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AN OVERVIEW OF TYPICAL METHODS AND RESULTS FOR BIOSENSOR REGENERATION

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ABSTRACT

Biosensors are excellent low-cost, portable instruments for detecting infections, proteins, and other analytes quickly. The worldwide biosensor industry is now valued over ten billion dollars per year, and it is a rapidly growing area of multidisciplinary study that is being heralded as a possible revolution in consumer, healthcare, and industrial testing. However, the expense of biosensors is a major impediment to their broad use. Many systems have been validated in the laboratory, and biosensors for a variety of analysts have been proved at the concept level, but many have yet to establish a compelling business case for adoption. Though there is a downward pressure on prices due to the development of cheaper electrodes, circuits, and components, there is also an increasing trend toward the development of multi analyte biosensors that is pulling in the other direction. One method to decrease the cost of some items is to allow them to be reused, lowering the cost-per-test.

KEYWORDS: *Biosensors, Kinetic Energy, Potential Energy, Receptors, Regenerators.*

INTRODUCTION

When the analyte interacts with the bioreceptor, a quantifiable signal is produced. Biosensors are typically characterized as a three-part system consisting of a bioreceptor, a transducer, and a signal processing unit. Sensors have been created for a wide range of analytes in areas such as medicine, food testing, environmental sensing, and process control monitoring in research and industry[1]. These sensors were created to replace conventional testing methods, which are typically technical in nature and require specialized knowledge and time, resulting in a substantial expense in their respective sectors. Although morecostly sensors are utilized in research, less priced sensors have the potential to reach a broader market. The present high prices

may be ascribed to the specialized nature of the equipment needed, as well as the dependence on high-grade analytical reagents and materials; a conventional system can cost thousands of dollars up front, while each sensor transducer assembly can cost up to eighty dollars A variety of methods are presently being explored to reduce the cost of biosensors[2].

On the one hand, enhanced printing using conductive polymer inks is being used in the creation of low-cost, disposable transducers and biosensor assemblies. This has shown to be effective in displacing costlier components and lowering system expenses. These disposable biosensors may be helpful in medical applications where cross contamination and cleanliness are concerns. Disposable sensors, on the other hand, are inappropriate for certain systems; if, for example, time course measurements are required, chip to chip variation may become a source of inaccuracy[3]. Similarly, extremely precise high-grade transducers are needed for certain applications, and the accompanying expenses cannot be avoided. In such cases, regeneration may be a significant strategy for reducing test costs. When it comes to creating biosensors that meet the requirements of the poor world, cost reduction is very essential. Biosensors for assessing food safety, water sanitation, and environmental testing are examples of proven biosensors that cater specifically to these requirements. Another critical need in the developing world is healthcare and diagnostic tools for illnesses that are presently causing high rates of death and morbidity due to avoidable causes[4].

More recently, a trend toward the creation of multi-analyte arrays of biosensors has emerged. Multiple biomarker analyses may theoretically offer a greater level of diagnostic confidence. Multi-analyte arrays, on the other hand, have the intrinsic requirement for more sophisticated transducer systems and data processing, which makes cost a major obstacle to their commercialization. Because each receptor: analyte pair will have its own unique binding physics and buffer systems that are optimized for one receptor: analyte pair may be a poor option for others, these multi analyte arrays may offer a special challenge when regenerating. A technique for repeated sampling is made possible by allowing biosensor renewal. Sensor-to-sensor variation is eliminated as a result, which is especially helpful when monitoring across time or probing comparable amounts of analyte. The development of impedimetric immune sensors is one area where chip-to-chip variation still poses a significant challenge. This problem may be completely solved by allowing regeneration While reviewing the research, it became clear that comparing the effectiveness of various regeneration plans was difficult[5]. This was owing to the fact that different definitions of regeneration were used across the literature. In our conclusion, we suggest a set of criteria for defining "biosensor regeneration" in order to establish a consensus across the field and guarantee that it is relevant to all fields of biosensor research.

