Pharmaco-Biotechnology and Nanotechnology

Pharmaco-Biotechnology and Nanotechnology:

Therapeutic Applications and Strategies

Edited by

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Pradipta Banerjee,
and Venkata Prasuja Nakka

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CHAPTER 1

A GUIDE TO THE DEVELOPMENT OF DIFFERENT KINDS OF BIOSENSORS IN THE AREA OF PHARMACOLOGY

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Abstract

A sensor is a device used for the qualitative or quantitative estimation of an analyte. A biosensor is a special class of sensor where a biological detector element is used. The key components of a biosensor are detector element (enzymes, antibodies, biomimetics etc.), transducer (electrochemical, optical, piezoelectric etc.) and signal processor. There are different strategies to identify the detector element corresponding to an analyte. There are three generations of biosensors based on the method of measurement and signal generation. The sensors have immense potential in medical diagnostics.

Keywords: Biosensor, Detector, Transducer, Enzyme, Biomimic

1. Introduction

A sensor may be defined as a device that is capable of detecting or measuring a particular property, thereby recording, indicating, or otherwise responding to it. The simplest chemical sensor is litmus paper, used to identify an acid or base, based on color change. On the other hand, pH paper, with a complex mixture of dyes, is used to estimate the hydrogen ion concentration of a solution. It should be noted that the best way of determining the acidity or alkalinity of a solution is to use a pH meter, an electrochemical device, which generates an electrical response that is

displayed on its digital screen. Litmus paper is an example of a qualitative sensor while the pH meter is a quantitative sensor.

There are at least five sensors built into the human body. The nose is a good quality gas sensor. The tongue can sense the tastes of different substrates and acts as a chemical sensor. The ears can sense vibrations and act as vibration/pressure sensors. The eyes are delicate color sensors. The fingers are used to sense temperature and other physical characteristics of materials.

Thus, the sensors could be broadly classified into two types based on their use: (a) qualitative (e.g. litmus paper) and (b) quantitative (e.g. pH paper or pH meter).

On the other hand, based on the parameters measured, sensors can be classified into (a) physical sensors (e.g., balance to measure mass) and (b) chemical sensors (e.g., equipment to measure blood glucose levels). Biosensors are a special class of chemical sensors that can detect/measure analytes using biological sensing components.

2. Biosensors

A chemical sensor responds to a substrate (an analyte) in a specific manner based on a chemical reaction. That reaction helps to identify or measure the type of chemical, qualitatively or quantitatively. Biosensors are considered to be a type of chemical sensor but are often categorized as a separate class. A biosensor is a device that detects or measures an analyte or group of chemicals by connecting a biological recognition/detector element to a transducer. The major difference between a chemical sensor and a biosensor is that in the case of a biosensor the recognition element is biological.

The IUPAC committee has defined a biosensor as “a self-contained integrated device, which is capable of providing specific quantitative and semi-quantitative analytical information using a biological recognition element (biochemical receptor), which is retained in direct spatial contact with a transduction element” [Biosensing for the 21st Century edited by Fred Lisdat, Springer, Berlin, DOI 10.1007/978-3-540-75201-1, ISBN 976-3-540-75200-4]. Professor Leland C Clark Jnr. introduced the biosensor concept through the invention of oxygen electrodes in 1956. In the published paper Clark and Lyons, 1962, proposed the term “enzyme electrode”.

3. Components of a biosensor

Figure 1 shows the different parts of a biosensor. The key components of a biosensor are the detection element and the transducer. The biological detection element interacts selectively with the analyte and is responsible for the selectivity of the sensor. The functioning of biosensors relies on responses generated by specific biochemical reactions caused by enzymes, immune systems, tissues, organelles, whole cells, etc. The response of the analyte with the detector element on the transducer surface is usually measured by optical, mechanical, thermal, or electrical signals.

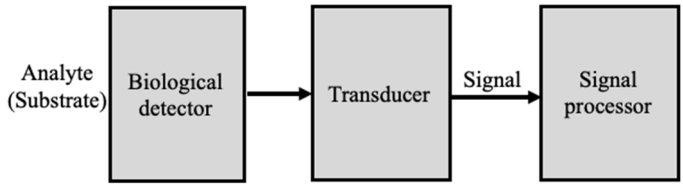

Figure 1. Schematic of a biosensor.

3.1 Detector element

In biosensors, the most common detector elements are enzymes. Other detectors include antibodies, nucleic acids, tissues, organelles, or whole cells.

3.1.1 Enzymes

Enzymes are proteins that are able to catalyze biochemical responses. The advantage of enzymes is their specificity. Complementary shapes, charge, the hydrophilic/hydrophobic characteristics of enzymes and substrates (Jaeger et al., 2004) and the spatial arrangement of molecules contribute to the specificity (Anfinsen, 1973). The activity of the enzyme is affected by inhibitors, activators, and cofactors. Its activity also depends on temperature, pH, and the concentration of substrate.

