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(54) Title: SENSOR DEVICES AND METHODS FOR USING THEM

(57) Abstract

Sensor devices for electrolytic analysis of liquid media, comprising a working electrode coated with an aminoacid polymer (preferably poly–lysine on platinum), advantageously with a diffusion–lowering barrier layer of an applied or deposited thin porous or permeable layer of organic polymer or inorganic material superimposed upon it which allows analytes to pass through. The barrier coating stabilises the poly–aminoacid coating against dislodgement by acidic media and the poly–aminoacid/covering barrier combination gives a sensor having enough stability to be heat–sterilised, good pH independence and selectivity with minimal interference by other components, and long user life. Preferred barrier materials are PVC, (which can be applied from solution, e.g. dip–coating) and diamond–like carbon ("DLC"), preferably 5μm thick or less. The electro–analytical procedure preferred is pulsed amperometric detection ("PAD"). The sensors are especially effective for ethanol determination, and applicable to monitoring and measurement of fermentation media with reduced interference by any sugars present.

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SENSOR DEVICES AND METHODS FOR USING THEM.

This invention relates to sensor devices and methods for their use, and more particularly to improved sensor devices useful for electrolytic analytical methods for the detection and determination of organic analytes, especially ethanol.

It is known to make and use a variety of electrolytic sensor devices incorporating one or more electrodes to produce a signal output from which specific analytes can be detected and measured. These electrodes can act in several ways, for example by detecting such conditions as oxidation, reduction, acidity/alkalinity (pH), electrical potential and current flow. The species which can be detected in this way include glucose, ethanol, and many other compounds.

The detection and measurement of ethanol is of great commercial importance, as the wine and brewing industries are very extensive and taxes and duties are payable to governments on the basis of measurement of the ethanol content of fermentation products. Consequently, there is a great demand for reliable devices for monitoring the progress and efficiency of alcoholic fermentation processes by measuring the content of ethanol in them, and also, in many instances, other properties of the fermentation media, for example the reducing sugar (for example glucose) content as it is fermented into ethanol. This monitoring is also desirable for other process or waste liquors or effluents.

Among the proposed sensor devices which have been proposed for carrying out such monitoring and measurement, many have contained enzymes - which act on the substrate chemical being evaluated and generates a different chemical which can be determined, thus providing means for determining the substrate chemical indirectly. Especially,

glucose oxidase has been used because it catalyses oxidation of glucose to gluconic acid -- producing hydrogen peroxide via oxygen reduction. the hydrogen peroxide is very readily and conveniently determined electrolytically.

Existing sensor devices are not entirely reliable or durable for the more demanding industrial uses, for example continuous monitoring of the whole fermentation cycle, and suffer from the disadvantage of being unable to survive the heat sterilisation steps which are so important in the fermentation industries. These often involve temperatures as high as 140 degrees C. (as in steam sterilisation) and enzymes are de-activated at such temperatures.

Therefore, current methods still rely on removing samples periodically from the fermentation process and determining the ethanol content - usually by specific density or chromatographic techniques. Clearly, this is inconvenient (as it does not give continuous measurement and may even contaminate the process media) and is slow. Therefore there is an unsatisfied need for an enzyme-free electrochemical sensor for ethanol and the like, and especially one which can withstand repeated steam sterilisation or rigorous chemical sterilisation, and thus can be part of the "clean in place" systems used industrially.

Ethanol is relatively inactive electrochemically (as some other organic compounds are also) and electro-oxidation typically requires strongly alkaline solutions, but by using platinum electrodes it is possible to detect ethanol in neutral or acidic solutions. Even so, the response signals are pH-dependent and are also weak, so they are susceptible to masking by background (capacitative) currents or "noise," and until now there has been no known sensor good enough for practical use.

We have now found that these problems can be overcome and improved performance can be obtained for the detection

of analytes electrolytically by using an electrode (especially a platinum electrode) coated with an aminoacid polymer (which may also be described as "a poly-aminoacid").

Thus according to our invention we provide an improved sensor device, useful in electrolytic analysis procedures, which comprises a working electrode carrying a coating of an aminoacid polymer.

