SOLID CONTACT BIOSENSOR BASED ON MAN-TAILORED POLYMERS FOR ACETYLCHOLINE DETECTION: APPLICATION TO ACETYLCHOLINESTERASE ASSAY

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A solid contact biosensor for Acetylcholine (ACh) based on host-guest interactions and potentiometric transduction has been designed and characterized. The biomimetic man-tailored host was synthesized using methacrylic acid as a functional monomer, ethylene glycol di-methacrylate as a crosslinker in the presence of benzoyl peroxide as an initiator. The imprinted beads were dispersed in 2-nitrophenyloctyl ether and entrapped in a poly(vinyl chloride) matrix. Slopes and detection limits are 55.2-59.6 mV decade^{-1} and 0.65-1.31 μg mL^{-1}, respectively. Significantly, improved accuracy, precision, good reproducibility, long-term stability, selectivity and sensitivity were offered by these simple and cost-effective potentiometric biosensors. A tubular version was further developed and coupled to a flow injection system for acetylcholine determination. This simple and inexpensive flow injection analysis manifold, with a good potentiometric detector, enabled the analysis of ~30 samples h^{-1} without requiring pretreatment procedures. An average recovery of 98.3 % and a mean standard deviation of 1.1% were obtained. The sensors were used to follow up the decrease of a fixed concentration of ACh$^+$ substrate as a function of acetylcholinesterase (AChE) activity under optimized conditions of pH and temperature. A linear relationship between the hydrolysis initial rate of ACh$^+$ substrate and enzyme activity hold 0.01- 5.0 IU L^{-1} of AChE enzyme.

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Introduction

Acetylcholine (ACh) serves an important function in the cholinergic system, where it acts as a neurotransmitter on cholinergic synapses.[1] The pharmaceutical preparation of ACh has many therapeutic utilities.[2] On the other hand, deficiency of ACh due to cholineacetyltransferase enzyme inhibition causes a disturbance in the transmission of nerve impulses, paralysis, and death.[3] Assessment of ACh is a challenging analytical problem because it is not UV-absorbing, fluorescent, electroactive or derivatize easily. Therefore, bioassays,[4] radiochemical methods,[5] liquid chromatography (LC) with enzymatic reactions[6-9] and LC with mass spectrometric detection[10-13] have often been employed, despite their tedious procedures. However, the aforementioned methods have several disadvantages such as long analysis time, high cost, and specialized personnel with laboratory facilities. On the other hand, the electroanalytical techniques provide many advantages such as simple instrumentation and short analysis time. Uni-, bi-, and tri-enzyme/mediators biosensors including chemi-luminometric,[14] amperometric,[15-17] conductometric[18] and voltammetric[19] methods have been used for monitoring AChs.

Potentiometric sensors are an important class of electrochemical sensors, which detect the relationship between the activity of analyte species and the potential response of the two-electrode system. Compared with other analytical techniques, ion-selective electrodes (ISEs) have some unique characteristics, such as small size, ease of operation, portability and low cost. For potentiometric determination of ACh, few potentiometric membrane sensors have been developed.[20-24] Some of these sensors involve the use of acetylcholine ion-pair complexes as electro-active materials that exhibit poor selectivity, limited range of linear response and long response time[20-22] and others involve macrocycle carriers.[23,24]

Molecular imprinting is one of the most promising approaches to achieving precise molecular recognition. The challenge of synthesizing man-made molecules which are capable of molecular recognition has drawn special attention to electrochemical sensors.[25] The sensing and transduction principles combined with the imprinting approach are used to make the imprinting process feasible thus giving us the detailed information about the recognition phenomenon occurring on the imprinting interface.[26]

Transducers based on potentiometric transduction comprise one of the most exciting areas of electrochemical analysis. Their appealing features, such as selectivity, sensitivity, and reproducibility, have drawn attention for the past couple of decades.[27-31] In this direction, the immobilization of biomolecules on the electrode surface for molecular recognition is a reasonable choice, thus gaining a great importance in the field of electrochemical sensors. The use of synthetic materials that imitate recognition characteristics of biological materials has been explored.[31,32] Particularly, molecularly imprinted polymers (MIPs) can be thought of as viable alternates to replace natural receptors. Bulk polymerization in the presence of a template is just one among many frequently used procedures for the fabrication of MIPs.[33-37] First, the preformed complex of the functional

monomer and template is copolymerized with an excess of the cross-linking agent in a porogenic solvent. This step results in a solid matrix of the highly cross-linked polymer. After template removal, molecular cavities featuring recognition sites are formed. These cavities are suitable for hosting the template compound used as the analyte in this step.

In this study, we have investigated the preparation of MIPs, new man-tailored hosts for Ach, based on imprinting technology. The polymers could be regarded as an artificial receptor to recognize ACh by shape recognition ability, non-covalent interactions as well as induced polarization between MIP and ACh. The newly synthesized sensors have been employed for rapid and sensitive measurements of AChE enzyme activities.

