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ISES USE TO DETERMINEVITAMINB9 BASED ON 1-AMINO-9-FLUORENONE

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Abstract: This paper presents a simple, rapid highly sensitive and selective sensor and novel method for determination of folic acid (FA) Folic acid based on 1-Amino-9-fluorenone as ion pair , DBS membrane electrode for dibutyl sebacate (DBS) as plasticizer . The liquid membrane electrode exhibits a rapid and almost Nernstian response for Folic acid over a wide concentration range 1×10^{-7}M to 1×10^{-2}M with a correlation coefficient of 0.998, a detection limit of this electrode is1×10^{-7}M and the slope of 24 ±1mV per decade. The sensor displayed a good selectivity for Folic acid with respect to number of common foreign inorganic. The proposed electrode was successfully applied as an indicator electrode for the potentiometric of Folic acid with volume as well as in the determination of Folic acid in drug. Optimum pH values for the release of bound folic acid were 5.0 to 7.0, respectively. The proposed method is highly selective and sensitive to folic acid. Finally, the new sensor was used to measure folic acid in industrial products.

Keywords: PVC- membrane, VitaminB9 -selective electrode, 1-Amino-9-fluorenone, Potentiometric.

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INTRODUCTION

Folic Acid, N-[p-{[(2-amino-4-hydroxy-6-pteridinyl)methyl] amino}benzoyl]-L-glutamic acid (as shown below), is chosen as the analyte for this investigation because it is an electroactive component of considerable biological importance It has long been recognized as part of the Vitamin B complex found in some enriched foods and vitamin pills. It is usually employed in the treatment or prevention of megaloblastic anaemia during pregnancy, childhood and other clinical situations often associated with alcoholism and liver diseases. A lack of folic acid gives rise to gigantocytic anaemia, associated with leukopaenia, devolution of mentality, psychosis, etc [(1)]. Folic acid is a water soluble vitamin, initially identified as an anti-anemia and growth factor. It is produced by plants (green leaves, algae) and micro-organisms (bacteria, yeast). In mammals, folic acid and its derivatives, the folates, serve as acceptors and donors of carbon units and are involved in amino acid and nucleotide biosynthesis Folic acid also prevents neural tube defects such as spina bifida, while its ability to lower blood homocysteine concentration, suggests that it might have a positive influence on cardiovascular disease. A role for this B vitamin in maintaining good health may, in fact, extend beyond these clinical conditions to encompass several others disorders (birth defects, several types of cancer, dementia, affective disorders, Down's syndrome etc). Folate is the generic term to indicate a group of compounds naturally occurring in food that have vitamin activity similar to folic acid, such as some polyglutamates. The terms folic acid and folates are often used interchangeably, but folic acid is approximately twice as bioavailable as the folates [(2)]. Excellent reviews and articles concerning various aspects of folic acid have been published [(3)]. This vitamin is essential for rapid cell growth like blood production, especially during pregnancy [(4)]. Vitamins are a group of organic compounds which are essential in very small amounts for the normal functioning of the body.Various natural sources like milk, grams, eggs, etc. are a huge storehouse of vitamins, which are useful in regulating the metabolic activity. Deficiency of vitamins induces different types of abnormalities in animals. The deficiency syndromes may be corrected by administering the vitamins from an external source (artificial or natural). This makes it necessary to quantify these vitamins in these sources to facilitate administration of appropriate quantities of vitamins. Folic acid or Vitamin B9 [(5)]. Folate, historically known as Will's factor and vitamin M, was isolated in an unknown chemical form in 1941. The term folate refers to the many different naturally occurring forms of the compound, all of which are made up of apteridine ring, p-aminobenzoic acid, and one to six glutamic acids moieties (Figure 1). Dietary folate is in the chemical form of pteroylpolyglutamate, and, in order for it to be absorbed, it must be cleaved or hydrolyzed in the small intestine to the monoglutamate. The chemical form of the pharmaceutical folate is pteroylglutamate, which does not need to be hydrolyzed in order to be absorbed. Therefore, synthetic folic acid supplements can provide better bioavailability than folate from natural sources[(6,7).]

