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DRUG ANALYSIS: A PERSPECTIVE OF POTENTIOMETRIC SENSORS

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ABSTRACT

The remarkable specificity of biological recognition processes has led to the development of highly selective bio-sensing devices. Electrochemical biosensors hold a leading position among the bio-probes currently available and hold great promise for the task of pharmaceutical analysis. They are inherently sensitive and selective towards electro-active species, fast and accurate, compact, portable and inexpensive. Among them potentiometric sensors are very attractive strategy that can be used for the direct measurement of ions, gases and bio-molecules in complex samples.

KEYWORDS: Membrane sensors Potentiometry Biosensors Ionophore Drugs analysis.

1 INTRODUCTION

Pharmacy is a medical branch Chat deals scientifically and practically with the medication and drugs. The functions of pharmacy involve research, obtaining of scientific knowledge about drugs, evaluating and drug quality control among others. The aim of all these pharmacy functions is to obtain a safe drug of high quality, dispense it in a required time and amount, so the drug can work as a preventive, diagnostic or therapeutic means. Drug control is a branch of pharmacy that ensures the quality, safety and effectiveness of the drugs.[1] In drug control, many techniques are used to follow its aim such as spectral, radiometric, electrochemical, separation methods. Although the methods of separation are too disclosed in most pharmaceutical control laboratories, the spectrophotometric and electrochemical ones are the most widely used. The electrochemical methods can be classified into two major groups: A) methods based on the electrode reaction (potenciometry, polarography, amperometry and and others), B) methods, where the electrode reaction is not decisive (conductometry).[2] Potentiometer is a technique based on a measurement of the potential difference between an indicator electrode and a reference electrode in solution, while the current is held at zero.[3] The potential obtained can be correlated with the activity of the ion to be measured.[3] Among the different types of electrodes indicators, it could be considered the ion selective electrodes (ISE´s) that are concentration cells. Special kind of indicator electrode are ion-selective electrodes (ISEs). They are made usually by a membrane selective to an ion and, through a process of electronic transduction the chemical potential difference is converted in an electrical potential. Nowadays, ISE´s are successfully applied in environmental, clinical, pharmaceutical analysis and also in monitoring process field. This work aimed to develop an ibuprofen selective electrode for its potentiometric determination in pharmaceutical products.

The aim of this work is to develop an ion selective electrode for its potentiometric determination in pharmaceutical products. Several membranes will be prepared, containing different ionophores as well as liphophilic species as additives and several mediator solvents. The ratio between the individual components will be changed in order to develop the membranes with better working characteristics. The electrodes will be evaluated in different conditions. The electrodes with the proper characteristics will be chosen for analytical applications. As a result, an environment friendly, low cost, fast and simple method should be proposed as an alternative to the more tedious generics methodologies.

2 Ion-selective electrodes (ISEs) are special kinds of indicator electrodes. An ISE is defined as an electroanalytical sensor with a membrane whose potential indicates the activity of the ion to be determined (the determinant) in a solution (the analyte). The membrane of ISE consists either of liquid electrolyte solution or of solid or glassy electrolytes that usually have negligible electron conductivity under the conditions of measurements.[7] ISEs allow the potentiometric determination of the activity of a certain ion in the presence of other ions.[8] Response of these sensors towards charged species is provided by ion-selective membranes, which can be prepared from different materials - glass (pH electrode), solid

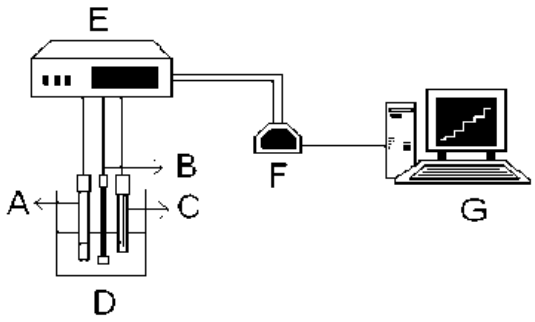
crystalline, ceramic or polymers.[9] Their selectivity is achieved either by means of material structure or by doping the membrane with specific ion-selective complexing agents. The most successful ISE is a glass pH electrode, used when pH is controlled or adjusted. The most widely employed selective sensors are electrodes with polymer membranes. Their character enables to adjust the properties of the sensor. Nowadays it is possible to measure up to 50 different ions. The improvement can be easily done by the 17 change of membrane composition and electrode construction, or by new methodologies of data interpretation.

