Synthesis, Spectroscopic and Electrochemical Characteristics of a Novel Schiff-Base from Saccharin and Tryptophan

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In the present work, a novel Schiff-base was synthesized by the reaction of saccharin with tryptophan and characterized by elemental analysis as well as UV-Vis, FT-IR, ^{1}H NMR spectroscopic data. The voltammetric behavior of Schiff-base was also studied on the hanging mercury drop electrode (HMDE) by using Square-Wave Voltammetry (SWV) and Cyclic Voltammetry (CV) techniques. The voltammograms of the Schiff-base exhibited two irreversible reduction peaks in Britton-Robinson buffer (pH 7.0-10.0) for the potential range from 0.0 V to -1.4 V. These peaks which appeared at more positive potentials than the reduction peaks of tryptophan and saccharin, may be assigned to the cathodic reductions of $C-N^+$ and $>C=N-$ moieties of Schiff-base.

INTRODUCTION

Tryptophan (Trp) is an amino acid and essential in human nutrition. Trp is also a precursor for serotonin, a neurotransmitter, and melatonin, a neurohormone [1]. In addition, Trp is an important and frequently used starting material in the chemical synthesis of a range of pharmaceuticals [2]. Some of its derivatives are potent drugs [3]. The complexes of Trp with metal ions have recently been receiving great attention. In a previous study [4], we have already reported the binary and ternary complexes of Trp with Cu(II).

Saccharin (*o*-sulfobenzimide) is widely used as an artificial sweetening agent [5-7]. The chemistry of saccharin has attracted attention because of its suspected carcinogenous nature [8,9] and potential use as an antidote for metal

poisoning [10]. A lot of saccharin binary and ternary complexes have been reported and investigated by means of electrochemical and spectroscopic techniques [11,12]

Schiff-bases are considered as a very important class of organic compounds. Azomethine compounds have wide applications in many biological aspects, proteins, visual pigments, enzymic aldolization and decarboxylation reactions [13-16]. Moreover, some Schiff-bases and their metal complexes exibit antibiotic, antiviral and antitumor agents' bilogical activity [17].

Limited information has been reported on Schiff-bases of saccharin [18,19] and tryptophan [20-24] in the literature. However, a novel Schiff-base of cysteine and saccharin has been recently synthesized and characterized by Çakır *et al*. [25]. Yet, nothing new on the interaction mechanism of saccharin with tryptophan is reported. In this study, we report the synthesis, characterization and voltammetric behaviour of Schiff-base of saccharin with tryptophan. The reaction of

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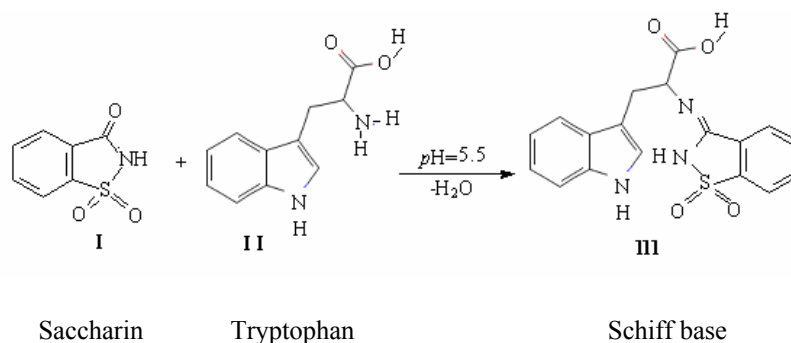
Scheme 1. The formation of the Schiff-base from the reaction of saccharin with tryptophan

saccharin with tryptophan proceeded spontaneously at room temperature in aqueous solution, one product of which was isolated at pH 5.5 in a 1:1 ratio (Scheme 1).

EXPERIMENTAL

Synthesis of Schiff-Base

The Schiff-base was prepared by the reaction of saccharin and tryptophan. Aqueous solution of tryptophan (0.01 M) was added to a magnetically stirred aqueous solution of sodium saccharin (0.01 M) in the stoichiometric ratio and then pH of the mixture was adjusted to *ca*. 5.5 with NaOH. The reaction mixture was continuously stirred with a constant temperature about 45 ºC for 4 h. After the evaporation of most of the solvent at room temperature for 7-8 weeks, a dark white solid compound was obtained. After the filtration processes, the compound was washed by ethanol and dried at room temperature.

The structure of Schiff-base ($C_{18}H_{15}O_4N_3S.3H_2O$) was confirmed by elemental analyses, UV-Vis, FT-IR and ^1H NMR spectra. The reagents and solvents used in this work were obtained from Sigma or Merck and used without further purification. Triply-distilled and deionised water was used in all solutions.

