Construction of ion selective membrane electrodes for the potentiometric determination of Atenolol in its pure form and pharmaceutical preparations.

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Abstract:

Two novel Atenolol (ATN) ion selective electrodes were constructed and used for the determination of ATN in its pure form and pharmaceutical formulation. The electrodes were based on the use of the ion association complexes of either phosphomolybdic acid (PMA) or Ammonium Reineckate (AR) as anions with Atenolol cation in a PVC matrix plasticizers with tri-butyl phosphate (TBP). The results of these electrodes showed stability (life time 45 and 48 days), near Nernstian response (slope 28.8 and 31.2 mV/decade) low detection limit (3.06×10^{-6} and 2.80×10^{-6} M) for ATN-PMA-TBP and ATN-AR-TBP electrodes respectively. For both electrodes the best concentration for internal filling solution was 1×10^{-4} M ATN and the linear range was 1×10^{-4}-1×10^{-1} M and found to be usable within the pH range 9.6 – 11.0 . This study also included the measurements of selectivity of these electrodes in the presence of common cations, anions and some drug excipients. The $K^{Pot}_{i,j}$ found to be less than 1. The electrodes were successfully applied for determination of ATN both in pure form and in tablet pharmaceutical preparation with recovery of not less than 97 %.

Introduction

Atenolol (ATN) (fig.**1**) [(R,S)-4-(2-hydroxy-3-isopropylaminopropoxy) phenylacetamide], $C_{14}H_{22}N_2O_3$ is medically used in the treatment of arterial hypertension, angina pectoris, cardiac arrhythmias [1,2], myocardial infraction and chest pains [3].

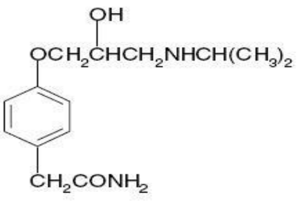

Figure (1): Structure of atenolol

Owing to the importance of atenolol many methods have been used for the determination of this drug. The most common method used is liquid chromatography [4-8], liquid chromatography – mass spectrometry [9,10], UV and visible spectrophotometric methods [11-14]. Methods such as diffuse reflectance spectroscopy [15], flow injection – chemiluminescence [16], titrimetry [17], fluorimetry [18], atomic absorption [19] and capillary electrophoresis [20,21] were also used. The adopted methods for the qualification of atenolol uses technique of HPLC [22], spectrophotometric [23] and titration with perchloric acid [24] in the American, British and European pharmacopoeia respectively. Potentiometric methods for the determination of ATN by ion selective electrodes based on PVC were also used. Arvand *et al* [25] constructed an electrode using phosphotungstic acid (PTA) as active material and acetophenone as plasticizer. This electrode showed linearity in the range 5×10^{-7} - 1×10^{-2} M and was found to be usable within the pH range 3-6 with response time about 8 sec., detection limit 1×10^{-7} M and life time > 2 months. PTA as active material was also used to prepare four electrodes with di-butyl phosphate (DBP), tri-butyl phosphate (TBP), O-nitro phenyl octyl ether (NPOE) and di-octyl phthalate (DOPH) as plasticizers [26]. The best electrode was that based on DOPH with linear range1×10^{-4} -5×10^{-2} M, detection limit was 5×10^{-5} and life time around 90 days. Atenolol was also determined by an ion selective electrode [27] incorporates PVC membrane with ATN tetrakis (p-phenyl) borate ion-pair complex exhibiting a Nernstian response in the concentration range 3×10^{-5} - 8×10^{-2} M with a slope of 56.5 ± 0.8 mV decade^{-1}, limit of detection of 1×10^{-5} M and working pH range of 3.0-9.0. PVC matrix membrane sensors responsive to Atenolol based on the complex of ATN cation with PTA anion is also described and characterized [28]. This sensor shows a slope of 34 mV/decade, limit of detection 8×10^{-7} M, response time 40 s for 1×10^{-3}, working pH ranges 3-7, concentration range 3×10^{-7} - 1×10^{-2} M and a life time of 6-8 weeks. In the present work and because of the advantages of simplicity, low cost, selectivity, low detection limit a novel ion selective electrodes were constructed based on ion pair complex of Phosphomolybdic acid *(PMA)* or Ammonium Reineckate *(AR)* as anions with Atenolol cation (ATN^+) in a PVC matrix plasticized with TBP.

