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Interaction of cisplatin with planar model membranes – dose dependent change in electrical characteristics

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Abstract

The drug cisplatin has broad antineoplastic activity against advanced testicular and ovarian cancers, epithelial malignancies, cancers of the head, neck, bladder, oesophagus and lungs. Peripheral neurotoxicity, ototoxicity and nephrotoxicity are its major side effects. The nonspecific action of this drug on the lipid bilayer architecture of membranes has been studied by following the effects produced on the electrical characteristics of model planar bilayer lipid membranes (BLM). The results confirm that the drug has a strong surface interaction with the zwitterionic polar head groups of the amphipathic phospholipids constituting the BLM. The permeability characteristics of cisplatin through the hydrophobic core are limited. Cisplatin does not fluidise the membrane sufficiently to cause its breakdown but creates small ion conducting defects on the membrane bilayer resulting in a marginal increase in ion conductivity. These results indicate that cisplatin exhibits a non-specific action on the lipid bilayer component of the membrane which might be partly responsible for its neurotoxic side effects.

Keywords: Cisplatin; Lipid bilayer; Zwitterionic lipid; Non-specific action; Electrical characteristics; Surface interaction

1. Introduction

The accidental finding of the cytotoxic effect of cisplatin has led to the investigation of several Pt(II) and Pt(IV) four and six coordination complexes for their chemotherapeutic potencies against solid tumours [1–5]. Cisplatin, carboplatin, oxaliplatin and a few other platinum complexes are currently administered in the treatment of testicular and ovarian cancers. Their interference with DNA synthesis and cell division by forming an adduct with DNA molecules has been well established [5–8]. Nausea, vomiting, sensory neuropathy and ototoxicity are the reported neurological side effects of cisplatin [9–11]. At higher doses, cisplatin causes peripheral neuropathy too but there is no evidence for central nervous system impairment [3]. Its suppressive sympathetic neuronal activity is evident from its reported action on adrenal chromaffin cells [12]. Cisplatin induced inhibitory action on resting leak conductance, transient depolarisation linked increase in conductance and enhancement of excitability by reducing

Abbreviations: BLM, bilayer lipid membrane; PC, phosphatidylcholine; PE, phosphatidylethanolamine; AC, alternating current; DC, direct current; RMS, root mean square; LCZ meter, inductance (L), capacitance (C) and impedance (Z) meter; CEC, Capital Equipment Corporation; IEEE, industrial standard also called general purpose interface bus (GPIB), a standard cable and protocol for connecting measurement devices to computers

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the threshold potential have been reported from electrophysiological patch clamp studies on cultured dorsal root ganglion neurones [13]. Cisplatin has been reported to attenuate voltage activated potassium and calcium current and thus interferes with Ca^{2+} activated chloride channels [9]. Interaction of cisplatin with membrane constituents could have modified the ion channel activity [11].

Earlier studies on neuroactive compounds such as insecticides, anaesthetics, tranquillisers, antidepressants, etc., have suggested a strong involvement of a nonspecific impeding action of these compounds on the lipid bilayer in their neurological effects. Popular insecticides like parathion, malathion, lindane, DDT and allethrin induce perturbations of membrane permeability and fluidise the membrane and the changes could be partially related to their primary insecticidal activity [14–16]. The major cause of the sedative side effect of the antidepressant imipramine has been attributed to its ability to penetrate membrane structures [17]. Studies on anaesthetics like halothane and enflurane have proved that the preferred position of interaction of inhalation anaesthetics with the lipid bilayer is the membrane interface [18,19]. Effects of alcohols on proton and potassium permeability of lipid bilayers have been qualitatively related to their anaesthetic activity [20]. The effect of amphiphilic drugs might be due to their interaction with the polar head groups of the membrane lipid bilayer [21]. These results give rise to the question whether the neurotoxic side effects of cisplatin could be due to a similar nonspecific interaction with the lipid bilayer component of membranes. According to some recent reports, cisplatin interacts with both cellular and model membranes involving acidic lipid constituents [22,23] and also with zwitterionic lipid rich azolectin model membranes, changing its resistance, surface tension and boundary potential [4]. Neuronal impairment could be due to electrochemical changes both at the membrane surface and at the inner core of the neuronal membrane. However, in spite of the significance of cisplatin interaction with membrane components, studies on cisplatin induced effects on model membranes simulating the lipid bilayer have been scanty [13,22]. Considering these facts, investigatory studies on cisplatin induced changes in the surface and interior electrical characteristics of bilayer lipid membrane (BLM) have been undertaken. Further, entry of cisplatin into target and nontarget tissues has been reported to be largely diffusional passage without the involvement of membrane associated proteins by some workers [3,5,12] and facilitated transport with the involvement of influx and efflux proteins by others [6]. So, the proposed study of nonspecific action of cisplatin on BLM could help to understand the in vivo permeability features of cisplatin.

