Microbiology Detection Methods of Piezoelectrics Bulk Acoustic Wave Sensor Research of Chitosan Properties

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Abstract. The piezoelectric bulk acoustic wave sensor technology has such unique advantages as high sensitivity, wide response spectrum, easy digitalization, simple structure and low cost and others, which are widely used in many fields of analytical chemistry, biochemistry, environmental monitoring, life science and molecular biology. This article has conducted research for theories and applications of several new systems on the basis of the tandem piezoelectric response to solution electrical conductivity and dielectric constant and the response of single-component piezoelectric bulk acoustic wave sonic sensors to the viscosity and density, which has broadened the application range of the piezoelectric bulk acoustic wave sensor applications in the life sciences and environmental monitoring. The article applies tandem piezoelectric sensor technology (SPQC) to study the adsorption properties of natural polymer chitosan to metal ions, and the influence from ion concentration, adsorbent dosage and degree of deacetylation of chitosan to chitosan sequestration performance has been investigated in the article. In addition, the determination of degree of deacetylation has been conducted with the application of tandem piezoelectric sensor technology in the article, this test method can effectively eliminate the influence of residual acid or residual alkali adsorbed by chitosan, making measurement results become more accurate and reliable.

Introduction

Since the 1990s, with the improvement of life quality, people are more concerned about their own health problems. Key application areas of analytical chemistry have begun to shift to the life sciences fields of medical science and bio-engineering and so on. The Human Genome Project is the most typical example. Food safety, disease prevention, environmental protection and monitoring, the fight against terrorism and so on all have posed a number of unprecedented challenges to the analytical chemistry, which requires analytical chemists to provide such new technology at the molecular level as studying life processes, understanding the impact of genetic modification, screening combinatorial chemistry synthetic drugs, on-site monitoring chemical and biological weapons. This tendency has been reflected very clearly in Pittsburgh meeting held in recent years, for example, in 2000, 42 branches are related to life sciences within 200 branches, in 2001, 53 branches are related to Life Sciences within 212 branches, whereas in 2002, 75 branches are related to life sciences within 227 branches.

Chemical and biological sensing technology is an important part of analytical chemistry, and it is consisted of chemistry, biology, electronics, optics, mechanics, acoustics, semiconductor technology, microelectronics technology, thin film technology and other subjects, which has been a integrative technology through mutual penetration and combination. The technology has been applied in many

areas such as life sciences, defense, chemical measurements and so on. Chemical and biological sensors are usually composed of two parts: the first part is the molecular recognition elements, which is composed by chemical and biological active substances with molecular recognition capacity; the second part is a signal converter, such as current or potential measuring electrode, thermistors, field effect transistors, optical fiber, the piezoelectric crystals and so on. Among them, the sensing technology using piezoelectric crystal as signal converter technology is called as piezoelectricity chemical and biological sensing technology.

Piezoelectric chemical and biological acoustic sensing is an emerging field for sensing testing. Compared with optical, electrical or thermal traditional sensor technology, it has a broad spectrum response, high sensitivity, easy to digitize and many other advantages. It applies the bulk acoustic wave device based on piezoelectric materials to realize interaction of thickness shear mode oscillation process and surrounding environment, the change of electrical impedance spectroscopy, frequency spectrum or phase and other parameters respond to such relevant physical and chemical properties of environment medium such as mass media, viscoelasticity, admittance, dielectric /rheological properties, ion / solvent transfer respectively and converts to the corresponding sensor detection signals to obtain one- dimensional or multi-dimensional information of component and property of relevant target species, in order to acquire a comprehensive, dynamic, real-time or in-place description for the determination of chemical, sensing life sciences, biology, pharmacy, clinical medicine and environmental science and other fields. In recent years, piezoelectric chemistry and biology acoustic sensing technology has presented a lot of advantages in analysis and characterization of biological system and environmental system, biological and microbiological process monitoring and other aspects, which has developed to be one of the most active fields in sensor technology research.

This paper presents a new method of analysis, which applies acoustic impedance analysis technology to study chitosan enzymatic degradation and conducts real-time monitoring of the non-specific degradation process of chitosan. The effects of pH value, temperature, enzyme concentration and substrate concentration on the degradation catalytic properties have been discussed. Effects of De-acetylation degree (DD) on degradation reactions of pepsin have been studied and the relationship between DD value and frequency shift response has been established, compared with other enzymatic test methods, the new method not only has no need to separate samples, but has simple and fast operation advantages, it can also conduct the real-time monitoring for the emzymolysis process of chitosan.

