IMPROVED AND ECONOMICAL SYNTHESIS OF CEFAZOLIN SODIUM

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ABSTRACT
Cefazolin sodium was synthesized using 7-amino cephalosporanic acid (7-ACCA) and H-tetrazole acetic acid (TAA) by acylation reaction in methylene chloride as a solvent and pivaloyl chloride as a catalyst. Various parameters involving synthesis such as duration, temperature, pH, catalyst (acidic and basic), coupling and activating agents were optimized. The product yield was more than 100% with 98% purity as per BP/ USP specifications. The material kept at 70°C and 75% of relative humidity for 72 h showed a slight change in general appearance from almost white to off white with 3-4% loss of weight due to removal of water, while pH of the material before and after the tests remained almost the same. The material complied well with pharmacopoeia specification. The described synthesis is found to be simple, efficient and economical and may be used by pharmaceutical raw material industry.

Keywords: Cefazolin sodium, 7-amino cephalosporanic acid, H-tetrazole acetic acid, Methylene chloride

INTRODUCTION
Cefazolin sodium, an antibiotic of 1st generation cephalosporin derivative, had been reported to be incompatible with amino glycosides, tetracycline and other antimicrobial agents including erythromycin gluceptate and polymyxin-B sulphate (Tintinali et al., 1995). It is more susceptible to Staplococcal β-lactamase than other cephalosporins (Mandell et al., 1940).
Chanfra et al. (1999) described a method for synthesis of an intermediate 2-mercapto-5-methyl-1, 3, 4-thiazolidine for the preparation of cefazolin. Kidwal et al. (1999) reported the pollution free microwaves assisted method for the synthesis of cephalosporins. Zbrozek et al. (1998) studied the stability and compatibility of cefazolin, cefazedone and cofactor. Much of the work reported was with respect to the antibiotics efficacy, bioavailability, kinetics and stability but its economical and industrial production was the least attempted. Efforts were made in these investigations to adopt different synthesis routs to obtain the target compound. The proposed method used 7-amino – cephalosporanic acid (7ACA) and H-Terazole acetic acid for the preparation of cefazolin sodium antibiotics.

MATERIALS AND METHODS
Chemicals
The materials used in this study included 7-aminocephalosporanic acid (7-ACA), pyridine, tetrazole acetic acid, pivaloyl chloride, methylene chloride, N, N-dimethyl acetamide, diethyl amine, N, N dimethyl formamide and acetone.

Instruments
The IR spectrum of compounds was recorded in nujol on IR spectrometer (Shimadzu IR 408 using a system of Shimadzu in wave number range 4000-650 cm⁻¹). Ultra-Violet spectrum was taken by UV-Vis spectrophotometer (Shimadzu UV-201). HPLC lc-6A chromatograph fitted with Lc-6A. gradient pump and SPD-6AV-UV-Vis detector was used.

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Methods

1. Formation of 7-ACA-TD salt
Mercapto-5-methyl-1,3,4-thiadiazole (28.0 g) was suspended in 220 mL of distilled water. Then boric acid (21.3 g) and suspended in 220 mL distilled water. Then triethyl amine (20.0 mL) was added in the above suspension. The contents were heated to 70-72°C and stirred vigorously to dissolve the materials. Then, 20.0 g accurately weighed 7-amino cephalosporanic acid (7-ACA) was added at this point and the mixture was kept at 70-72°C while stirring for 1 h. After this specified time, reaction mixture was cooled to 20°C and the pH of the solution was adjusted to 4.8. The reaction temperature was further lowered to 5-10°C and stirred for another half an hour.

2. Preparation of 7-ACA-TD salt solution
TDA salt (20.0 g) and 65 mL of dimethyl formamide were mixed at the temperature of 20-25°C. Later on, 8 mL of diethyl amine was added with continuous stirring for 10 min keeping the temperature constant.

Preparation of mixed acid anhydride
a) Formation of tetrazole acetic acid (TDA) salt
TAA (10.0 g) was added into 20 mL of IPA and stirred for 5 min. Then, 16 mL of triethyl amine (TEA) was added at this point followed by continuous agitation for 30 min at constant temperature of 65-70°C. Then reaction contents were cooled to room temperature followed by addition of 120 mL of methylene chloride. pH of the reaction mixture was adjusted to 7.2-7.5. The contents were brought to room temperature followed by addition of 5.0 mL dimethyl acetamide and 0.1 mL pyridine. Pivaloyl chloride (16 mL) cooled to a temperature of -45°C with the help of liquid nitrogen was added in the reaction mixture. Reaction mixture was stirred further for one and half an hour at temperature of -30°C. After that reaction content was brought to -50°C with the help of liquid nitrogen.