Biosensors are categorized in one of two ways: by signal transduction technique (optical, mechanical, or electrochemical) or by bioreceptor type. Catalytic sensors, which utilize enzymes, and affinity sensors, which use binding proteins or nucleotides, which includes immune sensors, are the two main groups that by bioreceptors fall under. Immunesensors are affinity sensors that detect the target analyte using antibodies or their derivatives. When discussing biosensor regeneration, it's critical to examine the bioreceptor's molecular contact with the analyte via mediating a specific response[6]. Enzymes serve as the bioreceptor in catalytic sensors, which process an analyte to produce a signal. Some of these enzymatic sensors do not need to be actively regenerated during regeneration since the analyte is consumed and the baseline signal is

ultimately restored. Though some studies have documented the re-use of these biosensors, this is not active regeneration, which is a critical difference to note in the field; this process is often referred to as passive regeneration. Another difference to make between biosensors is whether the assays directly measure the analyte or are part of a competitive assay[7].

Competitive assay-based sensors don't get their data directly from the analyte, but rather through the competitive binding or inhibition of a secondary process. The suppression of analyte detection must be evaluated with the inhibition of other stages in the test method that may potentially influence the signal when considering regeneration. Regeneration has been shown in a variety of systems, but the methods, reagents, and circumstances used in each study differ considerably. The different processes of regeneration are described in the following sections, and the most effective regeneration agents are evaluated. The attraction forces between the bioreceptor and the analyte must be overcome in all instances for regeneration to occur. When considering these forces in terms of thermodynamics, both an enthalpy and an entropic contribution must be taken into account. Because these forces are affected by the solvent environment, a regeneration buffer may be used to change them[8]. The entire energy of a thermodynamic system is defined as enthalpy. This energy may be dispersed in a variety of ways, including heat (kinetic energy) and potential energy, which can be expressed in a variety of ways, including chemical bonds and ionic or polar charges. A system will equilibrate to decrease total potential energy, according to the first rule of thermodynamics. Potential energy differences are often a significant factor in bioreceptor: analyte binding when examining interactions involved in biosensor functioning. Charge-charge interactions are frequently used to moderate interactions[8].

Depending on the iso-electric point of the amino acid residue, different amino acids may be positively or negatively charged at a particular solution. Using blood as an example (pH 7.4), there are acidic positively charged amino acids like asparagine and glutamine, as well as equivalent basic or negatively charged residues like lysine, arginine, and histidine in this environment. The bioreceptor binding region's tertiary structure is formed by these charged side groups. The interaction of charges is natural and tends to the system's lowest potential energy. Because charge is affected by the solvent environment, factors like ionic strength, pH, and the presence of competitor ions in the solvent can change the relative strength of charge interactions, allowing for more effective screening of enthalpy interactions between the analyte and the bioreceptor and thus assisting in biosensor regeneration. Typical decreases in enthalpy following antibody: antigen binding vary from as little as 26 KJ.mol-1 to more enthalpically driven interactions with changes as high as -130 KJ.mol-1 in extreme cases27. When compared to normal values for covalent bonds, which range from 200 to 400 KJ.mol-1, this is a significant shift in enthalpy. It's essential to remember that at extremely low ionic strengths, an antibody's binding may be promiscuous since any charge difference facilitates less selective binding, potentially lowering the binding species' overall stringency. High ionic strength environments, on the other hand, may screen antigen-antibody interactions and decrease binding[9]. The intrinsic chaos or disorder of a system is characterized as entropy.

According to the second rule of thermodynamics, a system's entropy will always grow, resulting in a more disordered system. This lowers the system's total potential energy; according to Gibb's Law, a process is spontaneous if the Gibb's free energy is negative. Gibbs free energy is the

difference between entropy and enthalpy. Though analyte binding is thought to reduce a system's entropy, entropic compensation occurs via mechanisms such as solvent displacement. The function of solvent molecules in the system must be considered to understand this. Because the free analyte is extremely disordered, the unbound state has a high entropy; however, the reduction in entropy when the analyte attaches is exceeded by the change in enthalpy, resulting in a negative Gibb's energy change, which explains why this is a spontaneous process. Certain systems, especially when dealing with hydrophobic analytes, have higher entropy upon binding, but this is less common. Because hydrophobic analytes cause ordered water caging at the solvent interface, this is the case. After that, a biosensor is regenerated. Biosensors have been created at the protein level. The detection of hydrophobic analytes that have been regenerated, such as fibrin.With regard to the solvent, the creation of hydration shells results in a highly ordered low entropy system, particularly near the interface, where any less order would be energetically unfavorable[10].

