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Photocatalytic oxidation of amoxicillin

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ABSTRACT

The photocatalytic oxidation of aqueous solution of amoxicillin was investigated and a reaction scheme is proposed for the formation of photoproduct which is in conformity with the composition and structure of the photoproduct established on the basis of analytical and spectral data. This study may help in removal of antibiotics by photocatalytic oxidation in pharmaceutical industry wastewater and also other types of wastewaters.

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KEYWORDS

Amoxicillin;
Penicillin;
Thiazolidine ring;
Azobenzene;
Sulphoxide.

INTRODUCTION

Amoxicillin belongs to the family of β -Lactam group of antibiotics and its chemistry provides a panoramic view of the photoreactions of aliphatic, aromatic and heterocyclic units. The functional groups present in amoxicillin are usually present in other pharmaceuticals also and this study may help in removal of such functional groups by oxidation in various types of pharmaceutical industry wastewaters.

The photoproducts are obtained by irradiating the sample with visible and UV light using ZnO, TiO₂, WO₃, Fe₂O₃, ZnS, and CdS as photocatalysts. The formation of various intermediate products in the reaction scheme has been proposed keeping in view the absence of amino and carboxylic group in the photoproduct. The amino group is oxidized to from -N=N- linkage by involving two molecules of substrate. The oxidation of S atom of thiazolidine ring to sulphoxide is

plausible in view of the hypsochromic shift of the CO-frequency (ν_{as}) of β -lactam ring of the penam structure of the substrate. Various semiconductors, viz. ZnO, TiO₂, WO₃, Fe₂O₃, ZnS, CdS were selected in the study because of their favourable band gaps. The effect of the nature of photocatalyst and the amount of photocatalyst on the percentage yield of the photoproduct was also examined. Further advanced studies by preoxidation of highly polluted waste pharmaceutical fermentation broth using wet air oxidation (WAO) is proposed. It has been studied as a possible method for the effective removal of organics^[1].

EXPERIMENTAL

The purity of all the chemicals used during experimentation was checked by their M.P./B.P. determinations. Sufficient care was also taken that the solvents were distilled before use. Throughout the course of the

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study double distilled water was used to prepare all the solutions. Before preparing any solution, this water was boiled for about 2 to 3 min. to remove traces of dissolved CO₂ and was then cooled in a stoppered vessel till it attained the room temperature. The pH of the solutions was noticed by a Toshniwal CI-54 pH-meter.

Amoxicillin (300 mg) was dissolved in 30 mL of double distilled water and to this 250 mg of photocatalyst was added. The photocatalyst did not dissolve and formed a heterogeneous system. The irradiation was then done by two ways: (i) the beaker containing the solution was irradiated with a light source of 200W tungsten lamp maintaining a distance of 20 cm between the light and the reaction vessel. The solution was constantly stirred magnetically during the course of irradiation. Stirring was sufficient vigorous to ensure that the suspension of semiconductor powder was exposed to incident light beam. A water filter was placed between the light source and the reaction vessel to cut off heat radiations. The temperature of the solution was also noted after regular intervals for checking that constant temperature is maintained throughout the reaction. (ii) Similar sample was prepared and irradiation was done with UV light in the photochemical reactor of Srinivasan Griffin Rayonet type. This reactor comprised of 8 UV tubes. These tubes were fitted inside the reactor. A magnetic stirrer attached with a cooling fan was provided. Two rods with clamps and boss heads were fitted on the reactor for holding the glass reactor to be suspended in the reactor for exposing to UV rays. When the UV tubes were lighted, the cooling fan was also started so that heat generated inside the reactor was dissipated. A top lid was provided for covering the reactor.

In both the above cases (i & ii), the progress of the reaction was assessed by TLC at regular intervals. The period of irradiation in visible and UV light were 12 h and 1 h, respectively. Progressively, when the product was formed the light source was removed and TLC was done which gave only one spot with a lower R_f value than that of amoxicillin. Stahl^[2] has described separation of antibiotics by TLC. Finally, after irradiation, the semiconductor was removed by filtration and the filtrate was concentrated under reduced pressure. The concentrated solution was then left for evaporation in a petridish placed in a P₂O₅ dessicator. The solid product isolated in the above manner was recrystallized from

alcohol followed by ether and obtained as orange coloured needle shaped crystals. Each experiment was repeated thrice for ensuring the correctness of the product obtained during the photocatalytic reaction.