The aminoacid polymer is used because it adheres well to the substrate surfaces (i.e. the electrode surface), and especially to platinum, and also because it greatly improves the performance of the active electrode in the electrolytic determination of substances (and especially ethanol) which are not determined so readily when the active electrode surface is bare.

However, although this electrode coated with an aminoacid polymer works well and shows a substantial improvement in performance over previously known electrode systems, we find that the layer of aminoacid polymer tends to be softened so that it can become loose and detached, wholly or partly, from the surface of an active electrode -- even a platinum surface -- in some liquid media. In particular, this can happen in prolonged contact with some solutions, samples, electrolytes, and the like, and especially in acid conditions; then, the aminoacid polymer tends to be dislodged and fall off the electrode.

This can be a disadvantage in use but, surprisingly, we have found that the sensors of our invention can be improved still further, so as to overcome the disadvantages mentioned above, by the use of a covering barrier layer over the layer of aminoacid polymer. Superimposing a covering barrier layer over the aminoacid polymer has an unexpectedly beneficial effect on the durability of the aminoacid polymer layer and the reliability of the sensor in use. It effectively stops this very undesirable effect

of loosening or detachment, and does so without any adverse effect on the electrode performance. Indeed, the electrode performance can be improved, and the improvement can be unexpectedly great. This enables it to be used in conditions for which it would not be entirely satisfactory without the coating by the barrier layer to protect it from exposure to the sample, for example under the conditions which can occur in fermentation liquors or effluents.

We do not know exactly what the mechanism for this improvement is, but we believe that it may be because the barrier layer is able to augment the performance of the aminoacid polymer by helping to retain a more stable micro-environment beneath it. Whatever the mechanism may be, however, we find that the aminoacid polymer layer is rendered unexpectedly more durable and stable in use by application of a covering barrier layer to help retain it in place.

Thus according to our invention we also provide an improved sensor device, useful in electrolytic analysis procedures, which comprises a working electrode carrying a coating of an aminoacid polymer upon which is superimposed a covering barrier layer.

The covering barrier layer may be any material which serves to maintain the layer of aminoacid polymer in place. It may be in the form of an applied or deposited layer or coating, which for most materials may be conveniently referred to in general terms as "a membrane" though, strictly, it can be continuous or discontinuous and may not have a true "membrane" structure. It should have diffusion-lowering properties, i.e. it should be able to restrict but not stop the passage of components of a sample. Thus it may be for example in the form of a thin layer or film which is sufficiently permeable to allow solutions and analytes to pass though and reach the layer of aminoacid polymer and, eventually, the electrode. For

this, it does not need to be very thick to achieve useful results, and it may be a separate pre-formed layer or film which is applied to the layer of aminoacid polymer, or it may be deposited upon the layer of aminoacid polymer, for example from solution.

The thickness of the covering barrier layer over the aminoacid polymer layer may be varied as found most appropriate having regard for the particular materials, analytes and conditions for which it is to be used. Layers only a few molecules thick (e.g. 10 molecular layers or less, down to even a single molecular layer) apparently can function well, though greater thicknesses can be used if desired - particularly when using a pre-formed membrane and applying it to the aminoacid polymer. These can be formed by dip-coating techniques and the like for coating materials which are soluble or dispersible to a sufficient degree.

The material of which the covering barrier layer is composed may vary, as its function is mainly physical rather than chemical one. The key factor appears to be that it has to be a diffusion-lowering barrier or membrane. This property can apply to many materials which will allow the analyte (especially ethanol) to cross it, for example cellulosic materials (cellulose, cellulose esters e.g. cellulose acetate), porous PTFE and even dialysis membranes, and the material may be homogeneous or anisotropic. When the sensor device is to be used in biological conditions, it is also desirable for the covering barrier layer to be made of a bio-compatible material.

