Formulation and Comparative Characterization of Methylcellulose, Carbomer and Petrolatum Gels for Topical and Transdermal Delivery of Zosyn (Piperacillin/Tazobactam) Antibiotics

Abstract

Piperacillin/Tazobactam otherwise known as Zosyn is a broad-spectrum combination antibiotic used to treat severe infections. It combines the high antibiotic activity of piperacillin with β-lactamase inhibitor properties of tazobactam that helps to restore the susceptibility of bacteria to the antibiotic and prevents resistance. Despite this interesting pharmacological profile, Zosyn is given intravenously because of its poor oral absorption and short half-life of between only 0.8 and 1.1 hours. However, this system of delivery is not convenient because it is invasive, requires continuous monitoring of patients, and incompatibility issues with other intravenous therapy. The purpose of this study, therefore, was to comparatively characterize an extended release gel formulation from Methylcellulose, Carbomer and Petrolatum and evaluate their suitability for topical and transdermal administration of the drug.

Methodology: Gels with 1%, 5% and 10% drug loadings were prepared with the three polymers. The physical characterization of the formulations was done for: drug release using dissolution apparatus; percutaneous absorption in rat skin using microporation technique and Franz cell apparatus. Drug stability studies were conducted at 25°C, 37°C and 45°C and at skin pH of 5. The formulations were also tested for chemical degradation under acidic/basic and oxidizing/reduction conditions similar to those found on the skin and in wounds with 0.01mol/l HCl, 0.01 mol/l NaOH, 0.1% H$_2$O$_2$, and 1M KI for 2 hours.

Results: The results showed that the gel with 10% drug loaded Methylcellulose formulation was more stable at temperatures studied and less susceptible to chemical degradation for all the chemicals used. The release of drug from the formulations was relatively high in the Methylcellulose (40.9%) and Carbomer (27.59%) gels but were limited in the Petrolatum (3.97%). Furthermore, transdermal delivery using microporation showed high permeation of 12.7%, 6.08% and 6.97% for the 10% drug loaded gels of Methylcellulose, Carbomer and Petrolatum respectively. The drug in the Methylcellulose was found to be subjected to higher degradation in basic medium by an oxidation pathway.

Conclusion: The 5% methylcellulose gel formulation containing 10% Zosyn loading was found to have the most potential for effective topical administration and extended-release transdermal delivery of the Zosyn.
Keywords
Extended-Release; Gel Formulation; Methylcellulose; Microporation; Transdermal Delivery; Zosyn

Introduction
Piperacillin/Tazobactam commonly known as Zosyn is a broad-spectrum combination antibiotic used to treat severe infections. It combines the high antibiotic activity of piperacillin with \( \beta \)-lactamase inhibitor properties of Tazobactam that helps to maintain the susceptibility of bacteria to the antibiotic and prevents resistance. As a result, the combination has increased the range of bacteria that piperacillin can kill [1]. With the advent of increasing drug resistance to most antibiotics, this combination is an example of successful strategy to fight the menace. It is bactericidal against numerous organisms including gram positive, gram negative, aerobic, and anaerobic microbes [2]. Nomura and coworkers reported as far back as 1997 that compared with ampicillin, Piperacillin/Tazobactam has up to 64- times stronger antimicrobial activity against all beta-lactamase producing bacteria [3]. The drug is currently approved by the Food and Drug Administration (FDA) as treatment for moderate-to-severe bacterial infections, nosocomial infections, postpartum endometritis or pelvic inflammatory disease, and skin and skin structure infections [4]. It is also used as an off-label antibiotic for uncomplicated intra-abdominal infections, urinary tract infections, bone and joint infections, septicemia, endocarditis, cystic fibrosis exacerbations, and surgical prophylaxis.