The yield of the presented reaction (Scheme 1) was within the range of 65-68%, m.p.: 255.5-257 ºC. Elemental analyses were carried out at TÜBİTAK Laboratories of Ankara Research Center. Anal. Calcd. for Schiff-base ($C_{18}H_{15}O_4N_3S.3H_2O$): %C, 51.05; %H, 4.96; %N, 9.93; %S, 7.59. Found: %C, 50.82; %H, 5.41; %N, 9.88; %S, 7.52.

Voltammetric Measurements

The voltammetric measurements were carried out using an EG&G PAR Model 394 with a computer loaded with the software package polarographic analyzer connected to an EG&G PAR Model 303A polarographic electrode (Princeton, NJ, USA). A three-electrode was employed, containing a hanging mercury drop electrode (HMDE) as the working electrode, an Ag│AgCl│saturated KCl as the reference electrode and a platinum wire as the auxiliary electrode. The solution in the electrochemical cell was stirred with a magnetic stirrer (305-PAR) and a stirring bar to provide the convective transport during the preconcentration step. All potentials are referred to the Ag│AgCl│KCl$_{sat.}$ electrode.

The voltammetric measurement of each solution was carried out in a Britton-Robinson buffer of pH 7.0-10.0 as supporting electrolyte. Before each experiment, the electrolyte was purged with nitrogen for 8 min. A known volume of standard solution of compounds was added to the polarographic cell, deaerated and then the square-wave voltammogram recorded.

Spectroscopic Measurements

The FT-IR spectrum (4000-400 cm^{-1} regional) was recorded in KBr pellets with a Jasco FT-IR 350 Spectrometer. The electronic absorption spectra in the 800-200 nm range were recorded on Unicam V2-100 UV-Vis spectrophotometer using 1 cm quartz cells. NMR spectra were measured in D_2O solutions using tetramethylsilane (TMS) as internal standard and recorded on a BRUKER AVANCE DPX-400 spectrometer.

RESULTS AND DISCUSSION

Voltammetric Studies

For the formation of the Schiff-base from the reaction taking place between tryptophan (as a primary amine) and saccharin (as a carbonyl compound) voltammetric techniques were employed. The electrochemical behavior of Schiff-base was investigated at pH range 7.0-10.0 in various supporting electrolytes using SWV and CV electrochemical techniques on an HMDE. The best results with respect to peak enhancement, baseline current and reproducibility were obtained in B-R buffer solutions.

SWV voltammograms of tryptophan and saccharin in Britton-Robinson buffer (pH 7.0), for the potential range from 0.0 V to -1.8 V produced a cathodic peak at -1.603 V and -1.420 V, respectively (Figs. 1A and 1B). These irreversible peaks are attributed to the catalytic hydrogen evolution [4,26].

The nature of electrochemical process of Schiff-base was studied by SWV and CV. Schiff-base gave two reduction waves in Britton-Robinson buffer (pH 7.0-10.0) for the potential range from 0.0 V to -1.4 V. As can be seen in Fig. 2, the CV of Schiff-base at pH 9.0 gave two irreversible reduction peaks at -0.820 V (E_{p1}) and -1.276 V (E_{p2}), respectively. These peaks at -0.820 V and -1.276 V were not seen in the voltammograms of free tryptophan and saccharin without an azomethine group (>C=N-) (Table 1).

At the same time, the Schiff-base was reduced at more positive potentials than free tryptophan and saccharin (Table 1). The effect of scan rate (v) on the voltammetric behavior of Schiff-base was studied. The peak current changes linearly with scan rate (v) according to the equation $i_p = Av^x$ [27]. The x values 1.0 and 0.5 are expected for adsorption-controlled and diffusion-controlled reactions, respectively [28,29]. The plots of $\log i_p$ $vs.$ $\log v$ are given in Fig. 3. When the potential was scanned at increasing rates from 200-1000 mV s^{-1}, the linear relationships between $\log i_p$ and $\log v$ were observed (Fig. 3). The experimental results are listed in Table 2. As can be seen in the table, the slope values of the former and second peaks indicate that the reduction currents of Schiff-base had contributions from both diffusion and weak-adsorption currents.

