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Verapamil Potentiometric Membrane Sensor for Verapamil Pharmaceutical Analysis. Computational Investigation

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Verapamil is a calcium channel blocker used in the management of angina, arrhythmia and hypertension. In this study a potentiometric liquid membrane sensor is used for simple and fast determination of verapamil in pharmaceutical formulation and urine. Computational studies were done electronically and geometrically on verapamil and tetraphenylborate before and after complex formation. These studies demonstrated tetraphenylbarate fits better with verapamil than potassium tetrakis. Thus, for the membrane preparation VER-tetraphenylborate complexes were employed as electroactive materials in the membrane. The wide linear range (10^{-5}-10^{-2} mol L^{-1}), low detection limit (8.2×10^{-6} mol L^{-1}), and fast response time (~30s) are characterizations of the proposed sensors. Validation of the method shows suitability of the sensors for application in quality control analysis of verapamil in pharmaceutical formulation and urine.

Keywords: Potentiometric sensor; PVC membrane; Verapamil; Computational Chemistry; Chemometrics; Density functional based tight binding (DFTB)

1. INTRODUCTION

Verapamil-based medications are prescribed for several heart and blood pressure indications. The fast acting formulations (verapamil hydrochloride and Isoptin) are taken for angina, as well as irregular heartbeat and high blood pressure. Its generic name is verapamil hydrochloride, 5-[N-(3,4-dimethoxyphenylethyl)methylamino]-2-(3,4-dimethoxyphenyl)-2-isopropylvaleronitrile hydrochloride (Fig. 1).

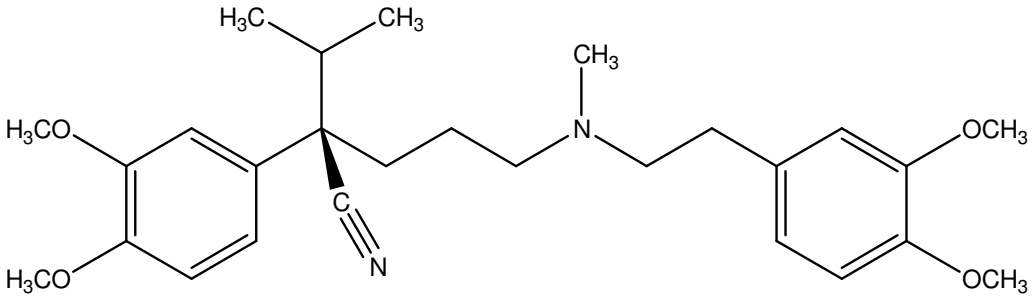

Figure 1. Chemical structure of verapamil

In medical practice verapamil is used in conventional tablet form with a minimal dose of 40 mg and a maximal dose of 180 mg, as well as in a slow release form in doses of 120 to 240 mg. Only 10–20% of the 90% of the dose absorbed from the digestive tract penetrates to the circulatory system in an unchanged form [1]. The remaining part of the verapamil dose undergoes a first-pass effect, mainly in the liver. In humans, verapamil is metabolized to more than six metabolites, according to a number of authors that are excreted mainly in urine [2].

Several methods have been employed to determine verapamil hydrochloride in biological specimens and pharmaceutical formulations. The determination in biological fluids normally requires the use of trace analysis techniques, mainly chromatography with fluorimetric [3] or UV [4].

Other methods include gas–liquid chromatography [5], capillary gas chromatography [6], potentiometry–conductometry [7], stripping voltammetry [8], atomic emission spectrometry [9], and mass spectrometry [10].

Potentiometric membrane sensors are playing an important role in pharmaceutical analysis [11-14] because of their simplicity, rapidity and accuracy over other analytical methods such as spectrophotometry and HPLC. Also, the other mentioned methods are elaborate, time-consuming and involve sophisticated equipment that might not be available in most analytical laboratories.

In this paper the interaction of verapamil with ion-pair reagents was studied by theoretical and calculative methods. According to the obtained results a verapamil ion-selective potentiometric membrane electrode can be developed based on the ion-pair compound of Verapamil-tetraphenylbroate (VER-TPB) as the electroactive substance. The proposed electrode was successfully applied for the determination of verapamil in pharmaceutical formulations and urine samples.

Computational chemistry and molecular modeling play an important role in modern drug discovery and electrochemical science [15-21]. Computational work is also valuable in drug development where medium-sized organic pharmaceuticals are selected as candidates and are then made in larger quantities. Instead of modeling interactions with macromolecules the prediction of molecular properties for small molecules is more essential in the development stage.

The strength of binding usually correlates with the target molecules tendency to the ionophore and several energy contributions may be responsible for the binding. It is believed that amongst these energies, electrostatic interactions play a dominant role in the process - at least in sequence preference and target molecule positioning [22,23].

