Pre-formulation Study of Salicylidine-cephalexin-Zn (II) dihydrate, a New Derivative of Cefalexin

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Pre-formulation Study of Salicylidine-cephalexin-Zn(II) dihydrate, a New Derivative of Cefalexin

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Key words
- cefalexin
- cefalexin complexes
- Schiff base complexes
- zinc complexes

Abstract

Background: Salicylidine-cefalexin-Zn(II)·$2H_2O$, a new derivative of cefalexin, has been reported to possess enhanced anti-microbial activity and lower toxicity than cefalexin. It is, therefore, desirable to carry out a pre-formulation study to determine its pharmaceutical properties which will be useful in conversion of the new molecule into various dosage forms.

Methods: The compound was synthesized by the previously reported method and characterized by elemental, Fourier-transform infrared and electronic spectral analyses. Crystallinity was determined by powder x-ray diffraction. Particle size distribution was determined by a laser-based sizer. Other properties including flow, density and compaction strength were determined by use of appropriate standard methods. The compound was also evaluated as a prodrug through dissolution study by the USP method.

Results: It was found that the new derivative is an amorphous powder with different bulk density, porosity, compressibility, plasticity and flow properties as compared to cefalexin. The amorphous character of the new compound suggests that it will have better bioavailability. The dissolution study indicated that this compound is hydrolyzed to produce cefalexin in water in a sustained manner, thus it will act as a prodrug in vivo. The release data fitted well into Highuchi model.

Conclusion: Various pharmaceutical properties essentially required for formulation of salicylidine-cefalexin-Zn(II)·$2H_2O$ into dosage forms were determined. This study has shown that the new drug would behave as a prodrug for cefalexin with better bioavailability.

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Bibliography
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Introduction

Cefalexin (cef) is a first generation cephalosporin antibiotic, which remained popular for about 40 years, for the treatment of infections involving streptococcal pharyngitis, otitis media, pneumonia, cellulitis, septic arthritis, and urinary tract. Now several bacterial strains have become resistant against this drug and it also has several adverse effects. In order to cope with these problems, medicinal chemists have been synthesizing cefalexin analogues and derivatives for better activity and broad spectrum [1–4]. One of the approaches that can be used to modify its structure is to prepare coordination complexes with metal ions [5]. Such complexes are expected to be more effective and less toxic as they may behave as prodrugs. Recently, we have reported synthesis and activity of the salicylidine-cefalexin-Zn(II)·$2H_2O$ (sal-cef-Zn), a derivative of cefalexin, showing greater efficacy and reduced toxicity [6]. In this paper, we wish to report its pre-formulation study, which determined the parameters to be considered for converting the new derivative into suitable dosage forms.

