The Influence of Excipients on The Physicochemical and Biological Properties of a Bactericidal, Labile Ester Prodrug in A Salt Form – A Case Study of Cefetamet Pivoxil Hydrochloride

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ABSTRACT

The article presents an innovative approach to a bactericidal drug design based on a cephem prodrug analogue – cefetamet pivoxil hydrochloride. The emergence of cefetamet pivoxil hydrochloride excipient systems (mannitol, hydroxypropyl methyl cellulose, pregelatinised starch, lactose monohydrate, magnesium stearate, polyvinylpyrrolidone) caused changes in the physicochemical properties of cefetamet pivoxil hydrochloride. They are significant for planning the development of an innovative pharmaceutical formulation. The biological activity profile of the prodrug was also modified. FTIR spectra were used to study interactions between cefetamet pivoxil hydrochloride and the excipients. The theoretical approach to the analysis of experimental spectra enabled precise indication of cefetamet pivoxil hydrochloride domains responsible for interaction with the excipients. The interactions between cefetamet pivoxil hydrochloride and the excipients resulted in some important physicochemical modifications: acceptor fluid-dependent changes in solubility and the dissolving rate as well as a decrease in the chemical stability of cefetamet pivoxil hydrochloride in the solid state, especially during thermolysis. The interactions between cefetamet pivoxil hydrochloride and the excipients also had biologically essential effects. There were changes in its permeability through artificial biological membranes simulating the gastrointestinal tract, which depended on the pH value of the acceptor solution. Cefetamet pivoxil hydrochloride combined with the excipient systems exhibited greater bactericidal potential against *Staphylococcus aureus*. Its bactericidal potential against *Enterococcus faecalis, Pseudomonas aeruginosa* and *Proteus mirabilis* doubled. The new approach provides an opportunity to develop treatment of resistant bacterial infections. It will enable synergy between the excipient and the pharmacological potential of an active pharmaceutical substance with modified physicochemical properties induced by the drug carrier.

KEYWORDS: Cefetamet Pivoxyl, pharmaceutical formulations, bactericidal activity, drug carriers

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INTRODUCTION

β-lactam analogues are still a predominant class of antibiotics applied to patients in hospitals as well as outpatients. Oral administration of β-lactam antibiotics is often limited by the increasing resistance of bacterial strains caused by long use of these drugs, their low chemical stability, poor bioavailability and peculiar flavour [1-3].

The introduction of prodrugs of penam, cephem and carbapenem antibiotics allowed the use of analogues characterised by better solubility (e.g. ceftaroline fosamil), greater bioavailability (e.g. tedizolid phosphate) and less bitter taste (e.g. cefuroxime axetil) [4-6]. Regardless of the subgroup of these analogues, their prodrugs originated in consequence of esterification of the carboxyl group in particular systems of bicyclic rings in penicillins, cephalosporins and carbapenems. The acidic form of β-lactam antibiotic prodrugs is activated by intestinal esterases. According to the definition, a prodrug is not supposed to convert into its pharmacologically active form until enzymatic or metabolic activation occurs. Due to the low bioavailability of β-lactam antibiotics the labile β-lactam bond responsible for the bactericidal potency of antibiotics may cleave and violate the cleavage priority of the ester bond in consequence of uncontrolled conversion of the prodrug into its active form within the digestive system [7]. Earlier studies showed that β-lactam bonds in acidic analogues from all subgroups of β-lactam antibiotics tended to break under the influence of various physicochemical factors [8-10]. Hydrogen and hydroxide ions as well as oxidising agents and temperature are the main determinants facilitating hydrolysis of labile ester and β-lactam bonds in solutions. Acid-base hydrolysis and oxidation of β-lactam analogues are rapid processes responsible for the degradation of antibiotics in the acidic environment of the stomach. These processes are induced by bacterial β-lactamases. During preformulation and formulation work the degradation of antibiotics may be catalysed by excipients. In the solid phase, the degradation of β-lactam analogues is induced by physicochemical factors such as humidity and temperature. The formation of degradation products different from those observed in acid-base hydrolysis shows that an increased rate of thermolysis at an elevated relative air humidity is an important limitation to the storage of β-lactam antibiotics. As outlined above, the presence of various degradation products and considerable lability of β-lactam antibiotics are vital safety aspects in antibiotic therapy. Specific products of β-lactam antibiotic degradation, such as haptens, cause the risk of anaphylactic shock [11].

Since labile oral active pharmaceutical ingredients (APIs) are required to be administered in the form of specified pharmaceutical formulations, it is necessary to examine the influence of excipients as agents modifying physicochemical properties crucial to pharmacotherapeutic safety and effectiveness (solubility, rate of release from drug-excipient systems, chemical stability and permeability) [12-15]. In view of this fact, it appears obvious that any modification affecting the physicochemical properties of labile prodrugs of β-lactam antibiotics requires detailed analysis.

The aim of this study was to assess the effect of the excipients on the solubility, chemical stability, dissolution profiles, permeability through artificial biological membranes and bactericidal activity of a specific prodrug of β-lactam antibiotics. Cefetamet pivoxil hydrochloride was selected as a model substance. Cefetamet pivoxil hydrochloride is an oral third-generation cephalosporin antibiotic. Its active form is cefetamet, which is obtained *in vivo* as a result of hydrolysis of the ester bond. The compound exhibits excellent potency against penicillin-sensitive *Streptococcus pneumoniae, Streptococcus spp, Haemophilus influenzae, Moraxella catarrhalis, Escherichia coli, Proteus spp., Klebsiella spp. and Neisseria gonorrhoeae* [16]. Cefetamet pivoxil hydrochloride is formulated only in tablets because of its intensive bitter taste and chemical instability in aqueous solutions.