The *trans* isomer of cisplatin (transplatin) reportedly possesses much less antitumour activity [3,11,13]. Therefore, it does not find widespread use in chemotherapy. Hence the focus of this study was on the more active and neurotoxic cisplatin.

2. Materials and methods

Phospholipid mix from hen's egg yolk was isolated following standard procedure [24]. Thin layer chromatography using a chloroform–methanol–acetic acid–water solvent system [24] in the ratio 25:15:4:2 was carried out on a glass plate spread with silica gel adsorbent containing calcium sulphate binder. The chromatogram, developed using iodine vapours and also standard phosphate stain [25], showed three distinct spots confirming the heterogeneity of the phospholipid mix. Earlier workers have shown that egg phospholipids contain a predominant fraction of phosphatidylcholine (PC) (74%) and phosphatidylethanolamine (PE) (15%) with lysophosphatides, sphingomyelin, plasmalogen and phosphatidylinositol as the minor ingredients [25]. The unfractionated phospholipid stock in chloroform (50 mg/ml) stored in nitrogen environment was dispersed in *n*-decane (2.5% w/v) after the evaporation of chloroform. This dispersion was prepared on a daily basis to form BLM.

A polymethyl methacrylate chamber with two 5 ml aqueous compartments divided by a 1 mm thick septum was used for the experiments. BLM was formed by a syringe (painting method) on a 1.2 mm diameter aperture made in the septum [26]. Electrical contact was through two non-polarisable $Ag/AgCl/Cl^-$ electrodes made following standard procedures [27]. The BLM unit was mounted on a vibration-free platform and provided with electrical shielding to avoid stray current pick-up [28].

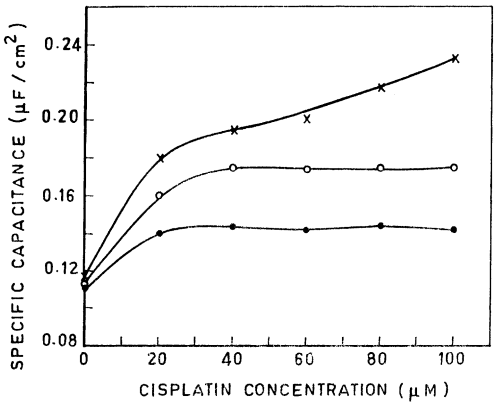

Fig. 1. Dose dependent change in AC capacitance by cisplatin in 0.01 M NaCl (●), 0.1 M NaCl (○) and 1.0 M NaCl (×) at an applied RMS voltage of 40 mV and an applied frequency of 40 Hz.

Aqueous unbuffered molar, decimolar and centimolar solutions of NaCl (E. Merck, India) with a pH around 7.0 and standard buffer solutions [24,25] of pH 6.0 and 7.0 containing 10 mM, 100 mM and 1000 mM NaCl constituted membrane 'bathing' media. The stabilisation of the membrane took about 10–30 min as evident from the recorded steady electrical characteristics. Cisplatin, procured from Dabur Research Foundation (India), was added in the aquated form in known micromolar quantities from an aqueous stock solution (0.5 mg/ml) prepared from chloride-free water. The drug was added in small aliquots up to a maximum cisplatin dose of 100 μM which is 10 times the reported clinically relevant dose [11].

The DC electrical characteristics such as conductance and capacitance were measured using the digital Keithley electrometer (Model 6517, USA) with IEEE interface and CEC test point data acquisition software. The AC characteristics were studied using a digital LCZ meter (Model 1061, Chen-Hwa, Taiwan) capable of applying 0.01–1.0 V at an increment of 10 mV. Applied frequency was varied in 30 steps from 40 Hz to 200 kHz. The capacitance, impedance and the phase angle difference of BLM were measured in both parallel and series modes. The capacitance, impedance and conductance are reported per unit area assuming that the membrane covers the entire aperture (1.2×10^{-2} cm^2). All the studies were done between 22 and 24°C.

It is a well-established fact that the electrical characteristics of BLM vary from one membrane to another [26,28,30,34]. Hence each experiment was repeated 5–7 times and the results presented are the mean values from these experiments. The variation in the electrical characteristics from one membrane to another was well within 10%.