Strategies to use an enzyme as a detector in biosensor:

a. Enzyme catalyzing a reaction: The Brenda enzyme database can be used to identify an enzyme corresponding to a substrate. With an example of glucose as the analyte, the steps have been shown in Figures 2–6. The steps are:

i. Open Brenda enzyme database (Figure 2).

ii. Write the name of the analyte, select the natural substrate option (Figure 3), and click "start search."

iii. The list of different enzymes will appear. Figure 4 shows a list of oxidoreductase enzymes (Enzyme classification number or EC No. starts with 1), while Figure 5 presents a list of hydrolases (EC No. starts with 2). Oxidoreductase enzymes usually use oxygen and the product is usually hydrogen peroxide. Thus, the monitoring of hydrogen peroxide can help estimate the substrate concentration. In the case of hydrolase, the products of hydrolysis or the potential of the system can be monitored. The specificity of the oxidoreductase type of enzymes is usually found to be better compared to that of hydrolase enzymes. Thus, oxidoreductase enzymes are usually preferred in sensors.

iv. The reaction corresponding to the enzyme and the substrate can be obtained by clicking the enzyme EC No. (Figure 5). The substrate may not be directly the one desired. In the case of alcohol dehydrogenase, the substrate may not be glucose directly. But glucose can work as it has a structural similarity with the substrate of the enzyme.

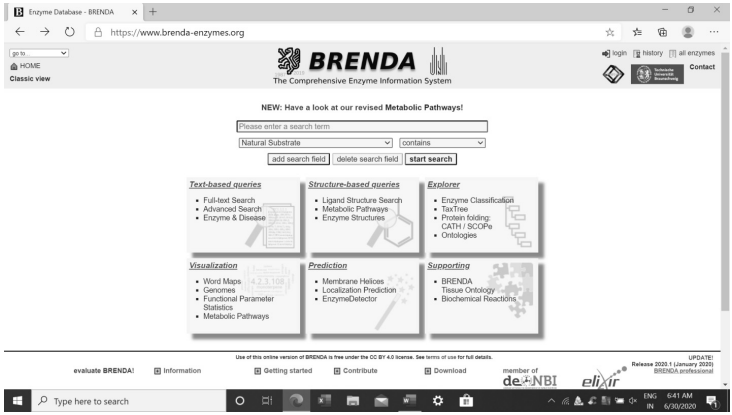

Figure 2. Brenda enzyme database.

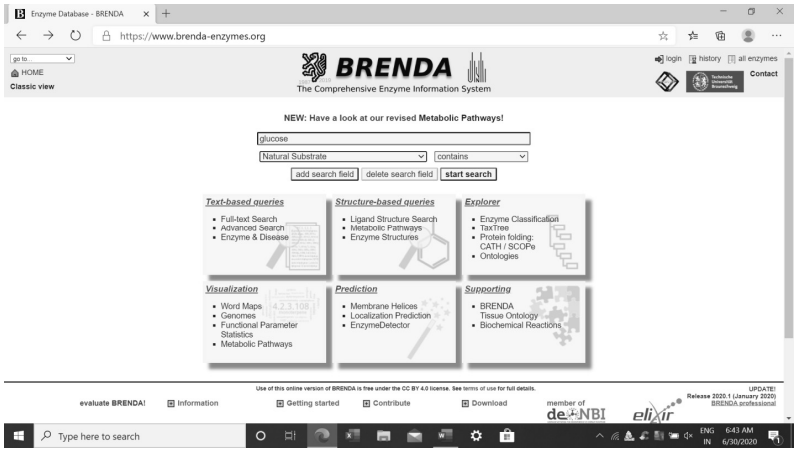

Figure 3. Search enzyme for glucose as substrate.

Figure 4. Identify oxidoreductase enzymes for glucose.

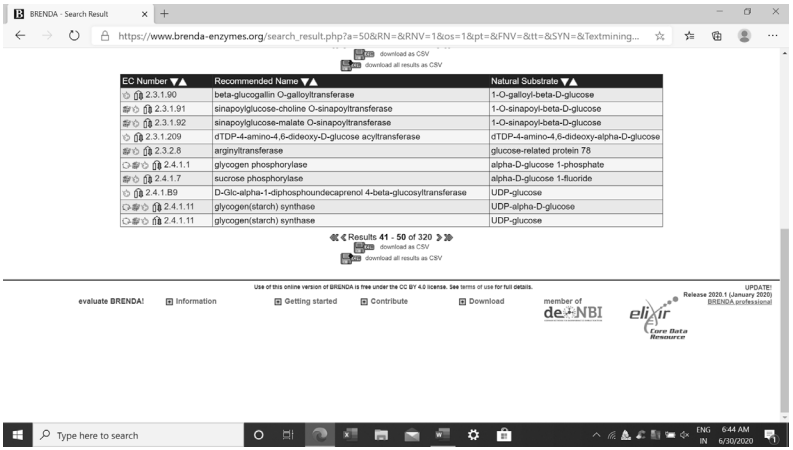

Figure 5. Identify hydrolase enzymes for glucose.