Binary systems of cefetamet pivoxil hydrochloride and selected excipients were prepared for the study. The first stage of the study involved assessment of the interaction between cefetamet pivoxil hydrochloride and the excipients in the aforementioned powder systems. Next, the influence of the excipients on the physicochemical properties and antibacterial activity of cefetamet pivoxil hydrochloride was investigated. These parameters provided reference for the powder systems of cefetamet pivoxil hydrochloride and the excipients.

2. Experiment

2.1. Materials

Cefetamet pivoxil hydrochloride ((6R,7R)-7-[[(2Z)-2-(2-amino-1,3-thiazol-4-yl)-2-methoxyiminoacetyl] amino]-3-methyl-8-oxo-5-thia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylic acid) was synthesised at the Department of Modified Antibiotics, Institute of Biotechnology, Warsaw, Poland. Cefetamet pivoxil hydrochloride is a white or almost white powder. All other chemicals and solvents of analytical grade were provided by Merck KGaA (Darmstadt, Germany). High quality pure water was prepared using an Exil SA 67120 purification system (Millipore, Billerica, MA). The following excipients were used to test the compatibility of cefetamet pivoxil hydrochloride: mannitol (Merck KGaA, Darmstadt, Germany), hydroxypropyl methyl cellulose (Sigma-Aldrich), starch (Colorcon, Harleysville, PA), lactose monohydrate (DFE Pharma, Goch, Germany), magnesium stearate (JRS Pharma, Rosenberg, Germany), and polyvinylpyrrolidone (Sigma-Aldrich).

2.2. Preparation of mixtures of cefetamet pivoxil hydrochloride and excipients

In order to investigate the apparent solubility, chemical stability, permeability through artificial biological membranes and bactericidal activity of a 1:1 mixture of cefetamet pivoxil hydrochloride with commonly used excipients (mannitol, hydroxypropyl methyl cellulose, pregelatinised starch, lactose monohydrate, magnesium stearate, polyvinylpyrrolidone), samples of cefetamet pivoxil hydrochloride were weighed into 5 mL vials.

2.3. Characterisation of mixtures of cefetamet pivoxil hydrochloride and excipients

Fourier transform infrared spectroscopy was used to identify interactions between cefetamet pivoxil hydrochloride and the excipients. Mixtures of cefetamet pivoxil hydrochloride with the selected excipients were prepared separately with IR grade KBr at a ratio of 1: 100. The corresponding pellets were prepared by applying pressure of 8 tonnes in a hydraulic press. Vibrational infrared spectra ranging from 400 to 4000 cm^{-1} were recorded with an FT-IR Bruker Equinox 55 spectrometer equipped with a Bruker Hyperion 1000 microscope. Changes in the positions and intensity of the experimental spectra of mixtures of cefetamet pivoxil hydrochloride and the excipients were identified by quantum chemical calculations based on DFT, which showed theoretical spectra of cefetamet pivoxil hydrochloride. The Gaussian 03 package was used for the calculations [17].

2.4. Changes in cefetamet pivoxil hydrochloride concentrations

An HPLC Shimadzu Prominence-i LC-2030 C with additional Nano Quantity Analyte Detector (NQAD) QT-500 was used for chromatographic separation. As the stationary phase a Kinetex C18 column (100 × 2.1 mm × 2.6 µm) (Phenomenex, Torrance, CA) was used. The mobile phase composed of ammonium acetate (10 mmol L^{-1}): methanol: acetonitrile (33:16:15 V/V/V). The flow rate of the mobile phase was 0.8 mL min^{-1}. Separation was performed at 30°C. The wavelength of the UV detector was set at 254 nm. The evaporator temperature of the NQAD was set at 40°C. Clean dry air was used to nebulize the eluent at a pressure of 276 kPa and condensing liquid was water. Separation was performed at 30°C. The HPLC-DAD method was validated for cefetamet pivoxil hydrochloride, including the selectivity of its degradation products formed by oxidation [18].

2.5. Apparent solubility of mixtures of cefetamet pivoxil hydrochloride and excipients

The apparent solubility of cefetamet pivoxil hydrochloride mixed with the excipients was measured with an Agilent 708-DS dissolution apparatus. A standard paddle method was used at 310 ± 0.5 K and a stirring speed of 50 rpm. Cefetamet pivoxil hydrochloride in its free form and physical mixtures of cefetamet pivoxil hydrochloride and the excipients weighed into gelatine capsules were placed in a spring in order to prevent flotation of the capsule on the surface of the liquid. The resulting samples were placed in 500 mL of media of simulated gastric fluids (pH 2.1) and phosphate buffer (pH 6.8) simulating the gastrointestinal environment. Dissolution samples (5.0 mL) were collected at specified time intervals. An equal volume of temperature-equilibrated media was replaced and filtered through a 0.45 µm membrane filter. The HPLC-DAD method was

used to measure the concentrations of cefetamet pivoxil hydrochloride in acceptor solutions [18]. The values reported in this article are arithmetic means of six measurements.

The model proposed by Moore and Flanner to compare dissolution profiles is based on two factor values, f_1 and f_2 [19-20]. The difference factor (f_1) measures the percent error between two curves over all time points. f_2 is a logarithmic transformation of the sum of squared error of differences between the test T_j and reference R_j system over all time points according to the formulas below:

$$f_1 = \frac{\sum_{j=1}^{n}|R_j - T_j|}{\sum_{j=1}^{n} R_j} \times 100$$

$$f_2 = 50 \times \log\left(\left(1 + (\frac{1}{n})\sum_{j=1}^{n}|R_j - T_j|^2\right)^{-\frac{1}{2}} \times 100\right)$$

where n is the sampling number, Rj and Tj are the percentage of the reference (cefetamet pivoxil hydrochloride) and test products (mixtures of cefetamet pivoxil hydrochloride and the excipients) dissolved at each time point j. Dissolution profiles are similar when the f_1 value is close to 0 and f_2 is close to 100 (FDA guidelines suggest that two profiles are similar if f_2 is between 50 and 100).