Polymer matrix: Polymer matrix provides the mechanical stability of the membrane. The choice of polymer depends on the requirements, such as biocompatibility, physiological fluids sample etc. One of the primary requirements of polymers creating the selective membrane is that their glass transition temperature should be below the room temperature.[10] There are some polymers that are used in potentiometry and meet this requirement, i.e. silicon rubber[11], several methacrylates[12], polyurethanes.[13] The most widely used polymer poly(vinylchloride) (PVC) must be plasticized. Plasticizer Plasticizers are solvents giving the convenient viscoelasticity to the membrane. In the ISE membrane, the plastisizer acts as a membrane solvent and also plays a role in the membrane selectivity and in the limit of detection. It can influence both extraction of an ion into organic phase and also the complexation with the ionophore.[14,15,16] The requirements that a plactisizer has to meet are: to be compatible with the polymer and to be a solvent for other membrane constituents. For PVC, 2-nitrophenyl octyl ether (oNPOE, polar) and bis(2-ethylhexyl)sebacate (DOS, apolar) are commonly used as plasticizers. Lipophilic ionic sites Lipophilic ionic sites are additives that play an important role in electrode selectivity. Chemically, lipophilic ionic site is a salt of non-exchangeable lipophilic anion/cation and exchangeable counter ion. They provide electroneutrality of the membrane with neutral ionic carriers, so that no significant amount of counter ions can be co-extracted into the membrane together with primary ion. Therefore, the membrane is permeable only for ions of the same charge sign as primary ion.[17] ISE membrane can eventually work without the additive, but there are some advantages on using the additive. The lipophilic ionic site in the ISE membrane keeps constant total concentration of the measuring ion in the membrane phase and can contribute to the membrane selectivity in the case that no ionophore is used or its present amount is insufficient. The addition of lipophilic salt without ion-exchanger properties, or lipophilic inert electrolyte, was initially suggested in order to reduce electrical resistance of ISE 19 membranes.[18] Later it was found that the addition of tetradodecylammonium tetrakis(4-chlorophenyl)borate also improves the selectivity of divalent over monovalent ions if membranes of low polarity and low site concentration are used.[19] Examples of some lipophilic additives in use are shown in Fig.1.

Fig. 1: Structure of some lipophilic ionic sites Ionophore Ionophore is a component of the ISE membrane that has a crucial role in the membrane selectivity. According to[9], ionophore is a compound that can carry a specific ion through a membrane. It forms a complex with the analyzed ion. The binding must be strong but reversible. In the ideal case, the ionophore binds only with the target ion - then we can say that the membrane is selective to this ion. It is important that the ionophore must also bind to the polymer matrix, so it cannot be washed away from the membrane but it must be retained within the structure. To meet this requirement, an ionophore must have a) binding centre to form the complex with the detected ion, b) lipophilic groups to bind to the matrix. Structures of several substances used to compose an ISE membrane hydroxypropyl-β-cyclodextrine chloro (5,10,15,20-tetraphenylporphyrinato) indium (III).

2.1. RESPONSE MECHANISM OF ION-SELECTIVE ELECTRODES Electrical response for ion-selective electrodes.

Principle of Ion-selective Membrane: Ion-selective electrodes are typically investigated under zero current condition by following cell set up.



Electrochemical measuring cell (Fig.1) is a device used in potentiometry measurements. It converts the chemical energy into the electrical one when a chemical reaction occurs in the cell. It consists of two galvanic half-cells. The half cell is representing by the electrode and a surrounding electrolyte. Indicator electrode and reference electrode, immersed in the solution of the analyte, are consider to be the two half cells of the electrochemical measuring cell.

