ABSTRACT

Biomembranes mainly composed of a bilayer of amphipathic phospholipids are the important components of all living organisms forming boundary between cells and cell organelles. The biomembranes control the material flow into and out of cell and they form selective barriers to ions and large sized molecules. These bilayer walls give a definite shape to cells and protect them.

The surfaces of bilayer lipid membranes are charged due to the presence of phosphate groups and nitrogen bases, which will attract the oppositely charged ions and form electrified interfaces at the internal and external surfaces of the cells leading to development of a potential across the cell membrane. A cell is said to be alive and perform biological functions only when there exists a potential difference across the cell membrane.The chemical nature of the cell membrane components and the events that occur at the interface or within the bilayer decide the permeability and structural properties of the biomembranes. The bilayer arrangement of phospholipid molecules in the cell membranes acts as a matrix, which incorporates different proteins and glycans to perform variety of functions.

Many model membrane systems that have evolved in the course of membrane research are very useful to characterize the structure and functions of the biomembranes the electrical properties of membranes and drug-membrane interactions. The pharmacological actions of many classes of drugs are brought about by their binding to ‘specific sites’ in membrane bound proteins. However, some liphophilic drugs also exhibit non-specific interaction with the membrane lipid architecture and their concentration in

the membrane phase might reach one order greater than that in surrounding medium. The phospholipid and protein molecules of biomembranes are sensitive to the presence of such lipophilic perturbants.

The model-membrane systems have garnered much attention because they provide a useful and interesting interface between the biological world and man-made materials and thus, have great potential for basic membrane and cell-biology research as well as a variety of biotechnological and biomedical applications.

The understanding of permeation of drugs and drug-like molecules through lipid bilayers and changes brought about by these molecules in the properties of the BLM have become an attractive topic in recent drug research. Drug molecules in solution typically form various species due to ionization, complexation, dissociation, ring opening and closing etc. These different forms interact with the bilayer membrane systems in different ways and exhibit different effects on the electrical properties of membrane.

Midazolam (MDZ), a tricyclic benzodiazepine used in anesthetic practice, differs from most of the "traditional benzodiazepines" in having a nitrogen atom in its additional ring structure. This nitrogen is not sufficiently basic to be protonated at physiological pH, but is basic enough to give water soluble salts when treated with strong acids. Its hydrochloride salt, midazolam hydrochloride ($MDZH^{+}Cl^{-}$) displays an important pH dependent ring opening reaction. Only few studies have been made on the interaction of benzodiazepines with model membranes and little work has been done on MDZ-BLM interaction. Studies employing supported BLMs on non specific interaction of drug molecules are also scanty.

In this work the interaction of MDZ with bilayer phospholipid membrane system was studied using planar lipid membranes and supported bilayer lipid membranes. The drug induced changes in the electrical properties of these BLMswere monitored using electrochemical impedance spectroscopy (EIS).

In solution the MDZ molecules exist in neutral, ionized and ion pair forms through complex equilibria. The extent of these forms in the solution is strongly affected by the ionic strength of the medium supporting the membrane. With increase in NaCl concentration in the bath, the concentration of ionized form of MDZ in the solution decreases due to common ion effect of Cl^- ion. Electrochemical impedance studies revealed that the charged form of MDZ imparted a tightening effect on the BLM surface by covering the unneutralized negative charges on the BLM surface whereas the ion pair and neutral forms of MDZ get partitioned into the interior hydrocarbon core of the BLM,imparting fluidizing effect.

The penetration of the drug into BLM has taken place from the initial dose. The adsorption of Cl^- ions on the surface neutralizes the most of the positive charges on the BLM surface while negative charges on the BLM surface is partially neutralized by the adsorption of Na^+ ions from bath solution at neutral pH. With decrease in NaCl concentration the neutralization of surface negative charge by Na^+ ions decreases and surface negative charge increases.

Stability of membrane systems was analyzed by measuring the electrical properties of BLM at various time intervals using EIS. The life time of planar lipid membrane is short, which is about 10 to 16 hours, hence the long term interaction of MDZ with BLM can’t be studied by this method. Hence, supported bilayer lipid membrane systems were used to study the

long term drug – membrane interactions. This system allowed us to study the changes in the electrical properties of BLMs at higher drug doses.

Agar gel supported BLM (sb-BLM) was used to study the long term interaction between MDZ and BLM by employing electrochemical impedance spectroscopy (EIS) and cyclic voltammetric (CV) techniques. The membrane was formed at the surface of freshly cut agar surface. sb-BLM was stable for 45 to 50 hours. The results of sb-BLM studies supported the results obtained in the classical planar lipid membrane system.

Glassy carbon supported bilayer lipid membrane (GCE-BLM) was also used to study the MDZ-BLM interaction in the presence of ferro-ferricyanide ions as marker ions in NaCl bath solutions by CV and EIS techniques at neutral pH using BR buffer. The membrane conductance decreased with decrease in NaCl concentration in the bath, which is an opposite trend observed in planar and agar gel supported BLMs. The core of the BLM has low dielectric constant (2.05). The smaller ions like Na^+ ions pass through the BLM and offer some ionic conductance for BLM. The marker ions also pass into the core of the BLM along with Na^+ ions. The dielectric constant of water is higher and these ions are well separated in aqueous solution. But in the core of the membrane the electrostatic attractive force between these two ions is not greatly reduced.

The Na^+ and marker ions in the core of membrane will exist in ion pair form whose size will be much larger and therefore the ionic conductance decreases inside the BLM phase due to lower ionic mobility. Cyclic voltammetric studies revealed that redox peak currents are linear with MDZ concentration from 80 to 600 µM in 0.1 M NaCl bath. Such type of linear relationship was not observed in 1.0 M and 0.01 M NaCl bath solutions.