2.6. Chemical stability of mixtures of cefetamet pivoxil hydrochloride and excipients

Mixtures of cefetamet pivoxil hydrochloride with the selected excipients (1:1) were prepared to test their compatibility. The mixtures were stored under stress conditions. The stability of the mixtures was investigated at increased RH (76.5%) at 348 K and in dry air (0% RH) at 363 K. At specified time intervals, determined by the rate of degradation, the vials were removed, cooled to room temperature and their contents were dissolved in distilled water and analysed with the HPLC-DAD method validated according to the ICH guideline [21].

2.7. Permeability of mixtures of cefetamet pivoxil hydrochloride and excipients

Differences in the gastrointestinal permeability of cefetamet pivoxil hydrochloride in its free form and in mixtures were investigated with the PAMPA method (parallel artificial membrane permeability assay). The system consisted of a 96-well microfilter plate and a 96-well filter plate. It was divided into two chambers: a donor at the bottom and an acceptor at the top, separated by a 120-µm-thick microfilter disc coated with a 20% (w/v) dodecane solution of a lecithin mixture (Pion, Inc.). Aqueous solutions of cefetamet pivoxil hydrochloride and mixtures of cefetamet pivoxil hydrochloride and the excipients (0.2 mg/L) were prepared in a different 96-well filter plate and added to the donor compartments. The donor solution was adjusted to pH 2.73 and 6.20 (NaOH-treated universal buffer). The plates were put together and incubated at 310K for 4h in a humidity-saturated atmosphere. After 4 hours of incubation the concentrations of cefetamet pivoxil hydrochloride in its free form and in mixtures with the excipients in the donor and acceptor compartments were measured with the HPLC-DAD method.

The apparent permeability coefficient (P_{app}) was calculated using the equation below:

$$P_{app} = \frac{-ln\left(1 - \frac{C_A}{C_{equilibrium}}\right)}{S \times \left(\frac{1}{V_D} + \frac{1}{V_A}\right) \times t}$$

where V_D – donor volume, V_A – acceptor volume, $C_{equilibrium}$ – equilibrium concentration, C_D – donor concentration, C_A – acceptor concentration, S – membrane area, t – incubation time (seconds). Compounds of $P_{app} < 1 \times 10^{-6}$ cm/s are classified as those of low permeability, whereas those of $P_{app} > 1 \times 10^{-6}$ cm/s are classified

as high-permeability compounds [22]. The P_{app} of cefetamet pivoxil hydrochloride in its free and complexed forms was compared with the ANOVA test.

2.8. Bactericidal activity of mixtures of cefetamet pivoxil hydrochloride and excipients

The Minimal Inhibitory Concentration (MIC) was calculated for each reference strain from the American Type Culture Collection and for clinical isolates. The MICs for cefetamet pivoxil hydrochloride and its mixtures with the excipients (1:1) were assayed using serial dilutions on a Mueller–Hinton liquid medium (Merck, Germany). A microbial culture of standardised optical density was used in the experiment. The method followed the standards of the National Committee for Clinical Laboratory Standards (NCCLS) [23].

3. RESULTS

The content of cefetamet pivoxil hydrochloride was analysed and checked for the presence of potential breakdown products in combination with the selected excipients (mannitol, hydroxypropyl methyl cellulose, pregelatinised starch, lactose monohydrate, magnesium stearate, polyvinylpyrrolidone). The chromatograms of the mixtures of cefetamet pivoxil hydrochloride and the excipients (1:1) did not reveal any peaks from breakdown products without chromophore structures (HPLC-DAD method) or non-chromophore ones (HPLC-NQAD method). The research results excluded the chemical instability of cefetamet pivoxil hydrochloride in mixtures with the selected excipients at the moment of preparation. Due to the fact that the formation of more hydrophilic and soluble compounds was excluded at this stage it was possible to assess the influence of the excipients on the lipophilic form of the prodrug in further part of the research.

The assessment of physical effects of the systems combining cefetamet pivoxil hydrochloride with the excipients was based on changes in FTIR spectra. The assessment of changes in the position of characteristic cefetamet pivoxil hydrochloride bands and their intensity was supported by the density functional theory. Therefore, first the bands which were significant to assessment of the identity of cefetamet pivoxil hydrochloride were identified (Fig. 2). Next, changes in the position and intensity of the characteristic bands were assessed when cefetamet pivoxil hydrochloride was added to mixtures with the selected excipients. The biggest changes in the cefetamet pivoxil hydrochloride spectra were found when it was combined with mannitol. There were much lesser changes when the prodrug was combined with polyvinylpyrrolidone (Fig. 1). The changes observed at 988 cm^{-1}, which corresponded to the C-O stretching vibrations in the pivoxil group, and the changes observed at 988 cm^{-1} and 1,038 cm^{-1}, which corresponded to the C-N and N-O stretching vibrations in thiazole and cepem rings, let us indicate 2-methoxyiminoacetyl] amino] group and (2-amino-1,3-thiazol-4-yl) structure as cefetamet pivoxil hydrochloride domains engaged in interaction with the excipients. There were no changes observed in the spectra when cefetamet pivoxil hydrochloride was combined with starch, hydroxypropyl methyl cellulose and lactose.

The second part of the research involved assessment of the influence of the selected excipients on the apparent solubility, chemical stability, permeability through artificial biological membranes and bactericidal activity of cefetamet pivoxil hydrochloride.

