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Monitoring of Cephalosporin C During Bioconversion

BHAGWANT S. DESHPANDE, SUDHA S. AMBEDKAR, AND JAIPRAKASH G. SHEWALE*

Research and Development, Hindustan Antibiotics Limited, Pimpri, Pune 411 018, India

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ABSTRACT

A simple method based on Schiff's base formation between *p*-dimethylaminobenzaldehyde and cephalosporin C is developed for estimation of cephalosporin C. The calibration curve was linear up to 500 μg of cephalosporin C. The application of the method in monitoring bioconversion of cephalosporin C to glutaryl-7-amino cephalosporanic acid is described.

Index Entries: Cephalosporin C; glutaryl-7-Amino cephalosporanic acid; 7-aminocephalosporanic acid; bioconversion.

INTRODUCTION

Cephalosporin C (Ceph C) is a bulk raw material for the production of 7-aminocephalosporanic acid (7-ACA), which is further processed to manufacture commerically important semisynthetic cephalosporins (*1*). In fact, the usefulness of semisynthetic cephalosporins has retained importance of Ceph C in the pharmaceutical industry. The Ceph C produced is totally converted either chemically, chemical-enzymatically, or enzymatically to 7-ACA (*2*). Witnessing the advantages of enzymatic methods for production of 6-aminopenicillanic acid (6-APA), production of 7-ACA using enzymes is becoming popular. Enzymatic process for production of 7-ACA involves oxidative deamination of Ceph C to glutaryl-7-aminocephalosporanic acid (GL-7-ACA) by D-amino acid oxidase (DAO) and hydrolysis of GL-7-ACA by glutaryl acylase (GA) to generate 7-ACA (*1,3*).

*Author to whom all correspondence and reprint requests should be addressed.

We describe here a colorimetric method for estimation of Ceph C and its application in monitoring the conversion of Ceph C to GL-7-ACA.

MATERIALS AND METHODS

Materials

Ceph C (purity 88–90%) was obtained from our Pilot Plant unit. Immobilized DAO and GA were prepared in our laboratory. *p*-dimethylaminobenzaldehyde (PDAB) was from E. Merck (Germany). All other chemicals were of analytical grade and obtained from local suppliers.

Estimation of Ceph C

To 1.0 mL of appropriately diluted aliquot of Ceph C solution, 3.5 mL of 0.05*M* acetate buffer, pH 2.5 and 0.5 mL of PDAB reagent (0.5% w/v in methanol) were added. The tubes were covered with marbles. The reaction mixture was heated in a boiling water bath for 45 min, cooled and volume was made to 5.0 mL with distilled water, if necessary. The absorbance was measured at 503 nm.

Conversion of Ceph C

Ceph C (90 mg) was dissolved in 30 mL of 0.1*M* phosphate buffer, pH 7.8 and added to 40 IU of immobilized DAO in a 100 mL capacity beaker. The reaction mixture was agitated at 35°C. The pH was maintained at 7.8. The samples were removed at different time intervals. Residual Ceph C was estimated as described above. GL-7-ACA formed was determined by incubating the samples for 60 min at pH 7.0 and 37°C with excess of GA. 7-ACA thus formed was estimated by PDAD method (*4*).

RESULTS AND DISCUSSION

Conversion of Ceph C to GL-7-ACA can be monitored by estimating either GL-7-ACA formed or residual Ceph C. Methods described for the estimation of Ceph C, such as iodometric, nicotinamide, hydroxylamine, glyoxal, UV absorbance, ninhydrin, enzymatic, and so on, are not used (*5–10*). This is because these methods suffer from one or more of the following disadvantages: cannot distinguish between Ceph C and GL-7-ACA, interference of liberated ammonia and laborious nature. Currently, HPLC is used for estimation of GL-7-ACA in this application (*11*). Only disadvantage of HPLC is that the samples need to be preserved in cold prior to injection due to thermolabile nature of both Ceph C and GL-7-ACA. Thus, a simple colorimetric method is desired for rapid analysis when samples are generated in large numbers particularly during development and optimization of processes.