According to theory, the full width, $\Delta E_{p,1/2}$, at half-peak height depends on the reversibility of redox couple. It should
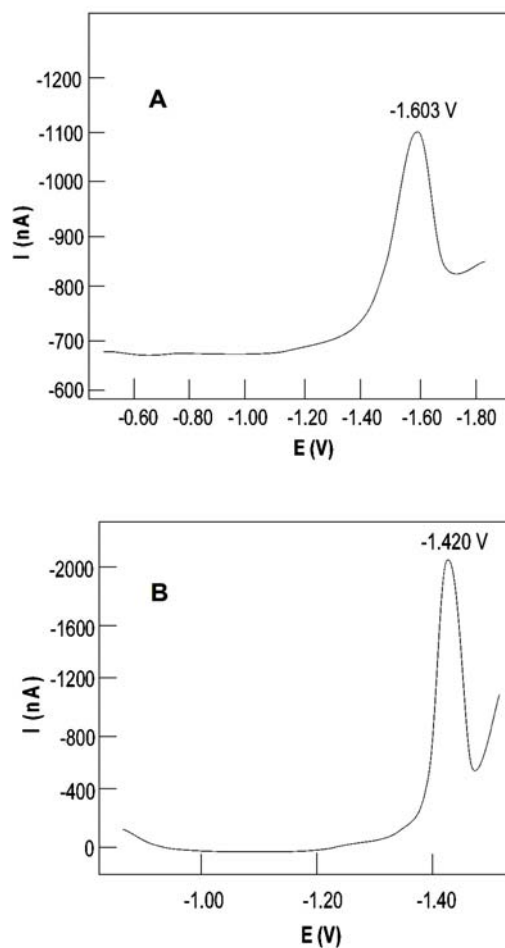

Fig. 1. Square-wave voltammograms of 1×10^{-5} M tryptophan (A) and 1×10^{-5} M saccharin (B) in Britton-Robinson buffer (pH 7.0). Experimental conditions: pulse height, 20 mV; scan increment, 2 mV; equilibrium time, 5 s; frequency, 100 Hz; drop size, large (HMDE).

be equal to $\Delta E_{p,1/2} = 90.6/n$ mV (at 25 °C) [30] for ideal Nernstian reversible reaction ($E_{pc} = E_{pa}$; when $n = 2$, $\Delta E_{p,1/2}$ should be 45.3 mV) and $\Delta E_{p,1/2} = 62.4/\alpha n$ [31] for irreversible electrochemical process (when $n = 2$ and $\alpha = 0.5$, $\Delta E_{p,1/2}$ should be 62.4 mV), where α is the transfer coefficient and n is the number of electrons transferred. From the CV voltammogram (Fig. 2), the half-peak width, $\Delta E_{p,1/2}$, values, of irreversible peaks at -0.820 V (E_{p1}) and -1.276 V (E_{p2}) were estimated to be 62.50 and 68.75 mV, respectively. In general, α is taken as 0.5 for an irreversible reaction [32] and thus by
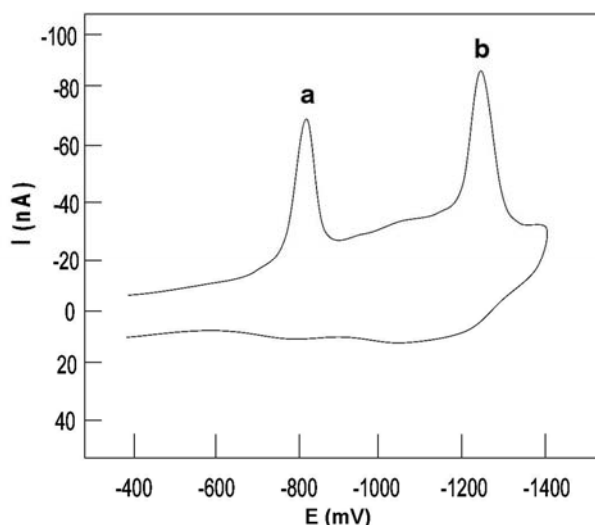
Fig. 2. Cyclic voltammogram of the Schiff base (4×10^{-5} M) in Britton-Robinson buffer (pH 9.0). Experimental conditions: scan rate, 200 mV s^{-1}; equilibrium time, 5 s; drop size, large (HMDE). (a) the reduction peak of C-N$^+$ moiety; (b) the reduction peak of >C=N- group of Schiff-base.
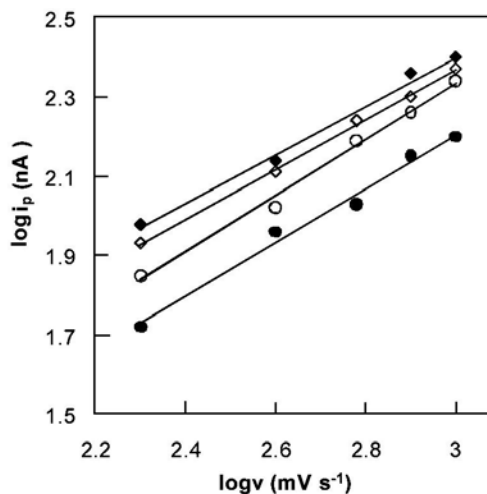

Fig. 3. The plots of logi_p vs. log v for Schiff-base. (●) The reduction peak of C-N$^+$ moiety at pH 7.4; (♦) the reduction peak of >C=N- group of Schiff base at pH 7.4; (○) the reduction peak of C-N$^+$ moiety at pH 9.0; (◊) the reduction peak of >C=N- group of Schiff-base at pH 9.0.