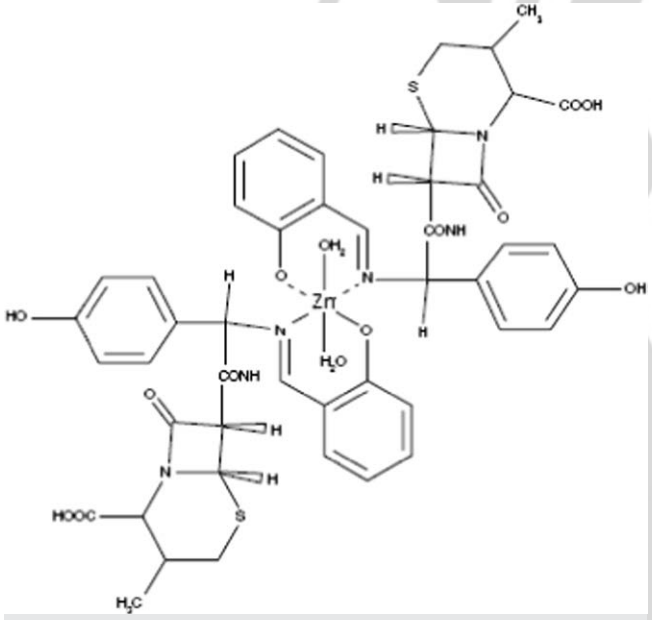
Materials and Methods

Materials

Chemicals used were: cefalexin monohydrate (Pharmagen, Lahore; Lot # 00213/108/2012), magnesium stearate (Linghuxinwang Chemicals, China; Batch # 20110701), zinc acetate dihydrate (Extrapure, E. Merck, Germany; Lot # 531A804400), salicylaldehyde (Extrapure, E. Merck, Germany; Lot # S5091440844), potassium hydroxide (Extrapure, E. Merck, Germany; Lot # B634632431), and methanol (Extrapure, E. Merck, Germany; Lot # 012K 137691089). All reagents and chemicals were of analytical grade and were used without further purification.

Preparation of salicylidene-cefalexin-Zn(II) • $2H_2O$

The complex was prepared in bulk quantity according to the previously reported method [6]. Briefly, cefalexin monohydrate (27.7912 g, 8.00 mmol) and potassium hydroxide (4.5000 g, 8.00 mmol), were mixed in methanol (100 mL) under constant stirring in a glass reactor. To this, salicylaldehyde (9.7696 g, 8.00 mmol) and zinc acetate dihydrate (8.7800 g, 4 mmol) were added one after the other. The reaction mixture was refluxed for about 3 h. The product was isolated after reduction of volume by evaporation. It was filtered off, washed with methanol and dried under vacuum. Yield: 84%. The complex was characterized by elemental analysis (CHNZn), decomposition point, FT-IR and electronic absorption (UV-vis) spectroscopic techniques. Elemental analysis: Found (calculated) C 54.93(55.11), H 4.46(4.42), N 8.26(8.36), Zn 6.46(6.52); decomp 261 °C; FT-IR (cm^{-1}): ν(OH) 3 395, ν(C=N) 1 625, $\rho_r(H_2O)$ 890, 847, $\rho_w(H_2O)$ 540, ν(Zn-N) 460, ν(Zn-O) 350; UV-Vis in DMF (nm/ε): ligand 210/7438, 251/53565. The complex was characterized to be similar to that previously reported; the structure is shown below.



Structure of sal-cef-Zn

Powder x-ray diffraction

Powder x-ray diffraction (PXRD) measurements were carried out in the 2θ range of 5–50 ° in steps of 0.02 ° by a diffractometer (D/MAX-II Rigaku Japan) equipped with a Cu-K_α source.

Particle size distribution

Particle size distribution was studied by using a Mastersizer 2000 (Malvern Instruments Ltd, UK). Slurry was prepared by dispersing the powder (2.00 g) in 20 mL ethanol-water (50:50) mixture. The slurry was sonicated to dissociate the agglomerates. Approximately 5 mL of slurry was used, after sonication, for each measurement. Size distribution was reported as 'span'; a narrower distribution would result in a smaller span. The span was calculated using the equation: Span = [$D(v, 0.9) - D(v, 0.1)/D(v, 0.5)$]. Particle size was reported as the mean of 3 measurements and expressed as $D(v, 0.1)$, $D(v, 0.5)$, and $D(v, 0.9)$, where D is particle size in µm based on the volume, v, of the particle, and 0.1, 0.5, 0.9 indicate the percentage (10, 50, 90% respectively) of the particles.

Moisture content

Moisture was determined by a Karl-Fischer titrator (799 GPT Titrino, Metrohm, Switzerland), using methanol as solvent. The Karl-Fischer method measures coordinated as well as lattice water alike [7].

True density

True density (ρ_{true}) was determined by the specific gravity method using 0.5 g of the sample in xylene and ρ_{true} was calculated as: $\rho_{true} = w/SG\,[(a+w)-b]$ [8], where w is the weight of the sample, SG the specific gravity of xylene, a is the weight of the bottle filled with xylene and b is the weight of the bottle filled with the powder and xylene. The determination was performed in triplicate and reported as the mean.

