

Full Paper

Potentiometric Detection of Clobutinol Hydrochloride Level using PVC Membrane Sensor and Relative Electronic Circuit

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Abstract- A potentiometric inexpensive sensor has been designed for detection of Clobutinol Hydrochloride level, which is used for curing non-productive coughs. The sensor is capable of doing direct detection of the drug level in the chemical solution using PVC ion-exchange membranes. The ion pair used as the sensing element was made through the interaction of Clobutinol Hydrochloride and Sodium Tetra-Phenyl borate. It is shown that the highest ion-selective electrodes in sensitivity were achieved by 10% ion pair, 45.5% dioctyl phthalate and 44.5% of PVC composition. The sensor response time is found to be less than 10 seconds, with a minimum detectable range of 4.0×10^{-6} M in 0.5×10^{-1} M – 1.0×10^{-5} M range.

For measurements, a sensing board in direct and permanent contact with the electrodes is used. This integrated measurement system is made of an ATMEGA16L micro-controller sampling, analyzing and data storage section which makes measurements easier and faster. It also helps with reducing the side effects caused by a traditional multimeter usage such as connection cables resistance.

Keywords- Potentiometric sensor, Clobutinol hydrochloride, PVC membrane, Ion-Selective electrode

1. INTRODUCTION

Clobutinol Hydrochloride called Silmot commercially, is one of the anti-cough drugs used for curing dry and non-infectious coughs. It is more specifically important for cases in which the source of coughs is not evident yet. The operation of the drug is by centrally suppressing coughs through medulla oblongata. It acts mostly like codeine in this case. The advantage is that the effective part of the composition has little side effects on the person's respiratory system. Fig. 1 shows the chemical structure of Clobutinol Hydrochloride.

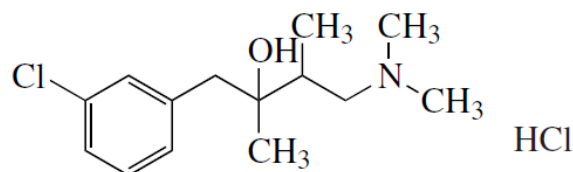


Fig. 1. Chemical structure of Clobutinol Hydrochloride [1]

Ion-selective chemical sensors designs work based on analyzing specific liquids in the solution. Several methods have been developed for measuring the drug levels in a solution. These can be categorized as optical, ultrasonic and electrochemical methods. A great advantage of electrochemical sensors is that they can be used for samples in solid, liquid and gas states. In this method use of solid electrolytes, and materials with high melting points, is the ideal case.

There are a lot of methods that can detect ions levels in an aquatic solution, like High Performance Liquid Chromatographic (HPLC) [2-4], Capillary Electrophoresis, Ultraviolet-Visible Spectroscopy (UV-VIS), Atomic Absorption Spectrometry (AAS), Inductively Coupled Plasma Mass Spectrometry (ICP), Fluorescence, Phosphorescence, and Voltammetry. All of these methods are highly selective. But there are tradeoffs in comparing different methods in terms of cost, method complexity, and measurement time. In this work ion selective electrodes, which is the most important category of potentiometric sensors, was selected. This is because this method is one of the simplest and fastest methods among others. These electrodes operate based on ion pair combinations as an electroactive material.

Abdel-Ghani et al. [5] made the sensor in flow injection condition in which they used Clobutinol Hydrochloride–Phosphotungstic acid ion pair. The average electrodes response time is reported about 30 seconds. In this research Clobutinol electrodes were made by PVC ion-exchange membranes, and the use of Sodium Tetra-Phenyl Borate reduced the response time to 10 seconds [6-10]. In this method potentiometer has less oscillations and solution has better stability.

An ideal ISE (Ion Selective Electrode) consists of a thin membrane across which only the intended ions can be transported. The transport of ions from a high concentration to a low concentration through a selective binding with some sites within the membrane creates a potential difference. When a neutral species diffuses from a region of activity, A_1 , to a region of activity, A_2 , it causes a potential difference which is proportional to ion activity logarithm according to Nernst equation. This can be calculated by:

$$E = \frac{RT}{zF} \ln \left(\frac{A_1}{A_2} \right) = \frac{0.05916}{z} \ln \left(\frac{A_1}{A_2} \right) \quad (1)$$

Where “z” is the charge of the species (including the sign). Membrane is basically non-porous, water insoluble and mechanically stable which its composition for voltage measurement is designed based on selectivity method.