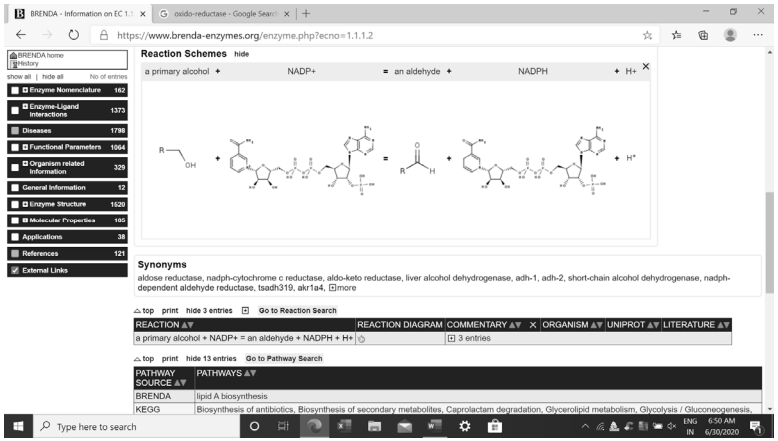

Figure 6. Identify a reaction scheme.

b. Deactivation of enzyme: The enzyme can be deactivated by different inhibitors or activated by different activating compounds. Thus, a change in the activity of the enzyme with the addition of an inhibitor or activating compound can help identify a chemical species. However, this method lacks specificity. Figure 7 shows the menu for finding the list of inhibitors or activating compounds. Figures 8–11 show different lists of chemical compounds that can increase or decrease enzyme activity. For example, NAD can enhance the response of the alcohol dehydrogenase enzyme (Figure 8). Figure 9 suggests

that Ca^{2+}, Mn^{2+}, and Na_2SO_4 can enhance the activity of the enzyme and thereby increase the response. Thus, these materials can be estimated by monitoring the increase in the activity of the enzyme in their presence. Figure 10 lists the inhibitors which can reduce the enzyme response. This property can be utilized to estimate the inhibitor concentration. For example, (1H-indol-1-yl)acetic acid can be estimated by monitoring the decrease in the response of the reaction of the alcohol dehydrogenase enzyme and its substrate. Figure 11 shows a strategy for estimating adenosine which will enhance the response of the alcohol dehydrogenase enzyme-substrate reaction.

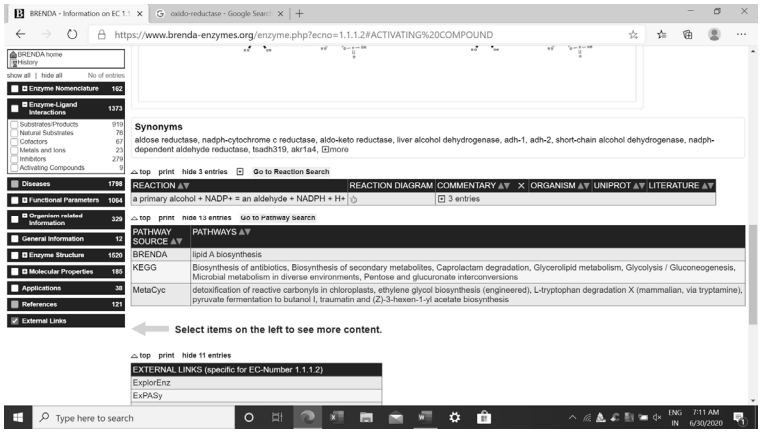

Figure 7. Options under Enzyme-Ligand interactions.

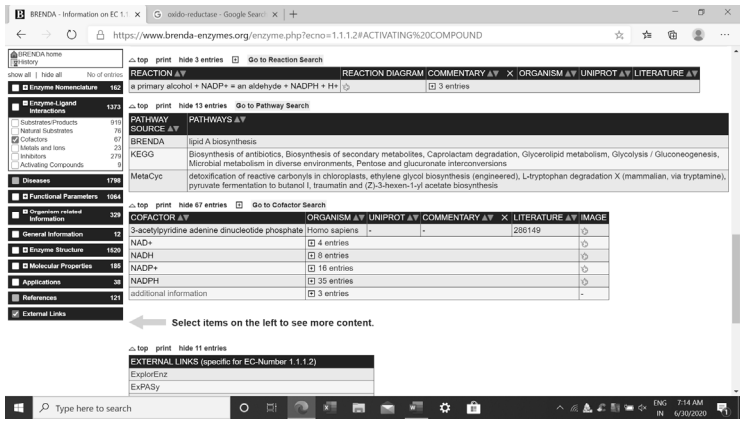

Figure 8. Cofactors.

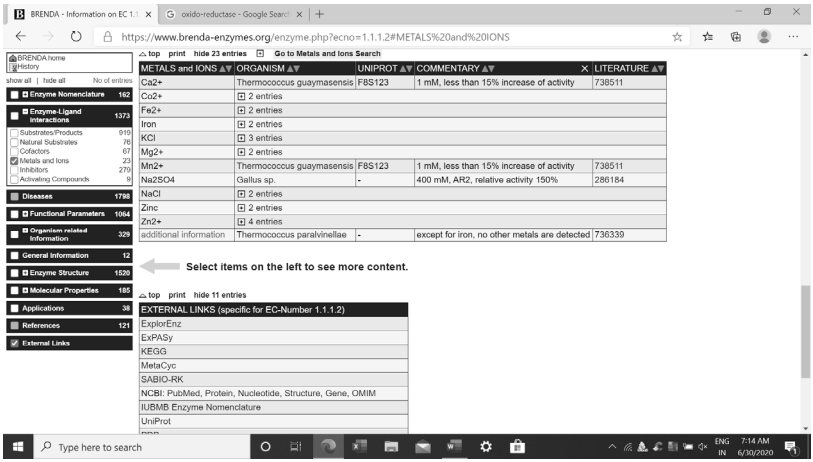

Figure 9. Metal ions.