Experimental

All potentiometric measurements were made at 25±0.1 ^{0}C with a Cole-Parmer pH/mV meter (USA model 59003-05). The assembly of the potentiometric cell was constructed as follows: Copper base | graphite | ACh selective membrane | buffered sample solution (PBS, 0.01 M, pH 7) ‖ electrolyte solution, KCl | AgCl(s) | Ag. The reference electrode was a Sentek, Ag/AgCl double junction reference electrode (UK model R2/2MM) filled with 4.0 M KNO_3 in the outer compartment. The selective electrode was prepared and designed to be suitable for static and hydrodynamic measurements. The design had no internal reference solution and epoxy-graphite was used as a solid contact. The pH values of the solutions were controlled by means of a combination glass pH electrode (Schott blue line 25, Germany).

The flow injection (FI) manifold consisted of a two–channel Ismatech Ms–REGLO model peristaltic pump, polyethylene tubing (0.71 mm internal diameter) and an Omnifit injection valve (Omnifit, Cambridge, UK) with a loop sample of 100 µL volume. An Orion (Cambridge, MA, USA) model 720/SA pH/mV meter connected to a PC through the interface ADC 16 (Pico Tech, UK) and Pico Log for windows (version 5.07) software were used for recording the potential signals.

All chemicals were of analytical grade and de-ionized water (conductivity <0.1 µS cm^{-1}) was employed. Acetylcholine chloride (ACh), Choline chloride (Ch), Creatinine (Creat), Potassium tetrakis (4-chlorophenyl) borate (KTpClPB$^-$), tri dodecyl ammonium chloride (TDMAC), 2-nitrophenyloctyl ether (*o*,NPOE), poly (vinyl chloride) of high molecular weight (PVC), and Ethylene glycol dimethacrylate (EGDMA) were purchased from Sigma Chemicals Co. (St. Louis, MO, USA). Methacrylic acid (MAA), Benzoyl peroxide (BPO), methanol and tetrahydrofuran (THF) were purchased from Fluka (Ronkonoma, NY). Acetylcholinesterase (type VI-S) from Electrophorus electricus (Electriceel, EC 3.1.1.7, 288 U mg^{-1} solid) was obtained from Sigma-Aldrich (Munich, Germany).

Polymer synthesis

Molecularly imprinted polymers with ACh were prepared by using acetylcholine (ACh, 1 mmol) as a template, MAA (4 mmol) as a functional monomer, EGDMA (20 mmol) as a cross-linking agent and acetonitrile (15 mL) as a porogenic solvent. The template-monomer mixture and solvent were transferred to a test tube and BPO (80 mg) as an initiator was added. The dissolved oxygen in the mixture was degassed by bubbling N_2 for 10 min. The tube was sealed and heated in a block heater at 70 ^{o}C for 5 h. The control blank polymers (NIPs) were prepared using an identical procedure but in the absence of the template. The polymers were obtained as brittle solids which were broken up, grounded in a mortar. The grounded polymers were washed to remove ACh with methanol/ acetic acid (9:1 v/v) to eliminate interfering compounds arising from the synthesis (template and un-reacted monomers). All polymers (MIP/MAA and NIP/MAA) were let to dry at ambient temperature, before their use as potentiometric transducers.

Binding Experiments

Binding experiments were carried out by introducing 20.0 mg of MIP and NIP washed particles in contact with 10.0 mL ACh^+ aqueous solutions ranging 10-70 µg mL^{-1}. The solutions were incubated overnight at static equilibrium at room temperature and the solid phase was then separated by centrifugation (3000 rpm, 15 min.). Free ACh^+ concentrations in the supernatant were measured by HPLC with a refractive index detector using calibration graph with ACh^+ standard solutions.[38] The amounts of ACh^+ bound to the polymers were calculated by subtracting the concentration of free ACh^+ from the initial ACh^+ concentration. The maximum binding capacity and dissociation constant for all synthesized polymers were calculated using Scatchard equation.

ISE membranes and electrodes measurements

The ACh-selective membranes for solid contact ion selective electrodes (ISEs) contained MIP/MAA [ISE I] or NIP/MAA [ISE II] (5.2 wt. %, 30 mg), *o*,NPOE (61.4 wt .%, 350 mg), and PVC (33.3 wt .%, 190 mg).The membranes were prepared by dissolving the components (in total, 570 mg) in THF (3 mL). The membrane solutions were cast into a conductive supports of conventional or tubular shapes and left to dry overnight for evaporating and yielding transparent membranes.

The sensors were conditioned by soaking in 1.0x10^{-3} M of ACh^+ aqueous solution for 12 h. The pH of the test solution was maintained at 7.0 by the addition of different aliquots from standard ACh^+ solution in 25 mL of 0.01 M PBS solutions. The potential of the test solutions was measured at different concentrations of ACh^+ in the range 1.0×10^{-7} to 1×10^{-2} M. The EMF was plotted as a function of the logarithm of ACh^+ concentration.