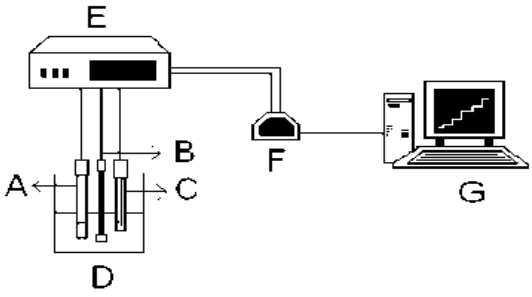


Fig. 1: Schematic diagram of an ISE measuring cell.

The total potential difference (electromotive force, EMF) rising between the ISE and reference electrode can be measured by millivoltameter or a multi-channel measuring station. The total potential consists of more contributing potentials, rising at each electrochemical interface:

EMF=Econst + EM + ED,ref

Econst refers to potentials that can be kept constant. EM (membrane potential) and ED,ref liquid-junction or diffusion potential) depend on the sample.

Liquid junction potential – rises because of different mobilities of ions in the sample and in the bridge electrolyte of the reference electrode. This potential can be kept constant by using concentrated bridge electrolytes with similar mobilities of cations and anions or can be prevented by using a double junction reference electrode.

Membrane potential – involves the phase boundary potentials at both interfaces and the diffusion potential within the ion-selective membrane. The diffusion potential depends on the sample, whereas the potential at the interface can be kept constant. The diffusion potential is significant if a considerable concentration gradients of ions with different mobilities arise in the membrane.[17] Otherwise the diffusion potential is zero, which is often the case for membranes that show theoretical Nernstian response.

The phase boundary potential (part of membrane potential) – arises from a charge separation caused by the non-uniform distribution for ionic species between the organic membrane and the aqueous phase.[17] The equation describing the membrane potential is the Nernst equation : EM=EI 0 +(RT/zIF).ln aI,s.

EI 0 - the standard potential when the activity of the main ion is equal to 1.
R - molar gas constant (8,31441 J mol -1 K-1)
T - absolute temperature (K)
Zi - charge of the ion I
F - Faraday constant (96484.56 C mol_1)
a - activity of the ion I

The potential of ISE selective towards ion I can be written as:
EI = EMF-ED, ref = EI 0 + sI log aI,S
Si = 2,303 RT/ zIF = 0.059/zI

Graphically, EI is a linear function with the slope sI . If the ion has a charge -1 (the case of ibuprofen), the theoretical slope of the linear function is 59mV.

Response mechanism: Ionophores in junction with lipophilic ionic sites are responsible for the ISE response. If the membrane contains no ionophore, but only lipophilic additive, extraction of ions from the sample to the membrane phase is the only potential determining process.[17] If the sufficient amount of ionophore is present in the membrane, complexation of the target ion by the ligand and membrane-solution ionic exchange determines membrane selectivity.[17] If there are more ions complexed with the ionophore, the difference between the stability constants of ion-ionophore characterizes the selectivity of the ISE.

2.2. Characterization of an ion-selective electrode selectivity

Selectivity

It is one of the most important characteristics of an ISE. Selectivity is an expression of the specificity of the membrane towards the primary ion in the presence of other ions in the solution that can potentionally interfere with the primary ion. It determines the applicability of the ISE for certain measurements. Quantitatively, selectivity is characterized by selectivity coefficient KA,B pot, which is a direct function of the difference of the individual potentials extrapolated to 1 M activity of ions A and B.[18] It is usually expressed as a logarithm of KA,B pot. Negative values indicate a preference of the ISE for the target ion relative to the interfering ion. Positive values of log Kxy show the preference of an electrode for the interfering ion. At KA,B pot = 1 the electrode responses equally to both ions. KA,B pot can be used to predict response functions in mixed samples.[19] IUPAC recommends these methods to measure the selectivity coefficient: SSM (separate solution method) – the potential is measured first in the solution of the possibly interfering ion (EB), and then in the solution of the primary ion (EA).