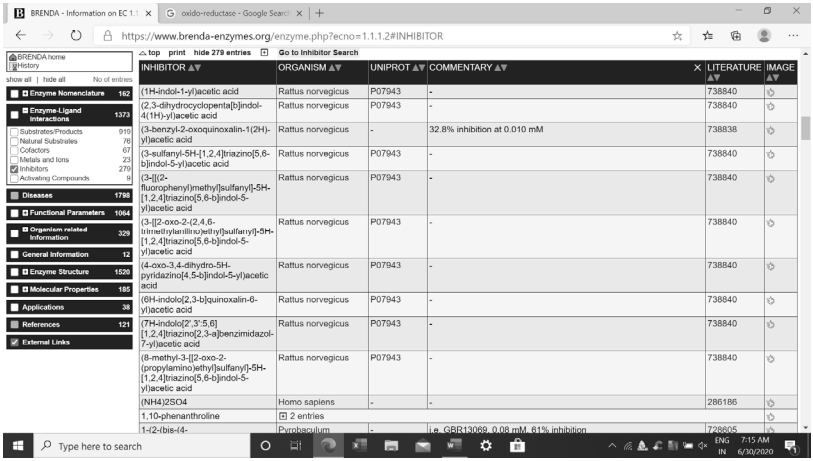

Figure 10. Inhibitors.

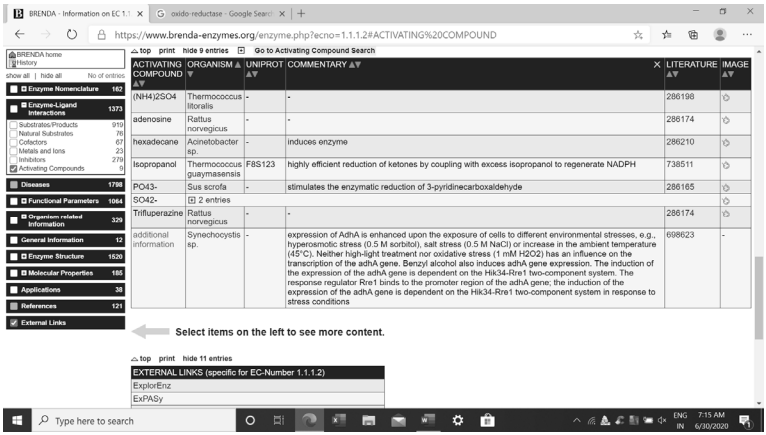

Figure 11. Activating compounds.

3.1.2 Antibodies

An antibody or immunoglobulin is a large Y-shaped protein used by the immune system. It can detect and prevent the attack of foreign objects. The antibody specifically binds to an antigen (Janeway et al., 2001). The antibody is usually tagged with enzymes or chromophores. The response of the enzyme or the chromophore is used to estimate the antigen content. Currently, aptamers are often explored in place of antibodies. The term aptamer is derived from the Latin word "Aptus," meaning "to fit," and the Greek word "meros" meaning "part." Aptamers are usually identified from a large pool of oligonucleotide or peptide molecules.

Strategy to identify an aptamer: Aptamers are conventionally identified through the process of SELEX (Systematic Evolution of Ligands by Exponential enrichment) (Cibiel et al., 2011). It takes a long time to develop an aptamer due to the trial and error involved. Thus, another approach can be followed. Based on the structural features of a molecule, a pharmacophore can be identified using Biovia Discovery Studio (Dassault Systemes, France). The pharmacophore is identified based on the following features of the analytemolecule: Hydrophobe, Hydrophobe_Aromatic, Hydrophobe_Aliphatic,Ionizable_Negative,Ionizable_Positive,Charged_Negative,and Charged_Positive. The pharmacophore can be docked with the analyte molecule. High positive values of CDocker Energy and CDocker interaction energy suggest that the pharmacophore can be used as a detector

element for the analyte. This strategy reduces the trial and error part and saves materials, time and money.

Figure 12 shows the menu for automatic pharmacophore creation. Figure 13 shows the nature of functional groups present in cineol identified by the automatic pharmacophore generation menu of Discovery Studio. In this case, hydrophobic groups have been presented with blue circles.

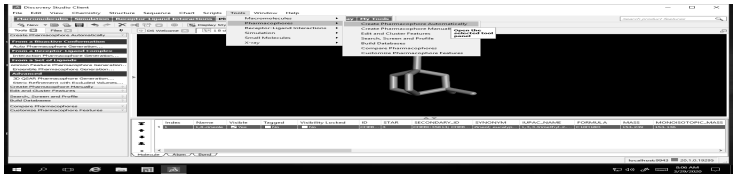

Figure 12. “Create Pharmacophore Automatically” menu of Discovery Studio.

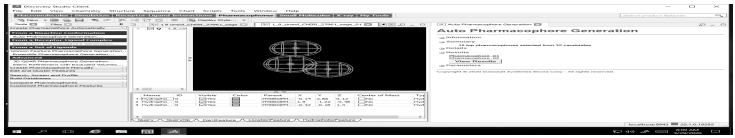

Figure 13. Nature of group.

Figure 14 shows a pharmacophore generated using Biovia Discovery Studio. The molecule shown in Figure 14 can be used as a detector element for the detection/estimation of cineol.

