In vitro Antioxidant Activity and Toxicity Studies of PANI Polymer

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Abstract

PANI polymer was prepared according to the procedure mentioned in the experimental part. Infrared (IR) Spectroscopic and X-ray diffraction were used to characterize the molecular structure of synthesized PANI polymer. The X-ray diffraction pattern reveals PANI structure indexed in a pseudo-orthorhombic lattice. The broadening of (110) reflection in the PANI sample has been analysed in terms of domain length and strain using a single-line method employing the Voigt function. The antioxidant activity of the PANI sample has been investigated using a total antioxidant assay by employing UV-visible spectroscopy. PANI exhibited effective antioxidant activity which has been found to increase with increasing concentration. Determination of the median lethal dose (LD_{50}) revealed that PANI polymer has no clear toxic effect on albino mice.

Keywords: PANI, Antioxidants, $LD_{50.}$

الخلاصة

تضمن هذا البحث تحضير بوليمر بولي انيلين تبعاً للطريقة المختبرية المتعارف عليها وشخصت بطريقة التحليل الطيفي تحت الحمراء وحيود اشعة (x–ray) وعند دراسة الفعالية ضد الاكسدة لهذا لبوليمر تبين ان له فعالية ضد الاكسدة تزداد بازدياد التراكيز. ان تحديد الجرعة النصفية القاتلة تبين ان البوليمر المحضر في هذا البحث غير سام عند حقن فئران الالبينو بها.

Introduction

PANI is in a group of π – conjugated polymers, its stability under various experimental conditions, its solubility in various solvents, and its interaction with acid or base, make it to be used as a material for a wide range of applications [1, 2]. PANI compounds have been widely used in different fields of medicine such as the development of artificial muscles [3], and controlled drug release [4], they have low cytotoxicity and low inflammation [5] and stimulation of nerve regeneration [6]. protection of humans against oxidative stress as well as prophylactic of cancer and other incurable diseases are important directions in medicine and biochemistry all over the world. oxidative stress arises in a biological system after an increased exposure to oxidants, a

decrease in the antioxidant capacity of the system, or both. It often leads to the generation of reactive oxygen species (ROS) including free radicals [7]. The free radical damage to the living tissue is already well established and recent studies indicate that the progression of various diseases such as inflammation, infection, cardiac and cerebral ischemia, reperfusion injury, neurodegenerative diseases, cardiovascular diseases, cancer, and aging are caused by the uncontrolled oxidation of lipids, proteins, and DNA in biological system [8]. Antioxidants play a major role in protecting biological systems against many incurable diseases. The antioxidants have been widely used in different fields of industry and medicine as substances, that interrupt radical–chain oxidation processes, improve general health, help cell rejuvenation, and prevent cancer [9]. The reduction of oxygen in tissue by:

$O_2 + e^- \rightarrow O_2^{\cdot -}$

$O_2^{\cdot -} + H^+ \rightarrow HO_2^{\cdot}$

$HO_2^{\cdot} + H^+ \rightarrow H_2O_2$

$H_2O_2 + 2H^+ + 2e^- \rightarrow 2H_2O$

Thus, antioxidant interaction with oxygen and the (ROS) proceeds to the general mechanism:

$R\text{-}H + O_2 \rightarrow R + HO_2^{\cdot} + e^- + H^+ \rightarrow R + H_2O$

Which, includes the preceding protonation reaction of oxygen by antioxidants [10].

In this paper, we present a detailed synthesis, characterization, antioxidant activity, and toxicity studies of PANI polymer. Infrared spectra and x-ray diffraction were used to characterize the molecular of the synthesized PANI antioxidant activity of PANI has been determined by using the total antioxidant activity method by employing UV–visible spectroscopy, and the concentration dependence of antioxidant material has been examined. In vivo, investigation of the toxicity of PANI has been determined by measuring the median lethal dose (LD_{50}), which indicated no clear toxic effect on albino mice used for this study.

Experimental details

1- Preparation:

The chemical polymerization of PANI in aqueous acidic solution was carried out by dissolving (11-5 gm) ammonium per sulfate in (250ml) of 1M HCL previously cooled to 0 c^0. The solution was added slowly by dropping wisely through (one hour) to (20ml) aniline dissolved in (250 ml) 1M HCL pre-cooled to 0 c^0.