The logarithm of the electivity coefficient is then:
log KA,B pot = (EB-EA)zAF/ 2.303RT + (1-zA/zB)log aA

Advantages

• speed and ease of determination,
• can determine a large array of interfering ion-selectivity coefficients very quickly,
• is used for simple flow-injection potentiometry applications (simple and well defined systems).

Disadvantages:

• does not account for any error due to multiple ion interaction,

• overly simplistic method for real solutions, often giving very different coefficients than other methods.[20]

FIM (fixed interference method) – The emf (electromotoric force) of a cell comprising an ion-selective electrode and a reference electrode (ISE cell) is measured for solutions of constant activity of interfering ion, aB, and varying activity of the primary ion. The emf values obtained are plotted vs. the logarithm of the activity of the primary ion aA. The intersection of the extrapolated linear portions of this plot indicates the value of aA which is to be used to calculate KA,B pot from the Nikolsky-Eisenman equation[21]:
KA,B pot = aA/aB zA/zB

Advantages
• accurate for a larger variety of systems than separate solutions,
• relatively simple to perform for a reasonable set of potential interfering ions of interest,
• method gives good (reasonable) data for most real world systems,
• coefficients translate fairly well to many observed application selectivity performance.

Disadvantages
• does not account for all multiple ion-ion interactions, only interfering ion analyte interference.[20]
MSM (mixed solutions method).

Advantages
• accurate for almost all stable systems, even if complex,
• more accurate than fixed interference solutions,
• method gives very good data for complex systems.

Disadvantages
• very cumbersome to perform if the system has any variance of the ionic background,
• laboratory technique and uncertainties of measurement are of great importance.[20]

SLOPE
It is the gradient of the line formed by plotting the electrode response in milivolts against the logarithm of the activity (or concentration) of the measured ion. The theoretical Nernstian slope at 25°C is 59.16 mV per decade for monovalent ions and 29.58 mV/dec for bivalent ions. In practice the slope is usually lower than the theoretical value, which calculates with ideal conditions that are not met in practice. The slope of an electrode can be determined by measuring the mV response in two standard solutions with concentrations (activities) of a1 and a2 and then calculated with:
m = (E1-E2) / ((Log(a1))-(Log(a2)))
or can be calculated from the calibration curve.

THE pH RANGE OF AN ISE
It is a range over which a change of pH will not cause a significant change in the measured voltage. The range of pH can be obtained from a graph of pH against potential, constructed at constant activity of the determined ion. The pH range is of an ISE is the plateau on this graph. Outside this pH area, a change in pH may cause a significant change in the measured mV. Consequence of this is the need of adjustment of pH of the sample with a buffer in the case that it differs from the pH of the standard solution.

DETECTION LIMITS
The response of an ISE is characterized by an upper and a lower detection limit. These values limit the analytical range of the electrode where the ISE shows Nernstian response. According the IUPAC recommendation, the detection limit is defined by the cross-section of the two extrapolated linear parts of the ion-selective calibration curve. Upper detection limit is a consequence of coextraction process of the primary ion and interfering ion from the sample to the membrane, thereby leading to a loss of membrane permselectivity.[17] Due to this process, the calibration curve of the response to the increase of the primary ion concentration has lower then Nernstian slope.

The lower detection limit occurs as the loss of Nernstian response slope at low primary ion activity. Depending on the type of ionophore, the ISE usually present detection limits of about 10-5 – 10-6 mol.L-1. 26.

Figure 2 represents a typical potentiometric response of a cation. Region I represents a response of the electrode to the concentration of an ion that is under the lower detection limit.

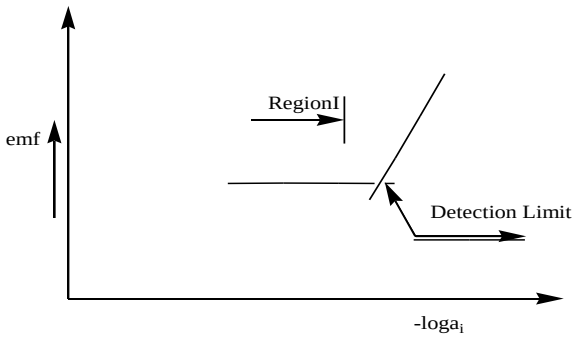


Fig. 2: The definition of the lower detection limit according to IUPAC recommendation.

