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Synthesis, characterization, spectrophotometric, structural and antimicrobial studies of the newly charge transfer complex of p-phenylenediamine with π acceptor picric acid

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ABSTRACT

Charge transfer complex (CTC) of donor, p-phenylenediamine (PPD) and acceptor, 2,4,6-trinitrophenol (picric acid) has been studied in methanol at room temperature. The CT complex was synthesized and characterized by elemental analysis, FTIR spectra, ^{1}H NMR spectroscopy and electronic absorption spectra which indicate the CT interaction associated with proton migration from the acceptor to the donor followed by hydrogen bonding via N^+–H···O^-. The thermal stability of CT complex was studied using TGA and DTA analyses techniques. The CT complex was screened for its antifungal activity against *Aspergillus niger* (Laboratory isolate), *Candida albicans* (IQA-109) and *Penicillium* sp. (Laboratory isolate) and antibacterial activity against two Gram-positive bacteria *Staphylococcus aureus* (MSSA 22) and *Bacillus subtilis* (ATCC 6051) and two Gram-negative bacteria *Escherichia coli* (K 12) and *Pseudomonas aeruginosa* (MTCC 2488). It gives good antimicrobial activity. The stoichiometry of the CT complex was found to be 1:1. The physical parameters of CT complex were evaluated by the Benesi–Hildebrand equation. On the basis of the studies, the structure of CT complex is [$(PPDH)^+(PA)^-$], and a general mechanism for its formation is proposed.

1. Introduction

Charge transfer complexes (CTCs) play a central role in bio-electrical and biological systems such as bactericides, fungicides, insecticides and various light-driven physical and chemical processes [1–5]. The Charge transfer interaction has been utilized for the estimation of different pharmaceuticals [6,7], micro-emulsion [8] and also as organic semiconductors [9]. The CT-complexes act as intermediates in a wide variety of reactions involving nucleophiles and electron deficient molecules. There exists a vast literature on theoretical [10,11] and experimental studies [12–20] in relation to the stoichiometry, structural, spectral, thermal and electronic properties of the CT-complexes.

The electron acceptor, picric acid (PAH) is known to form stable colored charge transfer complexes with many donors such as 7,7-bis(piperazino)-8,8-dicyanoquinodimethane, 8-hydroxyquinoline, 2,2′-bipyridine and 2,9-dimethyl-1,10-phenanthroline have been studied with the help of spectroscopic techniques like FTIR, ^{1}H NMR, TGA–DTA and UV–vis electronic absorption to obtain the stoichiometry, molecular structure and nature of interaction for the CT-complexes [21–24], single crystal studies of the crystal of CT-complexes formed between the reactions of picric acid and acenaphthene, 2-nitor aniline and phenanthrene have also been reported [25–27]. Interactions and stoichiometry of the proton transfer (charge transfer) reactions of p-phenylenediamine with p-chloranil and chloranil have been studied by the above-mentioned techniques [28,29]. The proton transfer interaction between O-phenylenediamine (OPD) tetracyanoethylene (TCNE) and PPD-xylidine were investigated in both solid and liquid states [30,31].

This paper presents studies of the charge-transfer interaction between p-phenylenediamine and picric acid in both liquid and solid states. The aim of the work is to determine the reaction stoichiometry, nature of bonding between PPD and PAH, and some physical parameters. By studying the antimicrobial activity of the CT complex, an important aspect in biological systems may be developed new type of antiproliferative agents. In addition, the nature and structure of the reaction product CT complex in both solution and solid can be estimated using the above-mentioned techniques.

2. Experimental

2.1. Materials

Analytical grade chemicals were used throughout. PPD was obtained from Merck, while PAH was obtained from CDH. Methanol

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(Merck), DMSO (Merck) and HCl (Merck) were all analytical grade (AR) and used without further purification. Tetracycline, Nystatin and agar were obtained from Hi-Media Mumbai.

2.2. Preparation of standard solutions

A 1×10^{-2} M standard solution of p-phenylenediamine (donor) and 1×10^{-2} M standard solution picric acid (acceptor) were prepared in methanol.

2.3. Synthesis of the solid CT complex

The solid CT complex [$(PPDH^+)(PA^-)$] was prepared by mixing 15 ml saturated solution of p-phenylenediamine (0.108 g, 1 mmol) in $CHCl_3$ with 15 ml of a saturated solution of picric acid (0.229 g, 1 mmol) in $CHCl_3$. A yellow color solution was obtained upon mixing that changed to yellow precipitate. The precipitate was filtered off and washed several times with $CHCl_3$ and dried under vacuum over $CaCl_2$. It was ensured that the product was not soluble in chloroform. The complex thus obtained was characterized by elemental analysis (theoretical values are shown in brackets): $[(PPDH)^+(PA)^-]$, ($C_{12}H_{11}N_5O_7$) CT complex (M/W: 337.25 g): C, 42.70% (42.74%); H, 3.22% (3.29%); N, 20.74% (20.76%).

2.4. Spectrophotometric analyses

When 3 ml solution each of the acceptor and the donor were mixed, a charge transfer complex was formed. The wavelength of maximum absorption of the resulting solution was determined. The CT complex of the 1:1 reaction mixture was kept over night at room temperature to form stable complexes, were analyzed. The maximum wavelength of the charge transfer complex was determined by spectrophotometer to be 236 nm.