The reaction mixture was then left for (3-4 hours) with continuous stirring. The precipitated green (PANI-HCL salt) was separated and washed consecutively with distilled water, methanol &diethyl ether and then dried for 48 hours under vacuum (10 mmHg). The (PANI-HCL) was further washed with DMF and dried following the same previous condition. The (PANI-base) was obtained by deprotoduation of (PANI-HCL) by adding 3% ammonia solution and stirring for 2 hours.

(PANI-base) was separated washed and dried. it is washed with methanol and diethyl ether and then dried. [11]

2-Structural characterization:

X-ray diffraction was used to characterize the molecular structure of synthesized PANI. X-ray diffraction pattern collected using a rigakuminiflex diffractometer with Cuk_{α}radiation (λ=1.5406 A^{o}) the angular range spread over the region between 10^{o} and 60^{o} in (2θ), insteps of (0.01^{o}). The X-ray measurements were undertaken with a view to understanding the structural details of the material as well as to evaluate the domain length (L) and microstrain in the PANI.

3-Antioxidant activity assay:

For total antioxidant activity assay [12], various concentrations of the prepared PANI (0.2-1.0 mg/ml)dissolved (100 µl) of (1- methyl –2 – pyrrolidain) is mixed with (2 ml) of linolic acid emulsion in sodium phosphate buffer (PH 6.6) in a test tube and kept in dark at (37^{o}C) to accelerate oxidation, after incubation for (15 h), (0.1 ml) from each tube is mixed with (70 ml) of 80% methanol in deionized water and the absorbance of the mixture is measured by using the Shimadzu 2450 UV-VIS spectrophotometer at the wavelength (516 nm) against ascorbic acid was used as a standard and the total antioxidant activity is expressed as equivalents of ascorbic acid, the total antioxidant activity is calculated as follows:

$$\text{Antioxidant activity \%} = (A_o - (A_1/A_o)) \times 100$$

Where A_o and A_1 is the absorbance of control and of test respectively.

4- Median lethal dose(LD50)

In this experiment Albino mice were used, BALB/C strain (male and female),36mouse were used and divided into six groups each group contain 6mice,(3 males, 3 female), the first group was considered as the control group which was administered orally with 1ml(0.1 solvent+0.9 Distilled water), while the other groups were administered 1ml of(o.1 of the solvent with polyaniline (pAni)+0.9Distilled water)contain different concentrations of polyaniline (0.5,1,3,5,and7 mg/kg), the mice watched for 72 hours and the mortality percent were recorded. The results are analyzed using the probit_analysis method to detect the LD_{50} value. [13]

Results and Discussions

1-Figure (1) shows the IR spectrum of PANI The most important vibrational bands at (3263) cm^{-1} due to the (N-H) str. Vibration. Which were characteristic secondary amine compounds. [14,15]. Moreover, the absorption band appeared at (3075) cm^{-1} due to the aromatic (C-H) str.Vibration[14,15].Also, the spectra appeared at (1585) cm^{-1} which were characteristic aromatic (C=N) str.vibration[14,16]. The peak at (1506) cm^{-1} was due to the aromatic (C=C) str.vibration which was characteristic of the aromatic ring (2) The spectra showed vibration bands between (1301) cm^{-1} and (1242) cm^{-1} Which be assigned to the vibration of the aromatic amine (C-N) str.[14,15]. Furthermore, the spectra at (1159) cm^{-1} attributed to the aromatic(C-N-C) band vibration. While the absorption band at (835) cm^{-1} is attributed to the (o.o.p) aromatic (C-H) band vibration. [15].

Str.= stretching , o.o.p. = out of plane

2-Figure (2) Shows the X-ray diffraction pattern over the entire (2θ) range from 10º to 60ºand the most intense (110) reflection peaks of the PANI sample. PANI exists in two different crystalline forms depending upon the method of preparation, namely emeraldine salt I (ESI) and emeraldine salt II (ESII). The (ESI) form can be indexed with a pseudo–orthorohombic cell with lattice parameters (a= 4.3 Aº), (b=5.9 Aº), (C=9.6 Aº) and (V=245 A^{o3}) [17]. All the main reflections observed in the x-ray pattern of PANI at (2θ) are (16.1º, 20.6º, 26.6º 29.0º 31.7º) as shown in Figure 1. Resemble the (ESI) crystalline form. A single–line method employing a Voigt function has been used to analyse the origin of line broadening in the X-ray diffraction pattern [18]. a well–annealed Cu powder sample was used for the correction of instrumental broadening. The contribution of crystallite size (referred to as domain length (L) in the case of polymers) and strain towards line broadening has been separately calculated as given in Table 1. Which is also includes the values of the d – d-spacing deduced from the angular positions (2θ) of the observed reflections using Bragg' 's formula. The domain length for (110) reflection in the case of (ESI) varies from 20 to 70 Aº [12], in the PANI sample is (38.69Aº) and the contribution of strain to the (110) reflection of the observed x-ray line broadening is (0.76%). The x-ray pattern thus reveals the conformation of the PANI backbone chain.