Experimental Instrumentations*:*

The following instruments were used:

1- JENWAY pH /mV meter 3310.

2- Reference Calomel Electrode (Fisher Scientific Company cat. No. 13-639-52).

3- (Silver- Silver chloride Electrode) as working electrode (Orion 90-02) .

4- Magnetic Stirrer with Hot Plate BIOSAN MSH 300.

5- Drying Oven / Soyokaze Isuzu Seisakusho Com. Ltd .

6- Ultrasonic with water bath UNISONICS model Fxp12.

7- Sartorius balance Model BL 210S.

Reagents:

All reagents were of analytical-reagent grade supplied by Fluka, BDH and MUMBI companies and deionised water was used throughout.

Solutions:

0.1M phosphomolybdic acid (PMA): prepared by dissolving 22.5760 gm of (PMA) in 100 ml of deionised water and solutions of concentrations from 1×10^{-7} – 1×10^{-2} M were prepared by appropriate dilution.0.1M Ammonium Reineckate (AR): prepared by dissolving 3.5542 gm of (AR) in 100 ml of deionised water and solutions of concentrations from 1×10^{-7} – 1×10^{-2} M were prepared by appropriate dilution.0.1M Atenolol (ATN): prepared by dissolving 2.663 gm of (ATN 100 ml of deionised water). Solutions of KCl, NaOH, HCl, NaCl, KBr, $CuSO_4.5H_2O$, Na_2HPO_4, Glucose, Mannitol and Fructose with concentrations ranged from 1×10^{-2} - 1×10^{-1} M were also prepared.

Preparation of ionic pair for ATN-PMA or AR electrode membrane:

90 ml of 0.1M (ATN) were dropped wisely, mixed with 90 ml of 0.1M (PMA) or (AR) and the mixture was continuously stirred for 15 min. and then filtered (Whatman filter paper No.42) and washed several times by deionised water and left for drying at room temperature (25 ºC) for two days. A yellow or pink precipitate insoluble in water was obtained and ground to a fine powder for each electrode respectively.

Preparation of liquid membranes for ATN drug:

The liquid membrane was prepared by mixing 0.1 gm of ATN-PMA or -AR ion pair with 0.45 gm of PVC (dissolved in 4 ml acetone + 4 ml THF). After this step of dissolution, 0.45 gm of TBP (as plasticizer) was added and mixed until a homogenous mixture was formed. The formed solution was gradually poured into a Petri dish [16] of 10 cm diameter and covered with a filter paper. This solution was then allowed to evaporate for two days at room temperature. The membrane was carefully lifted by a tong and kept in a refrigerator. The resulting membrane is of 0.3 mm thickness and is sufficient to provide about 8 membranes.

Construction of ion selective electrodes:

One end of PVC tube of 3 – 4 cm length was softened by a circular motion on a glass on which few drops of THF were added. A circular part of the membrane of larger diameter than the PVC tube was cut and glued onto the soft end of PVC tube (**fig 2**) using adhesive prepared from PVC + THF.

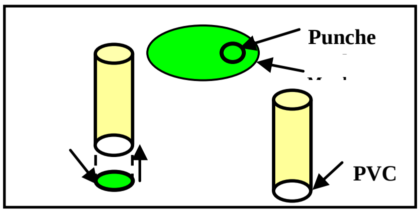

Figure (2): Construction of the membrane onto the PVC tube

The other end of PVC tube is attached to an empty and opened Ag/ AgCl electrode. This new electrode was filled by Atenolol as internal solution and connected with the saturated calomel as a reference electrode (**fig3**). Before use, this manufactured electrode was conditioned by immersing for 10 hours in 1×10^{-4} M ATN solution and kept in the same solution when not in use

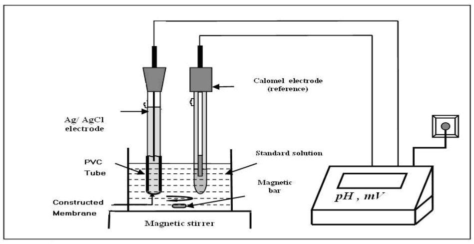

Figure (3): Parts of the constructed electrode and the electric cell

Results and discussion

Selective membrane of PVC containing a complex was prepared by reaction of Atenolol with an active materials PMA and AR, using TBP as a plasticizer. The properties of these electrodes including concentration of internal filling solution, pH range, effect of temperature, life time were investigated.

