Flow-through Chloroquine Sensor and Its Applications in Pharmaceutical Analysis

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Poly(vinyl chloride) membrane electrodes that responded selectively towards the antimalarial drug chloroquine are described. The electrodes were based on the use of the lipophilic potassium tetrakis(4-chlorophenyl)borate as ion-exchanger and bis(2-ethylhexyl)adipate (BEHA), or trioctylphosphate (TOP) or dioctylphenylphosphonate (DOPP) as plasticizing solvent mediator. All electrodes produced good quality characteristics such as Nernstian- and rapid responses, and are minimally interfered with by the alkali and alkaline earth metal ions tested. The membranes were next applied to a flow-through device, enabling it to function as flow-injection analysis (FIA) detector. The performance of the sensor after undergoing the FIA optimization was further evaluated for its selectivity characteristics and lifetime. Results for the determination of chloroquine in synthetic samples that contained common tablet excipients such as glucose, starch, and cellulose, and other foreign species such as cations, citric acid or lactic acid were generally satisfactory. The sensor was also successfully used for the determination of the active ingredients in mock tablets, synthetic fluids and biological fluids. The sensor was applied for the determination of active ingredients and the dissolution profile of commercial tablets was also established.

Introduction

Chloroquine (7-chloro-4-(4-diethylamine-1-methylbutylamino)-quinoline) has been the most widely used drug for more than 50 years in the prophylaxis and treatment of malaria.[1] The drug is effective against the erythrocytic forms of all types of malaria parasites, with the exception of resistant strains of *Plasmodium falciparum*, in South America and South-East Asia.[2]

Chloroquine in pharmaceutical preparations is determined using two standard methods: the British Pharmacopoeia[3] method which involves extraction into ether of the chloroquine in sodium hydroxide and back titration of excess base with hydrochloric acid; or the U.S. Pharmacopoeia[4] method which involves extraction into chloroform followed by spectrophotometric determination at 343 nm. These methods are far from being satisfactory; the main drawbacks being lack of selectivity and need to use potentially hazardous organic solvents. Other analytical methods such as spectrophotometric,[5] chemiluminescence,[6] spectrofluorometry[7] and liquid chromatographic (LC)[1,8,9] methods have also been reported. LC methods would be mandatory for the analysis of chloroquine and its metabolites in complex matrices such as body fluids.

Potentiometric methods offer lots of promise as an analytical tool in pharmaceutical quality control due to the inherent characteristics of the technique itself; direct, simple, involves cheap instrumentation, rapid response, not affected by color nor by presence of suspended particulates. Manual batch-type[2,10] and a flow-through type[10] chloroquine selective electrodes have been reported by our group and others. However, it can be anticipated that the lifetimes of these earlier sensors, especially under continous-flow conditions, will be relatively short due to the low lipophilicity of the sensing material used (sodium tetraphenylborate). Thus, in this study, we report on the development of an improved chloroquine potentiometric sensor that uses a more lipophilic ion-exchanger, potassium tetrakis(4-chlorophenyl)borate (KTPB). Its performance when used under flow-injection analysis (FIA) conditions over relatively long periods of usage was assessed. Characteristics of the sensor, especially its selectivity in the presence of diverse foreign species were also studied. Possible analytical applications of the sensor for quality control purposes such as the determination of active ingredients and establishing dissolution profiles of commercial tablets were demonstrated.

Experimental

Reagents

All reagents were prepared from analytical grade chemicals. Eighteen megaohm water purified by Millipore Q was used throughout. Chloroquine (CQ) diphosphate, dioctylphenylphosphonate (DOPP), bis(2-ethylhexyl)adipate (BEHA) and trioctylphosphate (TOP) were purchased from Sigma Aldrich. (St. Louis, USA). Polyvinyl chloride (PVC) of high molecular mass (M_w = 233000) and potassium tetrakis(4-chlorophenyl)borate (KTPB) were purchased from Fluka (Steinheim, Switzerland). Tetrahydrofuran, glacial acetic acid, starch and lactic acid were bought from Merck (Darmstadt Germany). Ammonium acetate and citric acid were obtained from Ajax. Chloroquine tablets were purchased from a local drug store.