DISCUSSION ON BIO SENSOR REGENERATION

The increase in entropy from released water molecules outweighs the slight decrease in entropy with regard to the analyte and receptor in these situations. Entropically driven binding must be minimized by negating the effects of hydrophobic areas in order to reverse these interactions; as a result, aliphatic detergents are often employed. In an aqueous solution, this allows for the disruption of water caging and the reduction of the hydrophobic effect at the analyte-bioreceptor interface, allowing regeneration. The solvent environment at a sensor interface is a crucial parameter that affects analyte: bioreceptor binding, as described above. The most common method for renewing biosensors is to change the liquid environment chemically. By withdrawing the transducer from any assembly and immersing it in a regeneration buffer, this may be done quickly. As a result, regeneration solutions are often made up of common chemicals, making sensor regeneration a low-cost option. Though it is a basic method, it may be improved with the use of a fluidic control system, or computerized control module; nevertheless, there is presently only little demonstration. This strategy may be crucial in the creation of a field-generable bio sensor. The following is a review of the most frequent chemical methods for biosensor regeneration that have been proven. In many cases, regeneration has been accomplished by using high or low pH buffers in the system. In order to avoid permanent damage to the bioreceptor, a low pH buffer will typically go no lower than ph. A high pH buffer, on the other hand, is often restricted to a pH of about 11 for the same reason. The system is affected in two ways as a result of this. To begin with, a change in pH affects the system's enthalpic state by altering the relative charges between the analyte and the bioreceptor. The charge distributions that preserve the bioreceptor's tertiary structure change when the side groups get ionized. Decoupling of the analyte from the bioreceptor is aided by structural denaturing.

Second, a change in pH causes a change in the environment's ionic strength, which may be used to screen receptor-analyte interactions. Strong electrolytes as Ca2+ 39 and NaCl may also be used to change the ionic strength. If a system is especially sensitive to changes in pH, this may be a better option for preventing permanently denaturing sensor components like the bioreceptor or changing the transducer's electrical state. Although the use of acidic / basic regeneration has been extensively documented, it has one drawback: it can only be utilized in systems where the changed pH will not interfere with the sensor signal. This makes pH regeneration in

electrochemical systems, where charge may influence the sensor's baseline output, especially challenging. Another important area where pH regeneration is not appropriate is the usage of extremely delicate bioreceptor proteins. If they are readily denatured, this would be a bad approach since the bioreceptor would be irreversibly damaged. The most significant benefit of employing acidic or basic regeneration is its cheap cost and broad applicability. In the regeneration of biosensors, detergents are often employed at low quantities.

In terms of structure, detergents are hetero-bifunctional molecules with two different regions: a polar head that is highly soluble due to its charge and an aliphatic non-polar tail. Due to their hydrophobic nature, the tail portions of the bioreceptor or analyte associate with comparable areas of the bioreceptor or analyte in an entropic ally-driven process. The polar head group extends into the aqueous phase, reducing repulsion and promoting analyte solubility45. Detergents may be a significant component of a regeneration buffer in some biosensor systems where hydrophobicity is a major factor in the interaction of the bioreceptor with the analyte, such as in the detection of hydrophobic analytes like 2-naphthol and 3-isobutyl-2-methoxypyrazin. Milder detergents, such as Tween®, are often employed, but low quantities of harsher detergents, such as SDS, have been utilized in the past. Detergents are helpful at low concentrations and to prevent pH extremes, but they may disrupt systems like self-assembled monolayers (SAMs) and should thus only be employed in systems with a solid transducer interface. The amino acid glycine is a commonly utilized regeneration agent for a variety of reasons, including assisting separation and minimizing harm to the bioreceptor. Glycine is a low-cost, widely accessible regent with a pH buffering range of 2-754. This buffering range is suitable for an acidic buffer that avoids pH extremes in certain areas. Because glycine is the simplest amino acid, it dissolves effectively in both aqueous and more hydrophobic environments and may easily mediate forces at a particularly hydrophobic interface, lowering the bound state's entropic favorability. Glycine is swatterionic in solution and serves as a moderate screening agent for charges at the interface, reducing enthalpic pressures between the bioreceptor and analyte once again. Because it is thermodynamically preferable, glycine prefers to attach to the surface of the bioreceptor and analyte. The bioreceptor is therefore partly shielded from harm induced by the changing pH environment when exposed to a regeneration buffer.While glycine is helpful in optical and mechanical sensor systems, it may have limited use in electrochemical sensor systems since the use of low pH may irreversibly alter the sensor output.