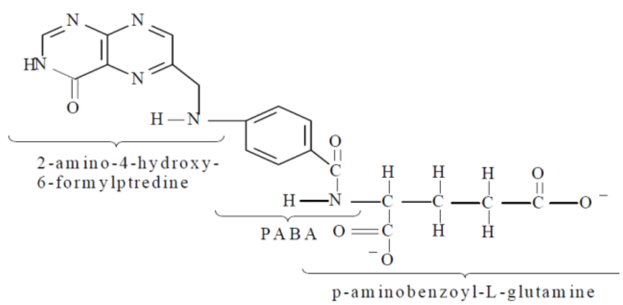

Fig.1 Structure of Folic Acid

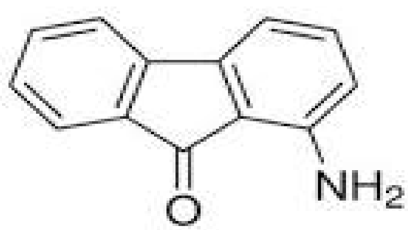

Fig.2 Structure of 1-Amino-9-fluorenone

Folic acid is a vitamin that has been used to treat macrocytic anemia without neurologic disease (8). The vitamin folic acid has received considerable attention in the 1990s because of its disease preventative role. The most significant literature exists in the area of periconceptional use of the vitamin to decrease the risk of neural tube defects (NTDs) such as spina bifida. It has also been suggested that folic acid may be effective in decreasing certain risks of cardiovascular disease and psychiatric illness, most notably dementia(9).

Analytical methods for folic acid include enzymatic methods, fluorimetry (10-14), spectrophotometry(15-17), chromatography (18- 23), chemiluminescence (24-26), phosphorimetry (27)and inductively coupled plasma-atomic emission spectroscopy (28). Electrochemical methods have been used to detect folic acid(29- 37) and to investigate its redox properties(38).

MATERIALS AND METHODS

Apparatus

pH/ mV meter Will be used for potentiometric measurement 25 ± 1ºC were obtained with an Jenway Model 4330. The potential measurements were made in stirred solutions at 25 °C(thermostat) using a magnetic stirrer. The reference electrode was filled with solution containing 10^{-2}M of KCl with 10^{-2}M Folic acid An Orion glass electrode was used to measure PH

Chemicals and reagents

Reagent: All chemicals were of analytical reagent grade unless otherwise stated and doubly distilled water was used throughout. Poly (vinyl chloride) powder. A PVC powder of molecular weight ~ 10,000 was obtained from Fluka (st .Louis, MO). Tetrahydrofuran (THF). A solvent with a purity of 99 %. Dibutyl sebacate (DBS) was obtained from Fluka (St. Louis, MO).Dioctyl phthalate was obtained from (Acros) with a purity of 98%. Acetic acid (Fluka). Sodium acetate (Fluka). Hydrochloric acid (Aldrich). Folic acid (Vitamin B9). Pyridoxine (Vitamin B6).Vitamin C (Ascorbic acid) was obtained from fisher with a purity of 99.8 % Cobalamin (Vitamin B12) .Sucrose was obtained from fisher with a purity of 99.9 %. Starch was obtained from fisher with a purity of 99.9 %. Maltose was obtained from fisher with a purity of 99.9 %.

Preparation of stock solutions

Preparation of solutions All solutions were prepared in bidistilled water acidic component which consists of 0.1M CH_3COOH 0.1M acetate buffer of pH 5 was prepared by mixing the stock of solutions of acetic acid and sodium acetate. Folic acid(Vitamin) was dissolved weighed amount of corresponding in bidistilled Water and less of 0.01 M HCl Stock solution of 1×10^{-2}M concentration were Folic acid. Diluting the stock solution working solutions of 1×10^{-2}M to 1×10^{-7}M concentration and Stock solution of 1×10^{-1}M concentration were potassium chloride dissolving weighed amount of potassium chloride diluting the stock solution of 1×10^{-2}M concentration.