3. Results

It is well established that the BLM with its hydrophobic core and polar head groups acts as an organic capacitator [29]. The dose dependent interaction of cisplatin on the AC capacitance of BLM at a constant applied RMS voltage of 40 mV and an applied frequency of 40 Hz in different ionic strengths of NaCl is presented in Fig. 1. Initially, a steep increase in capacitance was noted in all concentrations of NaCl (0.01 M, 0.1 M and 1.0 M). The capacitance then reached a saturation value above a cisplatin

Table 1
Dose dependent change in AC capacitance in buffered and unbuffered media containing 10 mM and 100 mM NaCl

Cisplatin dose (μM)	Membrane capacitance			
	10 mM NaCl		100 mM NaCl	
	Unbuffered	Buffered	Unbuffered	Buffered
0	0.1116	0.1177	0.1096	0.1262
20	0.1414	0.1482	0.1602	0.1752
40	0.1436	0.1490	0.1744	0.1871
60	0.1426	0.1481	0.1733	0.1913
80	0.1452	0.1475	0.1749	0.1993
100	0.1418	0.1488	0.1737	0.1994

The unit of specific capacitance is $\mu F/cm^2$.
The readings were taken at an applied RMS voltage of 40 mV and an applied frequency of 40 Hz.

concentration of 20 μM in 0.01 M NaCl and above 40 μM in 0.1 M NaCl. However, in 1.0 M NaCl bath medium, an initial steep increase in capacitance was observed up to doses of 20 μM which was followed by a sublinear increase for cisplatin doses up to 100 μM. The increase in capacitance was found to be maximal in 1.0 M NaCl and least in 0.01 M NaCl medium. Thus it is apparent that the ionic strength of the bath medium has a bearing on the cisplatin-BLM interaction.

Experiments with buffered solutions (pH 7.0) containing 10 mM, 100 mM and 1000 mM NaCl showed similar trends and the results are presented in Table 1.

Another important observation relates the extent of interaction of cisplatin when added symmetrically on both sides of the membrane and when it is added asymmetrically to the *cis* side only. The hydrostatic pressure developed due to unequal volumes on either side during asymmetric addition was offset by addition of an equal volume of distilled water on the *trans* side. Regardless of the mode of addition of cisplatin and ionic strength of the medium, an increase in capacitance was noted in all cases. From Fig. 2, it is seen that the per cent increase in capacitance, under similar conditions of applied RMS voltage and frequency, produced due to the addition of 100 μM cisplatin is considerably higher than that produced when the same quantity of cisplatin is added asymmetrically.

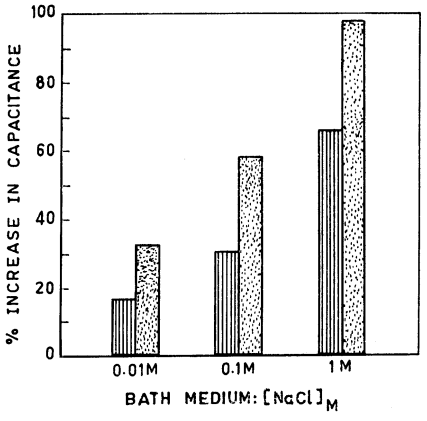

Fig. 2. Effect of symmetric and asymmetric modes of drug addition on the per cent increase in AC capacitance at an RMS voltage of 40 mV and an applied frequency of 40 Hz. Striped boxes indicate asymmetric addition and dotted boxes represent symmetric addition.

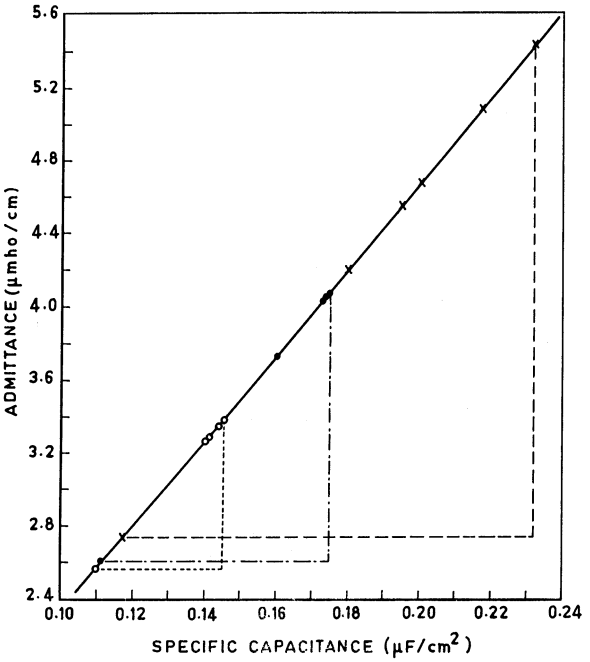

Fig. 3. Admittance–capacitance profile at 40 mV (RMS) in 0.01 M NaCl (○), 0.1 M NaCl (●) and 1.0 M NaCl (×).