RANGE OF LINEAR RESPONSE
This region corresponds to the range of linear response for the electrode. It is limited by the practical detection limits of the electrode. That range of concentration (or activity) over which the measured potential difference does not deviate from that predicted by the slope of the electrode by more that ± 2 mV.[22]

RESPONSE TIME
The length of time necessary to obtain a stable electrode potential when the electrode is removed from one solution and placed in another of different concentration.[22] There are many factors affecting the

response time: the electrode type, the magnitude and direction of the concentration change, the temperature, if interfering ions are present, if the sample is stirred when the potential is measured or the measurement is performed in static conditions. For ISE, it's generally quoted as less than 10 seconds.

LIFETIME OF ISE

It is a time after which the sensor starts to lose its characteristics. It is caused by leaching of the membrane constituents into the solution. The life span can be also affected by the concentration of the solution, the ionic strength and the environment conditions, such as the temperature, O2, CO2, the light intensity, etc.

3.2.6. ADVENTAGES AND LIMITATIONS OF ISE

ADVANTAGES

1. Linear response- over 4 to 6 orders of magnitude of A – a very low concentration of the analyte can be measured.
2. Non-destructive- no consumption of analyte.
3. Environment-friendly.
4. Short response time - in sec. or min., which makes these sensors appropriate for application in flow conditions systems.
5. The response is not affected by color or turbidity of the sample.
6. Simply device.

LIMITATIONS

1. Precision is rarely better than 1%.
2. Electrodes can be fouled by proteins or other organic solutes.
3. Interference by other ions.
4. Electrodes respond to the activity of uncomplexed ion, so it has to be ensured that there is a free ion in the solution (masking the ligands).

3 EXPERIMENTAL

Azelastine is a second generation H_1- receptor antagonist, anti-histamine and anti-inflammatory medication. It also has mast-cell stabilizing effects. Azelastine hydrochloride has been used both as a nasal spray and as eye drops.[23] The nasal spray is available in the market under different trade names i.e. Allergodil (in Europe), Rhinolast (in UK), Astelin and Astepro (in US), Azep (in Australia) etc. In India the drug is marketed under the brand names such as Azelast nasal spray (Sun pharma), Furamist – AZ nasal spray (Cipla) and Sarnase spray (Ranbaxy). Chemically azelastine is (±)-1-(2H)-phthalazinone, 4-[(4-chlorophenyl) methyl]-2-(hexahydro-1-methyl-1H-azepin-4-yl)-monohydrochloride.[24]

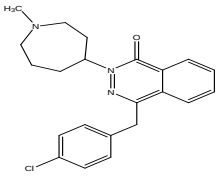


Fig. 3: Structure of Azelastine.

3.1. Reagents and Instruments used

All the reagents of analytical grade and were used as received. High molecular weight polyvinyl chloride (PVC), sodium tetra fuloro phenyl borate (NaTFPB), dioctyl phthalate (DOP), bis-(2-ethylhexylsebacate (BEHS), dioctylsebacate (DOS) and tetrahydrofuran (THF) were purchased from Merck. All metal salts were brought from Sisco lab (Mumbai India). Azelastine hydrochloride and its syrup were obtained from different local pharmaceutical factories. All solutions were prepared using triply distilled water. All potentiometric measurements were made at 25 ± 1ºC with a digital potentiometer (ECIL India)) using azelastine- selective membrane electrode in conjunction with an ECIL, India double junction Ag/AgCl reference electrode. A Stock solution of azelastine hydrochloride (0.1 M) solution was prepared by dissolving the calculated amount of drug in 20 mL water. The working solutions (1.0×10^{-6} to 1.0×10^{-1} M) were prepared by dilution of stock solution.