2. EXPERIMENTAL

2.1. Apparatus

For the experiments, Ag/AgCl potentiometric electrodes were ordered from Azar Electrode-Iran. For weighting chemicals and preparing solutions, AND GR-202 weighing system was used. Ion-pair compounds were heated by a Heidolph MR Hei-Standard heater.

In this work, for measuring the sensor voltages and selectivity detection, a MASTECH MS8222H multimeter was used. Also Behine pH Meter model Sat_2100 is used to adjust the pH level of the solutions at each step. To simplify sensor voltage measurements, an integrated on board readout circuitry is designed by the authors. The integrated system with sensor reduces measurements errors. It also makes the measurements easier because the electrodes are directly and permanently connected to a microcontroller based system. The results from the new read out system are compared with the voltages from multimeter at the end.

2.2. Materials and Reagents

In this work Clobutinol Hydrochloride with $C_{12}H_{23}Cl_2NO$ empirical chemical formula and 292.24452g/mol molecular weight was obtained from a local pharmaceutical manufacturer (Tehran, Iran). Tetrahydrofuran (THF), Sodium Tetra-Phenyl borate (NaTPB), Dibutyl Phthalate (DBP) (Merck. Co, Germany), Polyvinyl Chloride (PVC) (Fluka Co., USA) and other chemical materials and reagents were got from local manufacturers in Tehran, Iran. All materials had the highest available purity and were used without any modification.

2.3. Membrane Preparation and Electrode Assembly

2.3.1. Ion-pair preparation

To prepare the ion pairs from Clobutinol Hydrochloride drug, first 20 mg of it is solved in 2 ml of DI water in a beaker. In another beaker, 20.3 mg Sodium Tetra-Phenyl borate is solved in 2 ml of DI water. Then the content of the first beaker is added to the latter, and will let the combination enough time to sediment. Finally ion pairs are separated from the solution by filter papers.

2.3.2. Preparation of Membrane Electrodes

For membrane electrodes preparation, a plastic pipe with 3 mm internal diameter was immersed for 10 seconds in the mixture such that a semi-transparent micro-meter in thickness membrane is formed. Then the pipe is removed from the solution and is kept for about 10 hours at room temperature to dry. The inside and outside of membrane is filled with Clobutinol Hydrochloride 10^{-3} M after the membrane dried on the walls of the tube. Finally the electrodes containing the ion-pairs of Clobutinol Hydrochloride-Tetra-Phenyl borate (Cb-TPB) was prepared after it was dipped into the 10^{-3} M solution for 7 hours.

Before doing measurements, sensor resistance should be measured. This must be done after preparation and stability phases. For resistance measurement, concentrations on the membrane sides are not important. So 10^{-3} M and 10^{-7} M solutions were used for the internal and external sides respectively. If the final resistance became too high, it could be due to the membrane large thicknesses. But if it is just higher than 4 M Ω , it might be due to an unbalanced membrane which can be balanced by placing the cell in a solution with higher concentration for a few hours [6-10].

2.3.3. Standard Clobutinol Hydrochloride Solution Preparation

Solution of 0.5×10^{-1} M Clobutinol Hydrochloride was prepared by dissolving the calculated weight of pure drug in 3 ml of DI water. The solutions of 0.5×10^{-1} M to 1.0×10^{-5} M were prepared by appropriate dilution of the solution while keeping its pH constant.

2.3.4. Potentiometric Measurements and the Calibration Graph

ISEs are linear, non-destructive, non-contaminating and unaffected by color or turbidity. Nevertheless these electrodes are fragile and have a limited shelf life time and also limited response time to the activity of uncomplexed ions. The calibration graph is shown in Figure 2. The slope of PVC membrane electrode is found 52.1 mV per decade for the Clobutinol hydrochloride concentrations with a standard deviation of ± 0.6 mV. The sensors show a

linear response in $1.0 \times 10^{-5} \text{ M} - 0.5 \times 10^{-1} \text{ M}$ range. LD which is defined by the minimum Clobutinol hydrochloride concentration is $4.0 \times 10^{-6} \text{ M}$.