RESULTS AND DISCUSSION

Characteristics of photoproducts obtained by photocatalyzed reactions of amoxicillin was identified by various instrumental and other methods, e.g. elemental analysis and on the basis of UV-Vis, IR and NMR spectral studies and by analyzing their physical and chemical properties. The nature of the photoproducts obtained in presence of different semiconductors was found to be similar with regard to the above properties. The results of the blank experiments performed in absence of semiconductor photocatalyst and in presence of SiO₂, an insulator showed that amoxicillin is affected by irradiation with light, though the nature of the photoproduct is not homogeneous and photochemical decomposition in such a case is an extremely slow process.

The results of blank experiment with amoxicillin in presence of any semiconductor but in absence of light indicated that amoxicillin does not show any reaction by the mere presence of semiconductor. Interestingly, the products isolated from the solutions were obtained as white solids and TLC of each solution gave only one spot corresponding to the R_f value of the substrate amoxicillin itself.

Elemental analysis, viz. C, H and N of the photoproducts were performed using a C, H, N analyzer (Coleman Model 5621) which was first standardized with the help of benzoic acid (AR grade) and S by usual method of chemical analysis employed for element detection. It was found that the photoproducts contained C, H, N and S. It may be mentioned that these elements were present in the substrate also.

Some qualitative tests were also performed for various functional groups, e.g. carboxylic group, phenolic group, and amino group etc. by the specified methods. The results of these tests showed that carboxylic group and amino group were absent in the photoproduct whereas these groups were present in the original substrate.

Spectral data

For the identification of photoproducts, spectral

data analysis is necessary. Accordingly, UV-Vis, IR, and NMR analyses were conducted.

UV-Vis

The Beckman DU-6 UV-Vis spectrophotometer was used to record the UV-Vis spectra of the photoproducts.

The UV spectral data of the photoproducts of amoxicillin showed the absence of the absorption band due to carboxylic group which is observed at 203 nm in the spectra of amoxicillin. A strong and sharp absorption band at 362 nm in the solution spectra of photoproduct is caused by the appearance of a new chromophore, i.e. azo group formed by photooxidation of amino group. Lodha^[3] had also carried out the photooxidation of ampicillin in presence of various photocatalysts, the UV spectra of the photoproducts showed the sharp and strong absorption band around 362 nm that has been assigned to the azo chromophore formed as a result of photooxidation of $-NH_2$ group of ampicillin in presence of various photocatalysts.

The UV spectra of photoproducts of amoxicillin also showed the presence of strong absorption band at 211-215 nm which could be ascribed to the S=O linkage formed in the photoproduct due to oxidation of sulphur atom of thiazolidine ring.

IR

The IR spectra were scanned as KBr pellets and Nujol Mulls on Perkin Elmer 577 spectrophotometer/Nicolet ΔX FT-IR spectrophotometer in the range 4000-200 cm^{-1} .

The other carbonyl group of the amoxicillin such as $-C=O$ of β -lactam and the $-C=O$ of the carboxylic group were observed at 1780 cm^{-1} and 1680 cm^{-1} , respectively. The absence of band at 1680 cm^{-1} in the photoproduct showed the absence of carboxylic stretching vibrations. The assignment of the $\nu C-O$ of the β -lactam is in accordance with the references quoted by Clarke et al^[4] on the IR spectra of penicillins and the observations of Sheehan et al^[5,6] who extensively studied the IR spectra of β -lactams. It has been reported that when a β -lactam ring is fused to thiazolidine ring, the $-C=O$ absorption occurs in the range 1780-1770 cm^{-1} . In view of these observations, the absorption band around 1780 cm^{-1} observed in the IR spectra of amoxicillin can be assigned to β -lactam ring.