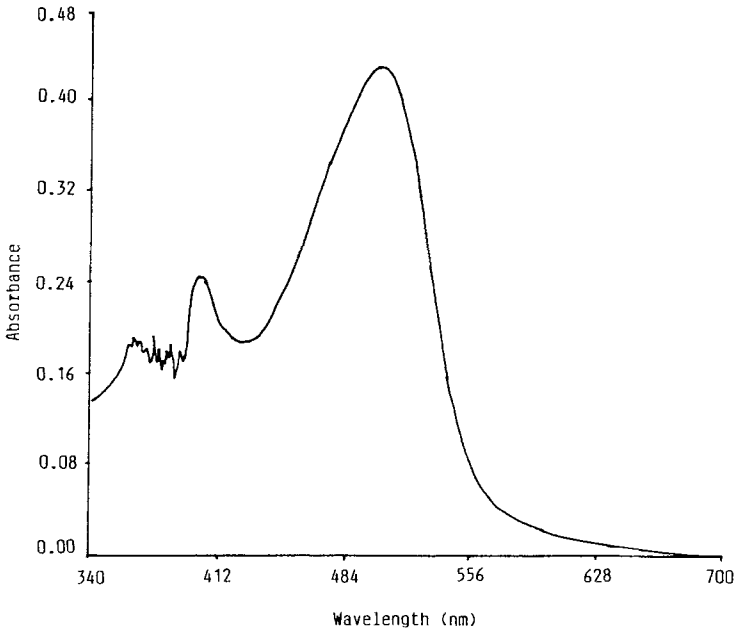

Fig. 1. Absorbance of the Schiff's base at different wavelengths.

Principle

Major structural difference between Ceph C and GL-7-ACA is the presence of alpha-amino group in the side chain moiety of Ceph C. Schiff's base formation between PDAB and alpha-amino group of beta-lactam intermediates, such as 6-APA, 7-ACA, and 7-amino desacetoxycephalosporanic acid (7-ADCA), is a documented reaction used in analytical determinations (*4,12,13*). PDAB forms colored Schiff's base with these intermediates instantly at ambient temperature and at acidic pH, 2.5-3.0. We have observed that PDAB does not form a colored Schiff's base instantly with Ceph C at ambient temperature. However, the colored Schiff's base is formed after prolonged incubation at room temperature and the reaction is accelerated by heating. This is because the adjacent carboxyl group in the side chain decreases the reactivity of alpha-amino group, which is overcome by heating.

The absorbance of color developed was recorded at wavelengths between 360 and 799 nm. The Schiff's base exhibits maximum absorbance at 503 nm (Fig. 1). A smaller peak of absorbance was observed at 400 nm, the wavelength near which the absorbance of Schiff's base between beta-lactam intermediates and PDAB is measured.

Effect of Heating on Schiff's Base Formation

The observation that heating acclerates the rate of Schiff's base formation between PDAB and Ceph C had lead us to study the relation between heating time and color development. The curve obtained was hyperbolic

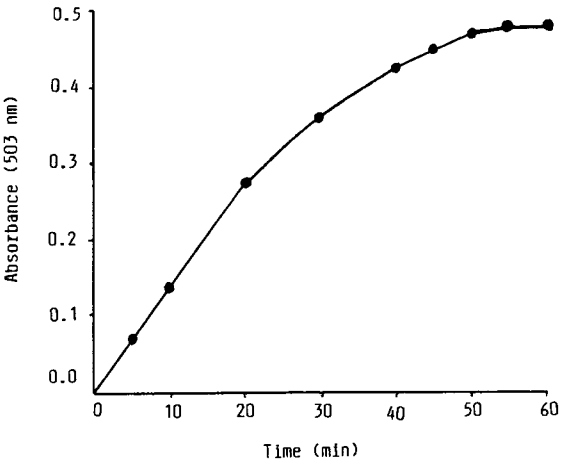

Fig. 2. Effect of heating time on color development.

and the reaction was complete after 55 min (Fig. 2). For routine estimations, heating for 45 min was practiced since 94% of the color was developed within this period. Compromise of 6% in sensitivity was made to reduce the evaporation losses that occur in the next 10–15 min. The color developed was stable for at least 8–10 h at room temperature.