It may be applied to the surface of the aminoacid polymer layer in a variety of ways, depending upon the nature of the particular material to be used to form it. The requirement is that the material should not prevent access of analyte from a sample under examination towards

the aminoacid polymer layer and the electrode (i.e. it should be permeable and not sealing) and should not attack or be attacked by either the sample or the aminoacid polymer. It also should be chosen to satisfy such requirements as compatibility with the aminoacid polymer and with any conditions in which it is to be used. The mechanism of action of the covering barrier layer is not clear, but its permeability towards analyte allows the electrode to function and its presence assists the action of the electrode system and can improve the measurements as well as improving stability.

Thus, it may be microporous, homogeneous or semi-permeable, and it may be made of any material with such properties. For example it may be an organic polymeric material, or alternatively it may be an inorganic material, which can be applied a thin and adherent layer to the surface of the aminoacid polymer to provide the desired properties.

We find that an especially useful material is a polyvinyl chloride (commonly referred to as "PVC") which very effectively maintains the aminoacid polymer layer in place and in contact with the working electrode.

Thus according to our invention we also provide an improved sensor device, useful in electrolytic analysis procedures, which comprises a working electrode carrying a coating of an aminoacid polymer which itself is covered by a coating of a polyvinyl chloride.

The PVC (polyvinyl chloride) may be any polymer (or copolymer) of vinyl chloride, as for example those made and available commercially. Preferably it should be free from any added plasticiser (an ingredient which is often present in some commercial products intended for uses such as moulding) but this is not essential and plasticised PVC can be used if desired. Such "un-plasticised" and "plasticised" PVC polymers are readily obtainable in

commerce, however, and it is necessary only for the quality and purity of any polymer to be checked, whether by its specification or labelling. The molecular weight of the PVC is relatively non-critical, and most commercial grades will be satisfactory in use. A typical molecular weight is in the range 10,000 to 200,000, but others may be used if desired.

The polyvinyl chloride applied as a coating superimposed upon the layer of aminoacid polymer may be of various forms and applied in various ways. Thus it may be for example in the form of a microporous or semi-permeable membrane, which may be pre-formed and then applied to the surface of the layer of aminoacid polymer. Alternatively, it may be formed in situ upon the layer of aminoacid polymer by conventional means of coating, for example deposition from solution or suspension in a liquid medium which does not damage or dislodge the aminoacid polymer.

When other polymeric materials are used instead of PVC, the comments on composition, mode of formation or application, and thickness of the layer or membrane are generally similar to those described for PVC. Variation of the nature of the material and whether or not it contains additive (e.g. is plasticised) may have an effect on their stability (mechanical and thermal), so it is advisable to check whether any particular material is fully suited to the particular conditions (especially heating) which may be encountered in any individual use for which they are intended. Choosing the optimum for any particular use is easily determined by simple trial. If desired, mixtures of materials may be used, or combinations of more than one covering barrier layer or membrane, provided the necessary access of analyte to the sensor device is not reduced so that the effective use of the sensor is hindered.

We have also found that a preferred and advantageous material is diamond-like carbon ("DLC"), because it is much

superior and allows the assembly to be smaller, simpler and more effective. Especially, as DLC is a relatively infusible inorganic material, it can render the coated sensor device more suited to heat-sterilisation as it is not liable to be affected whereas some organic polymers may be liable to melt.

Thus according to a further feature of our invention we provide an improved sensor device, useful in electrolytic analysis procedures, which comprises a working electrode carrying a coating of an aminoacid polymer upon which is superimposed a layer of diamond-like carbon.

Diamond-like carbon is already well-known in itself and described in the art, and commonly referred to as "DLC". DLC is a form of amorphous carbon or a hydrocarbon polymer with properties approaching those of diamond rather than those of other hydrocarbon polymers. Various names have been used for it, for example "diamond-like hydrocarbon" (DLHC) and "diamond-like carbon" (DLC), but the term "DLC" appears to be the most common. It possesses properties attributable to a tetrahedral molecular structure of the carbon atoms in it, similar to that of diamond but with some hydrogen atoms attached. It has been described in the art as being a designation for "dense amorphous hydrocarbon polymers with properties that differ markedly from those of other hydrocarbon polymers, but which in many respects resemble diamond" [J.C. Angus, EMRS Symposia Proc., 17, 179 (1987)].