Further, these workers have also pointed out that the oxidation of sulphur atom in fused β -lactam system introduces a strong hypsochromic shift of the $-C=O$ absorption. It is observed around 1800 cm^{-1} in such compounds. Morin et al^[7] have also pointed out that the higher IR frequency of strained β -lactam ring can be due to penicillin sulphoxide.

In the IR spectra of the photoproduct, there is no absorption band around 1760-1780 cm^{-1} but a strong absorption band around 1800 cm^{-1} is observed. It could be attributed to stretching vibration of $-C=O$ of β -lactam ring. The presence of the above band is direct evidence of the oxidation of the sulphur atom of thiazolidine ring and as a result of this oxidation the above mentioned $-C=O$ absorption has suffered a strong hypsochromic shift. The oxidation of the thiazolidine sulphur of Amoxy molecule during the photocatalytic reaction is further supported by the presence of absorption frequency characteristic of sulphoxide which have been observed as strong and sharp absorptions around 1050-1035 cm^{-1} due to stretching vibrations of sulphoxide (νSO). Archer and DeMarco^[8] have further supported these observations by giving IR frequencies of β -lactam at 1800 cm^{-1} and of sulphoxide at 1050 cm^{-1} . The frequency at 530 cm^{-1} can be attributed to deformation vibrations of sulphoxides. The SO deformation frequencies have been studied by Kresze et al^[9] at 535-497 cm^{-1} . However, according to these workers these frequencies are sensitive to the nature of substituents. Thus, the presence of strong absorption in the regions mentioned above in the spectra of the photoproduct can presumably be taken as presence of SO linkage which is complimented with the observation of hypochromic shift in the position of the $-C=O$ group of the β -lactam ring fused to thiazolidine ring.

The band at 750-810 cm^{-1} resulted from out of plane bending vibrations of two substituted benzene ring in case of the Amoxy and 780 cm^{-1} due to a trisubstituted benzene ring in the product. The change in the substitution of the benzene ring in the product could be due to the migration of the $-NH_2$ group in Amoxy substrate to the aromatic ring at meta position.

The frequency characteristics of azo group appeared at 1455 cm^{-1} in the photoproduct and it clearly showed the presence of aromatic azo-linkage. A second band due to symmetrical stretching of $-N=N$ group is also

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observed at 1200 cm^{-1} in the spectra of the photoproduct. Further, the absence of band at 2610 cm^{-1} due to $\nu_{\text{O-H}}$ (bonded) of the $-\text{COOH}$ group at 1545 cm^{-1} due to carboxylate anion stretching in the spectra of photoproduct shows the disappearance of $-\text{COOH}$ group from Amoxy during the photoreaction.

The absorption band at 1028 cm^{-1} in the spectra of Amoxy is attributed to the presence to the presence of $-\text{C-N}$ stretching (aliphatic). The conspicuous absence of the above mentioned absorption band in the IR spectra of the photoproduct was noticed in several samples obtained under identical conditions and on the basis of this, it was concluded that amino group is absent from the photoproducts.

NMR

The NMR spectra were recorded on JEOL FX 90Q spectrometer. The ^1H NMR spectra were scanned in DMSO-d_6 using TMS as an internal reference.

The NMR spectral data of penicillins have been largely studied in the literature. Manhas and Coworkers^[10] have also displayed the similar values for penicillins.

The ^1H NMR spectra of Amoxy and the photoproduct displays a singlet for the two methyl groups attached to the thiazolidine ring at $\delta 1.35$ and two doublets for the methane protons at $\delta 1.05$ and $\delta 1.45$. It has also been explained that oxidation of the sulphur atom to sulfoxide changes the environment of the β -lactam protons in penicillins.

Accordingly, the chemical shift value of the $-\text{CH}$ doublet increases to a little extent in the photoproduct. It can therefore be assumed that oxidation of sulphur atom has taken place; which has also been shown by UV and IR spectral studies. The two singlets for methylene protons appear at $\delta 1.68$ and $\delta 2.40$ in the spectra of photoproduct only. The spectra of Amoxy shows two doublets centered at $\delta 6.80$ and $\delta 7.25$ due to vicinal hydrogen typed protons H_a and H_b . The two protons typed H_a are chemically equivalent as are the two protons typed H_b . H_a and H_b are non equivalent to each other and their signals are split into a pair of doublets. The photoproduct on the contrary displays two doublets centered at $\delta 6.80$ and $\delta 7.35$ and a singlet at $\delta 8.10$ ppm due to three protons of tri substituted phenyl ring.

