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AN ION-SELECTIVE ELECTRODE FOR THE DETERMINATION OF PHENCYCLIDINE (PCP)

by

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An ion-selective electrode responsive to the protonated form of phencyclidine (PCP^+) is described. This electrode is based on incorporating PCP^+ as its dinonylnaphthalenesulfonate in a plasticized poly(vinylchloride) membrane. The electrode has very good selectivity with respect to inorganic and small organic cations. The detection limit is about $10^{-5.1}$ M. In addition to application for direct potentiometric analysis of PCP^+, successful use as an indicator electrode in potentiometric titration of PCP^+ at concentrations as low as 10^{-4} M is described.

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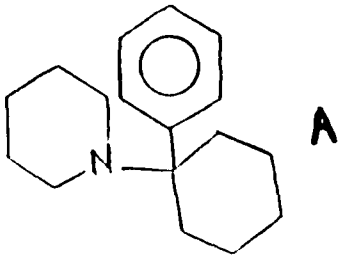
INTRODUCTION

Phencyclidine (N-[1-phenylcyclohexyl] piperidine,PCP)(A) was introduced



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in the late 1950's for use as a human anesthetic. Investigation by Greifenstein et al (1) and others (2,3) revealed however, that patients given PCP experienced severe and prolonged postoperative psychological disturbances. Although PCP was not approved for human use, it is currently used in veterinary medicine. Because of its psychological effects, PCP has gained popularity as an illicit "street drug" (4,5,6) and is known in the illegal market as "angel dust", "hog" or "the peace pill". In addition, it is often sold to unwary buyers as tetrahydrocannabinol,LSD, or cocaine (4). Because of its bizarre and often unpredictable effects and because it is often misrepresented in sale as other compounds, it has recently been called the most dangerous of all street drugs today (4).

The growing concern about phencylidine abuse is exemplified by the devotion of an entire recent issue of its research monograph to this topic by the National Institute on Drug Abuse (7) and by the increasing number of scientific publications concerning PCP (7-24, 35-39). A large percentage of these publications deal with fundamental questions concerning PCP's pharmacokinetics (8-11)and physiological effects (12-14). In addition, much effort has been devoted towards developing and improving methods for analysis of this drug (15-24, 35). These methods have involved the use of gas chromatography (16-20) mass spectrometry (16, 22-24, 35) liquid chromatography (21) and very recently radioimmunoassay (15).

We recently described an ion-selective electrode (ISE) based on the high molecular weight ion-pairing agent dinonylnaphthalenesulfonic acid (DNNS) (25) which has great selectivity for large organic cations relative to both smaller organic and inorganic cations. Because many compounds of clinical, pharmaceutical and toxicological interest are either high molecular weight cations or are converted to high molecular weight cations at physiological pH values, this ISE shows great promise for development of simple and inexpensive potentiometic assays for these species. Many of the common drugs of abuse including the

opiate alkaloids, cocaine, and PCP, are examples of the types of compounds which might be determined in this way.

Although ISEs for determination of some opiate (26) (as well as some non-opiate (27-30)) alkaloids have been reported, no electrodes responsive to cocaine, PCP, or other drugs of abuse have been described. For this reason, and as a result of the current interest in PCP, we decided to investigate the response characteristics of a DNNS-based PCP electrode. Initial results of this investigation are reported here.

Experimental

Electrode Preparation and Handling. DNNS based electrodes were prepared as described previously (25). Only conventional type polymer membrane ISEs (e.g. with internal reference) were prepared. The internal reference solution was 10^{-3}M phencyclidine-hydrochloride (PCP-HCl). All PCP-HCl solutions used were buffered at pH 5.0 with 10^{-3}M acetate buffer. The DNNS in the polymer membranes was converted to the PCP^+ (protonated PCP) form by soaking the electrodes in 10^{-3}M PCP-HCl for two d. When not in use, the electrodes were stored in 10^{-3}M PCP-HCl.