The cefetamet pivoxil hydrochloride dissolving rate in the presence of the selected excipients (mannitol, hydroxypropylmethyl cellulose, pregelatinised starch, lactose monohydrate, magnesium stearate, polyvinylpyrrolidone) was measured in an acceptor fluid of pH 1.2 and pH 6.8. The systems under analysis did not differ in the shape of the dissolving rate curves as was expected, the cefetamet pivoxil hydrochloride dissolving rate varied according to the pH value of the acceptor fluid. When the pH 1.2 acceptor fluid was applied, cefetamet pivoxil hydrochloride dissolved faster both in its free form and in mixtures with the excipients. The dissolving rate did not improve significantly when cefetamet pivoxil hydrochloride was combined with the excipients. When the phosphate buffer (pH = 6.8) was used for cefetamet pivoxil hydrochloride with or without the excipients, the dissolving rates were slower (Fig. 2).

As the ICH guidelines require that the compatibility of API-excipient systems should be assessed at higher

temperature and humidity, the next stage of the research consisted in assessing the influence of the excipients on the chemical degradation of cefetamet pivoxil hydrochloride at increased relative humidity (RH = 70%, T = 343 K) and dry air (RH = 0, T = 393 K) [21]. Regardless of the stress conditions, the degradation of cefetamet pivoxil hydrochloride in its free as well as in complexed form was a pseudo-first-order reaction described by the equation below:

$$\ln C_t = \ln C_0 - k_{obs}\, t$$

where C_t and C_0 are the concentrations of cefetamet pivoxil hydrochloride, at time t = 0 and t, respectively and k_{obs} is the rate constant of degradation reaction. The semi-logarithmic plots were linear and their slopes were equal to the rate constants of the reactions with the negative sign ($-k_{obs}$). There were no peaks originating from an additional degradation product in the HPLC chromatograms of API-EXP systems. By contrast, there were peaks on the chromatogram of cefetamet pivoxil hydrochloride in its free form. The analysis of changes in the concentration of cefetamet pivoxil hydrochloride under both conditions of degradation showed that HPMC and PVP had the strongest influence catalysing the degradation of cefetamet pivoxil hydrochloride (Fig. 3).

The comparison of the permeability of cefetamet pivoxil hydrochloride in its free form and in the excipient systems (the permeability coefficient values (P_{app} (A→B) and P_{app} (B→A)) through the artificial biological membrane system simulating the gastrointestinal tract showed that the diffusive permeability of API was reduced regardless of the excipients used. Interestingly, the pH value of the acceptor fluid was also particularly significant in this test. The greatest permeability of cefetamet pivoxil hydrochloride in its free form was noted at pH 2.73 (1.5661×10^{-5} cm/s). When it was combined with the selected excipients, the apparent permeability coefficients were significantly lower, i.e. PVP (0.1028×10^{-5} cm/s), HPMC system (0.07498×10^{-5} cm/s), mannitol system (0.0806×10^{-5} cm/s), lactose monohydrate system (0.0665×10^{-5} cm/s), magnesium stearate (0.0432×10^{-5} cm/s). When the pH value of the acceptor fluid was 6.20, the combinations with two excipients (mannitol and PVP) were characterised by greater permeability, but the permeability of cefetamet pivoxil hydrochloride in its free form was lower than in the pH 1.2 acceptor fluid (Fig 4).

Ten mostly Gram-negative bacterial species, including reference strains and clinical isolates, were analysed microbiologically to determine the inhibitory concentration of cephalosporin – cefetamet pivoxil hydrochloride (control sample) in combination with the excipients (mannitol, hydroxypropyl methyl cellulose, pregelatinised starch, lactose monohydrate, magnesium stearate, polyvinylpyrrolidone). The lowest values of the active substance concentration inhibiting the growth of bacteria were noted for the following species: *Proteus mirabilis* (reference strain and clinical isolate MIC=1.0 mg/L), *Klebsiella pneumoniae* (reference strain MIC=0.25 mg/L) and *Escherichia coli* (reference strain and clinical isolate MIC=1.0 mg/L). *Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella enteritidis* and *Salmonella typhimurium* were the most sensitive species to cefetamet pivoxil hydrochloride. The MIC value for these strains amounted to 125 or 250 mg/L. It is noteworthy that when the excipients were added, the MIC value changed in some cases. There was a difference between the group of compounds which could be a potential source of carbon for bacteria and those which microorganisms could partly metabolise or which could not be metabolised at all. When mannitol and lactose were applied, the MIC value increased for most of the strains. The only exceptions were the clinical isolates of the *Klebsiella pneumoniae* and *Enterobacter aerogenes* species as well as all the strains of the *Salmonella* genus. The MIC value for these microorganisms did not change in the variant where cefetamet pivoxil hydrochloride was combined with mannitol, as compared with the active substance on its own. When cefetamet pivoxil hydrochloride was combined with lactose, the MIC value increased for most of the strains (except those of the *Salmonella* genus), as compared with the active substance on its own. The MIC value decreased especially in the variants where cefetamet pivoxil hydrochloride was combined with polyvinylpyrrolidone or hydroxypropylmethyl cellulose. There were different MIC values observed for the following species: *P. aeruginosa*, *E. coli* (for reference strains and clinical isolates) and *Enterobacter aerogenes* (mostly for the clinical isolate). The biggest difference was observed for strains of the *P. aeruginosa* species. The MIC value of cefetamet pivoxil hydrochloride was 125 mg/L. When cefetamet pivoxil hydrochloride was combined with hydroxypropyl methyl cellulose, the MIC value amounted to 32 mg/L (Table 1).