There are no studies to date in recent literature that have used computational methods to evaluate drug selective ligands by electronic properties. The lack of work in this area is probably due to the inherent difficulties associated with doing calculations on a Drug-Ligand complex. One of these problems includes the lack of parameters for semi-empirical or empirical methods (even though the numbers of atoms in typical drug complexes indicate the use of lower level calculations being appropriate).

In this study the Density Functional Theory (DFT) atomic population analysis was used to measure the Ligand-Drug complex. This was achieved by looking at the ability of the ligand to change in atomic charges and bond length.

2. EXPERIMENTAL PART

2.1. Materials and Reagents

The chemical reagents (analytical grade) were: Sodium tetraphenyl borate (NaTPB), high-molecular weight polyvinylchloride (PVC), dibutyl phthalate (DBP), benzyl acetate (BA), nitrobenzene (NB), tetrahydrofuran (THF), and the chloride and nitrate salts of the used cations (Merck Co.). Verapamil and its tablet were obtained from different local pharmaceutical factories. All solutions were prepared using triply distilled water.

2.2. Apparatus

The glass cell, where the Verapamil-selective electrode was placed consisted of a R684 model Analion Ag/AgCl reference electrode as the internal reference electrode, and a calomel electrode (SCE, Philips). Both electrodes were connected to a Corning ion analyzer with a 250 pH/mV meter with ±0.1 mV precision.

2.3. Preparation of ion-pair compound

The ion-pair compound of Verapamil-tetraphenylborate (VER-TPB) was prepared by: 20 mL of 0.01 mol L^{-1} solution of verapamil hydrochloride mixed with 20 mL of tetraphenyl borate solution (0.01 mol L^{-1}) under stirring. The resulting precipitate was filtered off, washed with water and dried [13,14].

2.4. Preparation of the electrodes

The general procedure to prepare the PVC membrane was as follows: Different amounts of the ion-pair along with appropriate amounts of PVC, plasticizer and additive were dissolved in tetrahydrofuran (THF), and the solution was mixed well. The resulting mixture was transferred into a

glass dish of 2 cm diameter. The solvent was evaporated slowly until an oily concentrated mixture was obtained. A Pyrex tube (3-5 mm o.d.) was dipped into the mixture for about 10 s so that a transparent membrane of about 0.3 mm thickness was formed. The tube was then pulled out from the mixture and kept at room temperature for about 10 h. The tube was then filled with an internal filling solution (1.0×10^{-3} mol L^{-1} verapamil hydrochloride). The electrode was finally conditioned for 24 h by soaking in a 1.0×10^{-3} mol L^{-1} verapamil hydrochloride solution [24-27].

2.5. Standard Verapamil hydrochloride solutions

A stock solution of 10^{-1} mol L^{-1} verapamil hydrochloride was prepared by dissolving the calculated weight of pure drug in 25 mL water. The working solutions (10^{-6} to 10^{-1} mol L^{-1}) were prepared by serial appropriate dilution of the stock solution.

2.6. Emf measurements

The following cell was assembled for the conduction of the emf (electromotive force) measurements:

Ag–AgCl internal solution, 10^{-3} mol L^{-1} verapamil hydrochloride| PVC membrane | sample solution | Hg–Hg_2Cl_2, KC1 (satd.)

These measurements were preceded by the calibration of the electrode with several verapamil hydrochloride solutions (working solutions).

2.7. Computational methods

Calculations on the isolated molecules and molecular complexes were performed within GAUSSIAN 98 package [28]. Each species was initially optimized with PM3 method and then the optimized structures were again optimized with density functional theory (DFT), using the 6-31G* basis set. Full geometry optimizations and frequency calculations were performed. Each species was found to be of minima by having no negative values in the frequency calculation. The calculations gave internal energies at 0 K. In order to obtain gas phase free energies at 298.15 K it was necessary to calculate the zero-point energies, and thermal corrections together with entropies to convert the internal energies to Gibbs energies at 298.15 K [29, 30].

Frequency calculations on these structures verified that they were of true minima and provided the necessary thermal corrections to calculate H (Enthalpy) and G (Gibbs free energy). Finally, full optimizations and frequency calculations for each species were performed with the DFT/6-31G* [31,32].

The other one-electron properties (dipole moment, polarizability, energies of the frontier molecular orbital) were also determined at the B3LYP/6-31G* level. For the charged species the dipole moment was derived with respect to their mass center because for the non-neutral molecules, the calculated dipole moment depended on the origin of the coordinate system.

The stabilization energies of the selected complexes were determined with the help of the DFT calculations and calculated with a recently introduced method based on the combination of the approximate tight-binding DFTB with the empirical dispersion energy. The DFT methods are known to be inherently deficient for stacking interactions as they basically ignore the dispersion attraction [32-34]. As a consequence, their enlargement by an empirical dispersion term currently appears to be a very reasonable way to improve the major deficiency of the DFT method for the evaluation of the molecular complexes. It should also be mentioned that the interaction energies were obtained as the difference between the complex energy and the combined energies of the molecules in isolation [35].