Formation of piezoelectrics bulk acoustic wave sensor technology

For a long time, people think that it is impossible to conduct the stable oscillation or stop vibration for piezocrystal after the immersion of the solution since energy loss in crystal / solution interface is too large. Determination of substances in solution can be conducted through the indirect method, so the substance to be tested should be transform into gas for the determination, or it should be shifted to the gas phase after the reaction in liquid phase. This greatly limits the application of electrical sensing technology. In 1980, Japan's Nomura Group, the United States Bastianns and Konashl group have independently acquired the success of piezoelectric quartz crystal single-face liquid contacting vibration, and have applied such single-face fluid contacting apparatus for assay. However, due to start-up key was not found, so crystal can have start-up in only a small number of the liquid. Yao and other people have made a lot of pioneering research work for liquid phase

vibration of piezocrystal, in 1985, it has been reported the key to ensuring PQC start-up in the liquid phase, that is to PQC has temperature point for the failure of vibration, in order to achieve stable oscillation in water solution and more than 40 kinds of organic liquid, and he has also proposed technical approach to achieve stable oscillation of the crystal. Since then, the piezoelectric sensing technology has been applied in liquid oscillation. Subsequently, Yao and other people have conducted systematic study for laws and theories, the new plant design, component selection many other aspects related to the liquid phase of the piezoelectric sensor and have made a series of theoretical results.

In order to study physical factors affecting resonant frequency change in PQC liquid sensing system, the pioneers have done a lot of exploratory work.. Studies have shown that piezoelectric acoustic transducer not only has sensitive response to the quality, but also can conduct agile response to many other non-quality factors the solution, such as viscosity, density, electrical conductivity, dielectric constant. Nomura and Okuhara have first reported the empirical correlation between frequency shift and solution density and electrical conductivity; as for the organic phase, it is necessary to establish the correlation between the frequency shift and viscoelasticity and density of the solution. In 1985, Bruekenstein has applied the method of dimensional analysis to establish the correlation between frequency shift of PQC sensor and solution viscosity - density.

$$\Delta f_L = -2.26 \times 10^{-6} n f_0^{3/2} \sqrt{\eta_L \rho_L}$$

In order to broaden the application of the piezoelectric acoustic sensors, before and after 1991, Nomura and Zhou have independently developed a new bulk acoustic piezoelectric sensor - electrode disengaging piezoelectric sensor (ESPS). ESPS is set in one side of PQC, and a parallel electrode becomes the excitation electrode of PQC, and the liquid to be tested should be placed between the electrode and the piezoelectric crystal. ESPS not only retains the quality and density response of classic touch-hydraulic PQC, but also has a more sensitive impedance response characteristic. Yao and Shen also export the frequency shift formula and discuss the effects of equivalent circuit parameters, the nature of solution temperature on frequency response. In 1993, Yao further developed the tandem piezoelectric bulk acoustic wave sensor (SPQC). SPQC is consisted of a conductance electrode by PQC in series, PQC can have oscillation in vacuum, only the conductivity electrode is in contact with the solution. Compared with the classic touch liquid formula PQC, SPQC only responds to the change of solution conductivity and permittivity and has no response to the change of solution viscosity - density. Shen and other people have derived SPQC frequency shift formula and discussed the equivalent circuit model and parameters in detail. Recently, Huang and other people have reported the relationship between impedance parameters of SPQC sensor and capacitance of liquid phase and gel phase.

Microorganism determination

Human life is inseparable from the microorganisms in industry, agriculture, medicine, pharmacy and other fields, microorganisms have been widely used. Moreover, micro-organisms has made great contribution in the history of human science, especially in the development of life sciences, and plays an important role molecular biology, molecular genetics and even the contemporary genetic project. Therefore, the development of fast and simple measurement method of microbial analysis is very significant. However, the traditional method of measuring microorganisms are not only complicated but also have long determination cycle, such as cell count method, turbidity, dry weight

method and bacterial length measurement method, which can not meet the requirements for microbial rapid determination in practical applications. Therefore, a majority of microbiologists, physicists, chemists and other disciplines scientists take advantage of microbial optical, electrochemical, biochemical and physical properties to propose a series of new microbial rapid assay techniques.

The content of certain the microbial cell component is relatively stable, and therefore microorganisms can be determined by the measurement of these components.

(1) Plasmid fingerprinting is an indirect molecular biology technique used for plasmid analysis, including plasmid characteristics by AGE baterial monitoring and homology analysis for plasmid DNA by restriction endonuclease. The technology can monitor and trace bacteria infected in society. Many scholars have used the plasmid DNA analysis method to conduct survey for epidemiology and monitor the resistant bacteria.