Bulk and tap densities

The sample (10.000 g) was transferred to a 50-mL clean and dry graduated cylinder. The cylinder was lightly tapped twice to collect all the powder sticking to the walls. The volume, V_0 was then read directly from the cylinder. The cylinder was then tapped 500 times using tap density analyzer (Vanderkamp Model 10703, Vankel, USA) and the volume V_{500} was determined according to British Pharmacopoeia (BP) method. Bulk and tapped densities were calculated as the ratio of the weight to volume (V_0 and V_{500} separately).

Porosity

Porosity of the test material was calculated using the equation: $\varepsilon = (1 - \rho_{tap}/\rho_{true}) \times 100$ where ε, ρ_{tap} and ρ_{true} are porosity, tap density, and true density respectively. From these data, Carr's Index (CI) [9] and Hausner ratio (HR) [10] were calculated using the equations: $CI = [(\rho_{tap} - \rho_{bulk})/\rho_{tap}] \times 100 = [(V_0 - V_f)/V_0] \times 100$; $HR = \rho_{tap}/\rho_{bulk} = V_0/V_f$ where V_0 and V_f are initial and final volumes.

Flow

Flow was determined by 2 methods and the results were reported in terms of angle of repose (α) and flow rate ($g\ s^{-1}$). These parameters were determined by using the Powder Analyzer (Pharma Test PTB, Germany) according to the United States Pharmacopoeia (USP) method by using 10 g of the sample. All measurements were made in triplicate.

Preparation of compacts

Compacts (300 mg each) were prepared by use of a manual hydraulic press (Schimadzu, Japan) at different compression pressures in the range 6–60 MPa using a 13 mm die, flat-faced punches, and a dwell time of 30 s. Before each compression, die and punches were lubricated with magnesium stearate dispersion in alcohol (1% w/v). The compacts were stored over silica gel in a desiccator for 24 h for hardening [11]. The compacts were then weighed accurately and their thickness and diameter were measured with ±0.01 mm accuracy using a digital micrometer. The thickness was reported as the average of 5 measurements made at 5 different points between the 2 surfaces of the compact.

Density

Density measurements were made according to standard methods. Relative density for Heckel analysis was determined by the relationship: $\rho = \rho_{app}/\rho_{true}$, where ρ_{app} is the apparent density of the compacts at a particular pressure and ρ_{true} is the true density of the powder. The ρ_{app} was calculated from the ratio of the compact mass to its volume ($\pi r^2 h$, where r is the radius and h the thickness of the compact).

Hardness

Hardness of the compacts was determined after 24 h by an automatic hardness tester (Erweka TBH250 TD, Germany).

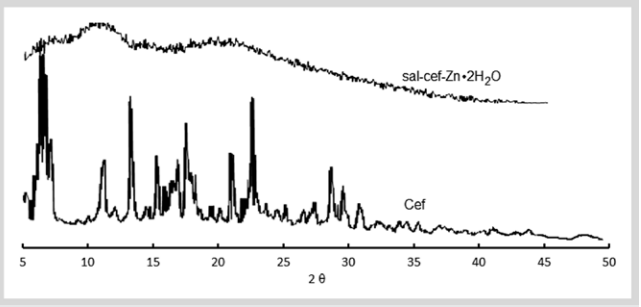


Fig. 1 PXRD spectra of sal-cef-Zn and cef.

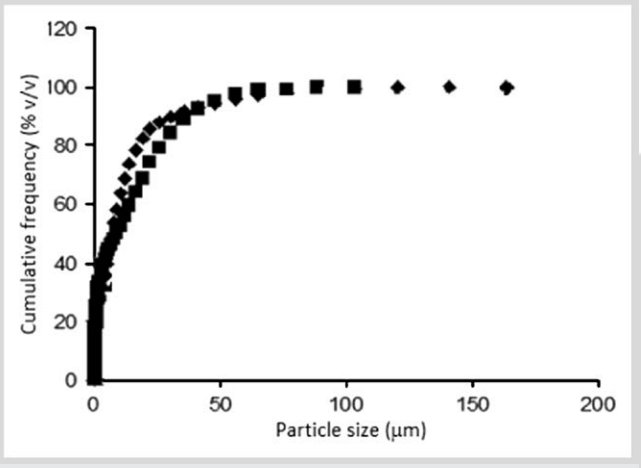


Fig. 2 Cumulative particle size frequency (volume) of cef (♦) and sal-cef-Zn (■).