However, in spite of the interesting pharmacologic and therapeutic profile of Zosyn, it is given as a continuous intravenous infusion because of its poor oral absorption and short half-life of between only 0.8 and 1.1 hours [5]. Piperacillin, a penicillin based beta-lactam antibiotic is hydrolyzed by water and degrades over time. The current recommendation after reconstitution is to use the solution immediately. Any unused portion should be discarded after 24 hours if stored at room temperature or 48 hours if refrigerated [4]. This makes the dosing of this antibiotic safe only in a facility setting, by infusion and mostly for severe infections only. However, this system of delivery is not convenient because it is invasive, requires continuous monitoring of patients, and incompatibility issues with other intravenous therapies. Furthermore, reconstitution of the commercial product with various diluents for infusion came with high levels of particulate matter formation that do not meet USP requirements [6]. Moreover, contamination by leachables and extractables has been noted to be high in admixtures for infusion with the changes in pH that come with dilution with the known diluents including normal saline, as well as presence of metal ions in container components. This contamination has been reported to lead to degradation of the antibiotics by acid hydrolysis, \( \beta \)-lactam ring opening [7], or epimerization [6]. It is therefore imperative to find a formulation that will optimize the pharmacokinetic/pharmacodynamics behavior of piperacillin/tazobactam to improve treatment outcome, and to prevent selection and spread of resistant bacteria strains [3]. The high activity level of the antibiotic reported by Normura and his coworkers, means that a smaller amount of drug may be needed for administration by non-parenteral routes [3].

Secondly, prevalence and incidence of wounds and their associated skin structure infections are on ascendancy in the United States. These are as a result of increasing numbers of elderly residents in long-term care facilities, people with diabetes or venous and pressure ulcers, burns and postsurgical wounds [9]. Skin and skin structure infections can become complicated and can pose serious therapeutic challenges that may require intravenous antibiotic therapy, surgical intervention, and hospitalization. Most often these conditions lead to increasing morbidity and mortality rates, as well as serious economic and healthcare burden [10]. Owing to the broad-spectrum activity characteristics of piperacillin/tazobactam, it has been recommended for skin and skin structure infections and wound dressing. It is effective in the treatment of infections caused by most of the bacteria responsible for wounds and skin structure infections.

Topical and transdermal drug delivery have become important classes of controlled-released systems, as such, their use in therapy is becoming more widespread. Topical delivery is often preferred to oral and other forms of drug administration in the treatment of cutaneous superficial infections and wounds [11]. If good drug release and penetration can be ensured, the sustained-release nature of transdermal drug delivery is usually preferred to the invasive intravenous administration. However, the therapeutic efficacy of topical/transdermal formulations depends on the nature of the vehicle. Vehicles influence the rate and extent of drug permeation into and across the skin [12].
ointment bases have been used in the delivery of many drug for topical/wound dressing and transdermal delivery. These vehicles have appropriate viscosity, satisfactory bioadhesion, and lack of irritation or sensitizing actions [13-15].

Cellulosic polymers are water-soluble and available in a range of molecular weights and degrees of substitution. These characteristics augur well for easy manipulation of chemical properties and concentrations to obtain a range of solution viscosities and other rheological properties. These polymers are widely used in a wide range of pharmaceutical formulations and have been found to be of low oral, dermal and inhalation toxicity. It is especially, used to produce thixotropic gels, emulsions and sprays. The polymers have been reported to be effective in enhancing drug permeation in human skin [16,17].

According to the Handbook of Pharmaceutical Excipients [18], Carbomers are cross-linked synthetic high-molecular weight polymers of acrylic acid. The homopolymers are those that do not use benzene as the cross-linking agent. The polymer is used in liquid or semisolid pharmaceutical formulation including creams, gels, lotions and ointment for topical delivery [19]. In contrast to linear polymers, higher viscosity does not result in slower release with carbomers [18].

Petrolatum is a purified mixture of semisoloid saturated hydrocarbons obtained from petroleum [18]. It is generally considered a nonirritant and nontoxic material. It is mainly used in topical pharmaceutical formulations as an emollient base and non-adherent wound dressing. It is also used in transdermal formulations because of its ability to moisturize the stratum corneum for easy permeation of drug molecules. The high hydrophobic properties preclude absorption of water and therefore, prevent microbial contamination and hydrolysis of incorporated drug compounds.

The purpose of this project, therefore, was to characterize a cost effective extended release gel formulation for topical administration for wound and skin structure infections and transdermal administration for extended delivery of the drug for systemic effect. Previous topical applications of piperacillin/tazobactam on keratitis, eye and wound infections due to Pseudomonas aeruginosa [20], and intra-vaginal treatment of bacterial infections found the antibiotic beneficial and nontoxic [21].

Materials Equipment

Materials

Zosyn (Tazicub® piperacillin/tazobactam) was obtained from Wyeth Pharmaceuticals Inc., Philadelphia, PA, USA. The Methylcellulose, Hydroxypropyl methylcellulose, Hydroxypropyl cellulose and Triethanolamine were obtain from Sigma-Aldrich, St. Louis, MO, USA. Carbomer Homopolymer B and Petrolatum were obtained from Spectrum Chemical Manufacturing Corporation, New Brunswick, NJ, USA. The Phosphate Buffered Saline (PBS), Hydrochloric Acid (HCL), Sodium Hydroxide (NaOH), Potassium Iodide (KI) and mineral oil were obtained from Fisher Scientific, Norcross, GA, USA. The Spraque Dawley rat skin hide was obtained from Carolina Biological, Whitsett, NC, USA.