Table 1. The Square-Wave Voltammetric Characteristics of Electro-Active Centers of the Compounds in Britton-Robinson Buffer (pH 7.0 and 9.0)

Compounds	E_{pc} (V) for C-N$^+$ (irreversible)		E_{pc} (V) for >C=N- (irreversible)		E_{pc} (V) for hydrogen wave (irreversible)	
	pH 7.0	pH 9.0	pH 7.0	pH 9.0	pH 7.0	pH 9.0
Tryptophan	-	-	-	-	-1.603	-1.628
Saccharin	-	-	-	-	-1.420	-1.435
Schiff-base	-0.760	-0.820	-1.234	-1.276	-	-

Table 2. The Cyclic Voltammetric Data Obtained from the Plot of logi_p (nA) vs. log$v$ (mV s^{-1})
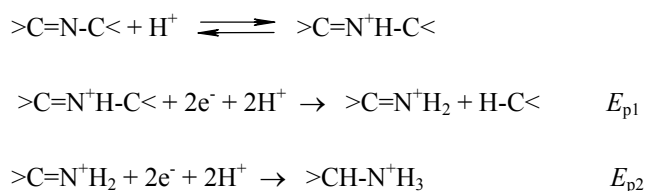
pH	C-N$^+$ Equation	>C=N- Equation
7.4[a]	logi_p = 0.6799 log$v$ + 0.1655 r² = 0.9884	logi_p = 0.6138 log$v$ + 0.5569 r² = 0.9882
9.0[b]	logi_p = 0.71 log$v$ + 0.2036 r² = 0.9919	logi_p = 0.6293 log$v$ + 0.4809 r² = 0.9985

[a][Schiff-base] = 8×10^{-5} M. [b][Schiff-base] = 4×10^{-4} M.

substitution of these values into the above equations, the n values for the reduction reactions observed at -0.820 and -1.276 V are equal to about 2.

The irreversible peaks at -0.820 and -1.276 V are considered as the cathodic reductions of C-N$^+$ and >C=N- moieties of Schiff-base, respectively. The C-N$^+$ bond is reduced in the protonized form only, but not in the free base [33]. Schiff-bases are also reduced in the protonated form [33]. The reduction peak of azomethine group was previously reported at about -1.2 V in Britton-Robinson buffer (pH 7) [34].

The potentials of the peaks at -0.820 and -1.276 V shift to more negative potentials with increasing pH from 7.0 to 10.0. The shift confirms the involvement of protons in the electrode reaction [35]. The relationship between the peak current and pH value of Britton-Robinson buffer was also investigated. The cathodic peak currents for these peaks decrease with the increasing pH from 7 to 10. If a redox reaction includes a protonation step, the currents become pH dependent. According to Lund's review [36], the reduction of Schiff-base may be interpreted as follows:

$$>C=N-C< + H^+ \rightleftharpoons >C=N^+H-C<$$

$$>C=N^+H-C< + 2e^- + 2H^+ \rightarrow >C=N^+H_2 + H-C< \qquad E_{p1}$$

$$>C=N^+H_2 + 2e^- + 2H^+ \rightarrow >CH-N^+H_3 \qquad E_{p2}$$

Spectrophotometric Measurements

Electronic spectra. The absorption spectra of tryptophan, saccharin and Schiff-base are shown in Fig 4. Electronic spectra were recorded in water between 200 and 400 nm. In UV-Vis spectrum of saccharin (Fig. 4), there are two maximum absorption bands (λ_{max}) at 201 and 266 nm which are related to $\pi \rightarrow \pi^*$ transitions. These bands are in accord with the literature data [37]. UV-Vis. spectrum of tryptophan (Fig. 4) exhibits three maxium absorption bands at 220, 245 and 277 nm, respectively. The aromatic amino acids are responsible for the ultraviolet absorption of most proteins with maxima between 275 and 285 nm [38]. Tryptophan is known to have a strong spectroscopic signature at *ca*. 270 nm that arises due to excitation of $\pi \rightarrow \pi^*$ transitions in the indole part

of the molecule [39]. All these transitions are also found in the spectra of the Schiff-base, but they are shifted towards lower and higher frequencies, confirming the coordination of saccharin with tryptophan.

