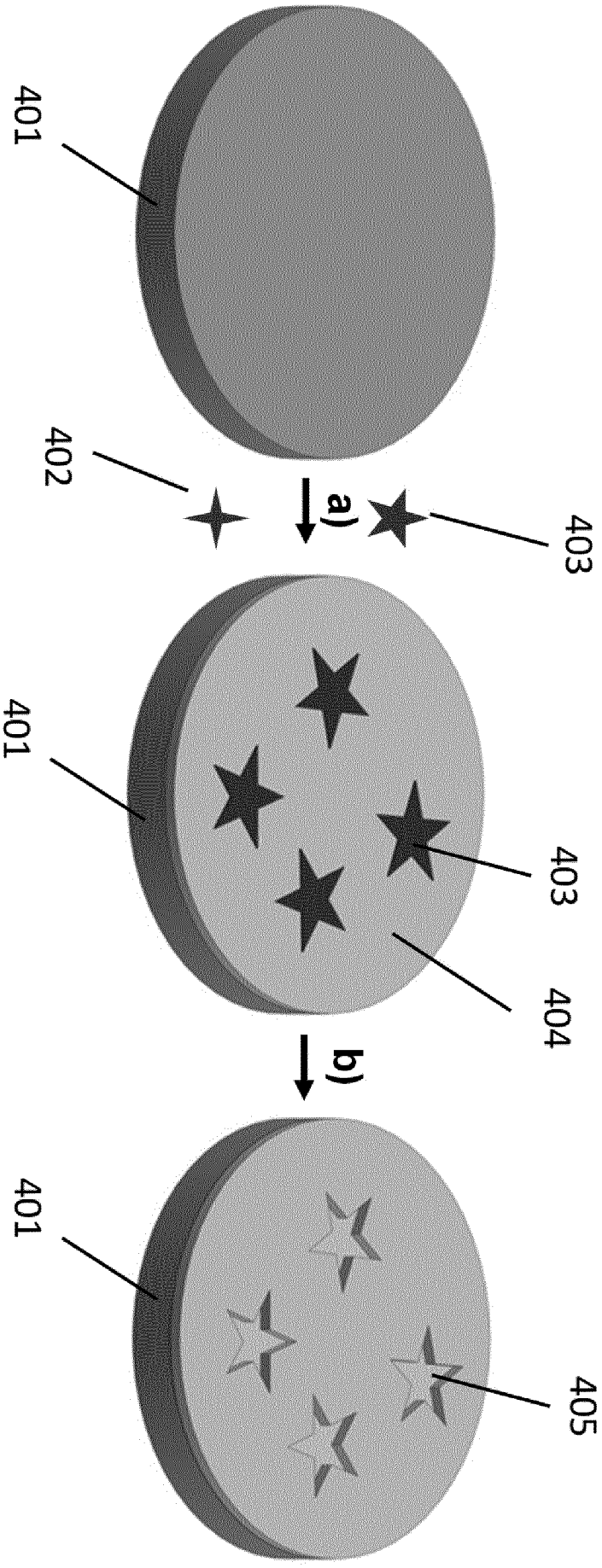

Fig. 4

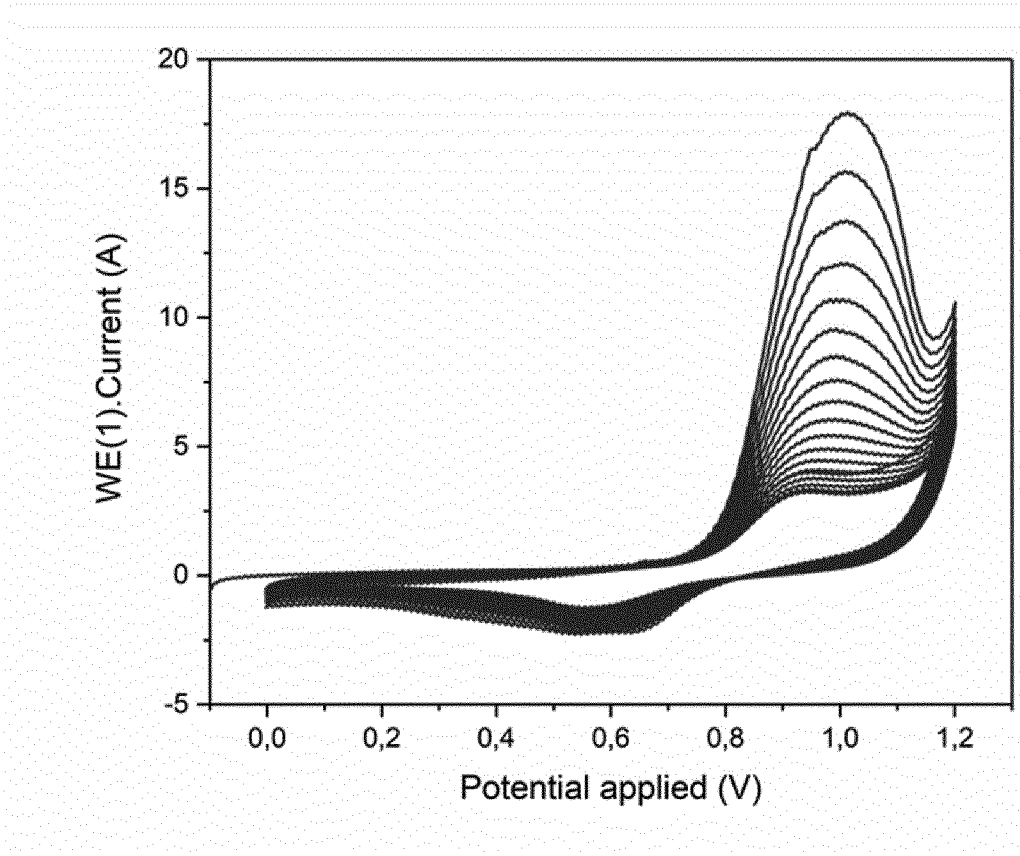
20
15
10
5
0
-5
WE(1).Current (A)
0,0
0,2
0,4
0,6
0,8
1,0
1,2