3-The total antioxidant activity of different concentrations of PANI is shown in Table 2. PANI which was exhibited effective antioxidant activity in this system. All the ground data were statically evaluated by Excel software and all the results were expressed as mean ± S.D. for five experiments in each P values of less than (0.05) were considered to indicate statistical significance. In the living system, free radicals are generated as part of the body's normal metabolic process. Antioxidants are radical scavengers that protect the human body against free radicals that may cause pathological conditions such as ischemia, anemia, asthma, arthritis, inflammation, neurodegeneration, Parkinson's diseases, mongolism, aging process, and perhaps dementias [19].

under most pathological conditions there is the generation of reactive oxygen species (ROS) and other free radicals, an increase in the antioxidant reserves of the organism can reduce oxidative stress and preparation of some of the antioxidant chemical compounds will help to develop new drug candidates for antioxidant therapy [20]. The total antioxidant activity of the PANI compounds was measured spectrophotometrically at (516 nm) [21]. PANI in the emeraldine oxidation state is comprised of half amine and half imine groups. A single monomer unit of emeraldine salt is capable of donating one hydrogen atom to the free radical, leading to its reduction (i.e. the emeraldine salt form gets converted into the fully oxidized pernigraniline state of PANI, which is no longer effective for scavenging free radical). The total antioxidant activity of PANI compounds increases with increasing concentration as shown in Table 2. , this is indicative of the fact that the emeraldine salt form has been fully oxidized and has lost its free radical scavenging activity. These results are consistent with those reported in the literature [21].

4-The study of LD_{50} for PANI is a very important step in detecting the harmful effect of PANI [22]. LD_{50} for PANI was found (4.25) mg/Kg shown in Figure 3. Additional studies and experiments are required to prove the importance of this compound PANI before being used as a drug in the future.

Table 1. (hkl) pseudo-orthorhombic, d spacing, domain length (L), and strain of PANI sample.

(hkl)	d (A^o)	L(A^o)	Strain(%)
010	5.477		
100	4.298		
110	3.343	38.69	0.76
111	3.073		
020	2.814		