3. RESULTS AND DISCUSSION

3.1. Theoretical Study

Molecular parameters are controlled by the molecular geometry. Optimization of geometry is the most important step for the calculation of interaction energy. The optimized geometries and numeration of the atoms of the studied molecules; TPB for NaTPB (Fig. 2), PTK for KTpClPB, VER for Drug (Fig. 3) and VER-TPB for Verapamil-NaTPB (Fig. 4) and VER- PTK for Verapamil-TpClPB are presented.

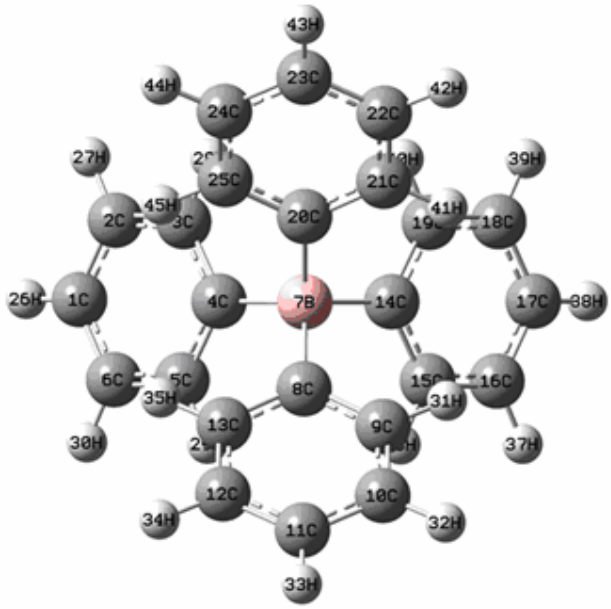

Figure 2. The full optimized structure of TPB

To obtain insight on VER tendencies for TPB and PTK as potential ionophores, DFTB calculations (B3LYP/6-31G*) were carried out. The interaction energy of the pair ΔE_{A-B} between molecules A (TPB or PTK) and B (VER) was estimated as the difference between the formed complex energy and the energies of the isolated partners. Interaction energies were corrected for the basis set superposition error using the counterpoise method [36,37]:

$$\Delta E_{A-B} = E_{A-B} - E_A - E_B$$

It was obtained to be -62.689 and -42.745 Kcal/mol for ΔE_{TPB} and ΔE_{PTK}, respectively. It indicates TPB is a more appropriate ionophore for VER sensor in comparison to PTK as it contributes to its higher interaction energy. The main discussions further on shall be on Verapamil-TPB interactions.