Flow injection set up

Transducers for flow injection analysis were prepared by mixing 30 mg of the sensing polymer, 350 mg of the plasticizer (*o*-NPOE), 190 mg PVC and 5.0 mg KpClTPB and dissolved in ~ 3 mL THF. Successive aliquots (200 µL) of the membrane were placed into a conductive support of graphite and epoxy resin, of conventional or tubular shape. This operation was repeated until a membrane with a thickness of approximately 0.1 mm was formed. The sensor was

conditioned by soaking in 1.0×10^{-3} M of ACh^+ aqueous solution for 12 h and was stored in the same solution when not in use. The sensor was placed in a beaker where a double junction Ag/AgCl reference electrode was placed downstream from the detector just before the solution went to the waste. A carrier stream containing 1.0×10^{-2} M PBS solution of pH 7.0 was pumped at a constant flow rate of 3.0 mL min^{-1}. To avoid slight pulsation originating from the peristaltic pump, grounding connection was made for flow system.

Potentiometric assessment of AChE activity

A volume of 45.0 mL of the pH 7.0 PBS solution was transferred into the thermostated vessel. The sensor was immersed in the solution in conjunction with a double junction Ag/AgCl reference electrode. After potential stabilization, a 2.5 mL of 10^{-2} M of ACh^+ working solutions was injected. When the potential stabilized again, 100 µL aliquots containing 0.01-5.0 IU L^{-1} of AChE enzyme was added. The potential kinetic curve was left to develop, and the maximum initial rate of potential change *(ΔE/Δt)* was graphically obtained using the rate portion of the curve. The initial rate was plotted as a function of the enzyme activity and the calibration curve obtained was then used for subsequent measurements of unknown enzyme activity. A blank experiment was carried out under similar conditions in the absence of the enzyme.

Results and discussion

In this study, our aim was to establish a simple, selective and sensitive analytical system based on MIPs for recognizing acetylcholine neurotransmitter. For this purpose, we proposed an electrochemical sensor utilizing the potentiometric determination method of ACh to the MIPs by electrochemical reaction. A schematic illustration of the molecular imprinting process is shown in Figure 1.

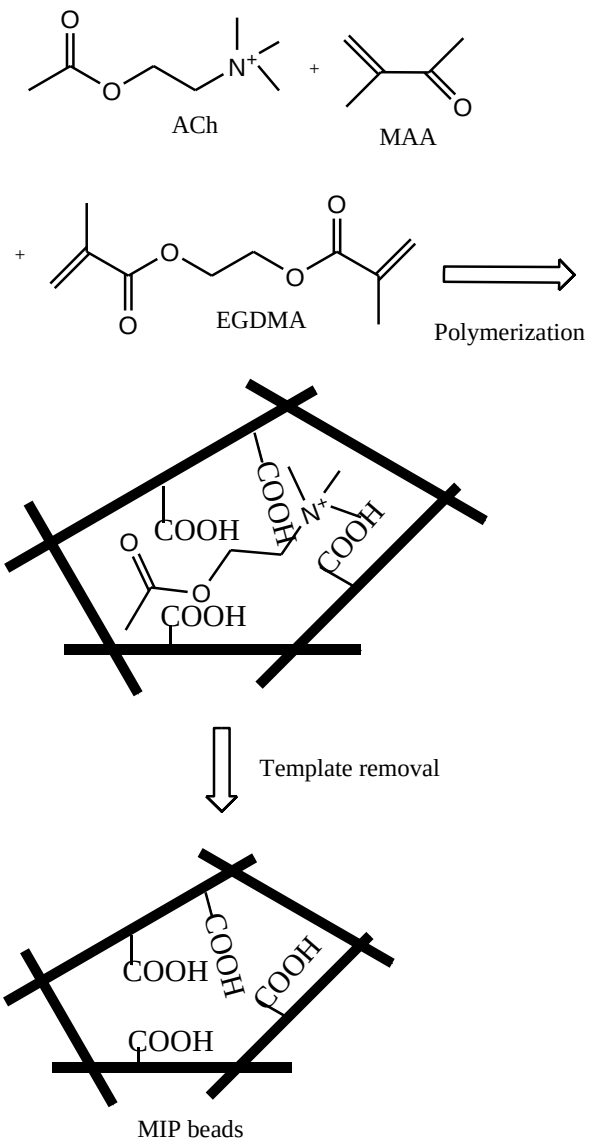

Figure 1. A schematic protocol for the molecular imprinting process.

Characterization of the MIP beads

The morphology for all prepared polymer beads was investigated and presented in the cross-sectional SEM and TEM images for the MIP/MAA and NIP/MAA (Figure 2). The non-imprinted polymers had more smooth and uniform shape than the imprinted polymers which had an irregular, rough morphology with small cavities. The regular structure of the non-imprinted polymer was due to the absence of specific binding sites in the polymers. The cavities in the MIPs were probably attributed to the imprinting effect or the introduction of ACh in the polymerization process. The specific surface area and pore volume impacted significantly on the efficiency of adsorption. The homogeneous and dense morphological structure is shown in the figure indicated that the imprinted process achieved a more highly cross-linked and porous structure. It thus provided a guarantee of a sufficient extraction performance of the MIP/MAA for ACh. Polymer surface area and porosity measurements were also carried out. BET and Langmuir surface areas for polymers were calculated and presented in Table 1. Binding experiments were performed by incubating fixed amounts of all MIPs and NIPs beads with different concentrations of ACh until equilibrium as reached and the free ACh concentrations were determined using HPLC method.[38] The resulting binding capacity of MIPs was calculated according to eqn. (1)

$$Q = \frac{\mu\, mol(ACh\ bound)}{g(MIP)} = \frac{(C_i - C_f)V_s \times 1000}{M_{MIP}} \qquad (1)$$

where

Q is the binding capacity of MIPs or NIPs (µmol g^{-1}),

C_i the initial ACh concentration (µmol mL^{-1}),

C_f the final ACh concentration (µmol mL^{-1}),

V_s the volume of solution tested (mL) and

M_{MIP} the mass of dried polymer (mg).