3. Acylation with mixed acid anhydride
Since the mixed acid anhydride is liable to temperature, the TDA solution step 1b was added in situ in 30 min through dipping funnel. The reaction mixture was agitated for 3 h according to the given scheduled (Table I) temperature and time period. The pH of the acetylated mixture was kept at 4.5 to 5.5.

4. Crystallization of cefazolin (Free Acid)
The reaction mixture contents of step 3 were transferred to 10 mL beaker and water (500 mL) was added. Two distinct layers were formed in beaker. The aqueous layer was separated and heated to 35°C while keeping the pH of the contents at 2.4 by the addition of hydrochloric acid (8.0 mL). The agitation was stopped and contents were cooled to 0-5°C. Precipitates formed were kept for 30 min. After this the agitation was started keeping the pH at 2.4 and temperature of the reaction mixture at 0-5°C. The agitation was continued for one and half hour at 0-5°C. The precipitates were filtered under suction, washed and dried.

Table 1 The scheduled temperature and time period

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Time period</th>
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<tbody>
<tr>
<td>-40 to -35°C</td>
<td>1 h</td>
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<tr>
<td>-35 to -30°C</td>
<td>1 h</td>
</tr>
<tr>
<td>-30 to -25°C</td>
<td>1 h</td>
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5. Preparation of cefazolin sodium salt
a) Sodium acetate solution
Sodium acetate trihydrate (8.3 g) was taken in a flask followed by addition of 18.0 mL of water. The contents were agitated to dissolve sodium acetate trihydrate. Isopropyl alcohol (40.0 mL) was added in it followed by agitation for further 30 min.
b) Cefazolin solution
Dimethyl acetamide (40.0 mL) was placed in a 100 mL flask followed by the portion wise addition of 23.0 g cefazolin (free acid) in a period of 10 min. The mixture was agitated for 30 min at room temperature (25°C) until the solution becomes clear.
c) Formation of cefazolin sodium
To the sodium acetate solution (5a) was added cefazolin solution (5b) drop wise in one hour at 10-15°C. The mixture was agitated for one hour at room temperature (25°C). The isopropyl alcohol (100 mL) was added in 30 min at 25°C. Then the reaction mixture was further stirred for half an hour at 5-10°C. The catalysts were filtered, washed with isopropyl alcohol (IPA) 80.0 mLx 2 and dried. The yield was calculated.

RESULTS AND DISCUSSION
The cephazolin derivative (cefazolin sodium) was prepared by different methods in making use of catalysts, temperature ranges and reagents. These methods were carried out experimentally to check out the yield and quality of cephazolin sodium. The different methods employed included silylation method and condensation method. Each method had shown its merits and demerits. But the synthetic method studied in these investigations revealed that this method is one of the economical and high yielding process in comparison to the already studied methods. A mechanistic scheme of the chemical reactions had been given in Figure 1. In step I conversion of 7-amino cephalosporanic acid (7-ACA) was carried out to form 7-ACA-TD by reacting with MMTD in presence of boric acid, at 70-75°C. In step II the compound thus formed was made soluble by reacting the acidic group in DMF with diethyl amine. The aqua
Figure 1: The scheme of synthesis of cefazoline sodium
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Soluble compound 7-ACA-TD solution was reacted with mixed acid anhydride which was obtained from salt of 1\(^H\)-Tetrazol acetic acid and pivaloyl chloride (step III and step IV). Acylation of 7-ACA-TD solution was mixed acid anhydride had been explained in step V. The compound obtained was crystallized and sodium salt was prepared by treatment with sodium acetate.

The synthesized material from all experimentation was subjected to analysis. It had been found that the material came out to be of the same general appearance of white powder throughout all the experimentation. The pH of the material was found to be around 5.0, which complied with the pH range stated in pharmacopoeias, BP/USP, 4.0 to 6.0. Water contents were calculated with an automatic K.F Titrator, which was calibrated shortly before use. The values were around 4.5, which were also within the range specified in pharmacopoeias (i.e. not more than 6.0%).

Assay of the title compound was determined by HPLC in accordance with the method stated in pharmacopoeia and was calculated against standard cefazolin sodium. The assay of dried sample of the product prepared by each experiment was about 98%, which showed the chemical consistency of the product. All results complied as specified in pharmacopoeias, BP/95%-102% and USP/89.1%-110.1%.

The material was also subjected to stress test. In stress test material was kept for 72 h at 70°C with the environment having 75% of relative humidity. There was slight change in general appearance from almost white to off white while pH of the material before and after the tests remained almost the same. Material before and after stress showed slight reduction in water contents while loss in assay was around 3-4%. The material compiled well with specification (In-house and pharmacopoeia) even after stress test.

CONCLUSION

In conclusion, it is stated that the analytical results including stress test confirmed the excellent quality of the products.

REFERENCES