3.2. Preparation of ion – pair compound

Ion-pair compound of azelastine-tetrafluorophenylborate (AZ-TFPB): About 20 mL of 0.01 mol L^{-1} solution of azelastine hydrochloride was mixed with 20 mL of NaTFPB solution (0.01 mol L^{-1}) under stirring. The resulting precipitate was filtered off, washed with water and dried.[25]

3.3. Fabrication of electrode

To prepare membrane electrode the membrane components i.e. azelastine-tetrafluorophenylborate (AZ-TFPB), plasticizers (DOP, BEHS, DOS) and PVC were added in THF (25 mL) and the solution was carefully dissolved to get a homogenous mixture. The resulting mixture was transferred into a glass dish of diameter 2 cm. The solvent was evaporated slowly until an oily concentrated mixture was obtained. A Pyrex tube of diameter of 5 mm was dipped into the mixture for about 10 s so that a transparent membrane of about 0.3 mm thickness was formed. The tube was then pulled out from the mixture and kept at room temperature for about 10 h. The tube was then filled with 0.01M azelastine hydrochloride as an internal filling solution. The electrode was finally conditioned for 24 h by soaking in a 0.01 M azelastine hydrochloride solution.[25] The following cell was assembled for the conduction of the emf (electromotive force) measurements; Ag–AgCl |internal solution, azelastine hydrochloride (0.01M)| PVC membrane | sample solution | Ag - AgCl, KC1 (std.) These measurements were preceded by the calibration of the electrode with several azelastine hydrochloride solutions.

4. RESULTS

The determination of bio-active components is a subject of great importance especially for pharmacy and medicinal point of view. The use of ion – selective electrode for the determination of target ion in solution provides high selectivity, high sensitivity, fast response

time and wide concentration range. Thus ion – selective electrode is one of the best method used for the detection of bio-active material in solution. In present study NaTFPB is tested as electroactive material for the selective determination of anti-histamine drug azelastine hydrochloride. The experiment is based on ion – dipole interaction between azelastine hydrochloride and tetrafluoroborate ion.[25]

4.1. Optimization of membrane ingredients

The response of membrane electrode in significantly depends on the membrane components. [26] In present study membranes of various compositions were fabricated and their potential responses were studded. After several experiments it was observed that the membrane with the composition of AZ - TFPB: DOP: PVC of 4%: 64%: 32% (w/w) gives the best possible results. The membrane without ion – pair does not show significant response towards azelastine hydrochloride. The use of plasticizers (DOP, BEHS and DOP) significantly improves the linear concentration range, and slope of calibration curve. The electrodes (table 1) with DOS and BEHS as plasticizer has a detection limit of 1.0 $x10^{-4}$ M, within the liner concentration range of 3.8 x 10^{-4} –1.0 x 10^{-1} M respectively for azelastine hydrochloride solution. The electrode no. 3 with DOP as plasticizer was found superior in terms of linear concentration range, detection limit, and slope of calibration curve. The electrode no. 3 works satisfactorily in the concentration range of 1.8 x 10^{-5}- 1.0 x 10^{-1} M, with detection limit of 1.0 x 10^{-5} and slope 35.5 ± 0.5 mV/decay of activity. Further increasing the amount of ion-pair does not improve the response characters of the electrode, thus membrane with the composition of ion-pair: PVC: plasticizer of 4%: 64%: 32% (w/w) was taken as the most optimized membrane. The best response character of electrode in presence of DOP as plasticizers is due to its high polarity which provides the best possible complexation environment within the solution.

5 CONCLUSION

In this work we have tested NaTFPB as potential carrier for the determination of anti-histamine drug azelastine in different pharmacological solutions. The proposed membrane electrode work satisfactorily in the concentration range of 1.8 x 10^{-5} – 1.0 x 10^{-1} M, has a detection limit of 1.0 x 10^{-5} M and fast response time of 8 seconds. The proposed electrode can be used for a period of 3 weeks in a pH range of 2.5 – 6.0. The selectivity of membrane sensor towards target ions over some common interfering ions was calculated by MPM method.

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