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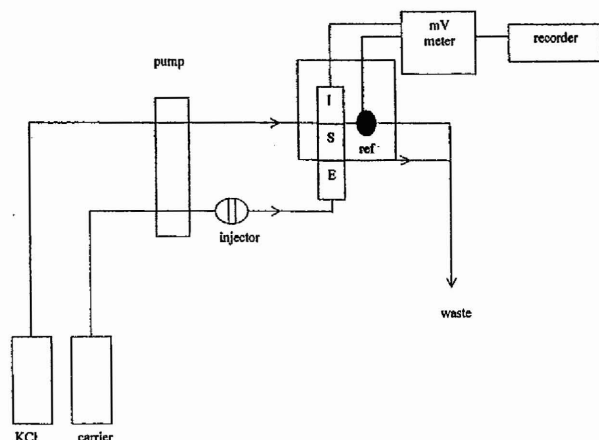E-mail: bahrud@usm.my

Fig. 1 FIA manifold used for the studies.

Table 1 Characteristics of batch-type chloroquine selective electrodes using different plasticizing solvents

Electrode No.	Plasticising solvent	Slope/ mV decade^{-1}	Detection limit/ M
1	BEHA	28.3	7.0×10^{-5}
2	TOP	32.3	5.0×10^{-7}
3	DOPP	31.2	7.0×10^{-5}

DOPP, dioctylphenylphosphonate; BEHA, bis(2-ethylhexyl)adipate; TOP, trioctylphosphate.

Table 2 Selectivity coefficients[a] of chloroquine selective electrodes using different plasticizing solvents

Interferent species, B	log K^{pot}		
	Electrode 1	Electrode 2	Electrode 3
Na$^+$	−0.47	−0.85	−0.11
K$^+$	−0.72	−0.01	−0.37
Li$^+$	−1.13	−1.90	−1.03
Mg^{2+}	−3.29	−3.06	−1.29
Ca^{2+}	−3.45	−2.77	−0.80
Glucose	−4.81	−5.88	−3.83
Quinine	1.76	1.96	1.86

a. Separate solution method.

Fabrication of chloroquine selective electrode and use

The preparation of the master membrane and its fabrication into assembled electrodes has been described elsewhere.[11] Electrochemical measurements were conducted with an Orion digital ionanalyser (Model 701A). A double junction reference electrode (Orion Model 90-02) was used. Batch electrode calibrations were effected by spiking with successive aliquots of known concentration of sample into doubly deionized water (20 mL). All samples were prepared in milliQ water. Chloroquine standard solutions were prepared fresh and each stock solution was protected from light by wrapping the flasks with aluminium foil, solutions were stored in a refrigerator when not in use. Selectivity coefficients K^{pot} were measured using the separate solution method at a cation concentration of 10^{-2} M, as described earlier.[12]

The FIA sensing membrane was prepared by dissolving 170 mg of PVC, 360 mg of plasticising solvent and 40 mg of KTPB in 5 ml THF. Four drops of the mixture were deposited on the electrode body[12] and the system was allowed to evaporate for 4 h. The membrane was conditioned for 8 h before use by immersing in 10^{-2} M chloroquine solution. Standards were prepared by dilution of 0.1 M stock solution.

FIA system and procedures

Solutions were propelled by a multi-channel peristaltic pump (Gilson Minipuls 3) through PTFE tubing (0.8 mm i.d.). Samples were injected into a Rheodyne Type Teflon rotary injection valve. A sampling volume and carrier flow-rate of 50 μl and 2.0 ml min^{-1}, respectively, were used. A flow-through cell of the wall jet design with an in-built reference electrode (Model FIP-3), purchased from Chemflow Devices, Thornbury, Australia, was used. Details of this flow cell have been described elsewhere.[12] The sample was injected into acetate buffer pH 6.5 (0.3 M) as the carrier stream. Potassium chloride (0.1 M) was used as reference solution to maintain baseline stability. The flow injection manifold is shown schematically in Fig. 1.