Potential applied (V)

Fig. 5

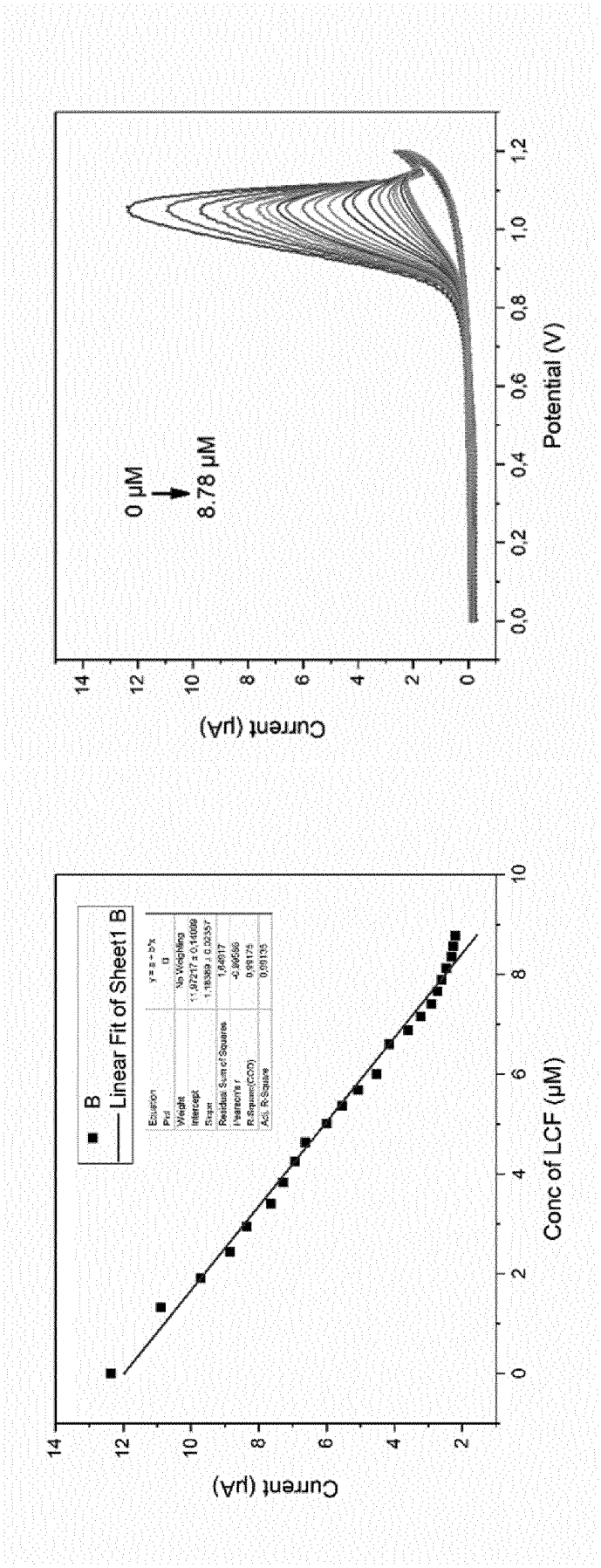
Current (µA)
Potential (V)
0 µM
8.78 µM
Current (µA)
Conc of LCF (µM)
B
Linear Fit of Sheet1 B