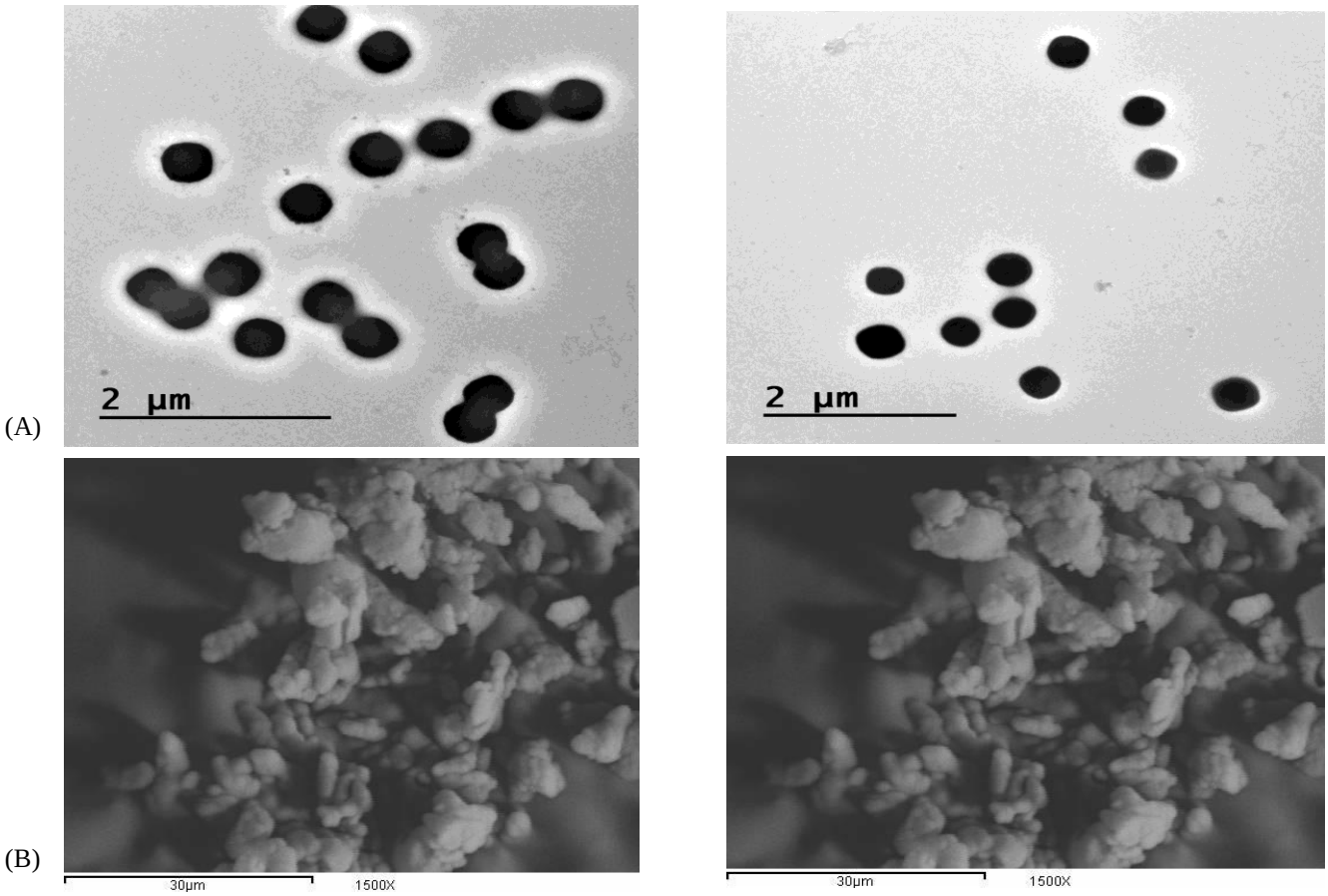

Figure 2. (A) TEM micrograph of MIP/MAA and NIP/MAA, (B) SEM micrographs of MIP/MAA and NIP/MAA under 1500 magnification.