On the other hand, the electronic absorption spectrum of the Schiff-base is characterized by six main bands at 202, 217, 238, 280, 292 and 309 nm, respectively (Fig. 4). The first three bands (λ_{max}) at 202, 217 and 238 nm can be assigned to the medium energy π-π^* transition of the aromatic ring, while two other bands (λ_{max}) at 280 and 292 nm are due to the low energy π-π^* transition. The last or the third band (λ_{max}) at 309 nm is due to the excitation of the electrons of the azomethine group [40] which corresponds to an intra-ligand n$\rightarrow\pi^*$ transition [41]. In summary, the UV-Vis. data presented above provide strong support for the formation of the Schiff-base between tryptophan and saccharin.

FT-IR spectra. The FTIR spectrum of tryptophan was introduced in Fig. 5. This spectrum accords with that previously reported for L-tryptophan [42]. As can be seen in Fig. 5, tryptophan has the IR absorption peak of the carboxylic acid group at 1675 cm^{-1} [43,44] and the deformation vibrations in the region between 700 and 500 cm^{-1} [42]. In Fig. 5, the vibrational bands of the indole ring in the low frequency region (1300-700 cm^{-1}) are clearly observed. The bands obtained at 1230, 1160, 1115 and 1045 cm^{-1} are assigned to the in-plane deformation of C-H (β_{CH}) in indole ring [45]. Another major characteristic in the IR-spectrum of solid L-Trp, belonging to bending out-of-plane indole CH was observed at 770 cm^{-1} [46,47]. This peak is also due to the out-of-plane deformation of C-H (γ_{CH}) in indole ring. For the formation of Schiff-base, this band is shifted to 750 cm^{-1}. The band located at 1375 cm^{-1} (Fig. 5) is assigned to the stretching vibration of C=C in indole ring [47,48]. Also, the IR-spectrum of Trp, shows an intensive peak at 3410 cm^{-1} of NH stretching vibration of indole ring (ν_{NH}) [49]. This signal was observed at 3400 cm^{-1} on the spectrum of Schiff-base. In addition, the intensive peaks of ν_{CH}(r) at 3100 and 3050 cm^{-1} are observed [49]. These peaks are shifted to 3090 and 3035 cm^{-1}.