The N-H protons of amide appear as a broad

ragged singlet around $\delta 5.39$ both in Amoxy and the photoproduct. The signal is affected by the magnetic and electric properties of ^{14}N nucleus to make it appear as a broad signal.

The sharp singlet at $\delta 3.88$ in Amoxy showed its disappearance from the photoproduct and it is most likely to be due to $-\text{NH}_2$ protons.

The absence of carboxylic proton signals in the NMR spectrum of the product which appeared at $\delta 11.0$ in Amoxy shows that it has been lost in the photocatalytic oxidation of Amoxy due to photocatalytic decarboxylation.

The effect of nature of photocatalyst on percentage yield

The % yield of the photoproduct shows that it depends on the type of photocatalyst employed. For samples irradiated under comparable conditions the % yield of the product in the visible light varied with the semiconductor in the order $\text{Fe}_2\text{O}_3 > \text{CdS} > \text{WO}_3 > \text{TiO}_2 = \text{ZnO} > \text{ZnS}$ and just in the reverse order in UV light, i.e. as the band gap increased the yield decreased when the source employed was visible light and when the source employed was UV light the yield increased with the increase in band gap of semiconductors. These observations are in accordance with the fact that Hematite (Fe_2O_3), CdS and WO_3 are more absorptive in the visible region whereas TiO_2 , ZnO and ZnS are mainly UV absorber or in other words the oxide having $\lambda_{\text{max}} > 400\text{ nm}$ absorb more effectively in visible range and oxide having $\lambda_{\text{max}} < 400\text{ nm}$ absorb efficiently in UV region.

Effect of amount of photocatalyst on percentage yield

The effect of amount of photocatalyst has also been studied on the yield of photoproduct. It is observed that as the amount of photocatalyst is increased, the yield is also increased but only upto a certain limit. In the present study, when the amount is increased beyond 0.25 or 0.30 g, the yield no longer increased. This is because initially for very small amount, i.e. less than 0.25 g, the surface area increased as the amount of photocatalyst is increased but after a certain value the surface area no longer increases (as the bottom of the vessel became completely covered with photocatalyst). Now only the thickness of the layer increased in-

stead of the surface area, so the yield of the photo-product remained constant as the amount of photocatalyst was increased.

CONCLUSIONS

Proposed structure

On the basis of the data presented by spectral, elemental and functional group studies, the following structure was proposed to the photoproduct obtained in the photocatalytic reaction of Amoxy:

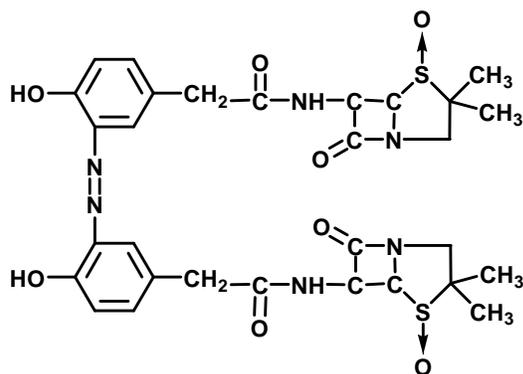
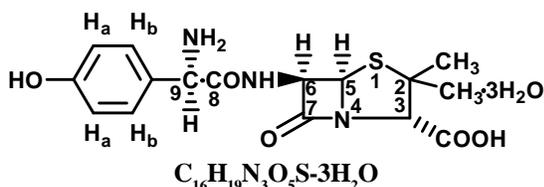