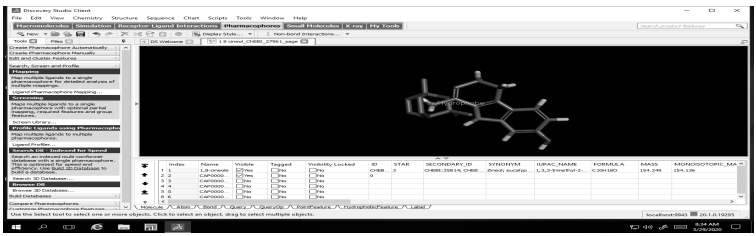

Figure 14. Pharmacophore of Cineol.

3.1.3 Nucleic Acid

A nucleic acid is a high-molecular-weight, complex biochemical macromolecule comprised of nucleotide chains that carry genetic information. The most common nucleic acids are deoxyribonucleic acid

(DNA) and ribonucleic acid (RNA). A few examples of nucleic acid-based sensors are listed in Table 1.

Table 1. A few examples of nucleic acid-based sensors.

Analyte	Nucleic acid	Reference
Microcystis spp. DNA Sequence	DNA	Erdem et al., 2002
Human Apolipoprotein E Genotypes	DNA	Marrazza et al., 2000
Uropathogens	DNA	Liao et al., 2006
Escherichia coli	RNA	Baeumner et al., 2003

3.1.4 Tissues/Cells

Animal/plant tissues can be used as biological detector elements. The use of tissues helps reduce the cost of biosensors but the selectivity of the sensor is lower compared to that of the enzyme sensors. In this case, the organism with the enzyme may be exposed to the substrate. The concentration of the substrate is increased slowly so that the organism can act as a detector element for the analyte. A few examples of tissue/cell-based sensors are listed in Table 2.

Table 2. A few examples of tissue/cell-based sensors.

Analyte	Tissue/cell	Reference
Pyruvic Acid	Pork Heart Tissue	Wu et al., 2006
Ethanol	*Agaricus bisporus* tissue	Huang et al., 2006
Phenolic compounds	*Agaricus bisporus* tissue	Topcu et al., 2004
Epinephrine	*Polyphenol oxidase* enzymes present in the fibers of palm tree fruits (*Livistona chinensis*)	Felix et al., 2006
Arsenite	*E. Coli* MC1061	Petani et al., 2003
Arsenate, mercury	*Pseudomonas fluorescence* OS8	Petani et al., 2002

3.1.5 Biomimetics

The basic problems in working with enzymes are their high cost and sensitivity to temperature, pH, etc. Biomimetics, i.e., synthetic prototype biomolecules, may have the same specificities and catalytic activities as enzymes and thus may have the potential to replace costly enzymes. A few examples of biomimetic based sensors are listed in Table 3.

Table 3. A few examples of biomimetic based sensors.

Analyte	Biomimic	Reference
Ethanol, propranolol, dopamine, and acetone	Dehydrogenase enzyme mimic	Kataky et al., 2003
Heat shock protein (HSP) 70	Biomimetic peptide	Mascini et al., 2006
[HOOC- C_6H_5-$C(CH_3)=NH_2$ (4-[(1E)-ethanehydrazonoyl] benzoic acid)	Acetylcholine esterase	Bhattacharyay et al., 2008

Strategy to design a biomimetic: A simple strategy for designing a detector element for a substrate is to find some functional groups in the analyte molecule and the distance between them. A molecule can act as a detector element if it has complementary functional groups corresponding to the functional groups identified in the analyte molecule. If the distance between the functional groups in the detector molecule is similar to that of the analyte, it may act as a detector element.

Figure 15 explains such a scheme.

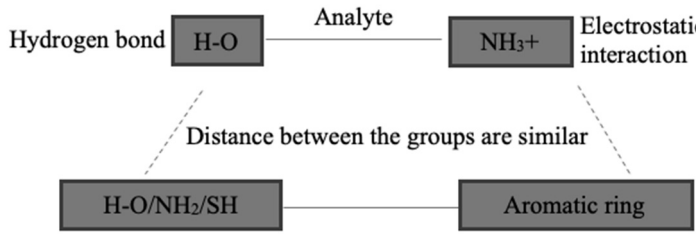

Figure 15. Scheme for designing a biomimetic as the detector element.

3.2 Transducers

The transducer is the heart of a sensor. It transmits the signal (electrochemical, optical, etc.) to the signal processor. Transducers may be subdivided into four main types:

3.2.1 Electrochemical Transducers

(i) Potentiometric: In this method, the potential of a cell is measured at a zero or constant current. The potential generated follows the Nernst equation. Hydrolase types of enzymes are usually used for potentiometric sensors. The concept of concentration cells is also employed in the potentiometric sensor.

(ii) Voltametric: In this method the voltage is varied and the current is measured. Usually, peaks are observed for oxidation and reduction potential. The height of the peak current is directly proportional to the concentration of the electroactive material and the scan rate. Oxidoreductase enzymes are often used in such sensors.

(iii) Amperometric: In this method, the current is measured at a constant potential. The applied potential is kept at a minimum value to reduce side reactions. The oxidoreductase enzymes are suitable for such types of measurements. Mediators (such as ferrocene) are used to reduce the potential. The potential is usually determined by cyclic voltammetry. Often a hydrogen peroxide-peroxidase combination is employed.