Test solutions

The sensor was evaluated for its performance in several mixtures. Their compositions were as follows.

Artificial tablet: mixture containing glucose (50% w/v), starch (25% w/v) and cellulose (20% w/v). Artificial biological fluid: mixture containing citric acid (25 mM), lactic acid (25 mM), ammonium chloride (1 mM), sodium chloride (140.2 mM), calcium chloride (1.1 mM), magnesium chloride (0.6 mM) and potassium chloride (4.1 mM). Artificial serum electrolyte: prepared by mixing NaCl (140.2 mM), CaCl$_2$ (1.1 mM), MgCl$_2$ (0.6 mM) and KCl (4.1 mM).

Analysis of chloroquine tablets

Five tablets were finely ground in a mortar. A portion of the powder was accurately weighed and dissolved in doubly deionized water in volumetric flasks and was placed inside an ultrasonic bath to ensure complete dissolution.

Dissolution profile

Sugar coated chloroquine tablets either containing 250 mg or 300 mg active ingredients were placed into the paddle apparatus (Pharmatest Dissolution Tester PTW II (Hainburg, Germany)) as described in US Pharmacopoeia.[4] The paddle speed was set at 100 rpm. A 900-ml volume of doubly deionized water was used as the dissolution medium. The temperature was maintained at $37 \pm 0.5°C$. Then, 25-ml of aliquots were withdrawn at every 2 min intervals for the initial 12 min and every 30 min thereafter, and analyzed using the FIA approach. All solutions were stored in wrapped bottles at 4°C prior to the FIA injection.

Results and Discussion

Batch electrodes

Chloroquine selective electrodes were obtained when used with the plasticising solvents studied. All electrodes exhibited Nernstian responses and detection limits typical of such a technique (Table 1). The selectivity values of the electrodes were evaluated against several common cations, glucose and quinine. With the exception of quinine, all these electrodes were minimally interfered with by the foreign ions studied (Table 2). Although quinine poses a source of interference, the two are not normally present together in pharmaceutical preparations.
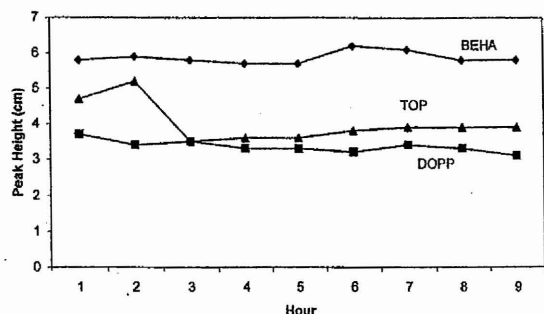
Fig. 2   Lifetime studies of chloroquine sensors in FIA mode.

FIA studies

The FIA conditions which had been used for the studies were as follows: injected volume, 50 µL; carrier stream, 0.3 M acetate buffer (pH 6.5); flow-rate, 1.5 mL min^{-1}. These conditions were chosen as compromises between peak sensitivity and sample throughput. The calibration curve was linear over 0.01 mM to 100 mM with a correlation coefficient of 0.9944. The detection limit (signal over noise, 3:1) was estimated to be 0.02 mM.

The lifetimes of the sensors were studied by continuously pumping for 9 h and repeatedly injecting 1 mM chloroquine standard solutions at every hour. Sensors containing BEHA as plasticizing solvent exhibited superior lifetime values and there was no noticeable reduction in peak heights over the period studied (Fig. 2). Indeed, it was noted that this sensor remain operational over 3 weeks of daily use. In contrast, sensors containing TOP showed a marked reduction in peak height at the 3rd hour on continous use. Thus, the sensor with BEHA was used for further studies.