Another well-known chaotrope is urea, which is often used in pH-sensitive settings because of its ability to maintain a neutral pH in solution. Urea was utilized to renew sensors that acquired their data via cyclic techniques, avoiding any changes to the tethering layer and signal disruption. Changing the temperature has a big impact on the structure and behavior of biological molecules like proteins and oligonucleotides. Temperature increases the kinetic energy of molecules, which may enable binding forces to be overcome. Although warming causes permanent denaturing and aggregation in most proteins, some groupings of proteins, as well as oligonucleotide base pairs in general, may be dissociated by increasing the temperature in a process known as melting. Base pairing between the nucleotides holds double stranded DNA dsDNA together at room temperature. The number of base pairs used to join the strands is directly proportional to the temperature at which the individual DNA strands acquire enough kinetic energy to overcome base pairing and split. The use of DNA Melting as a feasible technique for the regeneration of nucleic acid biosensors has already been shown. Other DNA structures, such as aptamers, may

be transiently denatured in the same way as double-stranded DNA can. This has been shown for sensor regeneration utilizing biosensors based on DNA:protein interactions. Though thermal regeneration has been shown to work with nucleotide-based bioreceptors, it is restricted to this kind of sensor since heating would destroy or denaturize the biological components of many other sensors.

Biosensors have been regenerated utilizing direct electrochemical techniques in a small number of investigations. By providing a negative potential to the sensor surface, reductive desorption of surface species was accomplished in these experiments. Though under-represented in the literature at the moment, possibly owing to its restricted application, this is an excellent solution to the issue of regeneration since it creates a very localized regeneration environment that can be carefully regulated. In one example of electrochemical biosensor regeneration, indium tin oxide electrodes were exposed to an electro reductive current to renew antibodies on the sensor surface. The transducer surface is a key concern when building a biosensor, since it is the physical substrate on which the sensor is built and to which the bioreceptor is connected. The regeneration method used is often influenced by the transducer used. Here's a quick rundown of the most popular transducer materials. Silica is also especially helpful since it can be manufactured to have smooth microscale surfaces.

Its chemical inertness prevents the regeneration buffer from reacting with the transducer surface, and its flatness allows the buffer to be readily wiped away from the sensor surface. An electrically conductive substrate is required for electrochemical sensors. Many contemporary examples do so by screen-printing carbon or metallic electrodes, which, although cost-effective, may lead to quality problems owing to the wide range of micro and Nano-topologies produced during the printing process. When attempting regeneration, especially when washing the electrodes, this local variance becomes troublesome, with rougher areas proving more difficult to renew. Other techniques for electrode manufacturing have been used to get a flatter surface, such as sputtering and vapor deposition of the conductive layer, both of which produce flat layers at the nanoscale. Extra expenses are unavoidably incurred as a consequence of such additional pre-processing. Though certain kinds of biosensors allow for direct bioreceptor conjugation to the transducer surface, there is frequently a loss of biological activity as a result; this is especially true when working with metal surfaces. Despite the fact that electrochemical biosensors are often touted as having a large potential effect in a variety of analytical areas (as the glucose biosensor's success has shown), many electrochemical sensors have yet to reach widespread popularity. Although regeneration may help improve the economic feasibility of these sensors, research has so far been restricted.

Despite the limited study, several results have been reported, with aerometric and potentiometric sensors being successfully regenerated. The aerometric sensor, for example, has been claimed to be re-used 1000 times with little signal loss despite the fact that, as previously stated, this is not "regeneration" per se. In potentiometric sensors, the current or potential changes when the analyte is present, allowing for calibration. Liu et al. utilized urea, a powerful chaotrope, to renew the sensor through 10 cycles with little signal loss in their most effective regeneration research. More instances of regeneration in potentiometric biosensors are provided, which are used in many cases to prevent the impact of strong acids or bases, which may permanently change the sensor's electrochemical characteristics. Though it's possible that the usage of Urea

influenced the signal over time by altering the charge properties of the biosensor surface. Due to its dependence on binding proteins, which have a considerably larger repertory than enzymes, electrochemical immune sensors may be designed for a far broader range of analytes. They're often tested impedimetrically, which is a highly sensitive technique that relies on the capacitive and resistive characteristics of the transducer surface. These sensors may either look at the change in these characteristics immediately after analyte binding reagent less sensors or employ reagents such HRP-tagged secondary antibodies or nanoparticles to increase the signal seen after analyte binding (reagent-based sensors). In any case, charge transport characteristics are critical in this method, and any regeneration buffers employed may change these charge-related features. Using slightly acidic glycine or mildly alkali regeneration buffer before neutralizing to reestablish a stable baseline signal, there have been a few documented instances of effective regeneration that has prevented permanent modification of the biosensor.