(iv) Conductometric: The change in impedance of the solution during the course of the reaction is monitored. The conductivity of the solution can be correlated to the analyte concentration. Hydrolase enzymes, biomimetics, etc. are used as detector elements.

3.2.2 Optical Transducers

These kinds of sensors offer flexibility. The techniques used include absorption spectroscopy, fluorescence spectroscopy, luminescence spectroscopy, internal reflection spectroscopy, surface plasmon spectroscopy, and light scattering. Colorimetry can sometimes reduce the cost of the sensor by the use of visual analysis or a low-cost colorimeter. Analysis of the RGB (red-green-blue) components of color can be calibrated with the analyte concentration. For example, 4 amino benzophenone can produce a red color during the reaction of H_2O_2 and peroxidase enzyme. The RGB

values of the color can be mapped with the H_2O_2/enzyme concentration which, in turn, can be mapped with the analyte concentration.

3.2.3 Piezo-electric Devices

In these devices, an electric current is generated due to the vibration of a crystal. The frequency of the vibration is affected by the mass of material adsorbed on its surface. Biomimetics or adsorbents can be used to dampen the vibration of the crystal with an increase in analyte concentration. The vibration will affect the current produced. Thus, by measuring the current the analyte concentration can be monitored.

3.2.4 Thermal Sensors

All chemical reactions involve heat production or heat absorption. The heat generated or absorbed can be measured by thermistors and can be correlated to the analyte concentration.

3.3. Generations of biosensors

There are considered to be three generations of biosensors.

(a) *First generation biosensors:* Here the normal product of the reaction is diffused to the transducer and causes the electrical response.

(b) *Second generation biosensors:* Here specific mediators are involved between the reaction and the transducer to produce an improved response. Ferrocene, quinones, quinoid-like dyes, organic conducting salts, and viologens are used as mediators.

Table 4 shows a list of common mediators and enzymes for electron transfer.

Table 4. List of common mediators and enzymes for electron transfer.

Enzyme	Mediator	Reference
Glucose oxidase	Ferrocene	Katrlik et al., 1997
Glucose oxidase	Vinylferrocene	Dulce et al., 1995
Glucose oxidase	Ferrocene carboxylic acid	Tian et al., 2002

(c) *Third generation biosensors:* Here the reaction itself is the main reason for the response and no product or mediator diffusion is directly or indirectly involved. Nowadays reduced graphene oxide is used as the matrix in such

sensors as it facilitates electron transfer. Since neither the mediator nor enzyme needs to be added, this design facilitates repeated measurements. Third generation biosensors are often used in Micro-Electro-Mechanical Systems (MEMS) technology. Table 5 lists some commercially available biosensors of different generations.

Table 5. Some commercially available biosensors of different generations [Vreeke, M. S., Electrochemical biosensors for affinity assays**, IVDT, July, 39-45 (1997)].**

Company	Analyte	Generation
Yellow Springs Instruments	Glucose, lactate, ethanol, lactose	First
Fuji Electric	Glucose	First
Nova Biomedical	Glucose, lactate	First
i-Star	Glucose, lactate, urea	First
Boisen	Glucose, lactate	First
Ciba	Glucose, lactate	First
Via Medical	Glucose	First
Boehringer Mannheim	Glucose	Second
Bayer (Matsushita)	Glucose	Second
Bioanalytical Systems	Hydrogen peroxide, glucose, lactate, choline	Third

4. Conclusions

Biosensors are a subset of chemical sensors and are often preferred for their selectivity and specificity. The major parts of a biosensor are the detector element and the transducer. The basis of selecting the detector elements and transducers has been described. The principles described may be used to develop a biosensor.

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CHAPTER 2

ETHNOPHARMACOLOGY: ISSUES, CHALLENGES, AND OPPORTUNITIES

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Abstract

Globally, ethnopharmacy is a buzzword in the pharma industry due to the global demand for medicines without side effects. As traditional and folklore medicine have gained popularity, we discussed issues related to ethnopharmacology, with a focus on quality control and efficiency measurements that should be taken while working with ethnomedicine. Furthermore, this chapter explains why modern technologies (OMICS) are necessary for understanding the molecular mechanisms of ethnomedicine. An emphasis was placed at the end of this chapter on the opportunities that exist in ethnopharmacology.

Keywords: Ethnopharmacology, Quality control, Omics, Alternative medicine and Natural resources.

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Introduction

We all know that with the growing population the demand for new medicines and therapeutic strategies is also increasing tremendously. In the recent global covid-19 pandemic, the whole world was looking forward to the development of therapeutic strategies either in the form of medicine or vaccines that can reduce and control the impact of the covid-19 infection. However, the way to address the global demand for new medicines, as well as complementary medicines that can minimize the complications arising from the target-specific drugs that already exist in the market, seems to be "ethnomedicine." The study of traditional medicine as well as folklore medicine and even sometimes the medicinal practices existing in indigenous populations is considered to be "ethnopharmacology" (1). This includes the study of phytoactive principles, herbal therapeutic potential, and assays of bioactive compounds present in plants used as medicine. If we look at the global scenario of ethnopharmacology both the Chinese and Indian traditional systems of medicine have strong roots in this. In addition to these two countries, middle-income countries like Mexico, South Africa, Ghana and Russia also use traditional medicine (2). It is interesting to note that the global market value of complementary and alternative medicine is increasing and is expected to reach 210 USD billion by the year 2026 (2).