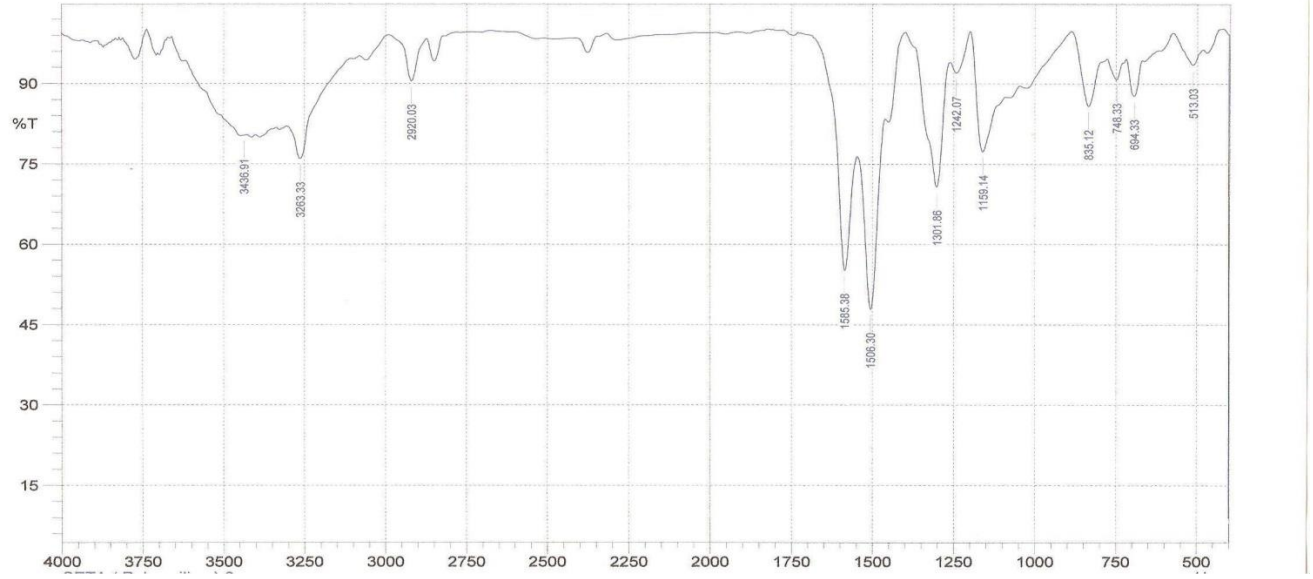

Figure 1.IR spectrum of PANI

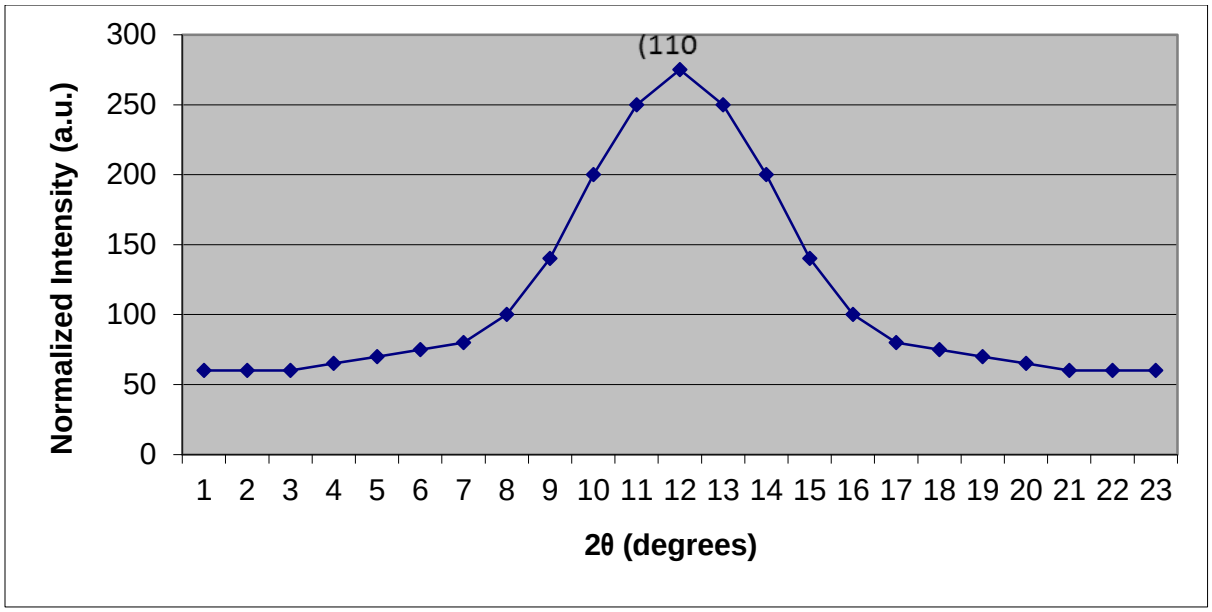

Figure 2.The X-ray diffraction pattern in the (2Θ) ranges from $10^{o}$to 60^{o} for the PANI sample. The peak has been included in a pseudo–orthorhombic lattice cell.