The formation and application of the diamond-like carbon (DLC) to the aminoacid polymer as coatings or films for the purposes of the present invention may be carried out by methods known in the art. It is usually formed by decomposition of carbon-containing compounds in gaseous or vaporised form (particularly hydrocarbon gases, for example propane, butane or acetylene) induced by radiation or electrical fields.

DLC itself is so well known that a full description of it is not needed here, but details of it and how it can be made are to be found in the literature, for example:-

(a) "Diamond-Like Carbon Applied to Bio-Engineering Materials;" A.C. Evans, J. Franks and P.J. Revell, of Ion Tech Ltd., 2 Park Street, Teddington, TW11 0LT, United Kingdom; Medical Device Technology, May 1991, pages 26 to 29.

(b) "Preparation and Properties of Diamondlike Carbon Films;" J. Franks; J.Vac.Sci.Technol. Vol.A, No.3, May/June 1989, pages 2307-2310;

(c) "Biocompatibility of Diamond-like Carbon Coating;" L.A. Thomson, F.C. Law, J. Franks and N. Rushton; Biomaterials, Vol.12, January 1991 (pages 37-40);

(d) "Categorization of Dense Hydrocarbon Films;" J.C. Angus; E.M.R.S. Symposium Proc., 1987, Vol. 17, page 179; Amorphous Hydrogenated Carbon Films, XVII, June 2-5 1987, Edited by P.Koide & P. Oelhafen.

(e) "Properties of Ion Beam Produced Diamondlike Carbon Films;" M.J. Mirtech; E.M.R.S. Symposium Proc., 1987, Vol. 17, page 377;

(f) "Diamond-like Carbon - Properties and Applications;" J. Franks, K. Enke and A. Richardt; Metals & Materials (the Journal of the Institute of Metals); and

(g) U.S. Patent No. 4490229; M.J.Mirtich, J.S.Sorey & B.A.Banks.

and also in our International (PCT) Patent Application No. PCT/GB 93/00982 (International Publication No. WO.93/24828) and corresponding European Patent Specification No.0647318.

The DLC coating may be made of a thickness which may be varied according to the particular requirements desired for the performance of the sensor and the system to be analysed. The thickness of the DLC coating or deposit may be in the range 0.01 to 5 μm, but thicker or thinner coatings may be used if desired. A convenient thickness is

in the range 0.5 to 5.0 microns, and preferably in the range 0.5 to 2.0 microns, is usually most suitable. As the thickness need not be very great, a typical and convenient coating deposit is one approximately 0.1 μm thick, but this is not necessarily the optimum for all purposes. The thickness in any particular case will depend upon such factors as the nature (physical and chemical) of the material upon which the DLC is deposited, and its porosity or permeability, and the particular characteristics appropriate to the intended use of the sensor.

The coating is conveniently carried out at a rate which allows the deposit to adhere to the aminoacid polymer and form a coating of the desired thickness - preferably also evenly coated so as to cover substantially all the surface without leaving any areas too thinly covered or even un-covered.

Surprisingly, the application of diamond-like carbon as a coating, applied over the aminoacid polymer, has an unexpectedly beneficial effect on the durability of the aminoacid polymer layer and the reliability of the sensor in use, and is better than any other material we know. Unexpectedly, it is very effective in providing protection for the aminoacid polymer layer, preventing its softening and detachment from the underlying metal electrode, without any unduly adverse effect on the electrolytic measurements. This allows effective use over long periods.

The diamond-like carbon coating not only gives stability to the aminoacid polymer layer but also provides a degree of selectivity in favour of ethanol against various potential interferents such as glucose. We are not sure what the mechanism for this is, but it may be associated with molecular size. Interference by sugars such as glucose in the detection and measurement of ethanol is a key problem in fermentations, so this selectivity effect of a diamond-like carbon over an aminoacid polymer

layer and the reduction of interference provides a great advantage for our invention.