The best part of ethnopharmacology is its holistic approach. In this system of medicine, the majority of the time, the practitioners are going to treat with plant extracts, and these plant extracts possess bioactive principles as well as the minerals and vitamins that will take care of and combat the unhealthy state of the patient. The literature survey in this field concludes that herbal medicine, being natural, has the potential to address the imbalance developed inside the living system. It is also a well-known fact that the major biological conditions for the development of diseases are linked to oxidative stress. This is nothing but an imbalance between the free radicals and antioxidant agents (3). It is interesting to know that the balance of both endogenous and exogenous antioxidant levels in the body is one of the major modus operandi of ethnomedicine.

With this brief background, the topics we will discuss in this chapter will explain the significance of ethnomedicine. Also, the readers will benefit from increased knowledge of the issues related to ethnopharmacology and the challenges being faced by this field. Finally, we will conclude with the opportunities that are available in the field of ethnopharmacology. Figure 1 shows some of the active principles derived from plant sources and their potential therapeutic efficiency.

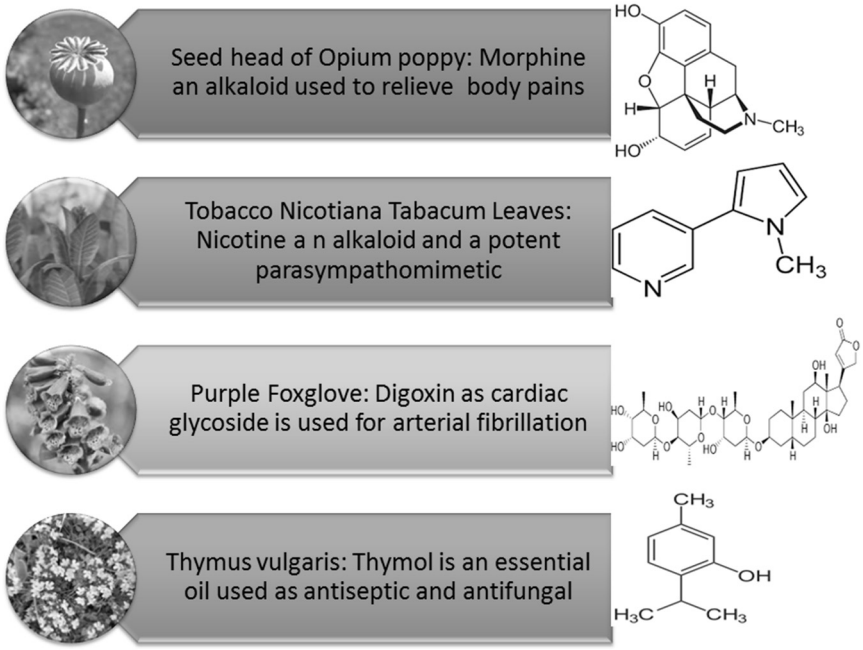

Figure 1. Bioactive principles isolated from plant sources and their therapeutic usage (the source of information obtained from Research Journal of Phytochemistry (ISSN 1819-3471), Front. Pharmacol and images obtained from flickr.com and pixabay.com).

1. Issues associated with ethnopharmacology

Ethnopharmacology is a combination of traditional knowledge and the efficacy of natural products (Figure 2). This section will mainly discuss the issues related to ethnomedicine.

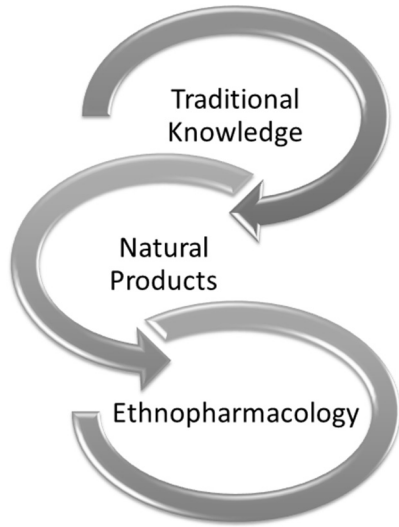

Figure 2. Association between traditional knowledge, natural products, and ethnopharmacology.

Quality

Since ethnomedicine is based on extracts or products derived from natural resources, especially plants, it is always challenging to meet and maintain the quality of the product. It is essential that the preparation of the extracts and herbal products are maintained under controlled hygiene conditions to avoid any sort of adulteration or contamination. Hence, it is important to remember that any sort of impurity in the form of heavy metals or toxic elements may cause unwanted complications and may lead to new disease conditions and that will worsen the existing disease state. It is a well-known fact that unless there is recognition by an approved government or authorized agency it is very difficult to develop and produce medicine. These agencies need clinical evidence to authorize the production of any medicine. To achieve these permissions any proposed drug has to prove its efficacy.