EMF Measurements. All electrodes protentials were measured vs. a double junction Ag/AgCl reference electrode; 0.1M NH_4NO_3 and 0.1M NaCl were used in the external junction for the calibration and titration experiments, respectively.

Calibration experiments were carried out using a microcomputer-controlled potentiometric analysis system (31). Calibration solutions were thermostated at $25.0^{o}\pm 0.1^{o}C$ using a Forma Scientific (Marietta, Ohio) circulating bath. Ion size parameters were obtained and selectivity coefficients and ISE detection limits determined as described previous (25).

The PCP electrode was used as the indicator electrode in potentiometric titrations of PCP^+ with sodium tetraphenylborate (TPB). The microcomputer controlled potentiometric analysis system performed all titrations (31). The TPB solution was standardized by potentiometric titration with a dodecyltrimethylammonium bromide (DoTAB) solution using a $DoTA^+$ ISE (25) as the indicator electrode. The DoTAB solution was standardized by potentiometric titration with primary standard $AgNO_3$ using a Ag wire as the indicator electrode.

Reagents. Pure PCP-HCl may be obtained from the National Institute on Drug Abuse, Rockville, Maryland. Quaternary ammonium bromides were obtained from Eastman (Rochester, NY); their purities were determined as described previously (25). Sodium tetraphenylborate was obtained from Aldrich (Milwaukee, Wis.). All other reagents were AR grade.

Results and Discussion

The critical response characteristics of the DNNS-based PCP^+ electrode are summarized in Table I. The highest concentration of PCP-HCl used for calibration was 10^{-3}M; it was not considered necessary to calibrate to higher concentrations. As the data indicates, nearly Nernstian response was obtained down to $10^{-4.2}$M. This lower limit of linear response and the detection limit of $10^{-5.1}$M are somewhat poorer than the 10^{-5}M and $10^{-5.9}$M lower limits obtained with the DNNS-based $DoTA^+$ electrode described previously (25). Why poorer linear and detection limits are obtained for the PCP^+ ISE is as yet unknown but may be related to the fact that PCP^+ is a protonated tertiary ammonium ion and $DoTA^+$ a quaternary ammonium ion. Studies are currently underway to see if this is, indeed, the case. It should be noted that the reproductibility measures cited in Table I represent data collected over a period of a month, indicating the high stability of the electrode.

Selectivity coefficients ($k_{i,j}$) for the PCP^+ electrode are presented in Table II. As was the case with the DoTA+ electrode (25) the DNNS-based PCP^+ electrode exhibits negligible interference from inorganic cations. In addition, the selectivity data again demonstrate the role solvent extraction parameters play in determining ISE selectivity (32) in that the selectivity coefficients for the monovalent ions increase with the molecular weight of the ion. A plot of $\log k_{i,j}$ vs. carbon number for the interfering ions is not linear, however, as was the case for the DoTA+ electrode (25). A linear relationship might be expected because an analogous plot of the logarithm of the extraction constant ($K_{ex.}=K_{ion\ pair} \times K_{distrib.}$) for a series of homologous alkylammonium-picrates vs. carbon number in the ammonium ions is linear (33). The primary ion (PCP^+) and interfering ions (tetraalkylammonium ions) used in this study, however, are not homologs but rather have significant structural and geometric differences. These differences probably account for the non-linearity of the $\log k_{i,j}$ vs. carbon number plot.

An apparently anomalous selectivity order is observed for the PCP^+ electrode for the two largest interfering ions. Despite the fact that PCP^+ (mol. wt. =244) is larger than TBA^+ (mol. wt. = 242) and $DoTA^+$ (mol. wt. = 228) the electrode is most selective for $DoTA^+$ and least selective for PCP^+. A similar selectivity reversal was observed for TBA^+ interference at the $DoTA^+$ electrode (25) and was explained in terms of the steric hindrance of the N^+ by the bulky n-butyl groups in TBA^+. This effect undoubtedly, at least partly, accounts for the preference of the PCP^+ electrode for $DoTA^+$ since the N^+ in PCP^+ is also sterically hindered.