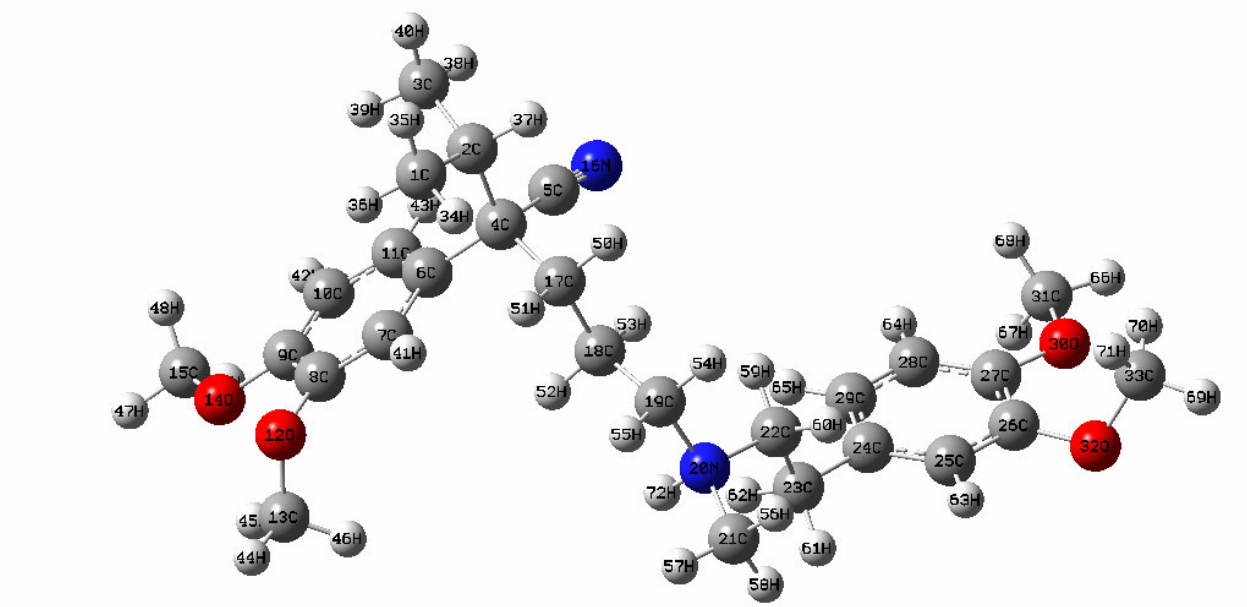

Figure 3. The full optimized structure of VER

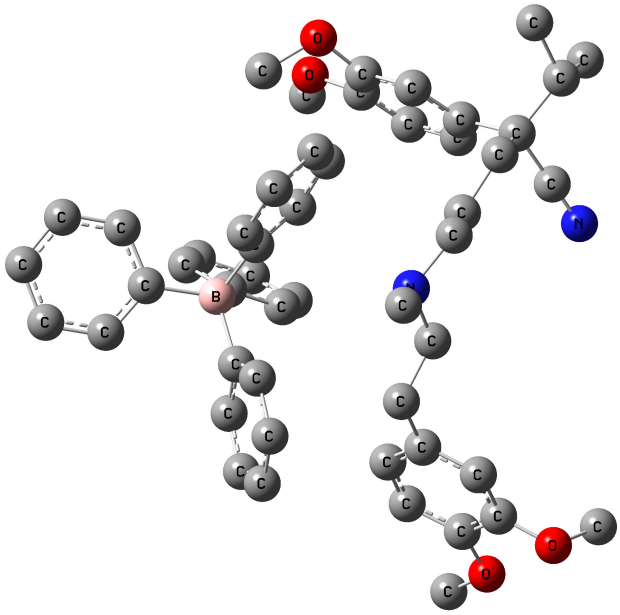

Figure 4. The full optimized structure of VER-TPB complex

Table 1. Significant computed atomic charges and bond length for verapamil before and after complex formation

Charges				Bonds(Å)		
	NO.	Verapamil	VER-TPB	NO.	Verapamil	VER-TPB
4	C	0.045	0.047	R(5,4)	1.154	1.154
5	C	0.054	0.056	R(5,16)	1.154	1.154
6	C	0.141	0.124	R(6,7)	1.396	1.398
8	C	0.103	0.107	R(6,11)	1.385	1.384
16	N	-0.207	-0.213	R(8,12)	1.409	1.408
20	N	-0.220	-0.230	R(9,10)	1.387	1.386
17	C	-0.095	-0.095	R(10,11)	1.389	1.390
18	C	-0.112	-0.112	R(12,13)	1.440	1.443
19	C	-0.003	-0.004	R(14,15)	1.434	1.434
21	C	-0.081	-0.085	R(19,20)	1.539	1.533
22	C	-0.002	-0.002	R(19,55)	1.091	1.090
35	H	0.074	0.067	R(20,21)	1.521	1.519
40	H	0.072	0.065	R(20,22)	1.541	1.538
46	H	0.047	0.068	R(20,72)	1.041	1.054
48	H	0.074	0.067	R(22,23)	1.549	1.548
50	H	0.071	0.064	R(23,24)	1.529	1.530
52	H	0.077	0.087	R(23,62)	1.088	1.087
54	H	0.109	0.093	R(21,56)	1.089	1.092
56	H	0.123	0.103	R(19,54)	1.091	1.090
58	H	0.124	0.137	R(27,28)	1.388	1.387
60	H	0.108	0.092	R(27,30)	1.393	1.397
59	H	0.112	0.109	R(30,31)	1.434	1.434
65	H	0.064	0.082	R(32,33)	1.442	1.441
66	H	0.090	0.081	R(22,59)	1.089	1.094
69	H	0.082	0.074			
72	H	0.274	0.299			
	NO.	TPB	VER-TPB	NO.	TPB	VER-TPB
1	C	-0.093	-0.087	R(3,4)	1.401	1.404
4	C	-0.068	-0.081	R(4,7)	1.643	1.648
5	C	-0.086	-0.109	R(7,8)	1.643	1.653
6	C	-0.078	-0.081	R(7,8)	1.643	1.654
7	B	0.232	0.224	R(7,14)	1.643	1.646
9	C	-0.086	-0.138	R(7,20)	1.643	1.641
10	C	-0.078	-0.090	R(8,9)	1.400	1.396
15	C	-0.086	-0.095	R(8,13)	1.401	1.411
23	C	-0.093	-0.085	R(9,10)	1.386	1.396
26	H	0.030	0.047	R(10,11)	1.385	1.377
33	H	0.030	0.047	R(11,12)	1.385	1.393
34	H	0.033	0.055	R(12,13)	1.385	1.379
35	H	0.042	0.062	R(20,25)	1.400	1.397
36	H	0.042	0.005	R(4,5)	1.400	1.398
29	H	0.042	0.035	R(4,3)	1.400	1.404
30	H	0.033	0.040	R(9,31)	1.082	1.081
		HOMO	-2.777	-8.852 for VER		
		LUMO	10.919	0.318 for VER		

Results presented in Table 1 show that interactions existing between the VER and TPB are mostly electrostatic. The most noticeable atomic charge changes are shown in Table 1. Charge changes in the ion pairs are localized on specific atoms that interact together in each molecule [38-41].