DNA probe technology. As for the successful detection of pathogenic bacterium of human and animal body, nucleic acid probe has made the monitoring for the cultured and progenitive bateria and the virus become possible. In recent years, DNA probes has become a very popular tool, which is widely used in microbial detection. In 1988, Miller and other people have applied anti-DNA-RNA antibody marked by alkaline phosphatase to detect DNA (probe) immobilized by latex and crossbred formed by RNA supplemented with DNA probe in the sample, which can achieve accuracy of measuring 500 microbial cells. To improve the sensitivity of the probe, someone abroad has designed a new method with a DNA probe hybridizing ribosomal RNA, sensitivity of RNA of the target sequence determined through the method is increased thousands of times compared with the determination of DNA, and 1 ~ 10 bacteria can be detected, so there is no need to culture bacterial for many microbiological testing.

Gene amplification technology, namely the polymerase chain reaction (PCR). This technique can be used to identify pathogens that grow slowly or are difficult to cultivate, the most successful identification is the identification for Mycobacterium tuberculosis. It can also be used to diagnose the pathogen that is difficult to be identified. Generally it refers to fermentation gram-negative bacillus and anaerobic bacterial. Niederhuaer and other people use PRC technology to detect food Listeria monocytogenes bacteria (L.Momoytogenes), it just needs a few hours to complete the detection of the bacteria. This method is very sensitive, measuring microorganisms as low as 10eclls / mL.

Determination of cell wall components. Glucan of bacterial cell wall contains several components, which can be used as an indication of bacterial biomass. Determination of gram-positive bacteria biofilms can be selectively hydrolyzed by phosphate concentrated hydrofluoric acid, which can be completed by analyzing cell wall acid. Gram-negative bacteria can be determined by measuring the lipopolysaccharide (LPS) component.

Response principle of piezoelectrics bulk acoustic wave technology

For a good quartz crystal, Cady and Bototm have derived an equivalent circuit model of PQC (BVD model) in impedance analysis. Its response to the impressed voltage or current may represent response to the impressed voltage and current from PQC. The equivalent circuit model consists of a dynamic arm and static arm connected in parallel. Dynamic arm includes three equivalent circuit

parameters, namely the dynamic resistance (R_1), dynamic inductance (L_1) and dynamic capacitor (C_1). Static arm comprises only a static parameter, i.e., the static capacitance (C_0).

As for impedance analysis, G (conductance) f (frequency) spectrum and B (susceptance) -f (frequency) spectrum can be recorded through scanning frequency throughout the resonance regions of PQC to calculate the equivalent circuit parameters of PQC to obtain change information of various physical parameters in monitoring process. Four equivalent circuit parameters of PQC have clear physical meaning, which are related with the nature of quartz crystal and the medium:

$$R_1 = \frac{t_Q^3 r}{8S\varepsilon^2}, L_1 = \frac{e^3 \rho_Q}{8A\varepsilon^2}, C_1 = \frac{8S\varepsilon^2}{\pi^2 t_Q{}^C}, C_0 = \frac{k\varepsilon_0 S}{t_Q}$$

Where t_Q, S are the quartz crystal thickness and area respectively; e is piezoelectric stress constant; r is the dissipation coefficient; ρ_Q, c and k are the density of quartz, and elastic coefficient and dielectric constant; e_0 is the vacuum permittivity. As for impedance analysis, R_1 is related with PQC dissipation energy in the medium and the supporting structure; L_1 corresponds to the inertia components of the resonator and is related with the quality of vibration, including the effects of the PQC coatings and the liquid medium in contact with coatings, C_1 corresponds to the mechanical resilience of crystal and the surrounding medium. C_0 derives from the stray capacitance between two parallel metal electrodes of the quartz wafer and support structure. R_1, L_1 and C_1 are related with the oscillation caused by the piezoelectric effect, but C_0 has no relation with the piezoelectric effect.