The aminoacid polymer may be a polymeric form of any aminoacid. Such compounds are well known in the art and may be obtained commercially or can be made by conventional methods for making or synthesising polypeptides and the like, for example by the techniques of automated synthesis.

If desired, the polymer may be a copolymer derived from more than one aminoacid, but it is usually more convenient to use a polymer of only one aminoacid, and the complication of using a copolymer is not necessary.

The polymer may be derived from various aminoacids, and the term "aminoacid" is used in its conventional sense to refer to aliphatic carboxylic (or poly-carboxylic) acids having one or more amino-groups as substituent on the aliphatic chain - optionally with other substituent groups. Preferably, the amino acid contains a secondary amino group as substituent, and especially preferred amino acids are di-amino mono-carboxylic acids, for example lysine (diamino-caproic acid) or ornithine (diamino-valeric acid) -- both of which are alpha-omega di-amino mono-carboxylic acids. In practice, we find that lysine is particularly preferred as being the most satisfactory one. The aminoacid may be natural or synthetic, but it is usually more convenient and suitable to use a naturally-occurring form, e.g. L-lysine.

The aminoacid polymer may be applied to the working electrode surface as a thin layer, using any conventional coating technique. The preferred method is to use an solution of the aminoacid polymer and apply this to the electrode by dip-coating. the solution is preferably and most conveniently an aqueous one, but if desired a solution in a non-aqueous solvent (or a mixture of solvents, with or without water present) may be used provided it is not harmful to the aminoacid polymer - for example by causing

it to precipitate out from solution prematurely.

The aminoacid polymer for use in the present invention can be defined in terms of its degree of polymerisation and/or its molecular weight -- as these two features are inter-dependent. This choice may also depend upon the particular aminoacid from which the polymer is derived. The molecular weight of the polymer can vary considerably, but in general should be at least 5000 and may be much higher, e.g. 100,000 or more. in practice, we have found a poly-lysine of molecular weight approximately 70,000 to be very suitable.

The layer of aminoacid polymer may be of a thickness in the range 10 to 100 microns (um) but thicknesses which are greater or less than this may be used if desired. We find that the thickness does not need to be very great, and may be limited to the amount of aminoacid polymer that can be easily deposited on the electrode, and may be as thin as a mono-layer.

The choice of aminoacid polymer, its molecular weight and the thickness of the layer used in any particular instance depends upon such factors as the specific aminoacid polymer used, the electrode surface and the readiness with which the polymer adheres to it, the particular conditions of its use and the solutions or materials in contact with it. The optimum should allow a sufficient current/potential to be measured with reasonable ease and convenience. These choices can be determined readily by simple trial.

Once it has been applied to the surface of the active electrode, the aminoacid polymer can be stabilised in place by drying and fixing it on the electrode surface (e.g. metal), for example by drying and heating, conveniently to a temperature of about 50 to 100 degrees C. The time of heating may vary according to the temperature used and the degree of fixation found most effective.

The aminoacid polymer is used because it adheres well to the substrate metal, especially to platinum, but it tends to be softened and loosened from the platinum metal electrode base by prolonged contact with liquids, especially acid solutions (as would occur in using the coated electrode in fermentation liquors or effluents), so it is rendered more durable by application of barrier layer over it.

The working electrode may be any of those known in the art to be usable as such in a system or medium to which this invention is applied. The preferred electrode material is platinum, because it has good reactivity towards ethanol and the aminoacid polymer adheres well to this metal, but the electrode may be made of any material with an electrochemically active surface -- for example any of those used in electrochemical analysis where the electrode surface is electrochemically polarised -- provided the polymer can adhere or can be sufficiently well retained in place while in use, as for example with a semi-permeable membrane or other form of covering barrier layer as described more fully herein. Examples of working electrode materials include platinum, gold, and alloys thereof, and carbon, or combinations thereof, but the preferred electrode material is platinum because it has good electrolytic reactivity towards ethanol and the aminoacid polymer adheres well to this metal. The electrode may be in any conventional form, for example sheet or wire, or as a coating deposited upon a substrate support or carrier, or any combination thereof. When a support or carrier substrate is used it may be any which is capable of retaining the active electrode surface material on it without interfering with the electrical or chemical properties or the measurement procedure.