Efficacy

The credibility and ability to attract a larger population towards using a medicine is always decided by the efficacy of the drug. Unless the drug has

a proven track record of efficacy during its medical and preclinical studies it is almost impossible to prescribe or even commercialize as a drug. In ethnomedicine, being native to ethnic groups, the efficacy of many herbal formulations and combinations are yet to be proven clinically. However, many herbal medicines are being prescribed by traditional practitioners in tribal populations. To meet the criteria of efficacy the studies of ethnopharmacology need to be conducted under controlled clinical trials and show their significance through recognized research publications. Even after proving the efficacy, the real issue is the competition that is put forward by the other medical systems.

Competition from other systems of medicine

Competition exhibited by other systems of medicine like allopathy and homeopathy are predominant and occupy a large portion of the global market. It is important to note that both the above systems of medication have proven their track record and these systems have emerged from a systemic teaching, learning, and research-oriented process. Some Asian countries, especially China, India, Thailand, and Sri Lanka, have strong folklore and traditional practitioners. If the first two issues, i.e., quality and efficacy, are addressed the issues of the competition will be automatically answered.

Preventive medicine

Globally, phytomedicine is considered preventive treatment rather than emergency medicine. Research on experimental animal models concluded that phytomedicines exhibit their protective role (4&5) in diverse situations. However, there are limited numbers of studies explaining the therapeutic potential of herbal medicine at the molecular level. Moreover, it is challenging to investigate the effect of crude extracts at the molecular level since they have multiple active principles, while treatment with herbal medicine is considered to be holistic rather than systemic or target specific.

Inadequate literature

There is a dearth of literature providing information about how exactly some herbal formulations show efficacy/mechanism of action. In the case of folklore medicine, the traditional knowledge of using specific herbs/extracts is transferred from generation to generation without any documented evidence. Owing to illiteracy and the inability to communicate with

mainstream society the knowledge of phytomedicine available within tribal populations remains unexplored. Many of these practitioners can identify the plant species by their appearance, fragrance, and habitats. Hence it is important to record and document the traditional practices of diverse ethnic groups and tribal populations across the globe and encourage them to share their knowledge with medical society so that it can be explored further.

In Ayurveda ("Ayurveda is formed from two words, *Ayuh* and *Veda*, where *Ayuh* means life and *Veda* means knowledge or Science. Thus, Ayurveda is a science of life and is the most traditional approach of healing which bridges a healthy balance between body, mind, or soul"; see https://main.ayush.gov.in), the main literature is available in Sanskrit but many agencies are trying their best to translate the literature into other popular languages like Hindi and English. Similar traditional knowledge exists in other ethnic groups of the world that needs to be collected and translated. Sometimes even if we translate the literature into a common language the challenge is to understand and translate the true intentions or purpose of the original practitioner.

Geographical locations

The geographical locations of many ethnic groups and also the sources of specific flora are not easily accessible due to fiscal and technical limitations along with communication barriers with mainstream society and among tribal societies. Using the latest technologies, it could be possible to allot geographical tagging/marking for quick and easy access.

Access and availability

There is diversity in the flora across the globe and it is important to identify the specific plant species that are used to prepare the extracts for specific diseases as mentioned in folklore. It is also important to note that access to rare species and plant parts is not always easy. Hence, this may limit the exploration of the therapeutic potential of a specific plant. Apart from that, preparation of the extract during any emergency across all parts of the world also limits the interests of the researchers to explore more in this field.

IPR-related issues

In general, industries are interested in developing and marketing products that already have proven intellectual property rights (IPR). This will provide

an added advantage to the manufacturers to avoid any unforeseen conflicts once they start development and even at the time of marketing the product. Due to a lack of awareness in traditional practitioners as well as tribal populations, these ethnomedicines remain unrecognized and many of them do not even have any patents or exclusive rights. Another dimension of not having adequate patents is that it deprives them of opportunities in this field. This is related to the need for scientific evidence and supporting documents concerning the therapeutic and druggability of a specific phytomedicine against a specific disease.

In addition to the above-mentioned issues, a few more issues directly or indirectly contribute to less recognition of ethnopharmacology as well as phytomedicine. Even today we found ethnopharmacology at the level of research programs, as some of the research institutes are specifically exploring phytomedicines and their therapeutic approaches to treat several lifestyle issues and congenital errors of metabolism. Even in many graduate and postgraduate curriculums we found ethnopharmacology as a small portion rather than as a separate program or course of study. Ethnopharmacology is being discussed under other major subjects like life sciences or biosciences. Due to this identity crisis at the level of education and training programs, ethnopharmacology remains a neglected subject with less demand. However, by addressing the challenges that are covered in the next section of this chapter it is possible to prove the potential of ethnopharmedicine.

2. Challenges to be faced and addressed

One of the major challenges faced by many researchers working in the field of ethnopharmacology is to prove the mechanism of action of herbal drugs. In other words, the therapeutic potential and also evidence showing the target-specific action of these phytomedicines is needed. The reason for asking for evidence such as a mode of action could be to minimize the unwanted side effects as well as to know the target tissue and site of action. It is well known that some of the drug molecules that are being used as therapeutic agents against specific diseases have some unwanted side effects (6). Also, when treating with phytomedicine the majority of the time whole extracts of the plant parts are being given as a medicine. It is important to note that these extracts contain several bioactive principles, and it is believed that these bioactive principles will work in a synergic manner to address the target disease. That is the reason why phytomedicine or treatment with phytomedicine is considered a holistic approach. It is