Table 1. General characteristic of some potentiometric acetylcholine membrane sensors

Ionophore	Slope (mV decade^{-1})	Linear range (M)	pH range	Detection limit (M)	Interference	Ref.
Acetylcholine dipicrylaminate	54.4	5.0×10^{-5}-1.0×10^{-2}	Not reported	3.0×10^{-5}	Choline (-1.35); Butyrylcholine (-1.02); Dopamine (-2.21); Tyrosine (-2.39); Aminobutyric acid (-2.82); Carbachol (-1.43); Amphetamine (-1.06); K^+ (-2.65); NH_4^+(-3.39)	20
Cucurbit[6]uril derivative	49.1	1.0×10^{-6}-1.0×10^{-3}	7.2	9.7×10^{-7}	Choline (-2.51); NH_4^+(-1.96); NMe_4^+(-1.93); NEt_4^+(-1.93); K^+(-1.57); Na^+(-1.83); Dopamine(-1.51); Ascorbic acid (-2.45)	21
Dioctyloctadecylamine	41.4	3.0×10^{-6}-	8.0	2.0×10^{-6}		22
N,N-didecylaminometh-ylbenzene	52.9	4.5×10^{-5} 1.0×10^{-5}-8.0×10^{-3}	8.0	5.0×10^{-6}	Not reported	
Tetrakis(*p*-chlorophenyl)-borate			6.0	1.0×10^{-5} 1.0×10^{-5}	Not reported	23
Dibenzo-18-crown-6	Not reported	Not reported	6.0	1.7×10^{-5}		
Calix[6]arene hexaester			6.0			
β-Cyclodextrin derivative	55.6	1.0×10^{-5}-1.0×10^{-2}	3.0-10	2.7×10^{-6}	Choline (-2.50); NH_4^+(-3.80); Citrate (-2.53); Li^+(-3.76); K^+(-3.89); Caffeine (-2.30)	24
MIP/MAA+TPB^-	55.2	1.0×10^{-5}-1.0×10^{-2}	3.0 – 9	4.5×10^{-6}	Glutamine (-1.52); Codeine (-1.37); Ephedrine (-1.45); Morphine (-1.50); Caffeine (-1.5); Quinine (-1.57); Histidine (-1.60); Choline (-1.62); Cysteine (-1.70); K^+ (-2.51); Ca^{2+} (-2.54); Mg^{2+} (-2.82); Ba^{2+}(-2.93).	This work

Table 2. BET and Langmuir surface areas.

Polymer	Surface area, $m^2\ g^{-1}$ (*r*) *	
	BET	**Langmuir**
MIP/MAA	3.7±0.3 (0.997)	4.4 ±0.5 (0.996)
NIP/MAA	2.5±0.2 (0.998)	2.8±0.3 (0.997)

*r = coefficient of correlation

The adsorption data showed that the binding capacity of MIPs and NIPs increased with the increasing of the initial concentration of ACh, reaching to saturation at higher concentrations. Under the same conditions, the adsorption capacity data of MIPs were always clearly higher than those of NIPs. This indicates that the ability of MIPs to bind ACh is better than that of NIPs probably due to increased number of binding sites in MIPs thus increasing its sensing properties over NIPs.[39] The binding data were further processed with Scatchard analysis[40] using Eqn. (2).

$$\frac{Q}{C_f} = \frac{Q_{max} - Q}{K_d} \qquad (2)$$

where

Q is the binding capacity,
C_f is the free analytical concentration at equilibrium (μmol mL^{-1}),
Q_{max} is the maximum apparent binding capacity and
K_d is the dissociation constant at the binding site.

The equilibrium dissociation constant is calculated from the slopes and the apparent maximum number of binding sites from the y-intercepts in the linear plot of Q/C_f versus Q. Scatchard plots of both MIPs and NIPs consisted of two distinct straight lines inferring the existence of high and low-affinity populations of binding sites (Figure 3).[41] The K_{d1} and Q_{max1} were 54.05 μM and 88.22 μmol g^{-1}, for the high-affinity binding sites of MIP/MAA beads. The K_{d2} and Q_{max2} 595.23 μM and 374.39 μmol g^{-1} for the low-affinity binding sites of MIP/MAA beads. The K_{d1} and Q_{max1} were 144.92 μM and 80.34 μmol g^{-1}, for the high-affinity binding sites of NIP/MAA beads. The K_{d2} and Q_{max2} 442.47 μM and 175.21 μmol g^{-1} for the low-affinity binding sites of NIP/MAA beads. By the evaluation of these data, it can be concluded that the adsorption of ACh onto NIPs is based on non-specific interactions because K_d and Q_{max} quantities of NIPs are less than the values calculated for MIPs.

Sensors characteristics

The synthesized MIP's were incorporated into the PVC membrane and were tested as sensing materials in the proposed potentiometric sensors. The potential response obtained with the sensors prepared with MIP/MAA and NIP/MAA membrane was given in Figure 4. As seen from the figure, the sensors exhibited linear potentiometric response towards ACh^+ ions over a range from 3.0 x 10^{-5} to 1.0 x 10^{-4} M, and detection limits of 1.31 and 11.7 μg mL^{-1}, for sensors based on MIP/MAA and NIP/MAA polymers, respectively.