According to our invention we also provide a method for electrolytic analysis of a liquid medium, which is

characterised in that the said liquid medium is contacted with with a sensor device as defined above.

The output signal, or changes in output signal, obtained from the sensor electrode can then be used as a measure of the analyte sought.

Analyte compounds which can be detected and determined according to the present invention should be volatile and may be polar or non-polar, and should have a molecule which is uncharged. By the term "polar" we mean a compound whose molecule has a finite dipole moment. Preferably it is a non-polar compound. Its degree of volatility is important, and we find that compounds of low volatility do not permeate readily through to the aminoacid polymer and the working electrode. In most respects, the volatility which is appropriate for the operation of our invention is found in organic compounds which have vapour pressures which give the compound a boiling point at normal atmospheric pressure (760 mm. of mercury) of not greater than 150 degrees C., and preferably not greater than 80 degrees C. The compound may be solid, liquid or gaseous, but provided it has have the required volatility, in terms described above, the invention is applicable to it. As a guide, the compound's molecular weight is relevant, and this should be less than 400, and preferably substantially less - especially less than 100.

The present invention and this method of analysis are especially applicable to the detection and determination of ethanol, partly because the ability of this compound to pass through the covering barrier layer is so surprising and partly because a facility to make reliable analyses for this compound is of such high commercial and industrial importance and exceptionally wide applicability.

This method of analysis is especially applicable to the monitoring, measurement and assessment of media in which ethanol is being formed or produced -- e.g.

fermentation media, in which ethanol is formed by alcoholic fermentation of sugars -- and for monitoring and controlling the progress of fermentation processes. Thus, it is especially useful for analysis of the media used in the course of making alcoholic beverages, as well as the "finished" alcoholic products, e.g. alcoholic beverages for which fermentation is not being continued before sale, storage or later treatment. It is also applicable to other alcoholic media, e.g. distilled or fortified spirits, whether intended for use as beverages or not, and for the study of waste materials which may contain or give rise to ethanol.

Thus according to our invention we also provide a method for electrolytic analysis, especially for the detection of ethanol in a liquid medium, which comprises contacting the said liquid medium with a sensor device as defined above.

The combination of the aminoacid polymer and diamond-like carbon results in a very desirable combination of properties --- including pH independence, stable adherence of the coating, permeability, heat-stability (which can allow the sensor device to sterilised by heat, and selectivity which can minimise interference by other components (e.g. sugars) in the detection and determination of ethanol. These properties make it eminently suitable for use in making continuous measurement of ethanol content in the progress of fermentation processes.

The mode of electrolytic analysis used to carry out the method of our invention is preferably amperometric analysis, which is well known and used in the art. In this, we find that problems arise because oxidised products formed from the analyte (e.g. ethanol) during the electrolytic oxidation which occurs during amperometric analysis, tend to adhere to the electrode (metal) surface and thereby impede further functioning of the electrode.

This passivation effect can be overcome, however, by using the known technique of pulsed amperometric detection ("PAD").

In PAD analysis the waveform of the electrical potential applied is cycled continuously between positive and negative voltages, and the current flow is measured at a predetermined point in the cycle for a specified duration. The average current generated during this specified measurement period is then plotted to give a response curve of current against time. This procedure has the advantage of cleaning the electrode (removing the troublesome oxidation products) and also giving more rapid and stable responses -- so making the resulting measurements easier to make and more reliable to use, for example in being more free from noise and interference. The voltages applied to the electrode in this procedure can be varied over a considerable range, for example between +0.7 and -0.6 volts, and the measurements taken during made during the "specified duration" part of the cycle can be at a voltage appropriate for the measurement itself - conveniently at about 400 mV. Likewise, the duration of the measurement and the parts of the cycle may vary, but usually can be taken so that the whole cycle takes about 1 to 2 seconds. These voltages mentioned here are those made with reference to a standard silver/silver chloride (Ag/AgCl) electrode.