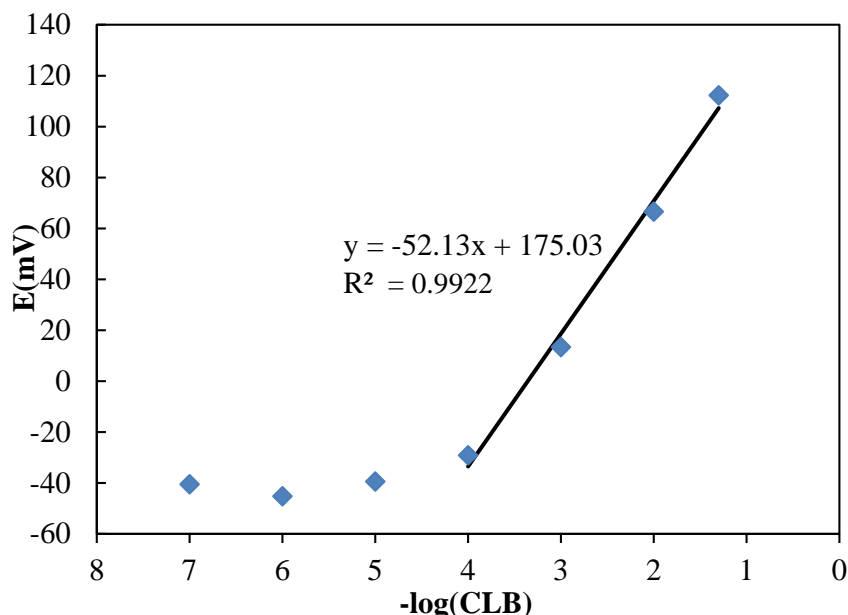


Fig. 2. Calibration graph for the proposed Clobutinol sensitive electrode based on 7 replicate measurements

2.3.5. Silomat Usage as an Application

Silomat tablets are the commercial name of Clobutinol Hydrochloride. The formulation of the tablets was *(RS)*-1-(4-chlorophenyl)-4-dimethylamino-2,3-dimethyl-butan-2-ol, and it was ordered from Boehringer Ingelheim in Germany. In this case powder of tablets with high purity were prepared & weighed for accurate measurements.

3. RESULTS AND DISCUSSION

3.1. Composition of the Membrane

For preparing the PVC membrane, different compositions were tested. The best sensitivity was obtained, when 44.5 mg of PVC powder, 10mg of ion pairs, and 45.5 mg of Plasticizer (DOP) were mixed. In this case the result was then solved in 2 ml of THF, then boiled, evaporated and shacked very slowly at 80° to obtain a homogenous solution. The details are shown in Table 1. As the table shows, 44.5:45.5:10 composition of PVC: DOP: Ion Pair gave the best Nernst response. The effect of DBP, DOP and ion pair contents were all monitored in improving the membrane functionality. As shown, the Clobutinol Hydrochloride drug in presence of 10% of ion pair and 45.5% of plasticizer causes the sensor

to have a good Nernst response with a 55.9 ± 0.6 Nernst line slope. It should be noted that the time to obtain results in the 6th row is much higher than the others and is about 4 min. The experiments showed that DBP and DOP are almost similar in terms of the electrodes voltage and measurements linear range. In these experiments NB and BA plasticizers are not used. The reason is because these were tested in a previous work [5] and better results were obtained by using DOP and DBP.