Fig. 6

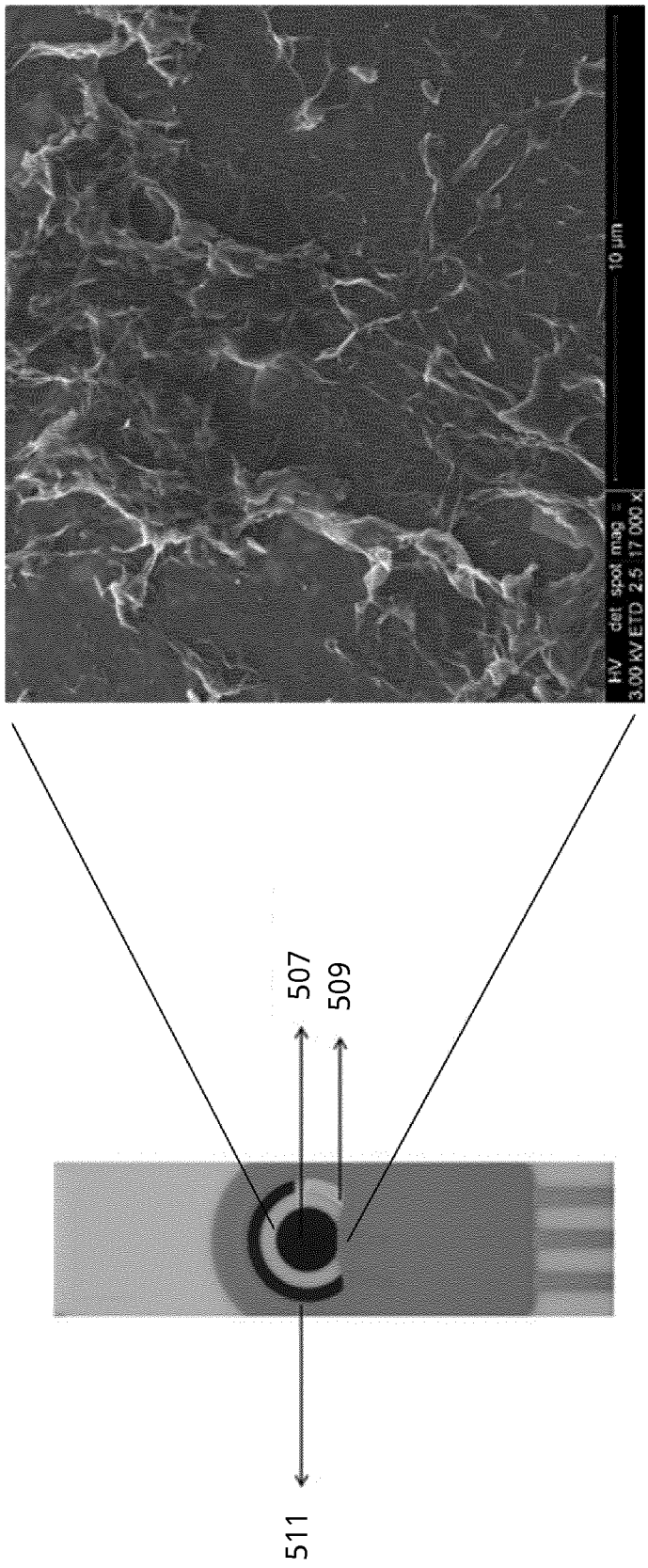

Fig. 7

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2019/0813 19

A. CLASSIFICATION OF SUBJECT MATTER
INV. G01N27/327 B82Y15/00 G01N33/04 G01N33/18 G01N33/543
A61B5/1486 G01N33/49 G01N33/493 A61B5/20 A61B10/00
A61F5/44

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
G01N B82Y A61B A61M A61F

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal , WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DONG CAI ET AL: "A molecular-imprint nanosensor for ultrasensitive detection of proteins", NATURE NANOTECHNOLOGY, vol. 5, no. 8, 27 June 2010 (2010-06-27), pages 597-601, XP055566261, GB ISSN: 1748-3387, DOI: 10.1038/nnano.2010.114 figures 1, 3 Section: "Abstract"; page 597 Section: "Methods"; page 601, column 1, paragraph 1 - paragraph 3 ----- -/--	1-56

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"L" document which may throw doubts on priority claim(s) orwhich is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
31 January 2020	11/02/2020

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Colasanti, Kathari na

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Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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X	DECHTRIRAT DECHA ET AL: "A screen-printed carbon electrode modified with gold nanoparticles, poly(3,4-ethylenedioxythiophene), poly(styrene sulfonate) and a molecular imprint for voltammetric determination of nitrofurantoin", MIKROCHIMICA ACTA, SPRINGER VERLAG, VIENNA, AT, vol. 185, no. 5, 23 April 2018 (2018-04-23), pages 1-9, XP036503830, ISSN: 0026-3672, DOI: 10.1007/S00604-018-2797-3 [retrieved on 2018-04-23] figure 1 Section: "Experimental"; page 261	1-56