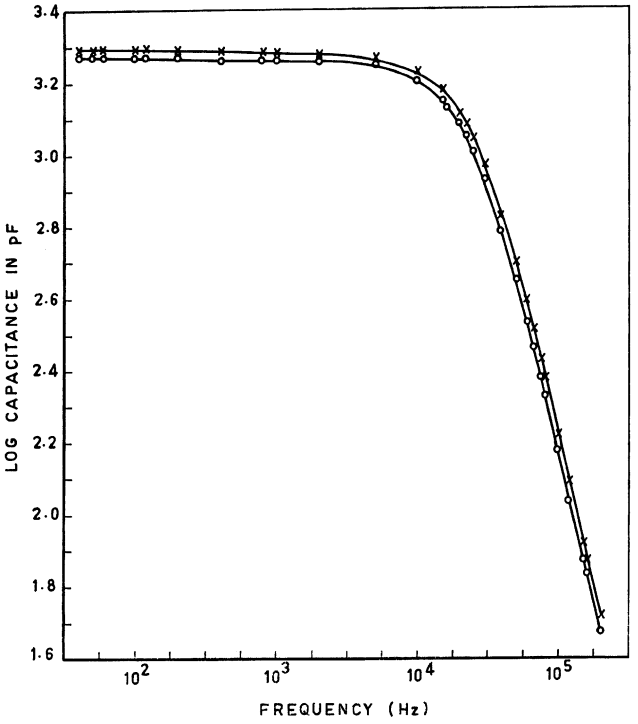

Fig. 4. Frequency dependent dispersion at 40 mV (RMS) of control (●) and drug infested (×) membranes in 0.01 M NaCl. The drug load is 100 μM in either compartment.

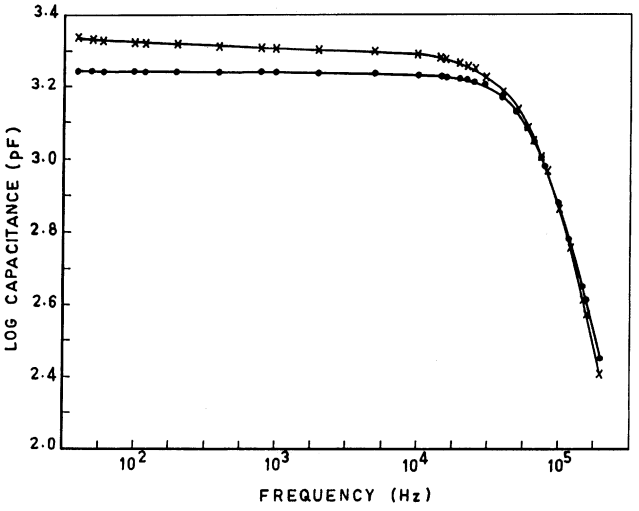

Fig. 5. Frequency dependent dispersion at 40 mV (RMS) of control (●) and drug infested (×) membranes in 1.0 M NaCl. The drug load is 100 μM in either compartment.

A plot of membrane admittance against membrane capacitance in NaCl bath media of different ionic strengths at 40 mV (RMS) and 40 Hz (Fig. 3) with cisplatin doses as the variant was linear. It is interesting to note that the linearity was not shifted in different strengths of NaCl, but the area under the curve increased with increasing NaCl concentration in the ratio of 1:4:10.

The frequency dependent dispersion of the membrane capacitance at a constant RMS voltage of 40 mV was studied in 0.01 M NaCl and 1.0 M NaCl for the bare membrane and membrane infested with 100 μM cisplatin. A Maxwell–Wagner type of dispersion occurred in both cases [30]. 100 μM was chosen because it is expected from the study of dose dependent change in capacitance (Fig. 1) that the maximum interaction in 1.0 M NaCl bath medium would be recorded at that dose while for 0.01 M NaCl, the saturation level would have been attained at the same dose. In 0.01 M NaCl, the frequency dependent dispersion pattern was observed to be the same for the BLM in the control and drug infested conditions. A small difference of 100 pF was maintained at all frequencies studied (40 Hz–200 kHz) as shown in Fig. 4 whereas in the 1.0 M NaCl medium, the capacitance curve for the bare and drug infested membranes synchronised above the applied frequency 60 kHz. But up to 60 kHz, the drug infested membrane maintained a higher capacitance (Fig. 5).

The trends obtained in the AC mode of operation were similar to those obtained in the DC capacitance