Table 1. Change of PVC membrane ingredients composition to obtain the best sensitivity

No.	Composition (%)				Slope* (mV per decade)	LR(M)	DL(M)
	PVC	Plasticizer	Ion-pair	NaTPB-additive			
1	30	DBP, 65	5	-	53.7 ± 0.5	$1.0 \times 10^{-5} - 0.5 \times 10^{-1}$	4.0×10^{-6}
2	30	DBP, 63	7	-	53.1 ± 0.3	$1.0 \times 10^{-5} - 0.5 \times 10^{-1}$	4.0×10^{-6}
3	44.5	DOP, 45.5	10	-	55.9 ± 0.6	$1.0 \times 10^{-5} - 0.5 \times 10^{-1}$	4.0×10^{-6}
4	46.5	DOP, 46.5	7	-	48.7 ± 0.5	$1.0 \times 10^{-5} - 0.5 \times 10^{-1}$	4.0×10^{-6}
5	45.5	DOP, 47.5	5	2	51.5 ± 0.2	$1.0 \times 10^{-5} - 0.5 \times 10^{-1}$	4.0×10^{-6}
6	45	DOP, 54	0	1	2.5 ± 0.7	$1.0 \times 10^{-4} - 1.0 \times 10^{-3}$	7.5×10^{-5}

*After 48 h of conditioning

3.2. Influence of Plasticizer and Sensing Components

The operating characteristics of the ISEs can be significantly modified by changing the relative proportions of the electrode membrane components. The main components of an electrode membrane of this type are PVC matrix, the plasticizer, and the ion-pair. Each membrane component plays a special role in the membrane function [11-15].

The plasticizer mainly acts as a fluidizer, allowing homogeneous dissolution and diffusion mobility of the ion-pair inside the membrane. The nature and/or the amount of the plasticizer must be properly controlled in order to minimize the electrical asymmetry of the membrane and to limit fouling of the sensor. In addition, the proper selection of the plasticizer allows the control of the value of the electrode/solution distribution ratio of the particular ion-pair. The analytical performance of such electrode is strongly dependent on a proper plasticizer-ion pair ratio. The nature of the plasticizer has a marked influence on the response slope, linear domain and also on the selectivity of the PVC membrane electrodes. Here, many plasticizer types as listed in Table 1 were tested. After their evaluation, DOP was chosen to be employed in the sensor construction, because it provided an effective linear

range. Both NB and BA have higher dielectric constant values than DBP & DOP, leading to the extraction of the polar interfering ions.

3.3. Influence of pH and Effect of Electrolyte Concentration

The electrodes response in sample solutions with same concentrations but different pH levels is measured. The valid pH range for measurement is the range in which electric potential stays almost the same. For this purpose the effect of pH on sensor response in a 1.0×10^{-3} M Clobutinol hydrochloride solution has been studied in the range of 1-14. In each step pH level is read by the pH meter which its probe is in contact with the solution. For pH adjustment, NaOH is used to increase pH level and HCl to decrease it.

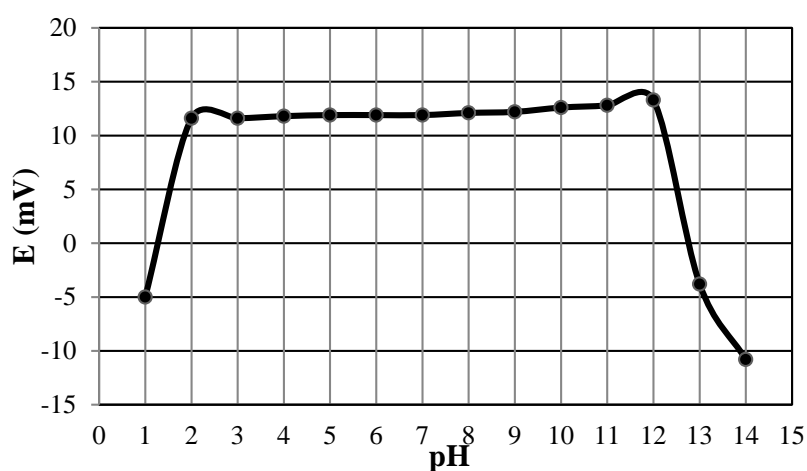


Fig. 3. Sensor response changes by increasing pH level from 1-14. The valid pH range for measurement is the interval in which the electric potential stays almost the same

3.4. Selectivity of the Electrodes

One of the most important properties of a sensor is its selectivity. It shows how much the sensor is capable of differentiating target ions from other ions. The influences of different organic and inorganic ions on the response of CLB sensors were investigated. These substances were selected based on tablets and drug composition.