Figure 1: Photoproduct of amoxy

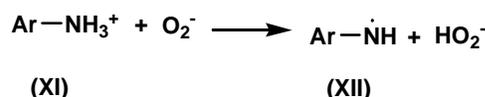
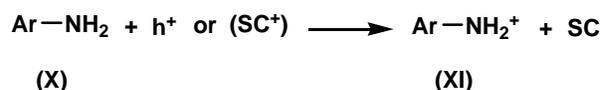
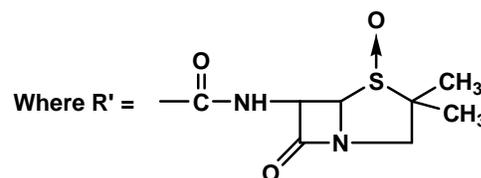
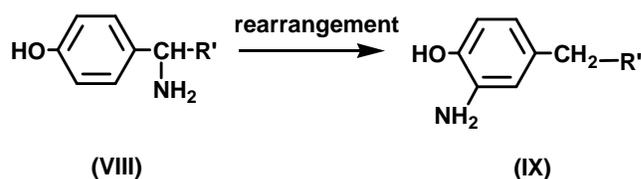
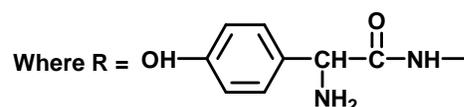
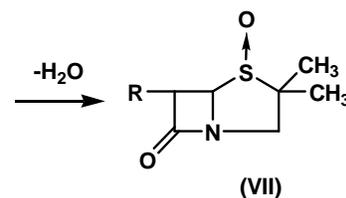
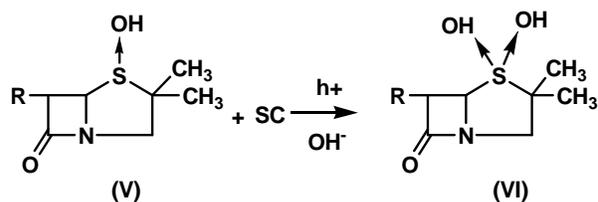
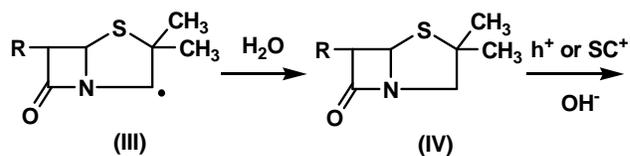
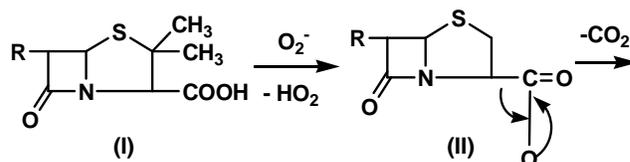
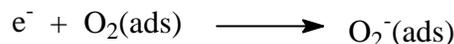
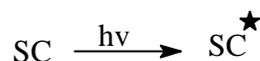
The molecular structure of Amoxicillin is as follows:



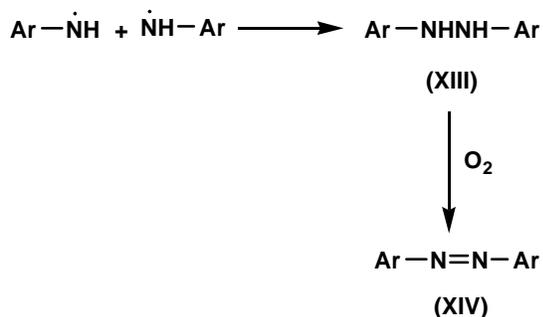
The photoproducts contain the phenolic group, but the $-NH_2$ group attached to phenolic moiety via C-9 is absent from the photoproduct which is indicative of the possibility of oxidation of the $-NH_2$ group. From the spectral data it was clear that $-NH_2$ group was oxidized to $-N=N$ group. Further negative test for carboxylic group indicates decarboxylation in photoproduct.