Preparation of electrode Membrane*:*

The component of electrode is a liquid potential determining membrane phase based on plasticized DBS and contact with a reference electrode of silver wire coated. The required amounts of the membrane ingredients 64% (Poly (vinyl chloride),1.79% 1-Amino-9-fluorenone ion per and 32.3% Dibutyl sebacate (DBS) as plasticizer) were mixed with1.79% folic acid and dissolved in 5mL of Tetrahydrofuran. The resulting mixture was transferred into a glass dish of 2cm in diameter. The THF content of the mixture was evaporated slowly. The selectivity coefficients were determined by the separate solution method.

Table.1 Selectivity coefficients of electrode

Interferent (M)	Selectivity coefficients $K^{pot}_{FA, M}$
Pyridoxine (Vitamin B6)	10. 17x1 0^-1
Maltose	9.5x10^-1
Cobalamim (Vitamin B12)	8.6X10^-1
sucrose	7.8x10^-1
Ascorbic acid (Vitamin C)	6X10^-1
Starch	1.2x10^-1

Table. 2 Optimization of membrane ingredients

Membrane type(Plasticizer)	Correlation coefficient	Slope mV/decade	Lower Limit of Detection (M)	Linear range(M)
DOP	0.96107	17.1	1×10^{-7}	1×10^{-7} to 1×10^{-2}
DBS	0.98389	24	1×10^{-7}	1×10^{-7} to 1×10^{-2}
PVC	0.95163	22	1×10^{-7}	1×10^{-7} to 1×10^{-2}

RESULTS AND DISCUSSION

Response time

The response time of the examined electrode was recorded by changing the Folic acid concentration $1x10^{-2}$M, $1x10^{-3}$M and $1x10^{-6}$M in to a stirred Folic acid solution. In this study, the practical response time of the sensor was recorded by changing the folic acid concentration in solution, over a concentration range of 10^{-2}M, 10^{-3}M and 10^{-6}M.The potentials vs. time traces are shown in Fig.3. It is noticed that stable potentials were attained within 21s. This short response time. In order to examine the stability of the potential reading of the electrode, it was also put in a 1.0×10^{-2}M of Folic acid. As the results, the electrode reaches its equilibrium response in a very short time 21s and remained constant for the response time and was remained unchanged by varying concentration and remained constant for approximately 6 min and Lifetime of the electrode there months.

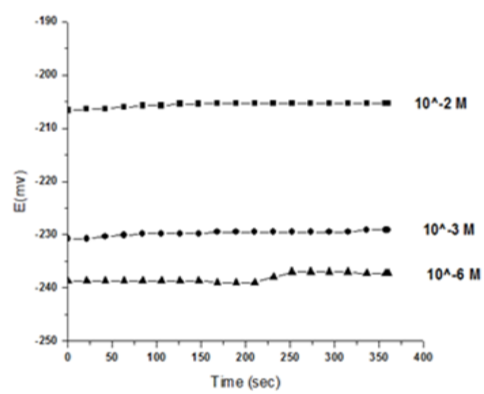

Fig. 3 Response time of the folic acid concentrations of 1×10^{-2}M ,$1X10^{-3}$M and $1x10^{-6}$M DBS membrane

Table.3 Performance characteristics of folic acid membrane sensor

PARAMETER	Folic acid
Slope, (mv/decade)	24.1
Correlation coefficient	0.98389
Linear concentration range,(M)	$1X10^{-7}$-$1X10^{-2}$
Life time	3 months
Lower Limit of Detection, (M)	$1X10^{-7}$
Response time, (S)	21
Working range ,PH	5-7

Effect of pH

The effect of pH on the response of membrane electrode was examined by use $1X10^{-3}$M of Folic acid. The potential responses indicate no significant changes at pH 5-7 in fig.4 but at higher pH than 7 the potential decreases . and the slope of the sensor respond decreased from 24.1 to 24mV per deca.