According to Table 1 interactions between the VER and the studied ligands correspond to the N20 results in the occurrence of the most significant changes in the atomic charges and also bond lengths For VER H72, H56, and H59, atomic charges changed from 0.274 to 0.299, 0.123 to 0.103 and 0.112 to 0.109, respectively, along with its bond lengths (N20-H72), (C21-H56), (C22-H59), which shifted from 1.041 to 1.054, 1.089 to 1.092 and 1.089 to 1.094, respectively.

High values of polarizability (160.606 and 195.136 for TPB and VER, respectively) prove its role on interactions amongst TPB and the Verapamil. The low values of dipole-dipole interactions (especially for that of TPB=0.0) show it does not play a significant role between TPB and the studied VER. Moreover, since the studied molecules are in form of ions electrostatic interactions should also be considered.

The highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) for TPB and the drug calculated at B3LYP/6-31G(d) level are displayed in Table 1. The eigen values of LUMO and HOMO and their energy gap reflect the chemical activity of the molecule. LUMO as an electron acceptor represents the ability to obtain an electron, while HOMO as an electron donor represents the ability to donate an electron. From Table 1 the results illustrate that charge transfer interactions occur between TPB and the VER because the HOMO energy of TPB is close to the LUMO energy of the drug. In this analysis, the effect of the TPB and VER charge is considerably higher.

3.2. Nuclear magnetic resonance (NMR)

For reliable assignment of NMR spectra of organic molecules, the calculation of chemical shifts associated with their magnetic nuclei is essential. H,N,O and C NMR experimental investigation is coupled with DFT calculation on the verpamil and complexes for NMR calculations purposes.

We used the GIAO (Gauge-Including-Atomic Orbitals) method. Implemented in the Gaussian package, using hybrid functional, in conjunction with 6-31G* basis sets.

As can be seen in Table 2 the main difference between our computational values are noted for N20 and O12 ,O14,C22 nuclei and 72, 52 protons(H) that these atoms are near the TPB as a negative charge source and minor differences for another H,C,O that are distant from TPB.

The expected chemical shifts for all the NMR active sites are shown in Table 2. For example N20 NMR shift change is seen from 218.560 to 193.994 ppm, C22 NMR shift change from 141.088 to 136.508 ppm, O12 from 236.475 to 245.525 ppm and H72 has a shift from 228.759 to 220.339 ppm. Additional chemical shift data, although required for determining VER-TPB assignments, were not used in the quantum-chemical structure determination.

In this investigation some of the atoms such as N20,H72,O12,O14,H52 of verapamil and B7,C8,C13,C19,C21 of TPB that are the nearest atoms together in VER-TPB have major chemical shift change. Accordingly, these NMR chemical shift changes in the ion pairs are localized on specific atoms that interact together in each molecule and show the most dominate electrostatic interaction between the drug and TPB.

Table 2. Significant Computed nuclear magnetic resonance (NMR) database for verapamil and TPB, before and after the complex formation.

Atom No.	VER	TPB	VER-TPB
N20	218.560	-	193.994
N16	1.328	-	-1.489
C19	130.756	-	131.406
C22	141.088	-	136.508
C21	151.156	-	152.698
O12	236.475	-	245.525
O14	228.759	-	220.339
H72	24.747	-	27.147
H52	31.282	-	30.984
B7	-	142.168	117.455
C8	-	91.637	26.228
C13	-	120.134	57.799
C19	-	120.929	63.409
C21	-	120.836	64.699
H33	-	26.946	25.945

3.3. Membrane composition effect on the potential response of the sensor

The potential response of a sensor is greatly related to the membrane constituents so the influence of membrane composition on the potential response of the verapamil sensor was studied. For this purpose different membrane compositions (shown in Table 3) were tested. As can be seen, the membrane with the composition of 30% PVC, 5% VER-TPB, and 65% DBP (no. 3) was the optimum one in the development of this sensor. This membrane composition was selected after many considerations.