The potentiometric selectivity coefficients of the Clobutinol hydrochloride sensor were evaluated by the matched potential method (MPM). In this method, the potentiometric selectivity coefficient is defined by the activity ratio of primary and interfering ions that give the same potential difference under identical conditions. First, a known concentration a'_A of the primary ion solution is added into a reference solution that contains a fixed concentration a_A of primary ions, and the corresponding potential ΔE is recorded. Next, solution of an interfering ion is added to the reference solution until the same potential change ΔE is

recorded. The change in potential at the constant background of the primary ion must be the same in both cases [16].

$$K_{AB}^{POT} = \frac{a_A' - a_A}{a_B} \quad (2)$$

In which a_B is the concentration of the interfering ion.

In this case some inorganic cations, sugars and amino acids were used as in [5]. The resulted selectivity coefficients are shown in Table 2. The selectivity coefficients show that the interferences are negligible in the performance of the electrode [17-21].

Table 2. Clobutinol Hydrochloride sensor selectivity coefficient for different compounds

Interfering ion	Log (K_{MPM})
Na ⁺	-3.5
K ⁺	-3.7
Ca ²⁺	-3.9
Mg ²⁺	-4.3
Glucose	-5.4
Maltose	-5.2
Fructose	-5.1
Vit.C	-5.3
Vit.B1	-5.2

The practical application of the electrode is for Clobutinol drug dosage detection. For this purpose the deviations in the dosage during drug preparation is found. The results of this measurement are shown in Table 3 for 5 different tablets which were selected randomly. The results are based on five replicate measurements.

Table 3. Clobutinol Hydrochloride determination of 5 random samples by the potentiometric sensor

Sample	Labeled Amount (mg/tab.)	Found by PVC Electrode (mg/tab.)
Sample 1	40	44.3±1.3
Sample 2	40	38.9±1.5
Sample 3	40	42.8±0.8
Sample 4	40	39.5±0.9
Sample 5	40	41.7±1.4

3.5. Lifetime, reproducibility and response time

The membrane lifetime is the time span that it responds to the related ions without a specific change in the responses. Some parameters like plasticizer loosening and ions leaking through the film or membrane into the sample limit the lifetime of the membrane. These cause instability in the selectivity of the electrodes which results in selectivity degradation of the membrane. Membrane lifetime is actually the time elapsed in which the ion pair contents of a membrane become less than its ionic contents [22-24]. The results of measurements for 10 consecutive weeks for monitoring lifetime are shown in Table 3. It should be noted that the slope of 54.6 ± 0.3 in this table is the result for the membrane test at the end of the first week, while slope of 55.9 ± 0.6 in Table 1 is the result obtained 48 hours after membrane preparation.

Table 4. PVC membrane electrode life time

Week	Slope (mV per decade)	DL (M)
First	54.6 ± 0.3	4.0×10^{-6}
Second	54.3 ± 0.2	4.8×10^{-6}
Third	52.3 ± 0.4	5.3×10^{-6}
Fourth	53.8 ± 0.2	8.5×10^{-6}
Fifth	52.9 ± 0.3	1.0×10^{-5}
Sixth	49.6 ± 0.4	3.2×10^{-5}
Seventh	46.1 ± 0.3	6.3×10^{-5}
Eighth	42.7 ± 0.4	2.4×10^{-4}
Ninth	39.5 ± 0.3	5.5×10^{-4}
Tenth	34.9 ± 0.5	1.5×10^{-4}

3.6. Electronic printed circuit board for measurements

For measuring the produced voltage by different solution concentrations, a PCB based measurement circuitry has been designed and implemented by the authors. The board is made to have a direct and permanent contact with the sensor electrodes to make an integrated system which makes measurements faster and easier than using a regular multimeter. This also reduces the measurement error created by the connection cables to a multimeter. The microcontroller-based system also helps in analyzing and storing the data and results on board. In this design first the detected voltage difference is amplified and then sampled by an ATMEGA16 microcontroller. It samples the voltage by its integrated 8 bit ADC inputs, stores samples on its internal memory and then shows the results on a 16×2 LCD.

The PCB layout of this circuitry is shown in Figure 4.