4. Discussion

The increasing bacterial resistance to a large number of different groups of antibiotics, including β-lactam antibiotics, is a serious clinical problem nowadays [24]. The effectiveness of treatment of bacterial infections with β-lactam antibiotics is significantly limited by their considerable chemical instability and low bioavailability, which limits their capacity to achieve the necessary bactericidal concentration after oral administration in vivo [25-27]. Prodrugs for β-lactam antibiotics are used in an attempt to reduce these limitations. However, it is necessary to remember that increased lipophility of a β-lactam analogue will reduce its solubility, modify its bactericidal effect and bioavailability. What is more, the combination of β-lactam antibiotics with the excipients may cause chemical changes resulting from lability of ester bonds (ester (at C-2) and amid (at C-7) bicyclic ring system) and physical effects resulting in significant physicochemical changes for the prodrug profile of action and its safety [28-29]. Therefore, it is important to define the effect of excipients on selected prodrugs, especially those with several labile bonds. Due to this fact, the main focus of the first stage of our study was to assess the potential physical and chemical changes in combinations of cefetamet pivoxil hydrochloride with the selected excipients such as: mannitol, hydroxypropyl methyl cellulose, pregelatinised starch, lactose monohydrate, magnesium stearate, and polyvinylpyrrolidone. The exclusion of chemical instability of cefetamet pivoxil hydrochloride should be associated with the fact that there are no acid forms of this prodrug, which would be more hydrophilic, easier to dissolve and more bactericidal. The fact that cefetamet pivoxil hydrochloride exhibited adequate chemical stability when it was combined with the excipients let us assess the influence of the excipients on the lipophilic form of the prodrug. The following characteristics were analysed: changes in the dissolving rate, permeability through artificial biological membranes simulating the gastrointestinal tract and bactericidal activity.

The FTIR assessment of physical interactions between the components of mixtures showed that two domains of cefetamet pivoxil hydrochloride were particularly engaged in interactions with the excipients, i.e. the 2-methoxyiminoacetyl] amino] group and the (2-amino-1,3-thiazol-4-yl) structure. Both groups exhibited considerable lipophility. Their interaction with selected macrostructures caused changes in the solubility of the lipophilic ester API form and its permeability through biological membranes. When cefetamet pivoxil hydrochloride was combined with mannitol and PVP, there were changes in the position and intensity of their spectra.

Differences in the dissolving rate of cefetamet pivoxil hydrochloride in acceptor fluids of different pH values may have been caused by different ionic forms of the prodrug and interactions with the excipients blocking the lipophilic group. It was reported that the maximum stability of cefetamet pivoxil hydrochloride was observed within the pH values ranging from 3 to 5 [30]. This observation suggests that when the pH value is acidic, cefetamet is relatively stable and more soluble. The in vitro hydrolysis of selected prodrugs in simulated gastric and intestinal fluids of different pH values showed that the prodrug was not hydrolysed in the stomach (pH 1.2.). When the pH value decreased, the prodrug ester started to degrade (about 10%) [31]. The degradation of the ester bond and formation of the hydrophilic form of cefetamet at pH 6.8 may slightly increase the prodrug solubility. However, under these conditions the ionic form of the prodrug will mostly become degraded, so the overall drug solubility will decrease. It is also necessary to note the fact that more noticeable changes in the dissolving rate profiles of individual combinations with the excipients at pH 6.8 may cause exchanges of ions.

During six months of the experiment the HPLC-UV method was used to assess the stability and compatibility of cefetamet pivoxil hydrochloride and its binary mixtures at 70°C and 70% RH as well as uncontrolled RH. The response was linear within the range studied (R^2 = 0.9882). The HPLC-UV method used for the quantitation of cefetamet pivoxil hydrochloride can detect thermal degradation and chemical interactions with excipients. In our analysis of the catalytic influence of the excipients on cefetamet pivoxil hydrochloride degradation there were no additional peaks except those observed during the degradation of cefetamet pivoxil hydrochloride. The concentration of cefetamet pivoxil hydrochloride in binary systems was slightly more reduced than the concentration of cefetamet pivoxil hydrochloride in its free form. Similarly, to other β-lactam analogues, humidity and temperature were important factors affecting the degradation of cefetamet pivoxil hydrochloride

in isolation and in combination with the excipients [32-34]. There was no alteration in the peak area. This indicates that the excipients were sufficiently compatible for use in solid dispersion of cefetamet pivoxil hydrochloride. Cefetamet pivoxil hydrochloride was the most degraded (approximately 9.17%, Table I) when it was mixed with hydroxypropyl methyl cellulose. Hydroxypropyl methyl cellulose, a coating agent and rate-controlling polymer for sustained release formulations, may react with the carbonyl (hydroxyl) group of cefetamet pivoxil hydrochloride and accelerate hydrolysis of the β-lactam combination [35]. There was a similar result when cefetamet pivoxil hydrochloride was combined with polyvinylpyrrolidone. This finding suggests that polyvinylpyrrolidone. catalysed the degradation of cefetamet pivoxil hydrochloride under the conditions of high humidity and temperature. Previous reports showed that polyvinylpyrrolidone was incompatible with several APIs due to the generation of hydrogen peroxide, which is reactive and can initiate radical chain reactions or react directly with APIs, inducing their degradation [36-37]. There have been numerous reports on the instability of the β-lactam ring in β-lactam antibiotics due to the presence of an oxidising factor [38-39].

The study on the cefetamet pivoxil hydrochloride permeability through artificial biological membranes simulating the gastrointestinal tract showed that the pH value strongly influenced the dynamics of cefetamet pivoxil hydrochloride absorption and the permeability of excipients. However, it is necessary to remember that the results of this study concern only the assessment of changes in the permeability through artificial biological membranes based on passive diffusion [40]. There have been suggestions that some carrier-mediated transport systems underlie the absorption mechanisms of amphoteric β-lactam antibiotics [41]. For example, the transport characteristics of aminopenicillins (ampicillin and amoxicillin), aminocephalosporins (cephalexin, cephradine and cefadroxil) and cefazolin were compared with the characteristics of an actively transported substance (D-glucose) and a passively transported substance (L-glucose) [42]. In view of the results of our study, we might suggest that at pH 6 the presence of excipients significantly increases the cefetamet pivoxil hydrochloride permeability based on passive diffusion. Interestingly, in the combinations where cefetamet pivoxil hydrochloride physically interacted with excipients (mannitol and polyvinylpyrrolidone) their influence on changes in permeability was the greatest. According to data presented in publications, cefetamet pivoxil exhibits significant positive food effect (40% vs 50%) after oral administration [43]. Hence, it is recommended that cefetamet pivoxil be taken with meals [44]. Therefore, our study might provide indirect evidence that selected excipients increase the absorption of cefetamet pivoxil hydrochloride.