Calibration Curve

The linearity of Schiff's base formation as a function of amount of Ceph C was examined by varying the quantity of Ceph C between 50 and 500 μg under assay conditions (Fig. 3). The results indicate that the reaction observed Beer's Law up to 500 μg of Ceph C. The methodology is sensitive enough to measure even 50 μg of Ceph C.

Monitoring of Bioconversion

The conversion of Ceph C to GL-7-ACA using immobilized DAO was performed as described in Methods. The values of residual Ceph C and GL-7-ACA formed are plotted in Fig. 4. The curve observed a typical enzymatic conversion pattern in which the rate is higher at the initial stages. A 95% conversion of Ceph C was achieved in 80 min; 75% of the conversion was complete in 45 min and it took another 35 min for remaining 20% conversion. The extent of Ceph C depletion was proportional to GL-7-ACA accumulation.

In conclusion, the developed method is sensitive and simple for determination of Ceph C and finds application in monitoring bioconversions. Storage of the samples prior to estimation is not required enabling processing of a large number of samples.

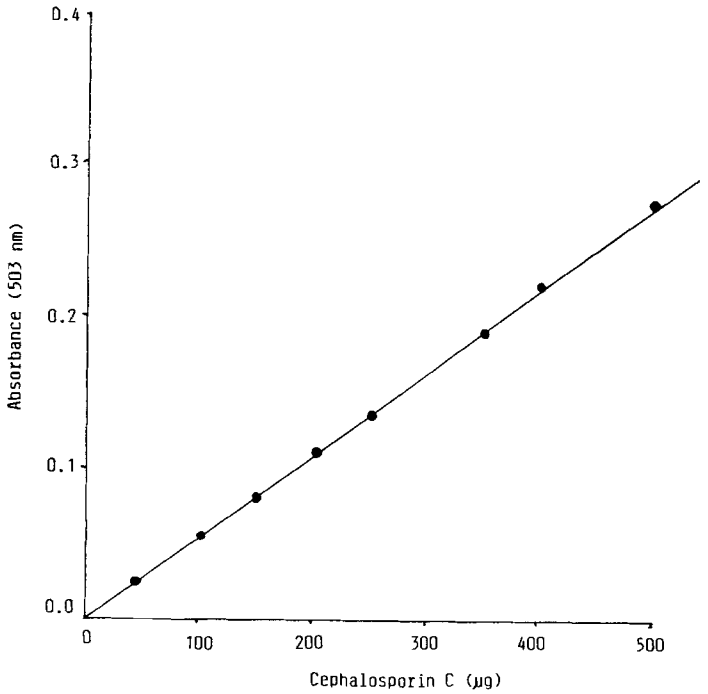

Fig. 3. Standard curve for estimation of Ceph C.

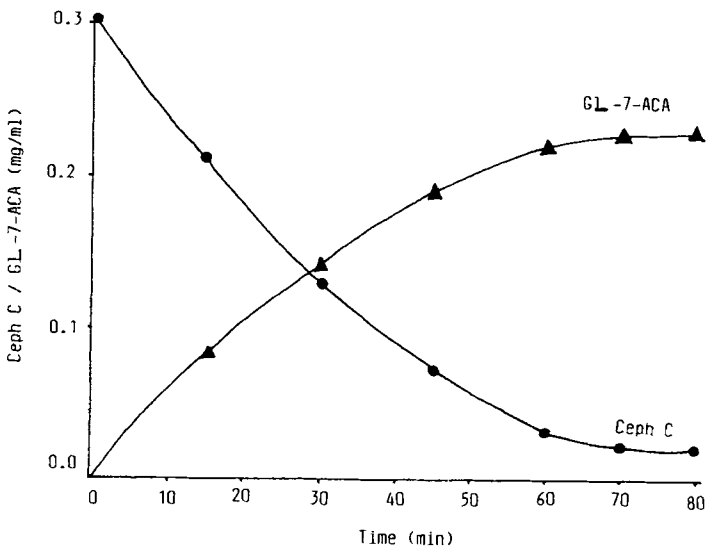

Fig. 4. Enzymatic conversion of Ceph C to GL-7-ACA.

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