CONCLUSION AND IMPLICATION

While several other biosensors have successfully shown regeneration, optical sensors have had the most success. Optical sensors have proved to be the most effective since regeneration methods that do not alter the sensor's optical characteristics have shown to be very easy to design. Though this is also true with acoustic biosensors, regeneration has proven more effective on a smaller scale, both in terms of the number of studies and the amount of time that sensors may be reused. The use of pH is the most frequently utilized example for protein regeneration: protein interaction based sensors and low pH glycine buffer are the most widely employed agents. Low/high pH pulses are a promising option for optical and acoustic sensor regeneration. Other regeneration methods have been found to renew certain sensors, including changing the temperature, ionic strength, and using powerful detergents. These should only be used in situations when the biophysics of recognition is a driving factor, such as with hydrophobic or strongly ionic analytes. The comparison of various regeneration methods reveals how additional kinds of biosensors may be regenerated.

REFERENCES

1. F. Bibi, M. Villain, C. Guillaume, B. Sorli, and N. Gontard, “A review: Origins of the dielectric properties of proteins and potential development as bio-sensors,” *Sensors (Switzerland)*. 2016, doi: 10.3390/s16081232.

2. R. Seitz, A. Hilger, and M. Heiden, “Bone Marrow, Peripheral Blood, or Umbilical Cord Blood: Does the Source of Allogeneic Hematopoietic Progenitor Cells Matter?,” *J. Blood Disord. Transfus.*, 2012, doi: 10.4172/2155-9864.s1-007.

3. J. H. He and Y. Liu, “A hierarchy of motion in electrospinning process and e-infinity nanotechnology,” *J. Polym. Eng.*, 2008, doi: 10.1515/POLYENG.2008.28.1-2.101.

4. D. Stephenson, D. A. Lockerby, M. K. Borg, and J. M. Reese, “Multiscale simulation of nanofluidic networks of arbitrary complexity,” *Microfluid. Nanofluidics*, 2015, doi: 10.1007/s10404-014-1476-x.

5. P.-Y. Chiou, T.-H. Wu, S.-Y. Park, and Y. Chen, “Pulse laser driven ultrafast micro and nanofluidics system,” 2010, doi: 10.1117/12.861757.

6. A. J. S. Ahammad, “Hydrogen Peroxide Biosensors Based on Horseradish Peroxidase and

Hemoglobin," *J. Biosens. Bioelectron.*, 2012, doi: 10.4172/2155-6210.s9-001.

7. V. H. Liu, C. C. Vassiliou, S. M. Imaad, and M. J. Cima, "Solid MRI contrast agents for long-term, quantitative in vivo oxygen sensing," *Proc. Natl. Acad. Sci. U. S. A.*, 2014, doi: 10.1073/pnas.1400015111.

8. T. Jiang *et al.*, "Response to comment on 'Interference-free determination of ischemia-modified albumin using quantum dot coupled X-ray fluorescence spectroscopy' [Biosens. Bioelectron. 51 (2014) 136-142]," *Biosensors and Bioelectronics*. 2015, doi: 10.1016/j.bios.2014.08.042.

9. S. Sang, Y. Zhao, W. Zhang, P. Li, J. Hu, and G. Li, "Corrigendum to 'Surface stress-based biosensors' [Biosens. Bioelectron. 51 (2014) 124-135] DOI: 10.1016/j.bios.2013.07.033," *Biosensors and Bioelectronics*. 2015, doi: 10.1016/j.bios.2015.06.035.

10. A. Kueng, C. Kranz, and B. Mizaikoff, "Erratum to 'Amperometric ATP biosensor based on polymer entrapped enzymes' [Biosens. Bioelectron. 19 (2004) 1301-1307] (DOI:10.1016/j.bios.2003.11.023)," *Biosensors and Bioelectronics*. 2007, doi: 10.1016/j.bios.2006.11.001.