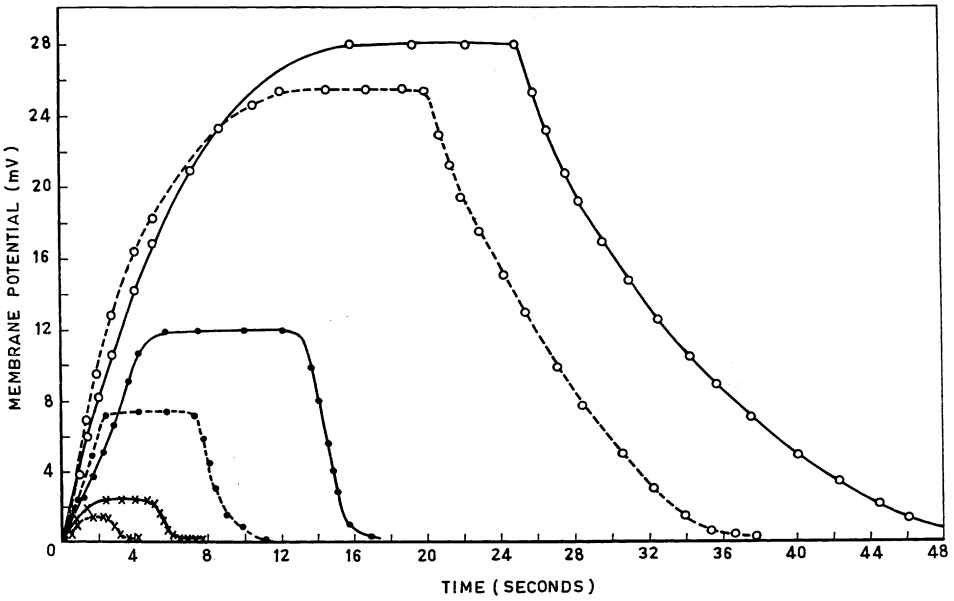

Fig. 6. Charge–decay profiles for control (solid lines) and drug infested (dotted lines) at 40 mV (DC) in 0.01 M NaCl (○), 0.1 M NaCl (●) and 1.0 M NaCl (×). 100 μM cisplatin is added to either compartment. The series resistance used for measurement in all cases is 1 GΩ.

studies. The DC capacitance was measured using the charge decay method [26]. We observed that the charging potential and the decay time depended on the membrane conductance and capacitance. The addition of cisplatin to the bath medium was found to alter both the charging potential and the time constant. As observed with the AC studies, the effect was most pronounced in 1.0 M NaCl and least in 0.01 M NaCl with the value in 0.1 M NaCl intermediate between the two. Fig. 6 summarises the effects produced by 100 μM cisplatin on the charging potential and time constant of the BLM in different NaCl media.

The cisplatin dose dependent change in membrane conductance also appears to be controlled by the ionic strength of the medium. The magnitude of increase in DC conductance due to loading of increasing doses of cisplatin was extremely small in the 0.01 M NaCl medium while it was considerable in 1.0 M NaCl and intermediate in 0.1 M NaCl. The plot of membrane capacitance against membrane conductance in different concentrations of NaCl followed a linear trend. However, the slope of the linear

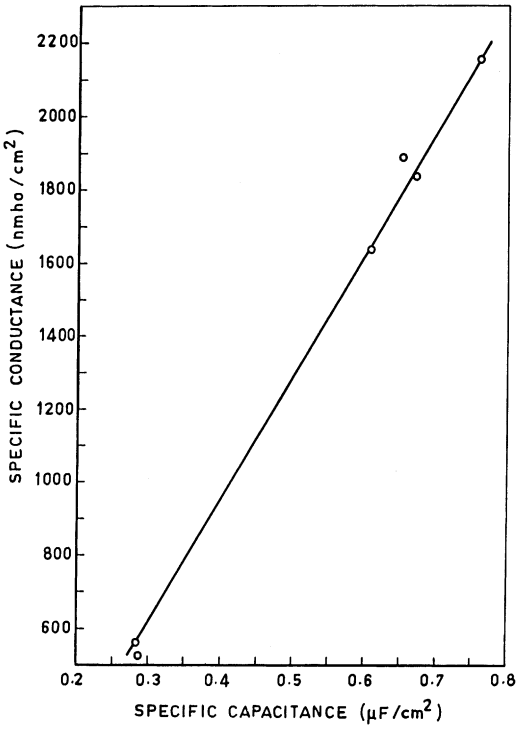

Fig. 7. Dose dependent capacitance–conductance profile of BLM at 40 mV (DC) in 1.0 M NaCl bath medium. The series resistance used for the capacitance measurements is 100 MΩ.

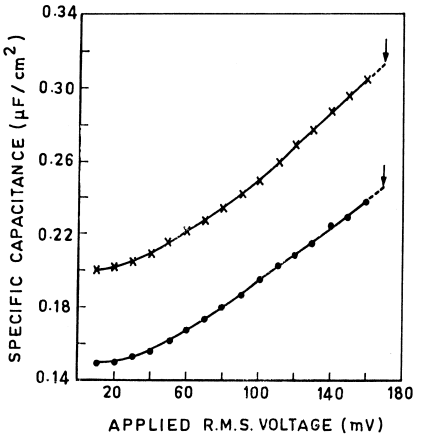

Fig. 8. Voltage dependent dispersion of membrane AC capacitance for control (●) and drug infested (×) membranes at an applied frequency of 40 Hz. The drug load in either compartment is 80 μM.

lines increased with increasing ionic strengths of the bath medium in the ratio 1:5:10. Fig. 7 shows the conductance–capacitance plot obtained at 40 mV (DC) in 1.0 M NaCl bath medium.