As far as the microbial activity is concerned, in comparison with older cephalosporins, such as cefalexin or cefaclor, cefetamet pivoxil is an oral cephalosporin with enhanced affinity for the target penicillin-binding proteins 1 and 3 and increased stability to beta-lactamases [45]. In our study there were high MIC values of cefetamet pivoxil hydrochloride noted both for reference strains and clinical isolates. It may have been caused by the fact that these strains produced extended spectrum beta lactamases (ESBL), which inactivate penicillins, especially 1st-3rd generation cephalosporins [46]. AmpC cephalosporinases are another type of beta lactamase which are responsible for the resistance of the following bacterial genera: *Acinetobacter*, *Pseudomonas*, *Enterobacter* spp., *Citrobacter* spp. and *Klebsiella* [47]. The analysis of the combinations of cefetamet pivoxil hydrochloride with the excipients showed differences between the group of compounds which might be a potential source of carbon for bacteria and the compounds which could not be fully metabolised by microorganisms. Mannitol and lactose increased the MIC value in most of the strains under investigation. Clinical isolates of the *Klebsiella pneumoniae* and *Enterobacter aerogenes* species as well as all the strains of the *Salmonella* genus were exceptions. The MIC value in the variant combining cefetamet pivoxil hydrochloride with mannitol was the same as in the active substance on its own. When cefetamet pivoxil hydrochloride was combined with lactose, the MIC value increased in most of the strains under investigation (except bacteria of the *Salmonella* genus), as compared with the active substance on its own. We can suppose that the increase in the MIC value was caused by the fact that these species were capable of metabolising excipients. In consequence, the populations of these species increased. Apart from that, some compounds, such as disaccharides and alcohols, may protect microorganisms from the unfavourable environmental stress generated by the presence of an active substance. However, it is noteworthy the MIC value decreased for some species, especially in the variants where cefetamet pivoxil hydrochloride was combined with polyvinylpyrrolidone or hydroxypropyl methyl cellulose. There were different MIC values observed for the following species: *P.*

aeruginosa, E. coli (for reference strains and clinical isolates) and *Enterobacter aerogenes* (mostly for the clinical isolate). The biggest difference was observed for strains of the *P. aeruginosa* species. The MIC value of cefetamet pivoxil hydrochloride was 125 mg/L. When cefetamet pivoxil hydrochloride was combined with hydroxypropyl methyl cellulose, the MIC value amounted to 32 mg/L. The authors of the study suppose that both the semi-synthetic cellulose derivative and the preparation composed of polyvinyl acetate, polyvinylpyrrolidone and stabilisers such as sodium lauryl sulphate and colloidal silicon dioxide may be toxic or they may inactivate and weaken microorganisms, thus making them more sensitive to the active substance.

CONCLUSION

The systems combining cefetamet pivoxil hydrochloride with excipients are examples where physicochemical and biological properties were modified to design modern oral forms and to search for a new approach so as to obtain effective antibiotic systems in the post-antibiotic era. According to the authors of the study, the modification of physicochemical and biological properties resulted in the following significant achievements: (i) the pH value of the acceptor fluid determined the influence of the excipients on the solubility of cefetamet pivoxil hydrochloride, (ii) the degradation of selected excipients had catalytic effect on the chemical stability of cefetamet pivoxil hydrochloride, (iii) there were pH-dependent changes in the cefetamet pivoxil hydrochloride absorption based on passive diffusion in combinations with selected excipients, (iv) there were changes in the bactericidal activity of cefetamet pivoxil hydrochloride in combinations with selected excipients. To sum up, the approach characterises an effective and viable bactericidal potential as well as physicochemical properties required for innovative dosage of pharmaceutical forms. In search for new chemotherapeutic solutions, this approach to the treatment of resistant clinical infections also has the essential advantage of safety in the administration of substances.

Conflicts of Interest

The authors declare that there is no conflict of interest.

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Table 1 Values of MIC for cefetamet pivoxil hydrochloride and its mixtures with excipients.

	Microorganism	CP	CP + HPMC	CP + lactose	CP + mannitol	CP + PVP	CP + starch
		mg/L					
1.	*Proteus mirabilis* ATCC 12453	1	1	4	4	0.5↓	4
2.	*Proteus mirabilis* clinical isolates	1	1	8	4	0.5↓	8
3.	*Klebsiella pneumoniae* ATCC 31488	0.25	0.25	8	1	0.25	1
4.	*Klebsiella pneumoniae* clinical isolates	4	2↓	8	4	2↓	1
5.	*Enterobacter aerogenes* ATCC 13048	2	2	16	4	1↓	2
6.	*Enterobacter aerogenes* clinical isolates	4	2↓	32	4	1↓	4
7.	*Enterococcus faecalis* ATTC 29212	2	2	32	4	2	1↓
8.	*Enterococcus faecalis* clinical isolates	4	4	32	16	4	1↓
9.	*Escherichia coli* ATCC 25922	1	0.5↓	32	4	0.25↓	1
10.	*Escherichia coli* clinical isolates	1	0.5↓	32	4	0.25↓	1
11.	*Staphylococcus aureus* ATCC 25923	125	125	250	250	125	32↓
12.	*Staphylococcus aureus* clinical isolates	125	125	250	250	125	64↓
13.	*Acinetobacter baumanii* ATCC 19606	8	8	16	16	8	8
14.	*Acinetobacter baumanii* clinical isolates	16	16	32	32	16	16
15.	*Pseudomonas aeruginosa* ATCC 27853	125	32↓	250	250	64↓	250
16.	*Pseudomonas aeruginosa* clinical isolates	125	32↓	250	250	64↓	250
17.	*Salmonella enteritidis* ATCC 13076	125	125	62↓	125	125	125
18.	*Salmonella enteritidis* clinical isolates	250	250	62↓	250	250	250
19.	*Salmonella typhimurium* ATCC 14028	125	62↓	62↓	125	125	125
20.	*Salmonella typhimurium* clinical isolates	250	62↓	62↓	250	250	250