Description of the Pulsed Amperometric Detection ("PAD") techniques are to be found for example in the following published references (among others):-

(a) J. Moracova, J. Stanek, J. Capkova & R. Lowcka; Rostlinna Vyroba, (1997), Vol. 43, 289-292.

(b) J.I. Yu, W.G. Huang & D.B. Hibbert; Electroanalysis, (1997), Vol. 19, 544-548.

(c) D.J. Tarnowski & C. Korzeniewski; Anal.Chim.Acta. (1996), Vol. 332, 111-121.

(d) W.K. Herber & R.S.R. Robinett;
J.Chromatography A, (1994), Vol. 676, 287-295.

The pH of the sample liquid contacted with the sensor devices of this invention for examination can have a considerable effect on the magnitude of the response signal when the aminoacid polymer is not coated with a covering barrier layer, especially PVC or DLC.

The coating layer of aminoacid polymer (e.g. poly-lysine) on the electrode overcomes this dependency upon pH, and the covering barrier layer (especially a PVC or DLC coating) secures the aminoacid polymer layer and gives it some selectivity. The combination thus enables the sensor to be stabilised and have a much lower pH dependency that electrodes coated with aminoacid polymer alone.

The principal advantage of our sensors (and especially those with a barrier layer coating over the aminoacid polymer - e.g. DLC, PVC etc.) is that they can be used in conditions of variable pH and be subjected to heat without their performance being destroyed. This enables them to be used for long periods of time and through many repeated use/cleaning/sterilising/re-use cycles, as is commonly done in commercial fermentation processes, especially in batch processes in which steam sterilisation is used between batches to avoid any contamination of the fermentation media.

The sensor devices and electrodes of our invention need not necessarily be made with a single electrode element, but can if desired be made in the form of a multiplicity of separate electrode elements. Such separate elements can be made small (as in a so-called micro-array or micro-electrode array), and may be connected electrically in any convenient way so that the signal output from them can be measured. For such arrays, the barrier layer (e.g. PVC or DLC) is especially useful, as it can provide a more viable, convenient and reliable way than

a restraining conventional membrane (e.g. of polycarbonate) for coating the aminoacid polymer to keep it in place.

An advantage of using multiple small electrode elements is that the surface of an array of them can be made to have various surface formations and configurations which can assist the construction and use of the device in practice. Also, such arrays can allow use of higher current densities, which can decrease background noise. For example, the surface can be made in a pitted form (i.e. a form in which the surface has a multiplicity of recesses or "pits" which are small enough to hold the aminoacid polymer and any barrier layer coating such as PVC or DLC). These recesses or "pits" can then, if desired, be provided with means over or around their entrances (especially "guard electrodes" - conveniently in the form of small rings or conducting regions to which an appropriate voltage potential can be applied) to affect or control the entry of charged solutes into the recesses and so to the underlying electrodes.

Calibration of our sensor device for use can be carried out in conventional manner, preferably by immersion of the device in samples of the medium which is to be monitored or examined (e.g. fermentation liquor). An isotonic or other buffer may be used, but it is preferable to use one which has an ionic strength similar to to that of the media in which the device is to be used.

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CLAIMS:-

1. Sensor device, useful for electrolytic analysis, which comprises a working electrode carrying a coating of an aminoacid polymer.
2. Sensor device as claimed in Claim 1 wherein a layer of material is superimposed upon the layer of aminoacid polymer to maintain it in place.
3. Sensor device as claimed in Claim 2 wherein the layer of material superimposed upon the aminoacid polymer layer to maintain it in place is a covering barrier layer, preferably a diffusion-lowering barrier layer.
4. Sensor device as claimed in Claim 3 wherein the covering barrier later is composed of an organic polymer, especially a polyvinyl chloride.
5. Sensor device as claimed in Claim 3 wherein the covering barrier later is composed of a diamond-like carbon ("DLC").
6. Sensor device as claimed in any of Claims 2 to 4 wherein the covering barrier layer is deposited upon the aminoacid polymer layer on the support electrode as a thin layer formed by dip-coating with a solution of the material of the covering membrane barrier.
7. Sensor device as claimed in any of Claims 2 to 7 wherein the covering barrier layer is of a thickness in the range 0.01 to 5 μm, for example in the range 0.5 to 5.0 microns, and preferably in the range 0.5 to 2.0 microns.
8. Sensor device as claimed in any of Claims 2 to 8 wherein the thickness of the covering membrane barrier is 10 molecular layers or less.
9. Sensor device as claimed in any of Claims 1 to 8 wherein the working electrode material is platinum.
10. Sensor device as claimed in any of Claims 1 to 9 wherein the aminoacid polymer is derived from an amino