Table 3. Optimization of membrane ingredients

Membrane no.	**Ion-pair (% wt.)**	**Plasticizer (% wt.)**	**PVC (% wt.)**	**Linear range (mol L^{-1})**	**Slope (mV $decade^{-1}$)**
1	3	DBP, 67	30	1.0×10^{-5} -1.0×10^{-2}	49.2
2	4	DBP, 66	30	1.0×10^{-5} -1.0×10^{-2}	52.4
3	5	DBP, 65	30	1.0×10^{-5} -1.0×10^{-2}	56.1
4	6	DBP, 64	30	5.0×10^{-4} -5.0×10^{-2}	48.3
5	5	NB, 65	30	5.0×10^{-4} -1.0×10^{-3}	19.5
6	5	BA, 65	30	5.0×10^{-3} -1.0×10^{-2}	13.7

The high verapamil extraction into the liquid membrane was a result of the elevated ion-pair tendency to exchange with the verapamil cations. From Table 3, the 5 mg ion-pair (VER-TPB) is the best amount for the optimum response. The second factor that allows verapamil ions to be extracted from an aqueous solution into the membrane as an organic phase is a plasticizer. After the evaluation of three solvent mediators (NB, BA and DBP), it was observed that they do not have the same results if the optimum composition is used. DBP, which is a low-polar solvent mediator, shows better response

than BA and NB. NB and BA have higher dielectric constant values than DBP leading to the extraction of polar ions, which have negative effects on the extraction of verapamil ions as a hydrophobic ion.

The presence of lipophilic anions in a cation-selective membrane was also considered. As seen from Table 3 the presence of such anions in a cation-selective membrane (which is based on an ion-pair) decreases the response behavior of the sensor.

3.4. pH effect on the electrode response

To understand the impact of pH on electrode response the potential was measured at two particular concentrations of the verapamil solution (1.0×10^{-3} mol L^{-1}), from the pH value of 2.0 up to 9.0 (concentrated NaOH or HCl solutions were employed for the pH adjustment). Fig. 5 shows the potential remained constant despite the pH change in the range 3.5 to 7.0 indicating the applicability of this electrode in this specific pH range.

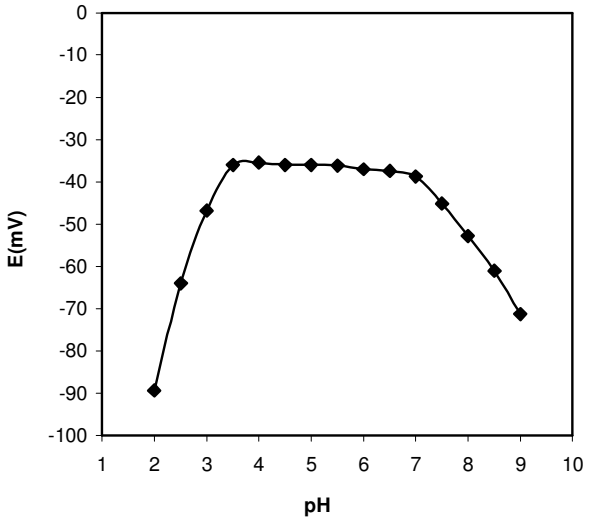

Figure 5. The pH effect of the test solutions (1.0×10^{-3} mol L^{-1}) on the potential response of the

On the contrary, relatively noteworthy fluctuations in the potential *vs.* pH behavior took place below and above the formerly stated pH limits. In detail, the fluctuations above the pH value of 7.0 can be justified by removing the positive charge on the drug molecule. The fluctuations below pH 3.5 were attributed to removing the ion-pair in the membrane.

3.5. Study of sensor properties

The properties of a potentiometric membrane sensor are characterized by parameters such as: measuring range, detection limit, response time, selectivity, lifetime and accuracy [42-45].

3.5.1. Measuring range

The measuring range of an ion-selective electrode includes the linear part of the calibration graph (Fig. 6). Measurements can be performed in this lower range but it must be noted that more closely spaced calibration points are required for more precise determinations. According to another definition, the measuring range of an ion-selective electrode is defined as the activity range between the upper and lower detection limits. The applicable measuring range of the proposed sensor is between 1×10^{-5} and 1×10^{-2} mol L^{-1}.

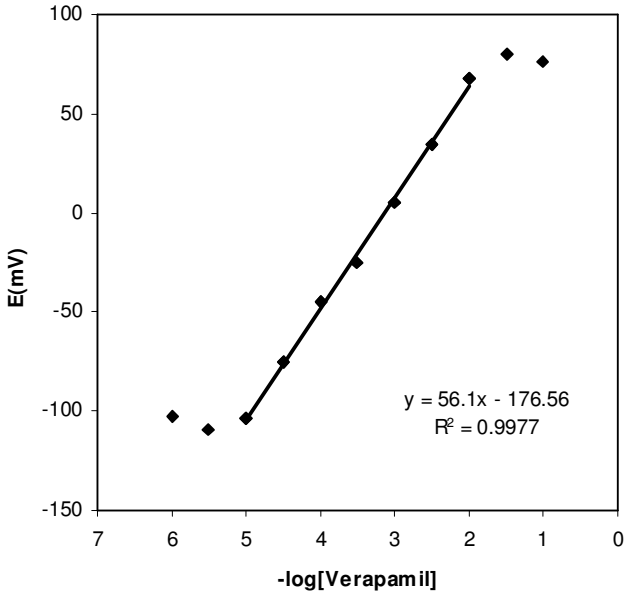

Figure 6. Calibration curve of the verapamil membrane sensor with the composition of membrane no. 3. The results are based on 8 measurements.