One common observation in all measurements pertaining to the dose dependent interaction of cisplatin with the BLM was that it took about 22–25 min for the drug–membrane interaction to take place, as was evident from the electrical measurements.

The dielectric strength of the BLM was not altered significantly by the addition of cisplatin as the breakdown voltage of the bare and drug infested membranes was found to fall in the same range in all bath concentrations of NaCl. Fig. 8 displays a plot of the voltage dependent dispersion of capacitance at 40 Hz for bare and drug infested membranes in 1.0 M NaCl. Both curves show a slight biphasic nature at lower voltages.

4. Discussion

The first part of the BLM the drug encounters is the polar head groups of the amphipathic lipids constituting the BLM. Cisplatin, being hydrophilic, is more likely to accumulate at the membrane–solution interface. The chemistry of cisplatin is typical of a platinum coordination complex. *cis*-Dichlorodiammine platinum(II) or cisplatin is a four coordinated square planar complex. When cisplatin is dissolved in

chloride-free water, the following aquation reactions take place:

$$[PtCl_2(NH_3)_2] + H_2O \rightarrow [PtCl(H_2O)(NH_3)_2]^+ + Cl^- \quad (1)$$

$$[PtCl(H_2O)(NH_3)_2]^+ + H_2O \rightarrow [Pt(H_2O)_2(NH_3)_2]^{2+} + Cl^- \quad (2)$$

$$[PtCl_2(NH_3)_2] + 2H_2O \rightarrow [Pt(H_2O)_2(NH_3)_2]^{2+} + 2Cl^- \quad (3)$$

The water medium can also supply hydroxo ligands which can take part in the aquation reactions.

$$[PtCl_2(NH_3)_2] + OH^- \rightarrow [Pt(Cl)(OH)(NH_3)_2] + Cl^- \quad (4)$$

$$[Pt(Cl)(OH)(NH_3)_2] + OH^- \rightarrow [Pt(OH)_2(NH_3)_2] + Cl^- \quad (5)$$

$$[Pt(H_2O)_2(NH_3)_2]^{2+} + OH^- \rightarrow [Pt(OH)(H_2O)(NH_3)_2]^+ + H_2O \quad (6)$$

It is a well-established fact that it is generally easier to replace a chloride ligand bound to Pt(II) than other ligands [31]. The rate constants for Eqs. 1–3 are reported to be 1.78×10^{-3} s^{-1}, 2.75×10^{-4} s^{-1} [22,23] and 7.2×10^{-7} s^{-1} [5] respectively. Since NH_3 ligand occupies a higher place in the spectrochemical series than both Cl^- and H_2O [32], replacement of NH_3 ligand does not take place under mild reaction conditions. The formation and existence of the monopositive and dipositive forms of cisplatin have been reported and well established in coordination chemistry [31] as well as in the intracellular medium where the chloride concentration is less than 100 mM [5,22].

When the system is supplied with an excess of chloride ions, the equilibrium is shifted towards the left and the reverse anation reactions take place [31]. The greater the number of chloride ions, the more feasible will be the formation of the neutral species.

In the present experiments, cisplatin was added from a stock solution which contained a mixture of all the above species. When cisplatin was added to the bath medium containing chloride ions, the reverse anation reactions occurred leading to the formation of the neutral dichloride and hydroxochloride forms depending on the availability of chloride ions in the medium. The pH of the unbuffered bath medium did not change drastically on addition of cisplatin and continued to be around neutral pH (7.0). At this neutral pH, Speelmans et al. have reported that the major species of cisplatin are the uncharged dichloride and hydroxochloride forms [22,23]. However, at lower chloride concentrations such as those in 0.01 M NaCl bath medium, a small amount of the charged monopositive form of cisplatin (about 20%) also exists [22,23]. The charged forms will be virtually absent in the 1.0 M NaCl bath medium where there is a large quantity of chloride ions available to facilitate the faster formation of the dichloride species. Reports of the existence of the neutral form of cisplatin in the blood stream where the chloride concentration is above 100 mM [5] further confirm these reactions.

The observed differences in the electrical properties of bare and drug infested membranes could be explained based on the drug induced effects on the ion conductance and the electrical double layer at the membrane-solution interface and also on the availability of the neutral dichloride form of cisplatin in the bath medium.

The polar head groups of the BLM act as charged plates of a capacitator enclosing the hydrophobic core which acts as a dielectric and is responsible for the capacitative nature of the BLM [26]. The polar head groups attract counterions from the aqueous bath medium resulting in the formation of an electrical double layer which is a well-documented phenomenon at any membrane–solution interface [30]. When the drug is added to the bath medium, the various forms of the drug (dichloride, hydroxochloride and the monopositive species) initially come into contact with the polar head groups and the counterion array. The neutral cisplatin molecule (either the dichloride or the hydroxochloride form) in the bath medium, being non-polar and non-conducting, might become entrapped between the electrical double layers thus acting as a dielectric sandwiched by two charged plates. This mini-capacitator will contribute to the total capacitance of the system resulting in the observed rise in capacitance. The for-

mation and existence of the neutral form of cisplatin being maximum and the availability of a large number of ions at the membrane–solution interface to form the electrical double layer in the 1.0 M NaCl medium may explain the maximum increase in capacitance observed in this medium.