It is not clear whether the preference of the electrode for TBA^+ relative to PCP^+ can be explained in terms of steric effects, however, since both ions have hindered N^+s. Further, the ability of PCP^+ to form hydrogen bonds in the aqueous phase undoubtedly lowers the distribution coefficient of the PCP^+-DNNS ion pair. This would, of course, be reflected in ISE studies by a lower selectivity coefficient for the tertiary ammonium ions relative to the quaternary ammonium ions. It is interesting to note, however, that in solvent extraction studies (33,34) on homologous series of primary, secondary, tertiary and quaternary alkylammonium-picrates, the extraction coefficients for ion pairs with the same number of carbon atoms in the alkylommonium ions, in general, decreased in the order tertiary> secondary>primary>quaternary. For example, for 12-carbon ammonium ions the extraction coefficients decreased in the order tributylammonium> dihexylammonium>dodecylammonium>tetrapropylammonium.

A possible explanation for the loss of affinity of the anion (picrate) for quaternary ammonium ions relative to protonated tertiary ammonium ions for the linear alkyl ammonium ions used in the solvent extraction studies (33,34) may be associated with the replacement of the very small hydrogen with a much larger alkyl group. That is, on going from a protonated ammonium to an alkylammonium a direction in space in which there is essentially no steric hindrance is lost. In PCP^+, however, the three rings probably create steric hindrance in all regions of space around the N^+ including that in which the hydrogen is directed. Therefore, the advantage of access to the N^+ obtained for protonated linear alky tertiary ammonium ions is not possible with PCP^+. Further work is needed to resolve these apparent anomalies.

Potentiometric precipitation titrations of PCP^+ with TPB were carried out using the DNNS-based PCP electrode as the indicator electrode. The results of titrations of PCP^+ at two different concentrations, shown in TableIII, indicate the feasibility of the titrimetric PCP method. These data show that despite the larger break in titration curve observed at the higher concentration, the accuracy and precision of the analysis are better at the lower concentration. Experiments are currently underway to determine why this unusual behavior is observed.

Conclusions

These initial investigations have indicated that PCP^+ can be quantitatively determined with the DNNS-based PCP^+ electrode described here. The electrode may be used for direct potentiometric analysis of PCP^+ (for example by standard addition techniques) or as an indicator electrode in a precipitation titration. The very low cost and east of operation of potentiometric instrumentation make potentiometric analysis of PCP^+ a highly desirable alternative and worthy of further investigation.

A comparison of the sensitivities of the potentiometric method and some other methods currently being used for analysis of PCP^+ is shown in Table IV. While the data from Table IV indicate that the potentiometric determination has the poorest sensitivity of the methods listed, a simple preconcentration procedure (which is done for several of the other methods) could lower the detection limit to around 20 ng/ml. Studies on human patients being treated for PCP intoxication (36,37) and on rats given single doses of PCP^+ for pharmacokinetic determination (11) show that levels in plasma urine, brain tissue, and adipose tissue remain well above 20 ng/ml for at least the first day after consumption or injection of PCP and sufficiently above 20 ng/ml for at least a week afterwards. Hence the sensitivity of the potentiometric methods if used with a preconcentration procedure should be sufficient for most clinical and pharmacological purposes.

While selectivity studies carried out thus far have shown that the PCP^+ electrode experiences essentially no interference from inorganic ions, it would be of interest to further investigate the selectivity of the electrode with respect to organic cations. Selectivity studies involving other cationic drug spieces and the metabolites of PCP (11, 35, 38) would be of particular interest.