acid containing a secondary amino group as substituent, preferably an di-amino mono-carboxylic acid, for example lysine or ornithine and especially L-lysine.

11. Sensor device as claimed in any of Claims 1 to 6 wherein the aminoacid polymer has a molecular weight of at least 5000 and especially 100,000 or more.

12. Sensor device as claimed in any of Claims 1 to 11 wherein the layer of aminoacid polymer is of a thickness in the range 10 to 100 microns (μm).

13. Sensor device as claimed in any of Claims 1 to 4 wherein the aminoacid polymer is on the support electrode as a thin layer, preferably formed by dip-coating with a solution of the aminoacid polymer.

14. Sensor device as claimed in any of Claims 1 to 5 wherein the aminoacid polymer, after application to the electrode, is stabilised in place, for example by drying and heating, e.g. to a temperature of about 50 to 100 degrees C.

15. Sensor device as claimed in any of Claims 1 to 14 wherein the sensor devices and electrodes of our invention are made using a multiplicity of separate electrode elements, for example as a multi-electrode array or micro-array.

16. Method as claimed in any of Claims 1 to 15 wherein the electrode surface is made in a pitted form (i.e. a form in which the surface has a multiplicity of recesses or "pits") small enough to hold the aminoacid polymer, especially with means over or around the entrances of such recesses or "pits" to affect or control the entry of charged solutes into the recesses and so to the underlying electrodes, especially "guard electrodes" - conveniently in the form of small rings or conducting regions to which an appropriate voltage potential can be applied.

17. Sensor device incorporating a layer of an aminoacid polymer on an active electrode surface, with or without a layer of material superimposed upon the layer of aminoacid polymer, substantially as described.

18. Method for electrolytic analysis of a liquid medium which comprises contacting the said liquid medium with a sensor device as claimed in any of Claims 1 to 17.

19. Method as claimed in Claim 18 wherein the mode of analysis used is amperometric analysis.

20. Method of analysis as claimed in Claim 19 wherein the analysis is carried out by pulsed amperometric detection ("PAD").

21. Method as claimed in any of Claims 18 to 20 wherein the analyte sought is ethanol.

22. Method as claimed in any of Claims 16 to 21 as applied to the monitoring, measurement and assessment of media in which ethanol is being formed or produced, for example in fermentation media, for monitoring and controlling the progress of fermentation processes.

23. Method as claimed in any of Claims 16 to 21 as applied to the monitoring, measurement and assessment of industrial media, e.g. process media, waste media or effluents and the like.

24. Method of analysis, especially electrolytic analysis, using a sensor device incorporating a layer of an aminoacid polymer on an active electrode surface, with or without a layer of material superimposed upon the layer of aminoacid polymer, substantially as described.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 98/03585

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 G01N27/30

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)
IPC 6 G01N

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 practical, search terms used)

C. 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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A	C. J. PICKETT: "BIOORGANIC REACTION CENTRES ON ELECTRODES. MODIFIED ELECTRODES POSSESSING AMINO ACID, PEPTIDE AND FERREDOXIN-TYPE GROUPS ON A POLY(PYRROLE) BACKBONE" J. CHEM. SOC., DALTON TRANS., vol. 14, 1994, pages 2181-2189, XP002066042 see abstract --- -/--	1

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

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"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
19 April 1999	25/05/1999

Name and mailing address of the ISA	Authorized officer
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INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 98/03585

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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A	US 5 281 319 A (KANEKO HIROKO ET AL) 25 January 1994 see abstract	1
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