In 0.01 M NaCl bath medium, the number of ions available at the membrane–solution interface is smaller. Also, the presence of a few monopositive species of cisplatin reduces the number of neutral cisplatin molecules available for the formation of the dielectric sandwich at the membrane–solution interface. The overall result is that there is an increase in the capacitance of the BLM due to a surface interaction of cisplatin but this increase is smaller than those observed in 0.1 M and 1.0 M NaCl media. Experiments carried out in buffered solutions of pH 7.0 containing 10 mM, 100 mM and 1000 mM NaCl also displayed similar trends. The results are presented in Table 1. Interaction of cationic forms of cisplatin with the acidic lipids phosphatidylserine and phosphatidic acid and isolation of such adduct products [22,23] have been reported earlier. The present experiments confirm that the drug also interacts with neutral zwitterionic lipids.

In 1.0 M NaCl, the interaction with the BLM surface progressively increases with added doses of cisplatin. It indicates that the binding sites on the membrane surface are relatively free of any adsorbed species, namely the charged form of cisplatin. This is similar to the results reported on the interaction of the neutral form of pentachlorophenol with phospholipid bilayers [33]. In 0.01 M NaCl bath medium, though the neutral species of cisplatin predominates, the charged form of cisplatin interacts electrostatically with the zwitterionic polar head groups of the BLM and the capacitance values soon become saturated. The onset of saturation in 0.1 M NaCl, where the number of ions in the bath medium is relatively greater, occurs at a higher dose of cisplatin. Since no experiments were carried out beyond 100 μM cisplatin concentration, possible saturation of capacitance values beyond the addition of 100 μM cisplatin in the 1.0 M NaCl medium could not be recorded.

The asymmetric addition of the drug results in a smaller change in capacitance than symmetric addition. This clearly indicates restricted permeability of cisplatin through the lipid bilayer. The difference in the percentage capacitance during symmetric and asymmetric addition rules out the possibility of the increase in capacitance being due to a change in the area or thickness of the BLM.

It is interesting to note that the per cent increase of capacitance in 0.01 M NaCl during symmetric addition is double that observed during asymmetric addition while it is nearly 1.8 times in 0.1 M NaCl and 1.4 times in 1.0 M NaCl. This shows that cisplatin has very low permeability through the membrane and hence the asymmetric addition produces approximately only half the effect on the increase in capacitance of symmetric addition. The possibility of formation of an additional capacitator on the *trans* side of the BLM is ruled out due to lack of drug molecules in the *trans* side during asymmetric addition.

In 0.01 M NaCl medium, the charged forms of cisplatin become anchored on the membrane surface by electrostatic attraction with the charged polar head groups of the BLM. Since cisplatin does not have any lipophilic end, its penetration into the lipid bilayer is not possible. Thus, in biological systems, influx of the charged form of cisplatin may involve a carrier protein. Such carrier proteins for influx and for rapid efflux in drug resistant cell lines have already been reported [8].

However, the permeability of the neutral form of cisplatin, which is the predominant form at neutral pH, through the BLM is feasible as generally neutral molecules are reported to possess greater permeability through the BLM than charged forms [34]. Moreover, as the concentration of the bath medium increases, some small ion conducting defects are formed on the compact bilayer architecture due to the additives (drug) as well as the number of ions in the medium, resulting in greater ionic flux [20,26]. Neutral cisplatin, being small, could squeeze through these defects in the BLM and reach the other side. This may explain the slightly higher per cent increase in capacitance in 0.1 M and 1.0 M NaCl bath media during asymmetric addition than the expected value which is half the increase observed during symmetric addition. As the number of ions is maximal in the 1.0 M NaCl medium, the deviation from the expected per cent increase during asymmetric addition is also maximal (expected: 50%, observed: 66%). These results indicate that neutral cisplatin might also serve

as an interstitial and substitutional impurity for the acyl chain core of the BLM.

Admittance is a function of capacitance at constant applied frequency and hence the plot of admittance vs capacitance (Fig. 3) is linear. However, the dose dependent admittance-capacitance profiles of cisplatin in 0.01 M, 0.1 M and 1.0 M NaCl media all fall on the same line indicating that the mode and site of interactions of cisplatin on the BLM are the same regardless of the concentration of the bath medium. The linear relation between drug induced change in membrane capacitance and admittance in all NaCl media (0.01 M, 0.1 M and 1.0 M) with the same slope confirms the better interaction of neutral cisplatin. The difference in the areas under the curve in the three bath media may be attributed to the amount of the neutral form of cisplatin and to the number of ions present in the bath medium and to the number of drug induced ion conducting defects created on the membrane matrix.