The performance of the sensor in the presence of tablet excipients such as sugars and binders was also studied. Results from the determination of 1 mM chloroquine in such mixtures indicate the satisfactory performance of the sensor (Table 3). The sensor was further evaluated for its ability to determine a fixed concentration of chloroquine (1 mM) in the presence of organic acids and metal ions, all at physiological levels. Results for the determination of chloroquine in all tested samples, with the exception of sodium chloride, are satisfactory (Table 4). Chloroquine at three different concentrations were spiked to synthetic tablet solution, synthetic biological fluid and artificial serum and amounts were determined by the FIA method. Excellent results were obtained from synthetic tablet, but less satisfactory results for biological fluids and for synthetic serum were found, especially at lower concentrations of the chloroquine (Table 5). On the whole, the results suggest that, although plasticized PVC membrane is a good sensor for tablet analysis, it is not suitable for clinical samples. Furthermore, the membrane surface was found to be cracked and swelled when it was continuously exposed to protein and urine for 8 h (scanning electron micrographs not shown).

The proposed FIA method was applied towards the determination of chloroquine in two types of antimalarial tablets. There was closer agreement of the results obtained by the FIA method when compared to the official US Pharmacopoeia[4] method and against the manufacturer's values (Table 6).

Dissolution profiles of two types of chloroquine tablets, determined using the FIA method are shown in Fig. 3. It can be readily seen that both tablets comply to the quality control requirements which stipulates that 75% drug must be dissolved

Table 3   Determination of 1 mM chloroquine in a few tablet excipients

Foreign species	Concentration, % w/v	Error, %
Glucose	50.0	1.9 (0.10)
Starch	25.0	4.0 (0.17)
Cellulose	20.0	4.0 (0.10)

Values in brackets denote standard deviations, $n = 4$.

Table 4   Determination of 1 mM chloroquine in several species at their physiological concentrations

Foreign species	Concentration/mM	Error, %
Citric acid	25.0	−3.30 (0.08)
Lactic acid	25.0	4.00 (0.17)
NH_4Cl	1.0	0.10 (0.14)
NaCl	140.2	12.3 (0.10)
KCl	4.1	1.5 (0.01)
$CaCl_2$	1.1	5.7 (0.05)
$MgCl_2$	0.6	7.3 (0.05)

Values in brackets denote standard deviations, $n = 4$.

Table 5   Determination of 1 mM chloroquine in synthetic mixtures and commercial tablets

Sample	CQ spiked/mM	Mean recovery, %
Synthetic tablet	5.0	96.8 (0.10)
	1.0	96.5 (0.10)
	0.2	100 (0.10)
Synthetic serum electrolyte	5.0	95.4 (0.10)
	1.0	87.0 (0.1)
	0.2	86.0 (0.10)
Synthetic biological fluid	5.0	90.0 (0.10)
	1.0	76.0 (0.10)
	0.2	68.0 (0.10)

Values in brackets denote standard deviations, $n = 4$.

Table 6   Results for the determination of chloroquine in commercial tablets

Sample	Manufacturer's claim/mg	Proposed FIA/ mg	USP method/ mg
Tablet 1	300	295 (0.1)	250 (0.1)
Tablet 2	250	236 (0.1)	215 (0.1)

Values in brackets denote standard deviations, $n = 3$.

within 45 min. The sensor possessed adequate sensitivity and selectivity, enabling it to be used for the determination of chloroquine in the dissolution vessel.

A PVC chloroquine-selective membrane containing bis(2-ethylhexyl)adipate (BEHA) and the lipophilic ion-exchanger KTPB was obtained. When operated under FIA conditions, the sensor exhibited adequate selectivity and sensitivity to be applied in the determination of chloroquine in drug dissolution and tablet testing. The method is rapid (sample throughput 90 samples per hour), simple and direct (does not require liquid–liquid extraction) and shows promise as a useful quality control tool in pharmaceutical testing.
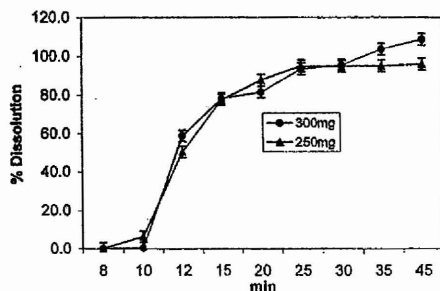
Fig. 3 Dissolution profiles of commercial chloroquine tablets.

Acknowledgements

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