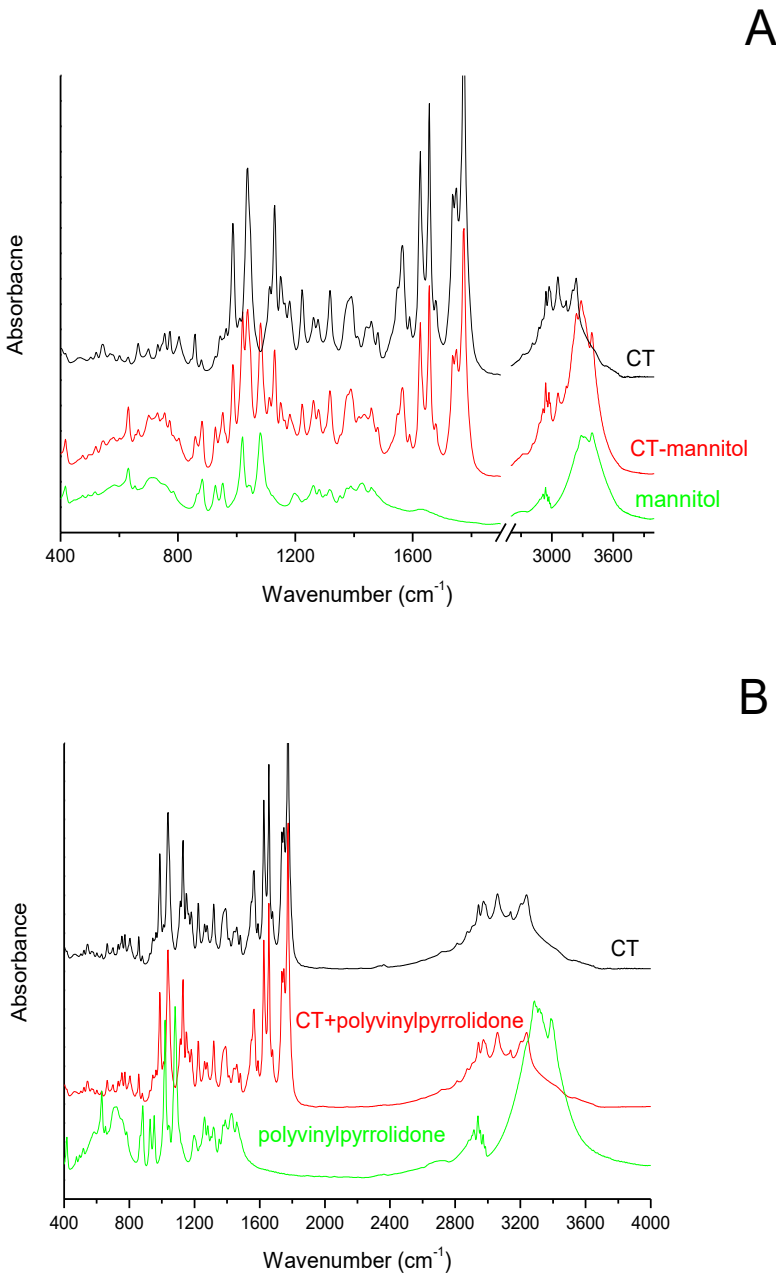

Figure 1 Spectroscopic interactions of cefetamet pivoxil hydrochloride in mixtures with mannitol (A) and polyvinylpyrrolidone (B).