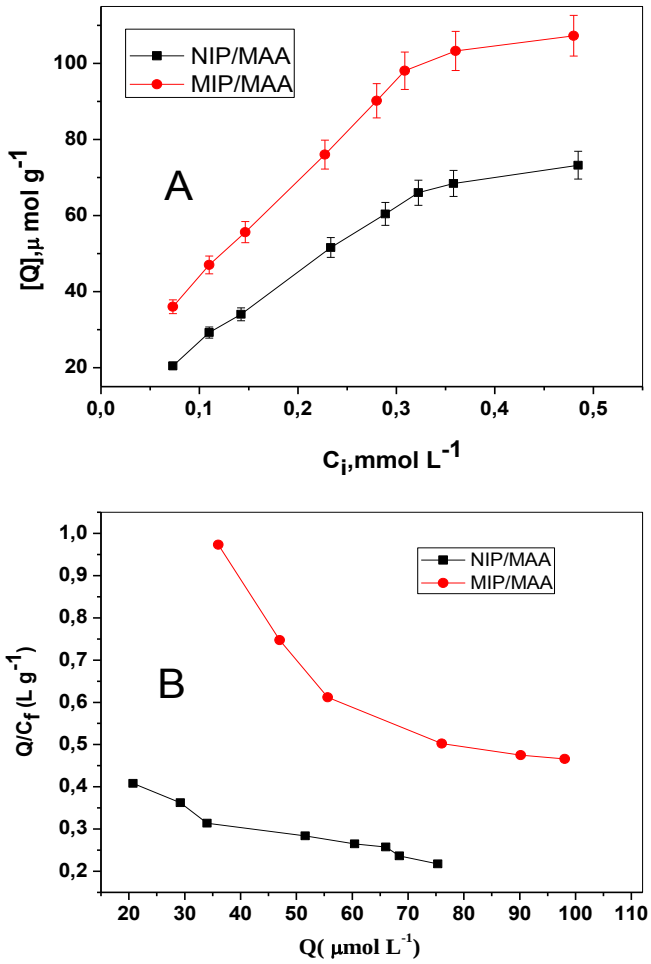

Figure 3. Binding isotherm (A) and Scatchard plot (B) for MIPs and NIPs (inset) .*Q* is the amount of ACh bound to 20 mg of polymer; *t*=25◦C; *V*= 10.00 mL.

All sensors exhibit near-Nernstian slopes of 59.6±0.9 (r^2=0.996) and 39.5±0.7(r^2=0.998) mV $decade^{-1}$, respectively.

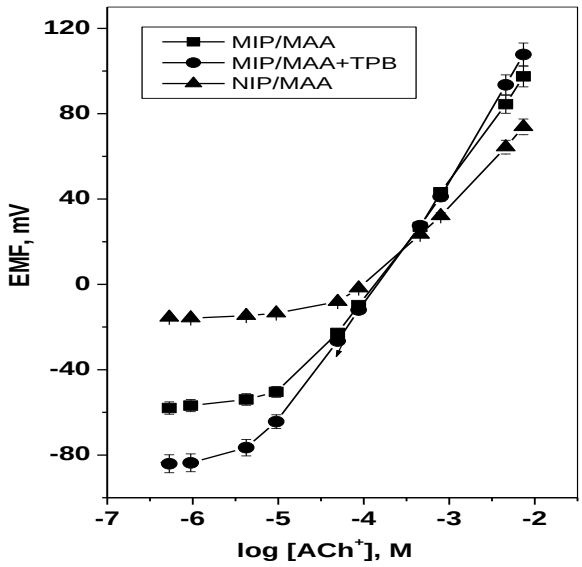

Figure 4. Potentiometric plot of acetylcholine membrane sensors in 1.0 x10^{-2} M PBS solution (pH 7.0).

Table 3. Potentiometric response characteristics of ACh membrane sensors

Parameter	MIP/MAA	NIP/MAA	MIP/MAA + TPB^-	MIP/MAA+$TDMA^+$
Slope[a] mV $decade^{-1}$	59.6 ± 0.9	39.5 ± 0.7	55.2 ± 0.8	15±0.8
Correlation coefficient (r^2)	0.996	0.998	0.999	0.998
Linear range, M	$3.0x10^{-5}$-1.0 x 10^{-2}	$1.0x10^{-4}$-1.0 x 10^{-2}	$1.0x10^{-5}$-1.0 x 10^{-2}	$5.0x10^{-3}$-1.0 x 10^{-2}
Detection limit, µg mL^{-1}	1.31	11.7	0.65	627.8
Working range, pH	3 - 9	3 - 9	3 - 9	-
Response time, s	<10	<10	<10	-
Lifespan, week	12	12	12	-
Precision Cv_w (%)	1.2	0.7	0.9	-
Between-day variability Cv_b (%)	0.8	1.3	1.1	-

[a] Average of six measurements

The effect of an addition of lipophilic salts or ionic additives upon the characteristics of conventional potentiometric sensors was also studied. A comparison between the membranes without ionic additive and that containing anionic additive (i.e. 30 mol % $TpClPB^-$ relative to the sensing material) showed that incorporation of $TpClPB^-$ in ACh sensors exhibited a slope of 55.2±0.8 mV $decade^{-1}$ with a linear dynamic range extended from 1.0 x 10^{-5} to 1.0 x 10^{-2} M and a detection limit of 0.65 µg mL^{-1}. The incorporation of cationic site additive (i.e. 30 mol % $TDMA^+$ relative to the ionophore) dramatically deteriorated the potentiometric response characteristics showing a slope of 15.0 ± 0.8 mV $decade^{-1}$, detection limit of 627.8 µg mL^{-1} and linear response range begins from 5.0 x 10^{-3} M.