A	COSIMINO MALITESTA ET AL: "MIP sensors - the electrochemical approach", ANALYTICAL AND BIOANALYTICAL CHEMISTRY, vol. 402, no. 5, 23 September 2011 (2011-09-23), pages 1827-1846, XP055566262, DE ISSN: 1618-2642, DOI: 10.1007/s00216-011-5405-5 page 1829 - page 1837; figure 1 Section: "Abstract"; page 259 Section: "Experimental"; page 261	7,8,38, 39

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	YING PAN ET AL: "Electrochemical immunosensor detection of urinary lactoferrin in clinical samples for urinary tract infection diagnosis", BIOSENSORS AND BIOELECTRONICS, vol. 26, no. 2, 15 October 2010 (2010-10-15), pages 649-654, XP055566440, AMSTERDAM, NL ISSN: 0956-5663, DOI: 10.1016/j.bios.2010.07.002 Section: "Abstract"; page 1 Section: "3.4. Electrochemical immunosensor assay of lactoferrin in infected urine samples"; page 7	20,46,48
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A	US 2016/183819 A1 (BURNETT DANIEL R [US] ET AL) 30 June 2016 (2016-06-30) figures 1-3, 5, 6 paragraph [0063] paragraph [0073] paragraph [0142] - paragraph [0152]	23-27, 53-56

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Information on patent family members

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PCT/EP2019/081319

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