The characteristic absorption bands of saccharin are C=O, S(=O)$_2$ and S-N-C. The C=O absorption of five-membered ring lactams is in the 1750-1700 cm^{-1} region [50]. The band at 1650 cm^{-1} corresponds to C=O stretching, whilst bands at 1600 and 1475 cm^{-1} are assigned to C-C stretching of the
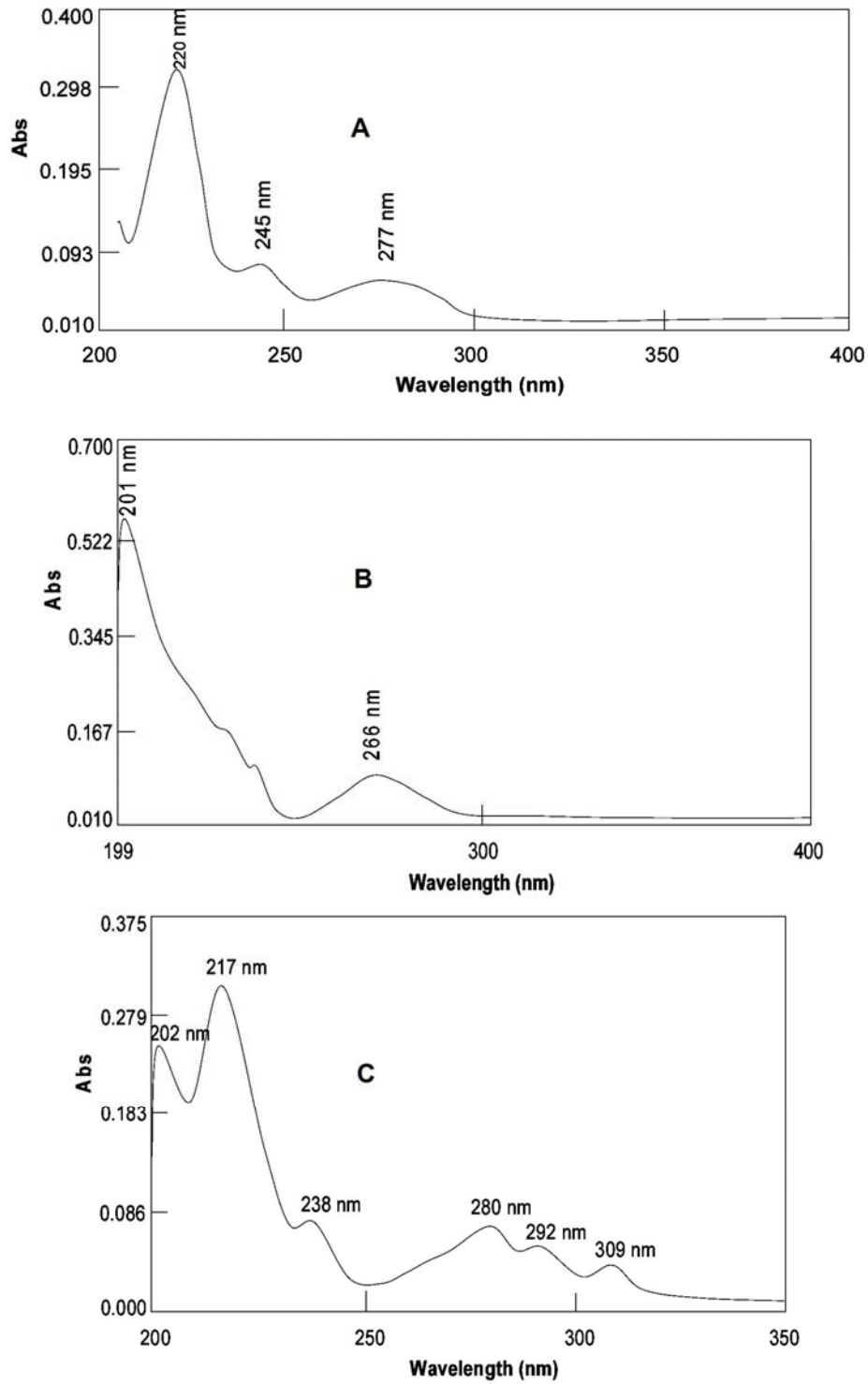
Fig. 4. UV-Vis spectra of A) Tryptophan (1.0×10^{-4} M), B) Saccharin (1.0×10^{-4} M), C) the Schiff-base solution (1.0×10^{-4} M) in water.
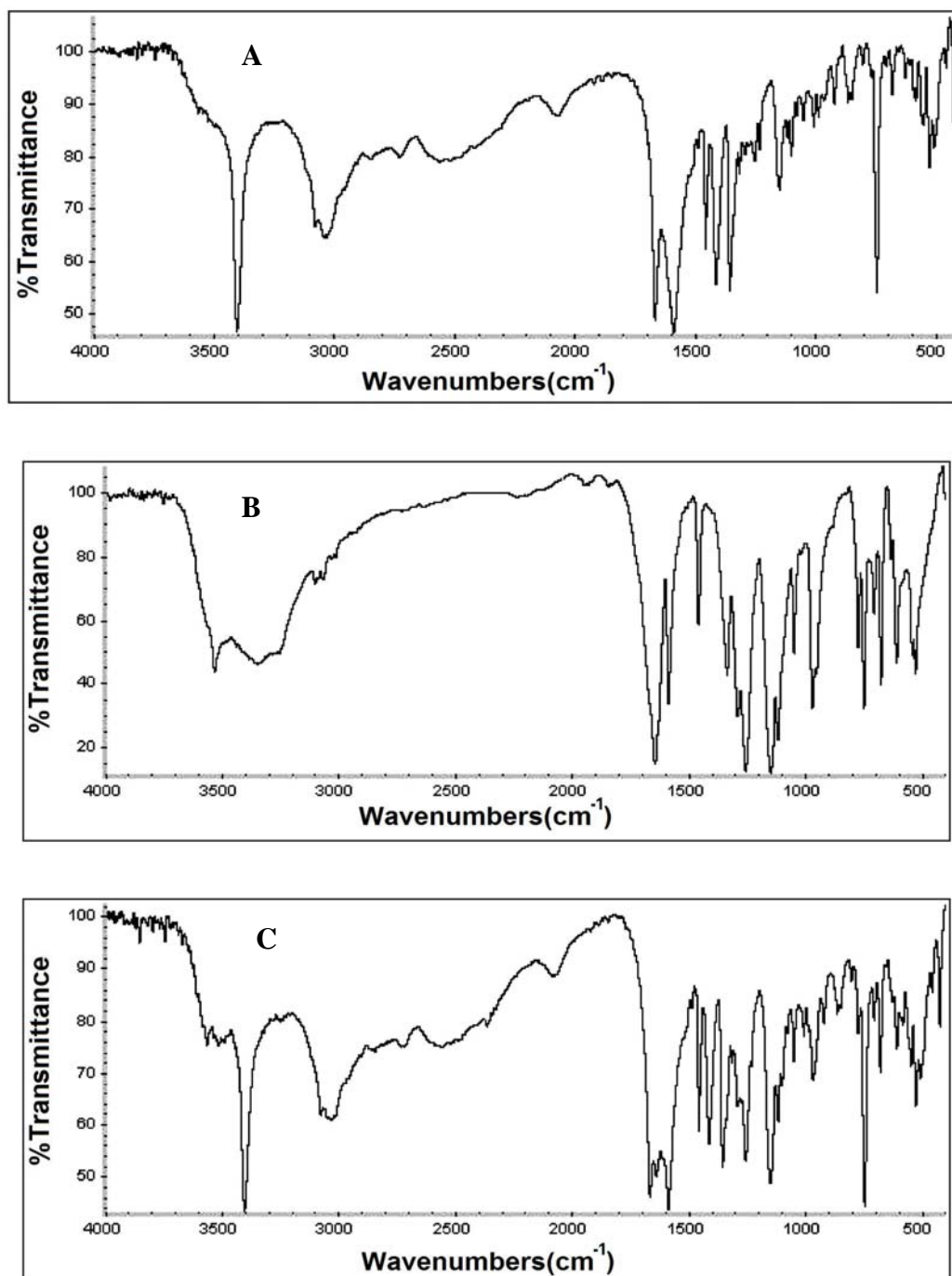
Fig. 5. FT-IR spectra of Tryptophan (A), Saccharin (B) and the Schiff-base (C).