Concentration of internal filling solution:

The concentration of 1×10^{-4}M ATN as internal filling solution showed the nearest value to the theoretical Nernestian slope thus it is adopted to be the suitable concentration (fig.4).

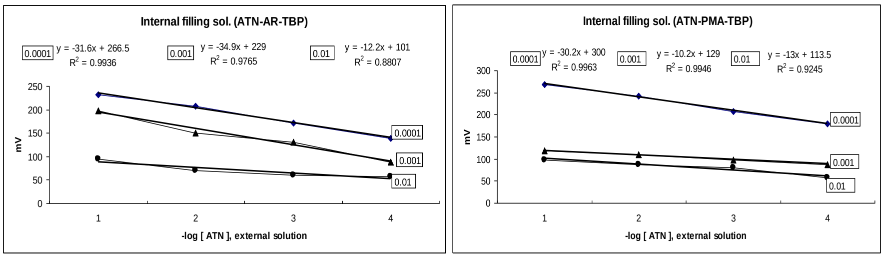

Figure (4): Effect of internal filling solution for ATN-PMA-TBP and ATN-AR-TBP electrodes.

Standard curve :

The results (fig.5) showed that the plot of potential (mV) versus –log [ATN] gives a linear regression relationship. The linear ranges, slopes and detection limit were 1×10^{-1}-1×10^{-4} M and 1×10^{-1}-1×10^{-4} M, 29.8 and 31.2 mV/decade, 3.06×10^{-6} and 2.80×10^{-6} M for ATN-PMA-TBP and ATN- AR- TBP electrodes respectively

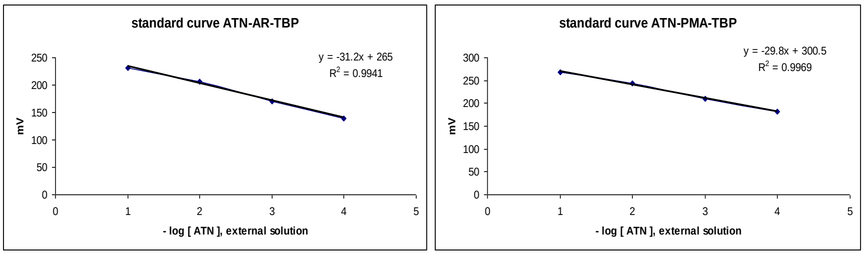

Figure (5): Standard curve of ATN using ATN-PMA-TBP and ATN-AR-TBP electrodes

It is worth noting that the addition of (0.1-1.0) ml of 1 M KCl onto the external solution did not change the mV readings by more than 2 mV. Also no improvement in sensitivity is obtained when mixing of 10 mg of either Cd or Ag with membrane composition.

Effect of pH:

A standard 1×10^{-1} M and 1×10^{-3} M of aqueous Atenolol solution were prepared and adjusted to the desired pH values with dilute HCl or NaOH solutions. The suitable pH range for both electrodes was 9.6-11. The increase of potential at pH of less than 10 can be due to the penetration of H^+ into the membrane surface [29]. At higher pH values (pH>11), free base precipitates and cause an increase in the concentration of unprotonated species resulting in a decrease of mV readings [30] and may also a result of penetration of OH^- ions into the membrane [31]. It is worthy of note that at pH's more than 11.0 and less than 9.6 a fluctuated mV readings were observed accompanied with hardening of the membranes (fig.6).

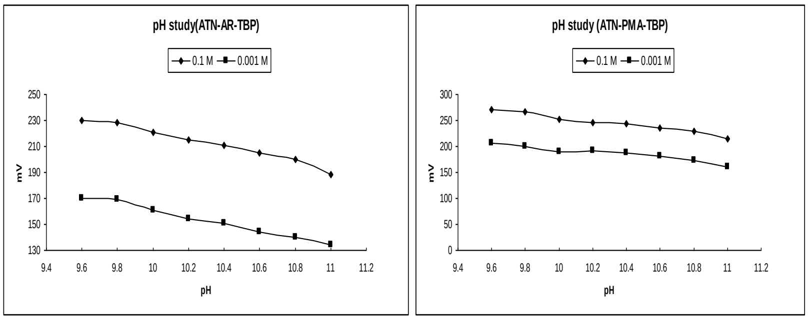

Figure (6): Effect of pH on the response of ATN-PMA-TBP and ATN-AR-TBP electrodes using two series of ATN solutions 1×10^{-3} and 1×10^{-1}M.