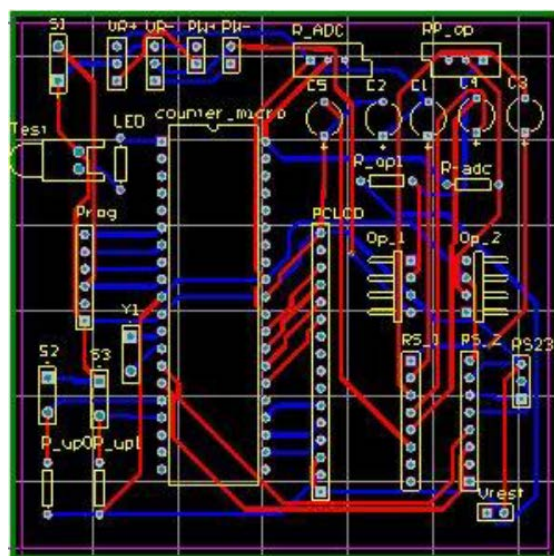


Fig. 4. PCB layout of the measurement board which uses an ATmega16 as a SOC to read out voltages, store data and show the results on a LCD

Table 5. Comparison between the voltages measured by the voltmeter and the microcontroller based readout board

No.	Conc. (Mol L ⁻¹)	Measured by Multimeter		Measured by ATmega16	
		Found(mV)	%RSD (n=7)	Found (mV)	%RSD (n=7)
1	1.0×10 ⁻⁷	- 40.5±0.7	1.7	- 42.3±0.8 t=1.51 F=2.81	1.9
2	1.0×10 ⁻⁶	- 45.2±0.8	1.8	- 44.9±0.3 t=1.58 F=2.51	2.0
3	1.0×10 ⁻⁵	- 39.4±0.6	1.5	-41.1±0.7 t=1.64 F=2.68	1.7
4	1.0×10 ⁻⁴	- 29.1±0.3	1.1	- 30.9±0.5 t=1.59 F=2.53	1.6
5	1.0×10 ⁻³	13.4±0.3	2.2	12.3±0.3 t=1.56 F=2.42	1.8
6	1.0×10 ⁻²	66.6±0.9	1.4	67.1±1.0 t=1.56 F=2.45	1.5
7	0.5×10 ⁻¹	112.3±2.1	1.9	113.7±2.5 t=1.48 F=2.21	2.2

*Values of t and F at 95% confidence level. Theoretical values: t=2.24, F=5.05

The voltages read by the proposed board for CLB determination in pharmaceuticals, are compared with the results from multimeter. These data are shown in Table 5. This experiment was repeated 7 times and the results were compared in terms of the average value of the detected voltages. The calculated F and t values did not exceed the theoretical values, indicating that there is no significant difference between both methods in considering the accuracy (t-test) and precision (F-test). More accurate comparison can be obtained by increasing the number of repetitions.

4. CONCLUSIONS

Using ion-exchange electrodes has several advantages. First of all they have a linear response. They are sustainable & superior in terms of their selectivity level, and lifetime. They are only sensitive to active ions instead of all ions in the composition.

In this method, a variety of potentiometric electrodes have been developed for Clobutinol hydrochloride detection. These sensors are very fast, and are sensitive (4.0×10^{-6} M level). They have a good Nernst response in the range of 0.5×10^{-1} M to 1.0×10^{-5} M. The sensor can be used in pharmaceutical formulation measurements. The sensor responses are based on ion-exchange mechanism. It was shown that Clobutinol Hydrochloride–Tetraphenyl borate has a better and more stable response over Clobutinol hydrochloride–Phosphotungstic acid ion-pair. It was also shown that for the Clobutinol hydrochloride electrodes, the best PVC membrane electrode is obtained by 10% of ion pair, 45.5% DOP and 44.5% of PVC.

Use of a microcontroller system for collecting and analyzing data in these measurements, reduced the effect of connection cables impedance to the multimeter, and made the measurements faster and easier. The sensor discussed in this paper is better in terms of its response time and chemical stability compared to the traditional ion-selective sensors. Also the use of an on-board electronic circuitry adds to the simplicity of the design.

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