The stability of these transducers was monitored continuously at 1.0 × 10^{-4} M of ACh^+ solution and evaluated for a period of 5 h, the potential drift noticed was ≤ 0.6 mV h^{-1}. The repeatability of the potential reading for the sensors was also examined by subsequent measurements in 5.0×10^{-4} M of ACh^+ solution immediately after measuring the first set of the solution at 1.0×10^{-4} M of ACh^+ solution. The standard deviations of measuring *emf* for 5 replicate measurements obtained are 0.8 mV for the solution of 1.0x10^{-4} M and 0.7 mV for the solution of 5.0x10^{-4} M. This means that the repeatability of potential response of the electrode is acceptable.

The time required to achieve a steady potential response within ±1.5 mV using the proposed sensors in 10^{-6} to 10^{-4} M ACh^+ solutions with a rapid 10-fold increase in concentration was <10 s. Replicate calibrations for each sensor indicated low potential drift, long-term stability, and negligible change in the response of the sensors. The sensors were conditioned in 10^{-3} M ACh^+ solution of pH 7.0 and stored in the same solution when they are not in use. With all sensors examined, the detection limits, response times, linear ranges and calibration slopes were reproducible to within ±3 % of their original values over a period of at least 12 weeks.

The influence of the pH on the potentiometric response of the proposed sensors was examined over a pH range of 2-10 for ACh^+ standard solutions of 1.0x10^{-4} and 1.0x10^{-3} M. The pH of the solution was adjusted with either hydrochloric acid and/ or sodium hydroxide solutions. The pH plot showed that the variation of solution pH over the range 3-9 has no significant effect on the potentiometric response for both MIP and NIP membrane based sensors. For pH<3 the sensor responses were severely influenced by H_3O^+.

Sensors selectivity

One of the most important parameters characterizing the analytical properties of each new transducer is its selectivity over many common ions. Therefore, the potentiometric selectivity coefficients of the sensors towards different organic and cationic inorganic species commonly associated in biological samples with ACh^+ were evaluated using the fixed solution method (FSM).[42] Potentiometric selectivity of the sensors was related to the preferential interaction of the mimic receptors with ACh^+ in 0.01 M PBS solution of pH 7.0 over many used common interferents. The selectivity pattern for the sensors was shown in Table 2.

Table 4. Selectivity coefficients ($K^{pot}_{ACh^+,j}$) of acetylcholine membrane based sensors.

Interferents, I	MIP /MAA	NIP/MAA	MIP/MAA+ TPB^-
Acetylcholine	0	0	0
Choline	-1.91	-1.74	-1.62
Codeine	-1.76	-1.45	-1.37
Morphine	- 1.80	-1.28	-1.50
Ephedrine	-1.67	- 1.40	-1.45
Caffeine	-1.57	- 1.29	- 1.50
Histidine	-2.03	- 1.85	-1.60
Glutamine	-1.85	- 1.54	-1.25
Quinine	-2.07	- 1.34	-1.57
Cysteine	- 2.12	-1.55	-1.70
Mg^{2+}	-2.96	-2.70	-2.82
Ca^{2+}	-2.75	-2.32	-2.54
Ba^{2+}	-3.12	-3.01	-2.93
K^+	-3.21	-3.00	-2.51

For MIP/MAA and NIP/MAA membrane based sensors, the selectivity order was in the order: ACh> Caffeine > Ephedrine > Codeine> Morphine> Choline> Glutamine > Quinine> Cysteine> Histidine > Ca^{2+} > Mg^{2+} > Ba^{2+} and ACh > Morphine > Caffeine > Quinine > Codeine > Ephedrine > Glutamine = Cysteine > Choline > Histidine > Ca^{2+}> Mg^{2+}> K^+ = Ba^{2+}, respectively. For MIP/MAA+TPB^- membrane-based sensor, the selectivity order was in the order: ACh > Glutamine > Codeine > Ephedrine > Morphine = Caffeine > Quinine > Histidine > Choline > Cysteine > K^+ > Ca^{2+} > Mg^{2+} > Ba^{2+}. Glucose, maltose, starch, talc, urea, and tween-80 at concentration level as high as 1000-fold excess over ACh^+ have a negligible effect on the accuracy of the results. Overall, the interfering effect of doubly charged cations was lower than that of singly charged ones. Compounds of positively

charged nitrogen atoms presented more similarities to the chemical structure of the main ion and their logarithm selectivity coefficients were always below -1.This suggested that only small interference from other quaternary ammonium salts or compounds of positively charged nitrogen atoms was expected and binding to ACh^+ was the most favorable process.

Potentiometric MIP sensor in an FIA setup

A tubular-type detector incorporating an MIP/MAA+TPB^- based membrane sensor was prepared and used under the hydrodynamic mode of operation for continuous monitoring of ACh. A linear relationship between ACh^+ concentrations and FIA signals was obtained over a concentration range from 1.0 x 10^{-4} to 1.0 x 10^{-2} M using 0.01 M PBS solution, pH 7 (Figure 5). The slope of the calibration plot was near-Nernstian (50.3 ± 1.9 mV $decade^{-1}$). The slightly lower sensitivity of the transducer in FI analysis may be attributed to several factors such as mass transport rate, sample dispersion and effect of contact time between sample and electrode. The limit of detection was 7.3 ± 0.3 µg mL^{-1} and the sampling frequency was 30-32 samples $hour^{-1}$.