2.5. Antibacterial activity

The antibacterial activity of newly synthesized CT complex was tested in vitro against two Gram-positive bacteria *Staphylococcus aureus* (MSSA 22) and *Bacillus subtilis* (ATCC 6051) and two Gram-negative bacteria *Escherichia coli* (K 12) and *Pseudomonas aeruginosa* (MTCC 2488) strains using disc diffusion method [32,33]. Media with DMSO was set up as control. The discs measuring 5 mm in diameter were prepared from Whatmann no.1 filter paper sterilized by dry at heat at 140 °C for 1 h. The sterile discs previously soaked in a concentration of the test complex were placed in a nutrient agar medium. The plates were invested and kept in an incubator for 24 h at 37 °C. The inhibition zone thus formed was measured (in mm) after 24 h. The screening was performed at 100 μg/ml concentration of test CT complex and antibiotic disc, Tetracycline (30 μg/disc, Hi-Media) was used as control.

Logarithmic serially twofold diluted amount of test CT complex and controls, was inoculated within the range 10^{-4}–10^{-5} cfu/ml. The cultures were incubated for 24 h at 37 °C and growth was monitored both visually and spectrophotometrically. The lowest concentration (highest dilution) required to arrest the growth of bacteria is regarded as minimum inhibitory concentration (MBC). To obtain the diameter of zone, 0.1 ml volume was taken each and spread on agar plates. The number of colony forming units (cfu) was counted after 24 h of incubation at 35 °C.

2.6. Antifungal activity

The newly synthesized CT complex was also screened for its antifungal property against *Aspergillus niger* (Laboratory isolate), *Candida albicans* (IQA-109) and *Penicillium* sp. (Laboratory isolate) in DMSO using standard agar disc diffusion method [34]. The synthesized CT complex was dissolved in DMSO and media with DMSO was set up as control. All cultures were routinely maintained on SDA and incubated at 28 °C. Spore formation of filamentous fungi was formed from seven day old culture on sterile normal solution, which was diluted to obtain approximately 10^5 cfu/ml. The culture was centrifuged at 1000 rpm, pellets was resuspended and diluted in sterile NSS to obtain a viable count 10^5 cfu/ml. The inoculum of non-sporing fungi *C. albicans* was performed by growing the culture in SD broth at 37 °C overnight. With the help of spreader, 0.1 ml volume of approximately diluted fungal culture suspension was taken and spread on agar plates. The fungal activity of CT complex was compared with Nystatin (30 μg/disc Hi-Media) as standard drug. The cultures were incubated for 48 h at 37 °C and the growth was monitored. Antifungal activity was determined by measuring the diameters of the zone (mm) in triplicate sets.

2.7. Analyses

The electronic absorption spectra of the donor (p-phenylenediamine), acceptor (picric acid) and the resulting CT complex in methanol were recorded in the region of 700–200 nm using a Intra 10 UV–vis spectrophotometer with a 1 cm quartz cell paths length. The FTIR spectra of the reactants and the resulting CT complex were recorded using KBr disc on the spectroscopic 2020 FTIR spectrometer, ^{1}H NMR spectrum of the CT complex, donor and acceptor were recorded in DMSO using Bruker DRX-300 NMR spectrometer and the thermal analysis (TGA and DTA) were carried out under nitrogen atmosphere with a heating rate of 20 °C/min for TGA and DTA using Shimadzu model DTG-60H thermal analyzers.

3. Result and discussion

3.1. Observation of CT bands

The spectrophotometric data were used to calculate both formation constant (K_{CT}), and extinction coefficient (ε_{CT}) of the CT complexes in the defined solvents based on Benesi–Hildebrand equation [35,36].

$$\frac{[A]_0}{A} = \frac{1}{K_{CT}\varepsilon_{CT}} \cdot \frac{1}{[D]_0} + \frac{1}{\varepsilon_{CT}}$$

where $[D]_0$ and $[A]_0$ are the initial concentration of the donor and acceptor, respectively and A is the absorbance of the CT band. The concentration of the donor in the reaction mixture was kept greater than acceptor, ($[D]_0) \gg [A]_0$) [35,36], and changed over a wide range of concentration from 0.4×10^{-4} to 1.5×10^{-4} M while concentration of the π acceptor (PHA) in each of the reaction mixture was kept fixed at 1×10^{-5} M. These produced solutions with donor: acceptor molar ratios varying from 4:1 to 15:1 as reported in Table 1. The electronic absorption spectra of 1×10^{-5} M PPD, 1×10^{-5} M PAH and charge transfer complex in methanol were recorded against methanol as reference are shown in Fig. 1. To obtain the electronic absorption spectra of CT complex, solutions of donor and acceptor were prepared in the same solvents. Straight line was obtained when $[A]_0/A$ was plotted against $1/[D]_0$ which supports the formation of the charge transfer complex as shown in Fig. 2. In this plot, the slope and intercept equals $1/K_{CT}\varepsilon_{CT}$ and $1/\varepsilon_{CT}$ respectively. The formation constant and molar extinction coefficient of the CT complex were obtained by calculation of intercept on y-axis and slope, and were found to be 2.181×10^5 l mol^{-1} and 2.398×10^6 l cm^{-1} mol^{-1} respectively. It is observed that new absorption peak appear in the UV region. In some cases multiple peaks were obtained, the longest wavelength peak was considered as CT peak [37], which is found at 236 nm. The change of the absorption intensities to higher values for complex on addition of