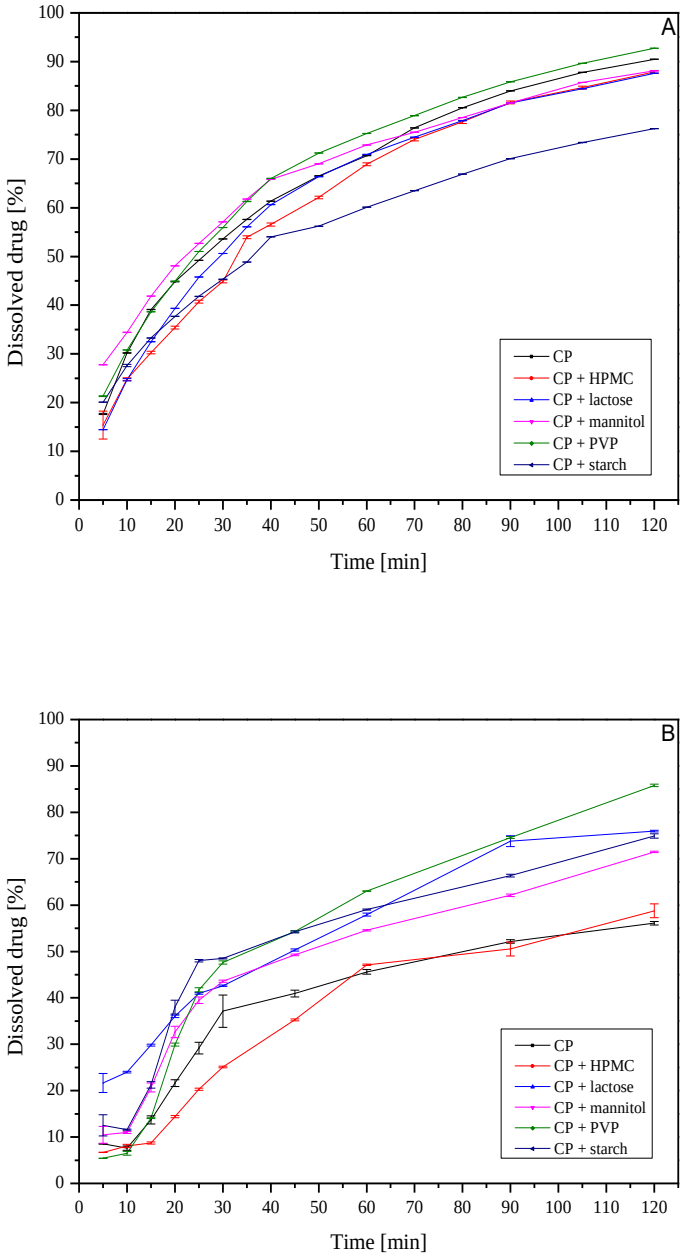

Figure 2 Plots of cefetamet pivoxil hydrochloride dissolution in pH 1.2 (A) and pH 6.8 (B).

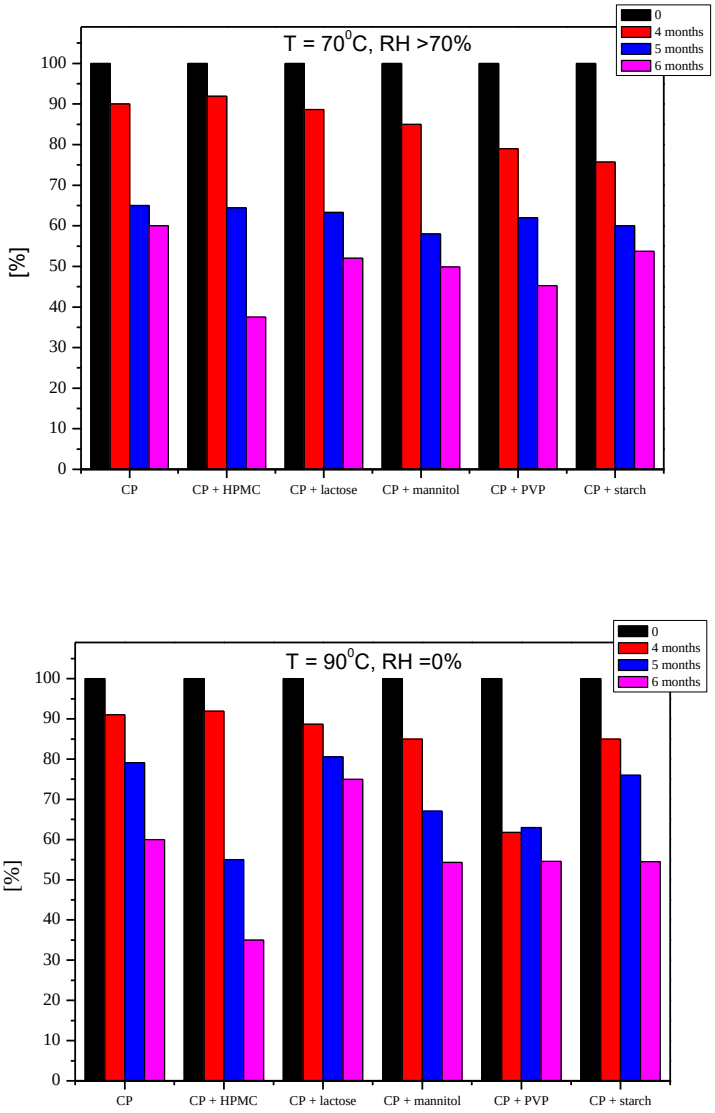

Figure 3 Chemical stability of cefetamet pivoxil hydrochloride in mixtures with excipients.

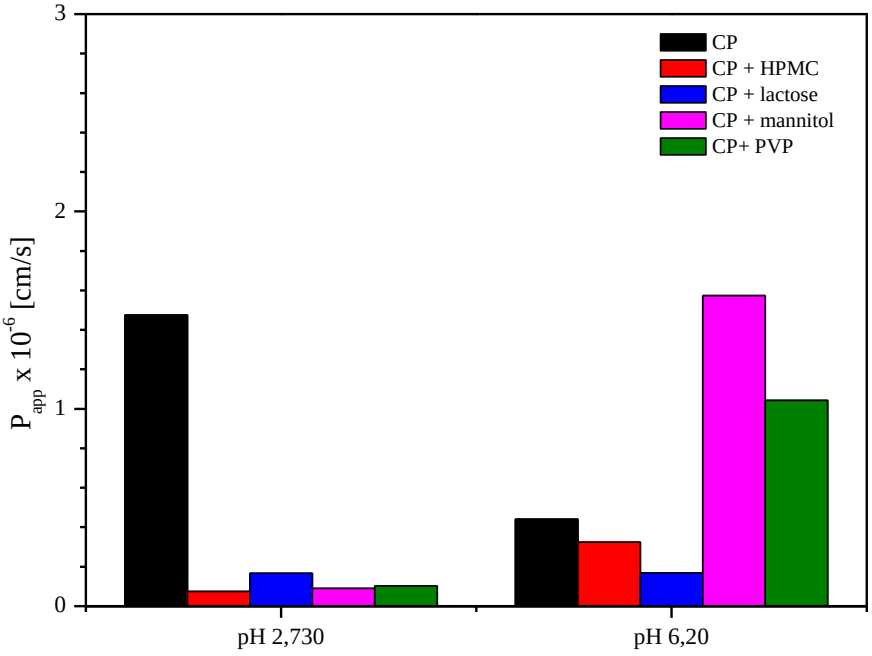

Figure 4 Permeability of cefetamet pivoxil hydrochloride in mixtures with excipients.