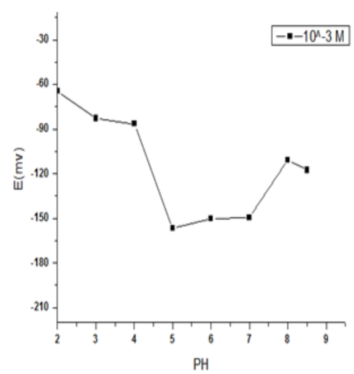

Fig.4 Effect of the pH of the test solution on the potential response of the $1X10^{-3}$ M DBS membrane

Analytical application of the electrode

Preparation of Solutions Samples:

Four tablet was powdered in a grinding mortar and then the whole powdered amount was transferred to a100ml were made up to the mark with diluting solution were prepared in bidistilled water .after that token Stock solution (1, 2, 3, 4,5,6,7,8,9and 01mL), were transferred to a 20mL volumetric flask and then were made up to the mark with diluting solution and draw relationship between potential and volume and concentration.

The membrane electrode can be successfully used to determine folic acid concentration in pharmaceutical preparations. Table.(4). shows the results obtained for determination in vitamin B9 using potentiometric (multiple-standard addition) method. Fig.5 and 6 show typical plots obtained for multiple $1x10^{-3}$M standard additive procedure. the results are in good agreement by another techniques and are within acceptable range of error. Table(4) show the sample analyzed by the standard addition method presented recoveries close to 99%(r=0.98), indicating that this electrode can be used for determination of folic acid in pharmaceutical samples.

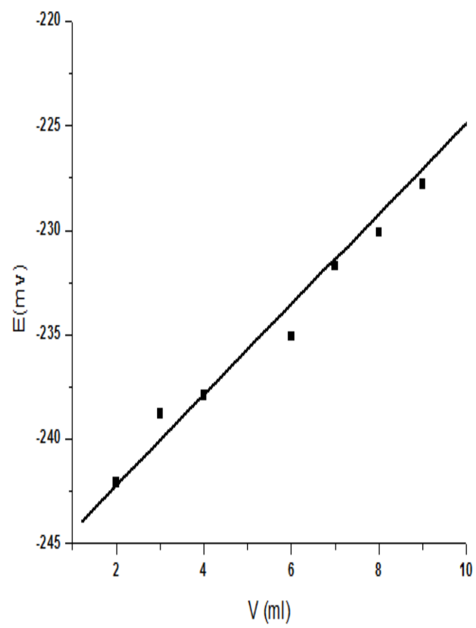

Fig.5 Relationship between potential and volume of the DBS membrane

Table (4) Results of optimization, precision and accuracy folic acid

S.N.	Parameter	Selected values
1	Correlation coefficient	0.98355
2	Regression equation	Y = -246.22667+2.14333 X
3	Standard deviation(SD)	0.72473
4	Number points	7

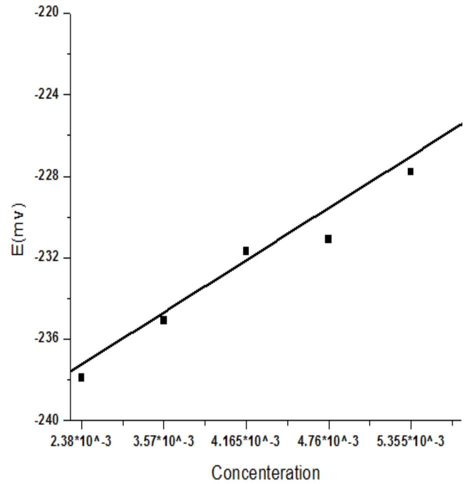

Fig.6 Relationship between potential and concentration of the DBS membrane

CONCLUSION

The data from the proposed sensors in this paper for Folic acid with those reported in literature is summarized in this paper. It can be concluded that the present studies have helped in developing the best sensors for the Folic acid. The propose sensors are very easy to prepare and show sensitivity and wide dynamic range. High selectivity, low detection limit and rapid response make these electrodes suitable for measuring the concentration of the Folic acid in wide variety of drugs.

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