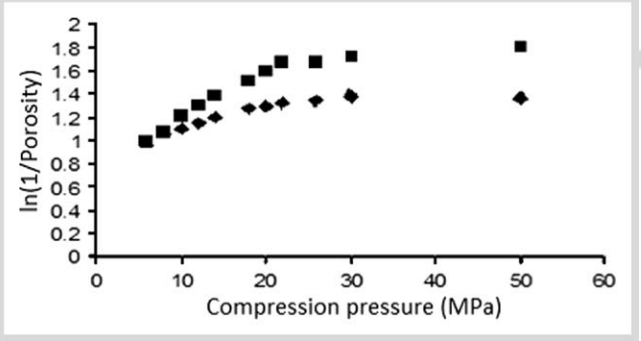


Fig. 3 Heckel plots of cef (♦) and sal-cef- Zn (■) compacts.

Heckel analysis

Porosity (ε) of the compacts was determined by the standard method and Heckel plots [12] were constructed by plotting $In(1/\varepsilon)$ against pressure P_y. Regression analysis was performed on the linear portion of the curve. A mean deformation pressure (P_y) was obtained from the slope of the line using the relationship: $P_y = 1/\text{slope}$. Statistical analysis was performed by use of Statgraphics® Plus software.

Evaluation as prodrug

The new derivative was evaluated as a prodrug by studying the release of cephalexin from the complex for 300 min by placing one capsule containing 180 mg of the test drug in each of the 6 vessels having water as a dissolution medium (900 mL) using USP dissolution apparatus II (Pharma Test, PTB, Germany) according the USP assay method. The samples (2 mL) were drawn at 10, 20, 30, 40, 60, 90, 120, 150, 180, 210, 240, 270, and 300 min after starting the apparatus and analyzed by measuring light absorption at 263 nm using a spectrophotometer (Pharmaspec UV1700 Schimadzu, Japan) according to the BP method. The amount of drug released from the derivative was expressed as percent of the total loaded drug. The kinetics of drug release was determined by fitting the data in zero order, first order, Higuchi and Hixon-Crowel models, and the release profiles were compared by dissimilarity (f_1) and similarity (f_2) factors [13].

Results and Discussion

Preparation of the cefalexin complex

The complex having light yellow color was easily obtained in bulk quantities with 84% yield according to the method used and the product was found to be similar to that already reported as indicated by the elemental analysis, FT-IR and UV-Vis spectra.

Powder x-ray diffraction

The PXRD spectra of cef and sal-cef-Zn are shown in **Fig. 1**. The sal-cef-Zn appears to exhibit amorphous character in contrast to the crystalline nature of cef. This change is expected to result in improved tabletting properties and bioavailability of the new derivative [14].

Particle size

The D value (µm) as determined by particle size analysis were: $D(v,0.1)$ 0.62, $D(v,0.5)$ 10.26, $D(v,0.9)$ 43.52. The values of span and specific surface area were 4.179 µm and 2.919 $m^2 g^{-1}$, respectively. This analysis indicates that particle size distribution of cef and sal-cef-Zn is largely comparable (**Fig. 2**). However, cumulative size range showed narrow span for sal-cef-Zn (4.179 µm) as compared to cef (4.273 µm) and the particle size of sal-cef-Zn was relatively greater than that of cef. This was also evidenced by better compactability shown by sal-cef-Zn as compared with cef (**Fig. 3**).

Density

Bulk, tap, and true densities of sal-cef-Zn observed were: ρ_{true} 1.30 $g\,mL^{-1}$, ρ_{bulk} 0.32 $g\,mL^{-1}$ and ρ_{tap} 0.47 $g\,mL^{-1}$. These values were higher than those of cef (ρ_{bulk} 0.21 and ρ_{tap} 0.35). This increase would eliminate the need for densification in tablet formulation. This also results in decreased bulkiness (the reciprocal of bulk density), which would reduce voluminous packaging requirements and use of bulking agents for formulations.