Effect of temperature:

The results showed that the appropriate working temperature is 25 °C for both electrodes (fig.7). The increase in potential with the increase of temperature is may due to the increase in surface area of the membrane resulting in easier permeation and equilibratio

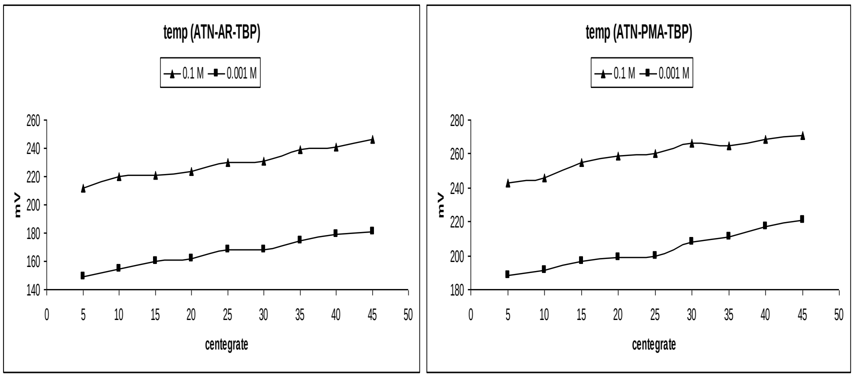

Figure (7): Effect of temperature on the response of ATN-PMA-TBP and ATN-AR-TBP electrodes.

Response time:

This time is defined as the time required for the electrode to reach a steady value within ± 1 mV of the final equilibrium value. The present study showed that the response time vary with the concentration of ATN and type of formulation (table 1).

Table (1): Response time of ATN-PMA-TBP and ATN-AR-TBP electrodes.

ATN Electrodes / Tablet	Conc. (M)	Response time (Sec.)
ATN-AR-TBP NAVOTEN 100mg	1×10^{-2} 1×10^{-3}	20 35
ATN-AR-TBP VASCOTEN 100mg	1×10^{-2} 1×10^{-3}	42 54
ATN-AR-TBP Tenordin 50mg	1×10^{-2} 1×10^{-3}	48 72
ATN-PMA-TBP NAVOTEN 100mg	1×10^{-2} 1×10^{-3}	32 45
ATN-PMA-TBP VASCOTEN 100mg	1×10^{-2} 1×10^{-3}	40 55
ATN-PMA-TBP Tenordin 50mg	1×10^{-2} 1×10^{-3}	56 67

These short response times indicated the fast equilibrium of the permeation process of the solution species with membrane ingredients.

Life time:

The electrodes displayed a constant potential readings ±1-5 mV from day to day and the calibration slopes almost did not alter over a period of 45 day for both electrodes (fig. 8). This short life time is due to the loss of plasticizer and the active material from the polymeric layer of the membrane [32].

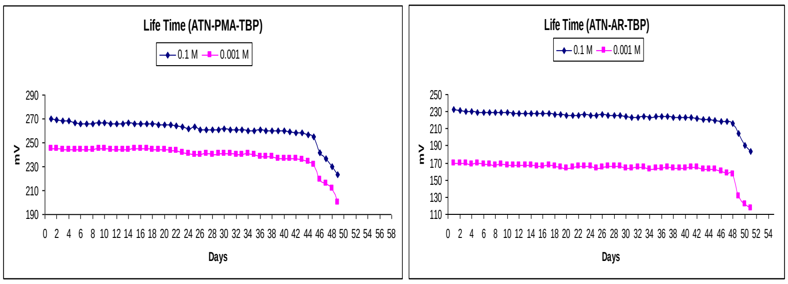

Figure (8): life time of ATN-PMA-TBP and ATN-AR-TBP electrodes

Selectivity:

The potentiometric selectivity coefficient $K_{i,J}^{Pot}$ of the two proposed electrodes were calculated in the presence of related substances using mixed solutions method [33] at 0.01M ATN and concentration range between 0.01- 0.1M for the investigated species. The selectivity coefficient $K_{i,J}^{Pot}$ was calculated from the following equation :

$$K_{ij}^{Pot} = \frac{a_i \cdot 10^{(Eij-Ei)/S} - a_i}{a_j^{Zi/Zj}}$$

Where $K_{i,J}^{Pot}$ is the potentiometric selectivity coefficient; $\mathbf{a_i}$ is the activity of **(i)** ion where the interfering ion is not present; $\mathbf{E_{ij}}$ is the potential of selective electrode for the **(i)** ions in a solution containing **(j)** ions; $\mathbf{E_j}$ is the potential of selective electrode for **(i)** ions when the interfering ions **(j)** are not present; $\mathbf{a_j}$ is the activity of interfering ions **(j)**. The following substances and species were investigated: *Glucose, Mannitol, Fructose,* Na^+, K^+, Cl^-, Br^-, $SO_4^=$ *and* PO_4^{-3}.The $K_{i,J}^{Pot}$ value represents the difference in potential in the presence of interfering ion (j) and when (j) is not present. When the value is less than 1 this indicates that the electrode shows low response to the interfering ions. The results of selectivity are shown on table (**2**). The $K_{i,J}^{Pot}$ values shows a very high selectivity of the electrodes towards the Atenolol species (AT^+).The inorganic species do not interfere owing to ionic size, mobility and permeability in comparison with ATN^+ while the reason for the sugars is mainly attributed to the difference in polarity and lipophilic character [25].

Table (2): $K_{i,J}^{Pot}$ values

Ions	$K_{i,J}^{Pot}$ *values*			
	ATN-PMA-TBP		*ATN-AR-TBP*	
	Conc. of Ions (M)		Conc. of Ions (M)	
	10^{-1}	10^{-2}	10^{-1}	10^{-2}
Na^{+1}	0.0107	0.0623	0.0387	0.0226
K^{+1}	0.0874	0.0672	0.1570	0.0844
Cl^{-1}	0.0529	0.0264	0.0793	0.0675
Br^{-1}	0.1046	0.0349	0.0961	0.0248
SO_4^{-2}	0.0959	0.0708	0.0509	0.0217
$PO4^{-3}$	0.0746	0.0472	0.0276	0.0124
Glucose	0.0804	0.0514	0.0389	0.0219
Mannitol	0.0911	0.0826	0.1706	0.1034
Fructose	0.0779	0.0562	0.0987	0.1109

Application*:*

Determination of ATN in its pharmaceutical preparation.

Ten tablets of Atenolol formulations (Vascoten Medochemie-Cyprus 100 mg and Navoten Aganta-India 100 mg) were weighted and finely crushed. An appropriate (1.0604 and 1.1099 gm) of this powder "equivalent to one tablet" were separately dissolved in 10 ml of ethanol and diluted to 100 ml with distilled water. In the same manner, eight tablets of Tenordin S.D.I-Iraq 50mg were finely powdered and 1.2050 gm was dissolved and diluted. These solutions were potentiometrically measured by the constructed ion selective electrodes using calibration method.

Table (3): Determination of ATN in its tablets pharmaceutical preparations using ATN-PMA-TBP and ATN-AR-TBP electrodes

ATN Electrodes / Tablet	Conc. (M)	Measured Potential (mV)*	Calculated Potential (mV)	RSD%	RE %	Recovery %
ATN-AR-TBP NAVOTEN 100mg	1×10^{-2} 1×10^{-3}	198.61 167.79	202.60 171.40	0.87 1.18	-1.97 -2.11	98.03 97.89
ATN-AR-TBP VASCOTEN 100mg	1×10^{-2} 1×10^{-3}	199.02 170.46	202.60 171.40	0.08 0.15	-2.77 -0.55	98.23 99.45
ATN-AR-TBP Tenordin 50mg	1×10^{-2} 1×10^{-3}	197.76 173.87	202.60 171.40	1.25 2.09	-2.39 1.44	97.61 101.44
ATN-PMA-TBP NAVOTEN 100mg	1×10^{-2} 1×10^{-3}	233.99 206.10	240.90 211.10	1.24 1.98	-2.87 -2.37	97.13 97.63
ATN-PMA-TBP VASCOTEN 100mg	1×10^{-2} 1×10^{-3}	235.53 208.61	240.90 211.10	0.93 0.53	-2.23 -1.18	97.77 98.82
ATN-PMA-TBP Tenordin 50mg	1×10^{-2} 1×10^{-3}	235.70 213.84	240.90 211.10	2.11 1.76	-2.16 1.30	97.84 101.30

***Average of four determination**

Table (**3**) shows that the constructed ion selective electrodes proved to be useful for the determination of ATN amount of tablet pharmaceutical preparations.