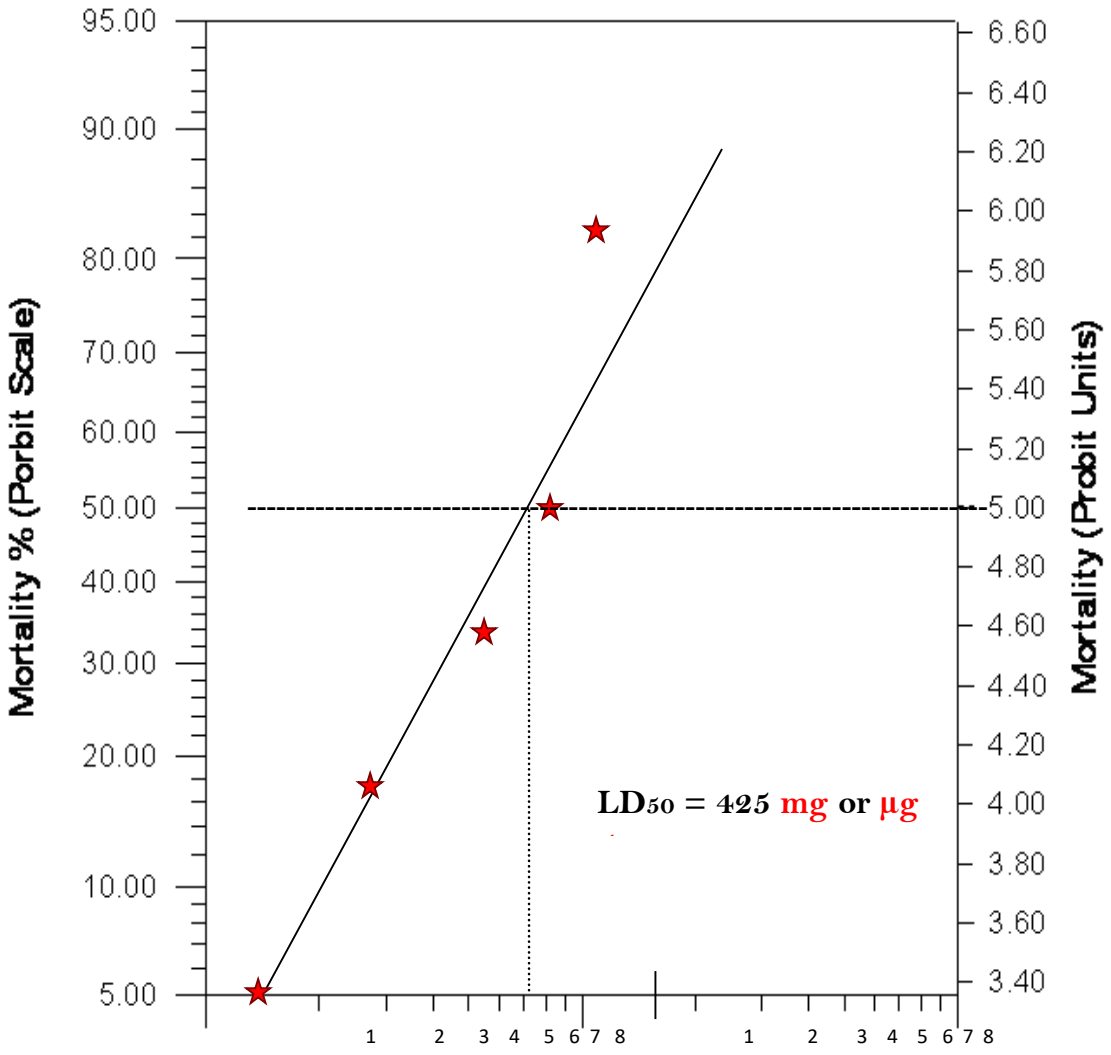

Figure (3) Midian lethal dose for PANI

Table 2. Total antioxidant activity and statical analysis of different concentrations of PANI

sample	Cons. Mg/ml	mean	Stander deviations	P value	Significant	Total antioxidant activity %
Ascorbic acid	0.2	2.042	0.412408	0.028506665	Sig	56.77591
PANI	0.2	3.0104	0.883018			
Ascorbic acid	0.4	2.1212	0.383198	0.020893437	Sig	59.3197
PANI	0.4	3.2412	0.714849			
Ascorbic acid	0.6	2.1798	0.378438	0.013921533	Sig	62.70887
PANI	0.6	3.3846	0.696773			
Ascorbic acid	0.8	2.2248	0.374717	0.01063	Sig	65.90862
PANI	0.8	3.4834	0.684047			
Ascorbic acid	1	2.286	0.896087	0.011677	Sig	70.6035
PANI	1	3.6118	0.736029			

Conclusions:

In the present work, we have investigated the antioxidant activity and toxicity of PANI polymer, the results of this study indicate that:

1-PANI polymer is not toxic as reflected by the measurement of (LD_{50}).

2-polyaniline polymer possesses powerful in vitro antioxidant activity and its antioxidant activity increases with increasing concentration. the PANI merits further investigation in animal models to confirm its antioxidant activity in vivo which may result in a modern drug.

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