Microbiological Assay of Amoxycillin and the Photoproduct: A Simple Method to Assess the Toxicity of Photoproducts of Antibiotics in Wastewater

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Abstract: **Antibiotics released from pharmaceutical industries, hospital wastewater and even disposed into domestic wastewater after use, reach wastewater treatment plants and surface waters and act as micropollutants. The ecotoxicity of these antibiotics has been studied widely. In the present paper the toxic effect of Amoxycillin, one of the commonly used antibiotic has been compared with the toxic effect of photoproduct of Amoxycillin. Photocatalysis of Amoxycillin has been carried out earlier for the oxidation of Amoxycillin from wastewater. Toxic effect of the photoproduct has been found to be reduced to 75% of the original substrate.**

1. INTRODUCTION

Numerous studies have investigated trace amounts of various pharmaceutical compounds in the aquatic ecosystems and in drinking water.[1-2] The biological process in the conventional wastewater treatment plant is unable to oxidize various antibiotics and other drugs present in Pharmaceutical industry wastewater as many of these drugs are biorefractory in nature. This treated water is creating various health hazards to aquatic animals as well as human beings over prolonged use and antibiotic resistant strains are being developed in their bodies. [3-7]

Various advanced oxidation processes as well as low cost adsorption methods have been proposed for complete or partial removal of antibiotics from pharmaceutical wastewater, thus removing or reducing the toxic effects of these drugs. [8-10]

The chemistry of Amoxycillin provides a panoramic view of aliphatic, aromatic and heterocyclic units and the functional groups present in amoxycillin are usually present in other pharmaceuticals also. The photocatalytic study of Amoxycillin was done using various semiconductors as photocatalysts by irradiating under UV and Visible light and the photoproducts were analysed on the basis of various instrumental studies.[11] Microbiological assay is one of the essential methods to assess the toxic effect of pharmaceutical drugs.

2. EXPERIMENTAL

The therapeutic efficacy of antibiotics can be demonstrated by the inhibition of microbial growth under standard conditions. Any change in the antibiotic molecule will be revealed by a reduction in the antimicrobial activity and this can be detected by microbiological assay.

In microbiological assay comparison of inhibition of growth of bacteria by measured concentration of the antibiotics to be examined is done with that produced by known concentration of a standard preparation of the antibiotics having a known activity.

For this the two conventional methods are:
1. Cylindrical Plate method or Cup-plate method
2. Turbemetric method or tube assay method

In the present study we have employed Cylindrical Plate method-

Cylindrical Plate method: This method depends on the diffusion of an antibiotic from a vertical cylinder or a cavity through the solidified agar layer of a petridish or a plate to an extent such that growth of added micro organism is prevented entirely in a circular area or zone around the cylinder or cavity containing a solution of antibiotic. Using the appropriate buffer solution, a solution of known concentration of the standard preparation was prepared along with the corresponding assumed concentration of antibiotics and photoproducts to be examined. These were followed by further dilution. The solutions so prepared were applied to the solid medium in sterile cylinder or in cavity. Left the dishes or plates standing for one to four hours at room temperature, as a period of pre incubation diffusion to minimize the effect of variation in time between the applications of different solutions. Finally incubated the medium for 18 hrs.at the particular temperature. For calculating the results accurately measured the diameter or area of the circular inhibition zones. The principal of antibiotics sensitivity test and microbiological assay has been well described by Gould. [12]

Composition of media used in assay of Amoxycillin and its photoproduct

Ingredient	g/L of water
Peptone	5.0
Yeast extract	1.5
Dextrose	1.0
NaCl	3.5
Dipotassium hydrogen phosphate	3.68
Potassium dihydrogen phosphate	1.32
pH (after sterilization)	6.95-7.05

RESULT AND DISCUSSION

Standard Preparation and Unit of Activity: A standard preparation is an authentic sample of the appropriate antibiotics for which the potency has been precisely determined by reference to the appropriate international standard.
The potency of the standard preparation may be expressed in international unit or in microgram per mg of the pure antibiotic.

Standard preparation: Amoxycillin
Activity units: 940 microgram per mg
(mg containing 1 unit: 0.001064)

The photoproduct of Amoxycillin was checked for its potency in a similar manner.

The potency calculated for photoproduct of Amoxycillin: 25%w/w (Potency has been calculated to the 100% potency assumed for original antibiotic)

The test organism recommended for Amoxycillin and its photoproduct: Micrococcus luteus

In the near future due to increased population size, rapid industrialization etc. water availability may reduce drastically and may result in increased concentrations of pharmaceuticals in wastewaters further leading to environmental degradation, ecological imbalance and increased toxicity and health risks. Antibiotic resistance would be a major health concern. It is therefore the need of the hour to upgrade the technologies and treatment procedures in wastewater treatment plants. Advanced oxidation processes like photo-fenton methods, cavitation methods, sonolysis, non-thermal plasma etc. have already been reported in literature for removal of diverse types of refractory pollutants in wastewater. Further, for improving the photocatalytic process, these methods in combination with heterogeneous photocatalysis can be a better tool [13]. These methods may also have the potential to be applied for commercial utilization. Further advanced studies using wet air oxidation (WAO) has been proposed for highly polluted waste pharmaceutical fermentation broth. It has been studied as a possible method for the effective removal of organics [14].

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