benzene ring [51]. Also, three bands at 1350, 1260 and 1150 cm^{-1} correspond to the stretching vibrations of the -SO$_2$-N-group [51]. The asymmetric and symmetric stretching vibrations of the SO$_2$ group in the FTIR spectrum of saccharin have already been reported at 1337 and 1179 cm^{-1}, respectively [52]. Another characteristic IR absorption for

saccharin is S-N-C which appears at 975 cm^{-1} [25] (Fig. 5).

The FT-IR spectra of the reaction product reveal that tryptophan and saccharin reacted with each other and resulted in the expected compound. The FT-IR spectrum of the reaction product reveals several points of forming of Schiff-base (Fig. 5). A new band appeared at 1645 cm^{-1} in the FT-IR spectra of the product. This absorption can be attributed to the stretching vibration of >C=N- group. The stretching vibration band (>C=N-) appearing at 1645 cm^{-1} is in good agreement with the values published for similar compounds [40]. Moreover, the IR absorption peak, belonging to the COOH group was observed at 1670 cm^{-1}.

Finally, there were shifts to higher wave numbers of $S(=O)_2$ stretch bands which appeared at 1425 cm^{-1} and 1255 cm^{-1}. The presence of a heteroaromatic type structure was best recognized by the presence of =C-H stretching vibrations [53], near 3030 cm^{-1}. In this study, the FT-IR spectrum of the Schiff-base raised the peak at 3035 cm^{-1} which was designated to C-H stretching vibrations. The characteristic absorption bands of saccharin in the 1300-900 cm^{-1} region also appeared at the spectrum of Schiff-base, but their intensities decreased.

The infrared spectrum of the reaction product (Fig. 5) shows changes in the position and profiles of some bands, as compared to those of the free tryptophan and saccharin which also displays a band at 1645 cm^{-1} that is attributed to ν(C=N) stretching frequency, suggesting the activeparticipation of tryptophan and saccharin.