3.5.2. Detection limit

By extrapolating the linear parts of the ion-selective calibration curve the detection limit of the ion-selective electrode can be calculated. In practice, detection limits for the most selective electrodes are in the range of 10^{-5}–10^{-6} mol L^{-1}.

In this work the detection limit of the proposed membrane sensor was 8.2×10^{-6} mol L^{-1} and was calculated by extrapolating the two segments of the calibration curve (Fig. 6).

3.5.3. Response time

The response time of an electrode is evaluated by measuring the average time required to achieve a potential within ±0.1 mV of the final steady-state potential, upon successive immersion of a series of interested ions, each having a ten-fold difference in concentration. It is notable that the

experimental conditions such as; stirring, flow rate, ionic concentration, composition of test solution, concentration and composition of solution to which the electrode was exposed before experimental measurements were performed, any previous use or preconditioning of the electrode and the testing temperature, have an effect on the experimental response time of a sensor [46-49].

In this work less than a 30 s response time was obtained for the proposed electrode when contacting different verapamil solutions from 1.0×10^{-5} to 1.0×10^{-2} mol L^{-1}.

3.5.4. Verapamil electrode selectivity

The selectivity of an ion-pair based membrane electrode depends on the physico-chemical characteristics of the ion-exchange process at the membrane. For example, sample solution interface, mobility of the respective ions in the membrane and on the hydrophobic interactions between the primary ion and the organic membrane [50].

The selectivity of the verapamil membrane electrode is related to the free energy of transfer of the verapamil cation between aqueous and organic phases. The response of the electrode towards different substances was checked and the selectivity coefficient values K_{AB}^{Pot} were used to evaluate the interference degree. The selectivity coefficient values were obtained using the Matched Potential Method (MPM) [51-53].

Table 4. Selectivity coefficients of various interfering compound for verapamil sensor

Interference	**Log K_{MPM}**
Na^+	-3.97
K^+	-3.78
Li^+	-4.43
Mg^{2+}	-4.82
Ca^{2+}	-3.95
Br^-	-4.25
Fe^{3+}	-4.12
glucose	-4.20
ammonium	-3.72
CO_3^{2-}	-4.31
NO_3^-	-4.25
Mg^{2+}	-3.96
HPO_4^{2-}	-4.22
Ca^{2+}	-3.57

The steps that need to be followed for the MPM method are: addition of a specified concentration of the primary ions (A, 10^{-2} mol L^{-1} of verapamil solution) to a reference solution (10^{-5} mol L^{-1} of verapamil solution), and the potential measurement. Then, the interfering ions (B, 10^{-2} mol L^{-1}) are consecutively added to the same reference solution until the measured potential matches the one obtained before addition of the primary ions. The selectivity coefficient defined by the matched

potential method, K_{MPM}, is equal to the ratio of the resulting primary ion activity (concentration) to the interfering ion activity, $K_{MPM} = \Delta a_A/a_B$.

The respective results are summarized in Table 4 depicting that the selectivity coefficient values of the electrode for all tested substances were in the order of 10^{-4} or smaller. Given the low coefficient values it was considered that the function of the verapamil-selective membrane sensor would not be greatly disturbed.

3.4.5. Lifetime

The average lifetime for most of the reported ion-selective sensors is in the range of 4–10 weeks. After this time the slope of the sensor will decrease, and the detection limit will increase. The sensors were tested for 8 weeks during which time the electrodes were extensively used (one hour per day). The proposed sensors can be used for six weeks. Firstly, there is a slight gradual decrease in the slopes (from 56.1 to 53.8 mV decade^{-1}) and then an increase in the detection limit (from 8.2×10^{-6} mol L^{-1} to 3.5×10^{-5} mol L^{-1}). It is well established that the loss of plasticizer (ionic site) from the polymeric film is due to leaching into the sample. This is the primary reason for limited lifetimes of the sensors.

3.6. Analytical application

3.6.1. Determination of verapamil in formulations

The proposed sensor was evaluated by measuring the drug concentration in pharmaceutical formulations. The recovery results are shown in Table 5. The drug concentration was determined with the calibration method. The results are in satisfactory agreement with the labeled amounts. The RSD was equivalent to 2.34%.