Validity of the proposed method:

As well as of good linear concentration range and low detection limit, the precision and the accuracy of the proposed methods were calculated for the analysis the ATN in its pharmaceutical preparations. The precision (RSD) is found to be not more than 2.11 and accuracy (Recovery %) is not less than 97 for both electrodes. Table (4) shows a comparison of some characteristics of the proposed electrodes with the literature

Table (4): Comparison of some characteristics of the proposed electrodes with the literature

Parameter	ATN-PMA-TBP (this study)	ATN-AR-TBP (this study)	Ref. [25]	Ref. [26]	Ref. [27]	Ref. [28]
Slope, mV/decade	29.8	31.2	---	35	56.5	34
Linear Conc. Range, M	1×10-4- 1×10-1	1×10-4- 1×10-1	5×10-7- 1×10-2	1×10-4- 5×10-2	3×10-5- 8×10-2	3×10-7- 1×10-2
Life time, day	45	48	> 60	45	15	45-60
Working pH	9.6-11	9.6-11	3-6	1.0-11.5	3-9	3-7
Response time, s	45-67 for 1×10-3 M	35-72 for 1×10-3 M	8	---	5-15	40
Recovery %	~97-101 for 1×10-3 M	~98-101 for 1×10-3 M	> 97	---	> 98	99.1
Lower limit of detection, M	3.06×10-6	2.80×10-6	1×10-7	1.8×10-6	1×10-5	8×10-7

Conclusion:

The proposed method introduced an ion selective electrodes for the determination of Atenolol based on PVC plasticized with TBP and using PMA or AR as active materials. These electrodes showed a successful application with low limit of detection and good recovery. The electrodes also showed fast response, good selectivity and reasonable working concentration ranges.

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بناء أقطاب غشائية انتقائية لتقدير الأتينولول مجهادياً في شكله النقي وفي مستحضراته الصيدلانية

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(تاريخ الاستلام: ١٧ / ٧ / ٢٠١١ ---- **تاريخ القبول:** ٢٦ / ١٠ / ٢٠١١)

الملخص:

تم في هذا البحث تصنيع قطبين انتقائيين لتقدير الأتينولول في شكله النقي وفي مستحضراته الصيدلانية بالاعتماد على معقد الترابط الأيوني المتكون بين أنيونات أما حامض الفوسفوموليبدك (PMA) أو رنيكات الأمونيوم (AR) وكاتيونات الأتينولول باستخدام ثلاثي فوسفات البيوتيل (TBP) كملدن ومتعدد كلوريد الفينايل كركيزة لها. بينت النتائج ثباتية الأقطاب (العمر ٤٥ و ٤٨ يوم) واستجابة قريبة من القيمة النيرنستية (الميل ٢٨,٨ و ٣١,٢ mV/decade) وحد كشف واطئ (٣,٠٦×١٠$^{-٦}$ و ٢,٨٠×١٠$^{-٦}$ مولاري) لكل من قطب ATN-PMA-TBP و-ATN-AR-TBP على التوالي . كذلك بينت النتائج ان التركيز الأفضل لمحلول الملئ الداخلي هو ATN ١×١٠$^{-٤}$ مولاري ومدى الخطية هو (١×١٠$^{-٤}$ – ١×١٠$^{-١}$) مولاري ومدى الدالة الحامضية المناسب هو ٩,٦–١١ ولكلا القطبين. وتضمنت الدراسة أيضاً قياس انتقائية هذه الأقطاب بوجود كاتيونات وأنيونات شائعة وبعض مسوغات الدواء ووجد ان قيمة $K_{i,J}^{Pot}$ لجميع الأصناف المدروسة هي أقل من ١. تم تطبيق القطبين المصنعين لتقدير الـ ATN بشكله النقي وكذلك في مستحضراته الصيدلانية وباسترجاعية لا تقل عن ٩٧%.