The observed large increase in membrane capacitance in 1.0 M NaCl medium may be partly due to the interior capacitance besides the surface capacitance [18,19]. But accommodated cisplatin with the bilayer core enhanced conductance but not inner capacitance. This is evident from the results depicted in Fig. 5. Generally, surface barriers for the entry of ions and polar molecules are relieved at higher frequencies [30]. In 1.0 M NaCl medium, the control and drug infested membranes show significant differences in capacitance initially. But at high frequencies ($>6\times10^4$ Hz), both curves converge indicating that the control and drug infested membranes have the same inner core capacitance. Thus the observed large increase of membrane capacitance is exclusively due to surface interaction. It could be concluded that the same dielectric loss mechanisms dominate the dispersion behaviour in both cases at high frequencies as has been reported for neutral pentachlorophenol [33].

In the 0.01 M NaCl medium, the electrostatic interaction of the small amount of monopositive cisplatin present in the medium with the polar head groups of the BLM interferes with the effective relieving of surface barriers at higher frequencies resulting in a small difference in capacitance being maintained throughout the frequency range studied.

The surface interaction of cisplatin is further substantiated from the voltage dependent dispersion studies of BLM in the presence of cisplatin, where irrespective of the charge status of cisplatin, the dielectric strength of the membrane was not altered significantly indicating that the drug does not fluidise the membrane in the concentration ranges studied.

The linear DC capacitance–conductance plot also favours an increased interaction of the neutral cisplatin over any charged forms. The increase in conductance with increasing cisplatin dose in 0.01 M NaCl indicated that the transport of any charged form of cisplatin through the BLM is minimal. However, neutral cisplatin, being non-conducting, cannot account for the observed increase in conductance in the NaCl bath media. So, the observed increase must be due to accelerated passage of chloride and sodium ions through some ion conducting defects created by the drug in the inner hydrocarbon core. As the neutral form of cisplatin is predominant under the experimental conditions, it is more likely to induce the ion conducting defects on the membrane matrix. As the number of ions is maximal in 1.0 M NaCl, the increase in conductance is also higher in this medium than in the lower bath concentrations. However, these defects caused by cisplatin on the BLM could not be large enough to fluidise the membrane architecture, which is evident from the observation that the dielectric strength of the BLM is not altered by cisplatin. This action of cisplatin is similar to that reported for the insecticide malathion with lipid bilayers [15].

The lack of any appreciable change in the dielectric strength of BLM due to cisplatin–BLM interaction could be explained based on the structure of cisplatin. Cisplatin is a simple, small and planar molecule whose voltage induced passage through the BLM or interaction with the polar head groups at the surface would not cause much perturbation of the membrane architecture partly because of its compact size and partly due to its relative difficulty in passing through the membrane. Hence, even the passage of neutral cisplatin does not fluidise the membrane but might only induce minor defects on the membrane matrix.

The observed voltage induced increase in capacitance of both control and drug infested membranes could be due to a combination of reduction in BLM thickness and an increase in the membrane area as reported by earlier workers [26]. The slight biphasic

nature of the voltage–capacitance profiles proves the necessity of a threshold voltage to promote the interaction of ions and the drug molecules with the membrane [26].

From the electrical characteristics, it could be established that the neutral form of cisplatin which is the predominant form at neutral pH shows the maximum tendency to interact with the BLM and increases the membrane capacitance considerably. It is also evident that the surface interaction of cisplatin is the dominant mechanism. Since all experiments were carried out with unmodified BLMs, the diffusion controlled passage of the neutral cisplatin through the lipid bilayer is strongly favoured in accordance with the reported in vitro and in vivo observations [3,5,12]. The present study highlights the fact that cisplatin also interacts with PC and PE apart from acidic lipids reported by some workers [22,23].

As discussed earlier in this paper, the neurological action of cisplatin comprises a nonspecific action on the lipid bilayer core structure. The present findings that cisplatin changes the surface capacitance and admittance of protein-free zwitterionic BLM in a dose dependent manner clearly indicate its nonspecific action. Earlier reports that acidic amphiphilic lipids strongly interact with the charged form of cisplatin [22,23] are also an observation of nonspecific action only. These nonspecific actions could initiate neuronal impairment in cancer patients being treated with cisplatin thus accounting for its neurotoxic side effects. As the lipid fraction has been reported to constitute about 75–80% of the nerve myelin dry weight [35], cisplatin, which could act on lipid bilayers even in the absence of any specific receptor protein, could possibly accumulate in the lipid rich nerve tissues resulting in the reported neurotoxicities.

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