It should be pointed out, however, that the selectivity required of an analytical drug sensor is highly dependent on the nature of the sample to be analyzed. For example, for analysis of illicit (39) or pharmaceutical preparations of PCP, selectivity with regards to other drugs is not required. The same situation prevails for pharmacokinetic studies in that only PCP would be administered to the test animal and therefore, PCP and its metabolites would be the only drugs present. Furthermore, in such studies selectivity with respect to PCP's metabolites may not even be necessary. For example, Done et al (8) pointed out that PCP's metabolites are not found in the blood, probably due to very rapid conversion to the glucuronides and excretion. Hence, interference by metabolites would not be a problem in plasma-PCP analysis. Furthermore, in as much as metabolites in the urine are present as the glucuronide complexes (8) metabolite interference would not be a problem in urine-PCP analysis either.

The important metabolites of PCP have been tentatively identified as 4-phenyl-4-piperidylcyclohexanol,N-(1-phenylcyclohexyl)-4-hydroxypiperidine, N-(1-phenyl-4-hydroxycyclohexyl)-4-hydroxypiperidine (35), 1-phenyl-1-aminocyclohexane, 1-phenyl-1-aminocyclohexanol, N-(1-phenylcyclohexyl)-2,4-dihydroxypiperdine (38), N-(1-4-hydroxyphenylcyclohexyl)-piperidine (11). Since these compounds are either hydroxylated analogs of or much smaller molecules than the parent compound, good selectivity for PCP relative to these species can be anticipated. This is because addition of hydroxyl or or decreasing the size of a molecule lowers its extractability which in turn should lower its selectivity coefficient relative to the parent molecule.

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TABLE I - Critical Response Characteristics of DNNS-based PCP^+ Electrode.

Slope (mV/log a)	59.21 ± 0.59[a]
Standard deviation (mV)[b]	0.091
Intercept (mV)	392.41 ± 1.80[c]
Lower limit of linear range (M)	$10^{-4.2}$
Detection limit (M).	$10^{-5.1}$

a Standard deviation in slopes obtained for multiple calibrations over 30 day period.

b Average of standard deviations obtained from least squares analyses of individual calibration curves.

c Standard deviation in intercept obtained for multiple calibration.

TABLE II - Selectivity Coefficients for the DNNS-based PCP^+ Electrode.

Interferants	$k_{i,j}$
Mg^{2+}	$< 10^{-4}$
Ca^{2+}	$< 10^{-4}$
H^+	$< 10^{-4}$
Na^+	$< 10^{-4}$
K^+	$< 10^{-4}$
TMA^+ [a]	1.4×10^{-4}
TEA^+ [b]	8.6×10^{-4}
TPA^+ [c]	0.031
DTA^+ [d]	0.68
TBA^+ [e]	1.7
$DoTA^+$ [f]	7.1

a Tetramethylammonium b Tetraethylammonium c Tetrapropylammonium

d Decyltrimethylammonium e Tetrabutylammonium f Dodecyltrimethylammonium

TABLE III - Results of Titrations of PCP^+ with Sodium Tetraphenyborate (TPB) at Two Concentrations.

	Vol PCP(ml)	[PCP](M)	[TPB](M)	Rel.Std.Dev.[a]	Rel.Error[a]
Analysis 1	25.0	1.00×10^{-3}	6.00×10^{-3}	0.60%	-1.01%
Analysis 2	40.0	1.00×10^{-4}	1.00×10^{-3}	0.21%	+0.55%

a Data represents average of four titrations at each concentration

TABLE IV - Comparison of Sensitivities of Various Methods for Quantiation of PCP.

Instrumentation or Methodology	Approximate Detection Limits[a]	Prior Extraction From Biological Sample	Reference
RIA[b]	0.5	No	15
GC[c]	60	Yes	19,20
GC[d]	100	Yes	18
GC-MS[e]	1[f]	Yes	35
CIMS[g]	300[f]	Yes	22
Potentiometry	2000	h	This Work

a. For cases where detection limits were not quoted, value is estimated from procedure.

b. Radioimmunoassay.

c. Gas chromatography-flame ionization detector.

d. Gas chromotography-NP detector.

e. Gas chromotography - mass spectrometry.

f. Preconcentration required to achieve this value.

g. Chemical ionization mass spectrometry (no GC separation)

h. No biological samples studies.

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