Proposed mechanism of photocatalysed reaction of amoxicillin

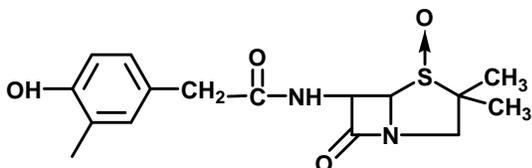
The plausible mechanism of the photoreaction proposed on the basis of the structure of the photoproduct established after a careful qualitative analysis for its functional group and interpretation of UV-Vis, IR and NMR spectral data can be presented as under:



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Where Ar =



Where, SC= Semiconductor, CB= Conduction band, VB= Valance band, h^+ = hole, e^- = electron.

In the above photochemical reaction the initial steps lead to the formation of $e^- - h^+$ pair by the excitation of semiconductor photocatalysts. The adsorbed O_2 is utilized in trapping e^- as superoxide ion and this process is well confirmed in many references cited in the literature, like Fox and Duley^[11], Ikezawa and Katal^[12]. In many such reactions, the yields of the oxidized products are drastically reduced in N_2 atmosphere which confirmed the participation of O_2 in these reactions.

Decarboxylation of (I) in the presence of H_2O takes place as shown in the reaction scheme. Decarboxylation is indicated by the absence of the absorption peaks associated with $-\text{COOH}$ group in UV, IR and NMR spectra. The substrate gives effervescence with NaHCO_3 but the photoproducts did not respond to the test of carboxylic group. The mechanism of decarboxylation is shown by Kraeutler and Bard^[13] in photocatalytic reactions. The oxidation of S of thiazolidine ring (IV) to sulphoxide occur by OH^\cdot radicals.

The oxidation of β -dichloroethyl sulphides to sulphoxide and sulpone has been shown by Fox et al^[14]. Oxidation of penicillins to sulfoxide by a variety of methods to a single sulfoxide has also been shown by Archer and DeMarco^[8]. The mechanistic aspect of the oxidation via OH^\cdot radicals which are formed when holes trapped at semiconductor surface reacts with OH^- in aqueous medium has been discussed^[15]. The spectral data about the nature of the photoproduct also confirms the formation of sulphoxide. The forma-

tion of azo- linkage can be explained by the photooxidation of amino group of (VIII). The presence of azo-linkage peaks in the photoproducts were indicated from its UV and IR spectra. The disappearance of $-\text{NH}_2$ peaks was confirmed from its NMR spectra. Hama et al^[16] and Kumar and Kumar^[17] have reported the photooxidation of aniline to azobenzene in presence of ZnO and CdS respectively.

The migration of $-\text{NH}_2$ group to ortho and meta position of the benzene ring under irradiation was noticed by Ninomiya and Naito^[18] in case of benzylamine. The $-\text{NH}_2$ moiety is attached to C-9 of the substrate (VIII). Its migration proposed a meta amino moiety (IX). The formation of ortho substituted phenolic moiety is forbidden in substrate because of steric reasons.

In the final conclusion it can be stated that the reaction scheme proposed above is in conformity with the composition and structure of the photoproduct established on the basis of analytical and spectral data. The formation of the $e^- - h^+$ pair and species involved in oxidation like superoxide ion and the OH^\cdot radical is based on the well established mechanisms reported in the literature. The formation of various intermediate products has been proposed keeping in view the absence of amino and carboxylic group in the photoproduct. The $-\text{COOH}$ group is decarboxylated whereas amino group is oxidized to form $-\text{N}=\text{N}-$ linkage by involving two molecules of substrate. The oxidation of S atom of thiazolidine ring to sulfoxide is plausible in view of the hypsochromic shift of the CO-frequency (ν_{as}) of β -lactam ring of the penam structure of the substrate. The above conjecture is further supported from the appearance of a conspicuous and characteristic absorption at 211-215 nm due to sulphoxide group in the UV spectra of the photoproduct obtained from substrate.

Further, for improving the photocatalytic process, the combination of heterogeneous photocatalysis with ultrasonic irradiation, photo-Fenton reaction, ozonation or electrochemical treatment can be a better tool^[19]. Finally the application of photocatalysed semiconductors has been successfully used for remediation of wide variety of contaminants containing compounds like alkanes, phenols, aromatic carboxylic acids, dyes halogenated alkanes and alkenes etc. in aqueous solution^[20].

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