Table 5. Results of verapamil assay in formulation by the verapamil membrane sensor

Samples	**Stated content per tablet**	**HPLC* (mg/tab.) n=5**	**Found ****	**t-test** (P=0.05; $t_{theoritical}$=2.20)
VERAPAMIL HCL-Rouz Darou (sample 1)	80 mg	80.2±0.3 mg	80.4±0.3 mg	$t_{experimental}$= 1.18
VERAPAMIL HCL-Rouz Darou (sample 2)	80 mg	80.5±0.2 mg	80.8±0.3 mg	$t_{experimental}$= 2.10
VERAPAMIL HCL-Rouz Darou (sample 3)	80 mg	80.4±0.2 mg	80.3±0.4 mg	$t_{experimental}$=0.60
VERAPAMIL HCL-Sobhan (sample 1)	80 mg	80.2±0.3 mg	80.4±0.2 mg	$t_{experimental}$=0.73
VERAPAMIL HCL-Sobhan (sample 2)	80 mg	80.3±0.2 mg	80.2±0.3 mg	$t_{experimental}$=1.20
VERAPAMIL HCL-Sobhan (sample 3)	80 mg	80.5±0.2 mg	80.7±0.2 mg	$t_{experimental}$=1.94

* High Performance chromatography; the results based on five replicate measurements
** The results are based on eight replicate measurements

3.6.2. Recovery of verapamil from urine samples

In order to investigate the applicability of the new sensor for determination of the drug in biological fluids it was applied to the recovery of verapamil from urine samples. A 2.5 mL of 10^{-4} mol L^{-1} verapamil solution was transferred into a 10-mL volumetric flask. After addition of 2.5 mL of urine samples, the solution was diluted to the mark with water. The determination of verapamil solution content was done using the calibration method by the proposed sensor. The recovery of three replicate measurements was found to be 103.3%, 106.5% and 103.7%, respectively.

3.7. Validation of the method

The linearity, detection limit, precision, accuracy, and ruggedness/robustness were the parameters which were used for the method validation.

As mentioned before, the measuring range of the verapamil sensor is between 1×10^{-5} and 1×10^{-2} mol L^{-1}. The detection limit of the sensor was calculated as 8.2×10^{-6} mol L^{-1} (4 µg/mL).

3.7.1. Precision

The parameters of the repeatability and reproducibility were investigated in order to assess the precision of the technique. For the repeatability monitoring, 8 replicate standards samples 4, 40, 4000 µg/mL were measured. Then, the mean concentrations were found to be 4.07, 40.4, 405.3 µg/mL with associated RSD values of 1.5, 1.08, and 0.58%, respectively. Regarding the inter-day precision, the same three concentrations were measured for 3 consecutive days providing mean verapamil concentrations of 4.06, 40.3, 404.4 µg/mL and associated RSD values of 1.94, 1.15, and 0.45%, respectively.

3.7.2. Accuracy

For determination of the accuracy of the method three samples of two different tablets of the verapamil hydrochloride was analyzed with an official method and the proposed sensor. The results are shown in Table 5. At 95% confidence level the calculated t-value did not exceed the theoretical t-value indicating no significant difference between the proposed methods and the reference method.

3.7.3. Ruggedness/Robustness

For ruggedness of the method a comparison was performed between the intra- and inter-day assay results for verapamil obtained by two analysts. The RSD values for the intra- and inter-day assays of verapamil in the cited formulations performed in the same laboratory did not exceed 2.83%. On the other hand, the robustness was examined while the parameter values (pH of eluent and

laboratory temperature) were slightly changed. Verapamil recovery percentages were good under most conditions and did not show any significant change when the critical parameters were modified.

4. CONCLUSIONS

1. In this study it is observed that different types of interactions exist between drugs and ligands. The DFTB method was employed in this case as the considered molecules were in the form of ions that resulted in ion pair formation. This technique was used as it considers dispersion energies in addition to those calculated by DFT for further investigations.
2. These theoretical calculations help select appropriate ionophores and also predict their selectivity for different drugs. The contribution of the VER-TPB charge change is much more than those of the VER-PTK charge change that cause Chemical shifts changes in the VER-TPB which are localized between specific atoms for example N20 and B7 that interact together in each molecule.
3. After a series of experiments involving the use of VER-TPB ion-pair complexes along with several plasticizers in the membrane design, it was concluded that the verapamil sensor exhibited excellent analytical performance characteristics. It demonstrated an advanced performance with a fast response time (~30 s), lower detection limit of 8.2×10^{-6} mol L^{-1} and pH independent potential responses across the range of 3.5–7.0.
4. The high sensitivity of this sensor allowed for the determination of verapamil in pharmaceutical analysis. The theoretical calculations are accurate and suitable methods to obtain interaction energies and therefore helped choose better ion pairs. Additionally, employing these methods finds the center of interactions in the target molecule and ionophore.

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