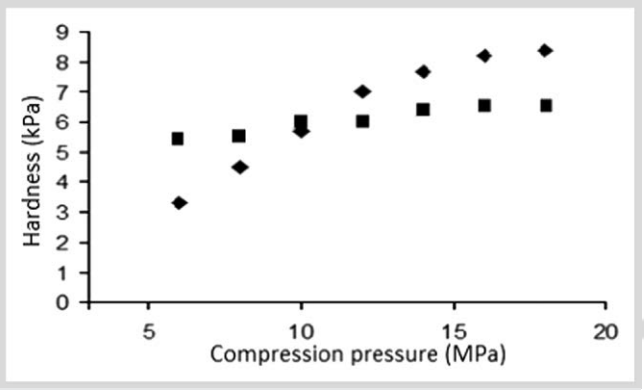


Fig. 4 Hardness of cef (♦) and sal-cef Zn (■) compacts.

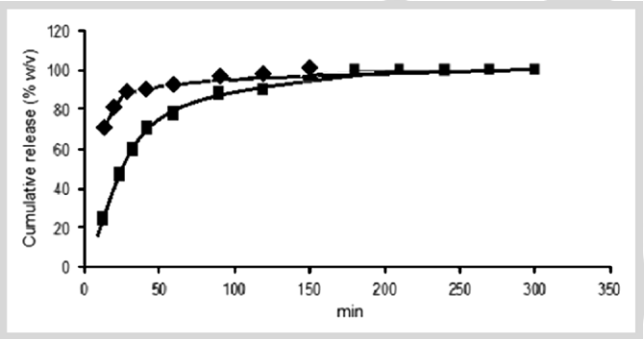


Fig. 5 **R**elease profiles of cef from capsules of the cef (♦) and sal-cef-Zn (■).

Porosity

The sal-cef-Zn (63.55%) showed decrease in porosity as compared with cef (76.54%) suggesting that the new material will exhibit lesser compressibility as evidenced by lower Carr's compressibility index (sal-cef-Zn: 31.50, cef: 40.00). This was the only negative effect of derivatization observed in this study.

Flow

Flow is typically determined by powder properties including density, surface area, moisture content, particle shape, particle size, and size distribution. The results of angle of repose (24°), Hausner ratio (1.46) and Carr's index (31.35%) [15] are consistent with the flow rate ($10\,g\,s^{-1}$), in case of sal-cef-Zn, as determined by flow meter. On the other hand cef did not flow through at all. Thus a substantial improvement was observed in flowability on derivatization.

Heckel analysis

Heckel analysis was performed to study the effect of applied pressure on the changes in relative density of the powder bed during compaction [16]. The Heckel plots for cef and sal-cef- Zn are shown in **Fig. 3**. The results of the regression analysis performed on the linear portion of the Heckel plot of sal- cef-Zn were: $R^2 = 0.9875$, slope = 0.0419 and mean deformation pressure (P_y) = 23 MPa. The slopes of the Heckel plots indicated that plasticity/ductility improved markedly on derivatization of cef.

Hardness

The relationship between the hardness of the compacts and the applied pressure is shown in **Fig. 4**. These results show the formation of weaker compacts by sal-cef-Zn. This property correlates well with the results of porosity data as discussed above.

Moisture content

Moisture content (bonded and adsorbed water) of the new material was 9.00% w/w, which is higher than the parent drug (6.00% w/w). The enhanced moisture content is understandable as the new derivative contains 2 water molecules as a part of the formula. Moisture content is known to have a significant effect on physical-mechanical properties, which play an important role in the tableting process [17]. The presence of free moisture on particle surface helps in the reduction of friction between the particles leading to increased fluidity. On the other hand, adsorbed water lowers the surface energy of crystals and enhances adhesion with the matrix as well as the die surfaces of the compression machines, which may be the cause of lower tablet strength observed here.