NMR spectra. The ^{1}H NMR spectra of the Schiff-base are consistent with the proposed structure (Fig. 6). The ^{1}H NMR spectrum recorded from D_2O solutions of tryptophan was reported by Selvakannan *et al.* [54]. The peak at 3.4 ppm (doublet) corresponds to the methylene protons and the one at 4 ppm corresponds to the α-protons in the tryptohan molecule [54,55]. Moreover, the multiplet peaks around 7-8 ppm represent the aromatic protons in the tryptophan molecule [54,55]. Compared with the ^{1}H NMR spectrum data of pure tryptophan [54], the ^{1}H NMR spectrum of the Schiff-base exhibits a slight up-field chemical shift of all protons. According to this observation, it can be said that the electronic environments of the tryptophan protons change with the formation of the Schiff-base. The peaks at 3.8 ppm correspond to the protons attached to the neighboring α-C to the C=N group of Schiff-base which are shifted relative to the value of pure tryptophan (4 ppm). Accordingly, it can be claimed that the reacting of tryptophan with saccharin occurs by the primary amine group. At ^{1}H NMR spectra of the Schiff-base (Fig. 6), the peaks around 7-8 ppm correspond to the aromatic protons in the molecule. Ramesh and Brown reported that
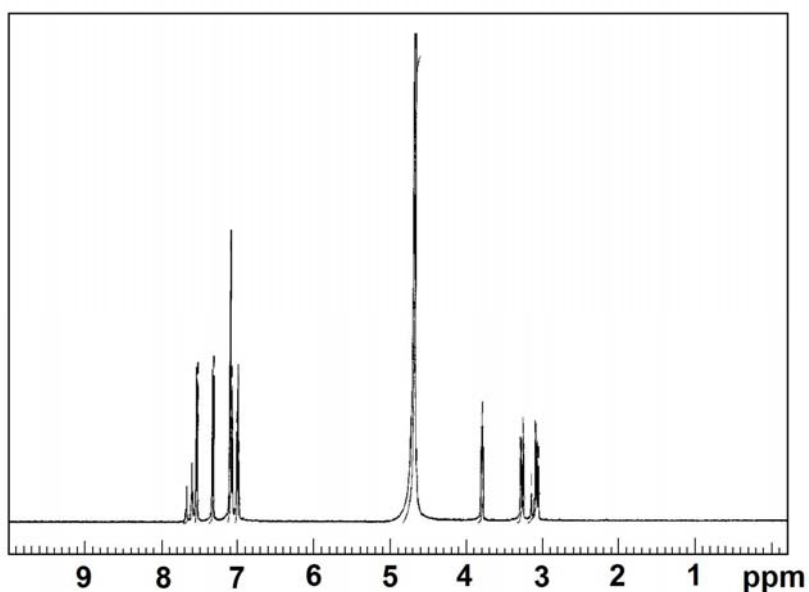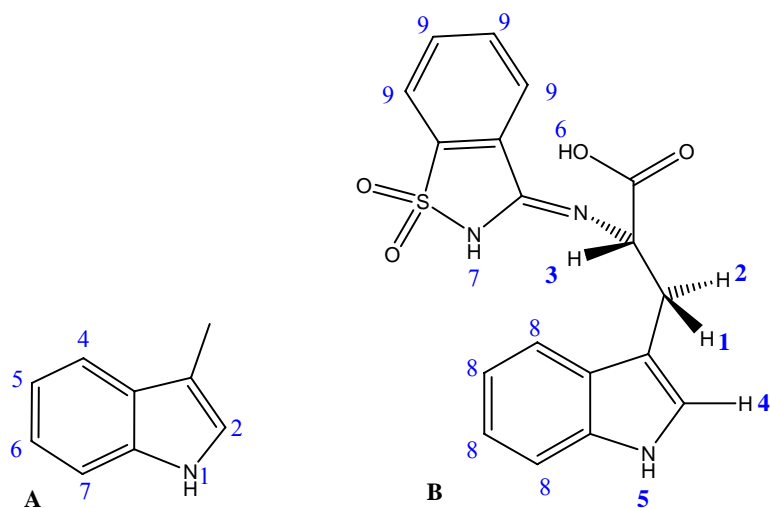

Fig. 6. The ^{1}H NMR spectrum of the Schiff-base.

Scheme 2. The numbered protons A) indole ring of L-tryptophan; B) Schiff-base

except for NH, other proton resonances of aromatic indole ring (Scheme 2A) of L-tryptophan after binding to TRAP (*trp* RNA-binding attenuation protein) shifted to high-field [56]. The peaks (which belong to the aromatic protons in the indole ring) at 7.33 (H2), 7.73 (H4), 7.21(H5), 7.29 (H6) and 7.55 ppm (H7) shifted to 7.10, 7.04, 6.80, 6.63 and 6.95 ppm, respectively [56]. Considering this characteristic feature of tryptophan binding on the ^{1}H NMR spectra of the Schiff-base (Fig. 6), the peaks at 7.10, 7.55, 7.01 and 7.34 ppm also support the formation of the Schiff-base between tryptophan and saccharin.

H1 and H2 protons (Scheme 2B) are split into a doublet (the signals at 3.1 and 3.3 ppm) in a manner that an AB pattern is formed. Then, these protons from doublets *via* their interactions with H3 proton. Since their interaction constants are different, the appearances of these peaks are also different. As a result of the interaction of H3 proton at 3.8 ppm with H1 and H2, two doublets coincide and so, the splitting, similar to a triplet, is formed. The signals which belong to the aromatic groups, are seen in the region of 6.9-8.0 ppm. The signal at 7.12 ppm shows the H4 proton. The peaks at 6.97-7.15 ppm are due to H8 protons of tryptophan moiety while the signals at 7.3-7.7 ppm correspond to the H9 protons of saccharin moiety in the Schiff-base molecule. H5 and H6 acidic protons are not seen due to deuterium exchange. Moreover, H7 proton is observed as a broad singlet at 3.15 ppm.

CONCLUSIONS

A novel Schiff-base of saccharin with tryptophan was synthesized and structurally characterized by ^{1}H NMR, UV-Vis., FT-IR spectroscopy, voltammetry and elemental analysis. As evidenced by the analysis of UV-Vis spectrum of the Schiff-base, a good intra-ligand n→π* transition from the excitation of the electrons of the azomethine group was found. These observations on the binding of saccharin to tryptophan, which are in line with other studies, will contribute to a better understanding of the behavior of saccharin in biological systems.

ACKNOWLEDGEMENTS

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