Bulk acoustic wave analysis technology is applied for enzymatic degradation catalytic characteristic research of chitosan

Chitosan is a kind of biodegradable natural polymers and is mainly from the deacetylation of chitin. The content is only next to cellulose, which has biodegradablity, biocompatibility, and antibiosis immunity, affinity with protein and other biology and physicochemical characteristics. Therefore, applications of chitosan as a functional biopolymer has attracted widespread attention in many areas, especially in pharmaceutical engineering, environmental protection, food industry, and biomedicine. However, high molecular weight chitosan often cause the invalid absorption in its low solubility and body in the aqueous phase, which greatly limits its application in various fields. In addition, high molecular weight chitosan is more viscous, leading to problems in its practical application. However, compared with high molecular weight chitosan, low-molecular weight chitosan can not only be absorbed and used by the body effectively, but also has a number of advantages over high molecular weight chitosan function, especially pentosaccharide chitosan

has attracted a lot of attention in inhibiting tumor. In addition, low molecular weight chitosan has better water solubility, which will greatly facilitate its application in various fields, and therefore, the preparation of low molecular weight chitosan (oligosaccharides) has attracted sustained attention. So far, the method for preparing oligosaccharides can be divided into three categories: acid hydrolysis, oxidation and enzymatic degradation. In contrast, the enzyme degradation method has such advantages as milder degradation conditions, easier control of product distribution, and no secondary pollution and minimal changes in the chemical properties and so on. Thus, enzymatic degradation method is a superior and more promising method for preparing oligosaccharides, it is attracting more and more world attention from researchers. So far, it has been reported that many enzymes have a chitosan matrix degrading activity, such as: lysozyme, lipase, lysozyme, cellulase and protease.

Pepsin is an important digestant and is widely used in pharmaceuticals and medicine. It had been reported the chitosan degrading activity from pepsin, but there are some researches on the effects of different degradation conditions on the degradation activity of porcine pepsin, and there has no relevant reports so far.

Bulk acoustic wave impedance analysis technology is a kind of piezoelectric process sensor technology, which can not only provide the dynamic resistance (R_1) of quartz products towel body pressure sensor, but also can provide other important parameters of the sensor, such as: the resonance frequency (f_0), dynamic inductance (L_1), the dynamic capacitance (C_1) and static capacitance (C_0) and so on, each parameter has a clear physical meaning. Bulk acoustic wave impedance analysis technology can give information reflecting the multi-dimensional information of physical and chemical properties of research system, and can conduct real-time monitoring for the various property change of the research system. Because the dynamic resistance of PQC sensor can react to the change of solution viscosity and density. Therefore, changes in dynamic resistance (ARI) can be used to reflect enzymatic degradation characteristics of chitosan under the different degradation conditions. This method is put forward based on the constant change of viscosity change in the degradation process of chitosan. By comparing the dynamic resistance curves under different experimental conditions, the effect of PH value, temperature, enzyme concentration, substrate concentration and the degree of deacetylation of chitosan (DD) on the enzymatic activity of pepsin has been investigated.

Conclusions

This paper presents a new tandem piezoelectric bulk acoustic wave sensing method (SPQC) for measuring the degree of deacetylation of Chitosan and conducting real-time monitoring for the chitosan adsorption process to the copper ions. NaOH amount consumed between two turning points in the changing curve by virtue of frequency acquired in the process of titration is applied to calculate DD value of chitosan. This measurement method can effectively eliminate the influence of residual acid or alkali residues absorbed by chitosan, making measurements more accurate and reliable. Compared with conventional potentiometric titration, SPQC technology can be applied for the determination of the degree of deacetylation of the chitosan, and the turning point is more obvious and the terminal point is easy to be judged. In addition, the method is also used for the real-time monitoring of chitosan metal adsorption process sucessfully. The article discusses the effects of adsorption conditions on the adsorption properties of chitosan. This is the first time to

investigate chitosan adsorption to metal ions on the basis of solution conductivity change. Compared with the traditional spectrophotometry, atomic absorption spectrometry and other methods, it is unnecessary to separate samples for SPQC method, and experimental apparatus and operation are all very simple, and its analysis cost is quite low, and it can also be used for real-time monitoring process. Therefore, this method can be applied more widely in the process analysis for the interaction between chitosan and metal ions, dyes, amino acids or nucleic acids.

According to the solution density change caused in the process of high molecular weight chitosan degradation by Porcine pepsin, bulk acoustic wave impedance analysis technology can be applied to monitor real-time changes in dynamic resistance, the pH, temperature, enzyme concentration and effect of enzymatic activity of pepsin from substrate concentration have also been investigated and discussed respectively. The article also studies the effect of the degree of deacetylation of chitosan on AR and response value to acquire the regression equation between dynamic resistance change and the relationship of the degree of deacetylation. Compared to other enzymatic assay method, it is unnecessary to separate samples for bulk acoustic wave impedance analysis technology, and the operation is simple and quick, and it can also conduct real-time monitoring for emzymolysis process of chitosan. Therefore, this study not only examines catalytic degradation of porcine pepsin to chitosan in more detail, which has provided useful information for large-scale preparation of pepsin low molecular weight chitosan. The article also proposes a new analytical method for enzymatic degradation of chitosan.

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