Evaluation as prodrug

This study was carried out to see if the complex under investigation can be used as a prodrug. It was performed by monitoring the release of cef from the complex in dissolution apparatus. The release profile is shown in **Fig. 5**. A comparison of the release profiles of cef from the derivative and the parent drug was made by use of dissimilarity and similarity factors, f_1 and f_2. The values of f_1 and f_2 obtained were 32.8246 and 8.4534 respectively, which indicate different release profiles of the 2 materials. The release data were fitted into various models; the R^2 values were: Zero-order 0.7789, First-order 0.3964, Highuchi 0.9416 and Hixon-Crowel 0.4274. The results indicate that release pattern from the derivative follows Highuchi model and as such the new compound can be considered as a prodrug with sustained release characteristics.

Conflict of Interest

There is no conflict of interest involved in this study.

References

1 *Brown AG*, *Roberts SM*. Recent advances in the chemistry of β-Lactam antibiotics. The Royal Society of Chemistry; London: 1984
2 *Brain EG*, *Eglington AJ*, *James BG et al.* Structure – activity relationships in cephalosporins prepared from penicillins. 2. Analogues of cephalexin substituted in the 3-methyl group. J Med Chem 1997; 20: 1086–1090
3 *Balkan A*, *Ertan M*, *Saraç S et al.* Synthesis and antimicrobial activities of some new tetrahydro-2H-1,3,5-thiadiazine-2-thione derivatives of cephalexin. Arzneimittel Forschung 1990; 40: 1246–1249
4 *Hornyák M*, *Hartmann JF*, *Sztaricskai FJ et al.* Beta-lactam antibiotics functionalized with gem-bisphosphonates. Acta Pharm Hung 1999; 69: 213–217
5 *Iqbal MS*, *Ahmad AR*, *Sabir M et al.* Preparation, characterization and biological evaluation of copper(II) and zinc(II) complexes with cephalexin. J Pharm Pharmacol 1999; 51: 371–375
6 *Iqbal MS*, *Bukhari IH*, *Arif M*. Preparation, characterization and biological evaluation of copper(II) and zinc(II) complexes with Schiff bases derived from amoxicillin and cephalexin. Appl Organometal Chem 2005; 19: 864–869
7 *Baudry DB*, *Dormond A*, *Durisa F et al.* Lanthanide bis (trifluoromethanesulfonyl) amides, synthesis, characterization and catalytic activity. J Fluorine Chem 2003; 121: 233–238
8 *Okhamafe AO*, *Azubuike CPC*. Direct compression studies on low-cost cellulose derived from maize cobs. J Pharm Sci & Pharm Prac 1994; 1: 26–29

9 *Carr RL*. Classifying flow properties of solids. Chem Eng 1965; 72: 69–72
10 *Hausner HH*. Friction conditions in a mass of metal powders. Int J Powder Metall 1967; 3: 7–13
11 *Krycer I*, *Pope DG*, *Hersey JA*. An evaluation of the techniques employed to investigate powder compaction behavior. Int J Pharm 1982; 12: 113–134
12 *Heckel RW*. An analysis of powder compaction phenomena. Trans Metall Soc AIME 1961; 221: 1001–1008
13 *Costa P*. An alternative method to the evaluation of similarity factor in dissolution testing. Int J Pharm 2001; 220: 77–83
14 *Yang W*, *Johnston KP*, *Williams RO III*. Comparison of bioavailability of amorphous versus crystalline itraconazole nanoparticles via pulmonary administration in rats. Eur J Pharm Biopharm 2010; 75: 33–41
15 *Shah RB*, *Tawakkul MA*, *Khan MA*. Comparative evaluation of flow for pharmaceutical powders and granules. AAPS PharmSciTech 2008; 9: 250–258
16 *Sebhatu T*, *Alderborn G*. Relationships between the effective interparticulate contact area and the tensile strength of tablets of amorphous and crystalline lactose of varying particle size. Eur J Pharm Sci 1999; 8: 235–242
17 *Rees JE*, *Hersey JA*. The strength of compacts containing moisture. Pharm Acta Helv 1972; 47: 235–243