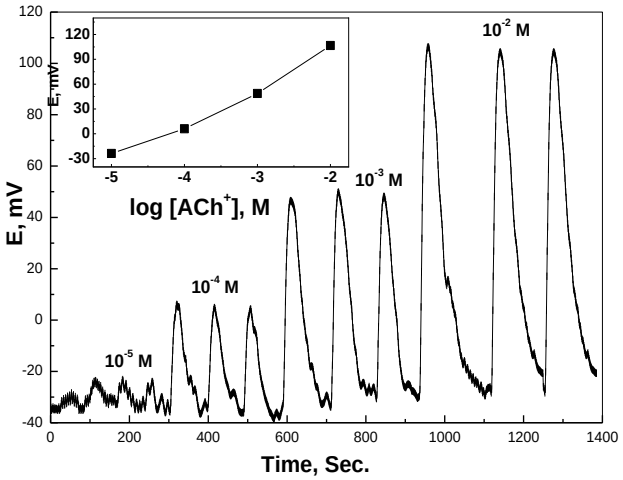

Figure 5. Typical FIA peaks produced by injection of 100 µL aqueous solutions of standard ACh into a stream of 10^{-2} M PBS solution pH 7 flowing at 3.0 mL min^{-1}

Kinetic monitoring of the acetylcholine hydrolysis

Acetylcholinesterase (AChE) terminates the transmission of impulses in the cholinergic synapses through the rapid hydrolysis of acetylcholine (ACh) to choline (Ch).[43]

For the estimation of K_m and V_{max} of the enzymatic reaction was carried out by ACh sensor using 0.5 IU L^{-1} of the enzyme to each concentration of ACh^+ from 0.01 to 1.0 mM and monitoring the potential change. It was found that lower substrate concentrations did not significantly increase the measured initial rate. This can be attributed to the low sensitivity of the sensor at low concentration levels of ACh^+ ions. A 5.0×10^{-4} M of ACh^+ solution was used in all subsequent AChE measurements. This concentration level offered a measurable change in the reaction rate at low enzyme activity, a better linearity of calibration plot, and a fast response of the sensor. As shown in Figure 6, it provided values of 7.9 x10^{-5} M and 61 mV min^{-1} for the characteristic parameters of the enzymatic reaction, K_m, and V_{max}, respectively. This value of K_m is close to the magnitude as that obtained previously.[44]

The effect of AChE concentration on the initial rate of the enzymatic reaction was also studied using an ACh concentration of 5.0 x 10^{-4} M, and varying the concentration of enzyme in the range of 8.0 x10^{-6} to 5.0x 10^{-3} U mL^{-1}.

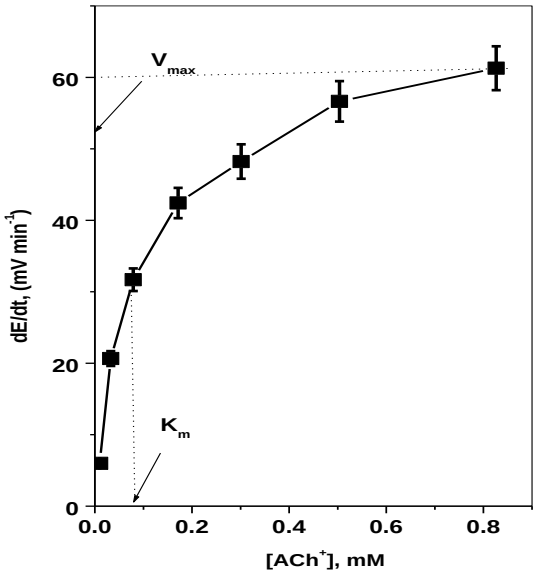

Figure 6. Michaelis-Menten plot of the hydrolysis of acetylcholine.

A linear relationship (r = 0.9994) was obtained in a concentration range of 1.0 x 10^{-5} to 5.0 x 10^{-3} U mL^{-1} with a detection limit of 1.0 x10^{-5} U mL^{-1}.

Conclusions

The Molecular imprinting technique was employed to produce ACh host-tailored sensors for potentiometric transduction. The performance characteristics of the sensors showed stable, sensitive and selective potential responses towards ACh^+ ions over the concentration range of 3.0 x 10^{-5}- 1.0 x 10^{-2} M with a limit of detection 1.31 µg mL^{-1} and a slope of 59.6±0.9 mV $decade^{-1}$. The addition of an anionic additive to the membranes showed a slope of 55.2±0.8 mV $decade^{-1}$ over the concentration range of 1.0×10^{-5}–1.0×10^{-2} M and detection limits of 0.65 µg mL^{-1}. Advantages of these sensors include the simplicity in designing, short measurement time, good precision, high accuracy, high analytical throughput, low limit of detection and good selectivity. The selectivity for acetylcholine over choline, the fast response, and low drift of the proposed sensors developed permit the assay of AChE activity via the hydrolysis of ACh^+.

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