Equipment

This Disket Dissolution Apparatus system model 2100C, USP Dissolution apparatus 1, with rotating basket (used for the release studies) was obtained from Disket Inc., North Brushwick, NJ, USA. The dialysis membranes with molecular weight cutoff of 6,000 to 8,000 Daltons and a flat width of 40 mm were obtained from Spectrum Laboratories, Rancho Dominequez, CA, and USA. UV/Vis Spectrophotometer Shimadzu UV-1650PC (used to determine drug concentrations) was obtained from Beckman, Fullerton, CA, USA. Brookfield DV-E Viscometer (used to determine the viscosities of the cellulose gels) was obtained from Brookfield Engineering Laboratories, Middleboro, MA, USA. The Hotpack Environmental Chamber models 100001434 and 448110 used for the solid state stability studies were obtained from SP Scientific, Stone Ridge, NY, USA. Vertical Franz Diffusion apparatus model V6A-01-00228 was from PermeGear Diffusion Cells and Systems. The heat exchanger Model SC-100 was obtained from Thermo-Fisher Scientific, Asheville LLC, NC, USA. The microporator Dermaroller HC model C-8 was from Dermaroller™, Germany.

Methodology

Zosyn quantification standard curve

A stock solution of Piperacillin/Tazobactam “Zosyn” was prepared. A serial double dilution of the stock solution was performed to produce solutions of the following concentration from 50%, through 25%, and 12.5% to 0.1953125%. Three samples of each dilution were introduced into a cuvette and the average absorbance were calculated using a UV/Vis Spectrophotometer Shimadzu UV-1650PC at 300 nm and 296.3 for Piperacillin and 233.3 nm for Tazobactam respectively. A comparative analysis of the respective standard curves and coefficient of determination (R²) were done to
select the best wavelength for quantifying the drug in the formulations.

Preparation of Zosyn gels

Six different formulations of the gels and ointment bases at different drug concentration were investigated to optimize the qualities based on the following parameters:

1. Physical appearance.
2. pH.
3. Viscosity.
4. Spreadability.
5. Extrudability.

Gel formulations

Gel formulations using three different bases were prepared. The bases were Methylcellulose, Carbomer homopolymer type B, and Petrolatum. Gels of concentration of 0.5, 1, 2, 3, 4, and 5 percent weight by volume were prepared for comparative physical characterization of the Methylcellulose and Carbomer bases. The 5% gel formulations of the two bases were selected with the petrolatum for the dissolution test.

Carbomer homopolymer Type B gel

The gels were prepared by mixing different quantities of the Carbomer in purified water with stirring (120 rpm) for three hours at room temperature. Secondly, appropriate quantities of triethanolamine were added to the gel bases and the pH was adjusted to 7.0. The gel bases were allowed to stand overnight to allow trapped air to escape and to allow for effective cross-linking by the triethanolamine. Although for topical and transdermal administration, the skin pH of 5 would have been ideal, formulation of the gel at that pH will lead to over-neutralization that can lead to decreased viscosity and permanent instability that cannot be reversed. Maximum viscosity and clarity occur at pH 7. As such the pH maintained at 7 for efficient preparation and stability of the gel.

Petrolatum gel

Appropriate quantities of Zosyn were levigated with few drops of mineral oil and incorporated into white petrolatum base.

Methylcellulose

To select the right type of cellulose base for the gel, a comparative viscosity study at concentrations of 0.5, 1, 2, 3, 4, and 5 percent of Hydroxypropyl methylcellulose, Hydroxypropyl cellulose and methylcellulose was performed using a Viscometer at skin temperature of 32°C. Based on the viscosity results, the methylcellulose was selected as the gel base for the formulation.

0.5 grams of methylcellulose was dissolved in enough purified water and heated to 58°C to prepare 10 ml of the 5 percent weight by volume gel base. Serial dilution of 5 ml of the 5 percent gel was performed to prepare gels of 0.5, 1, 2, 3, 4, and 5 percent weight by volume gel formulations.

Drug loading

Zosyn is composed at an 8 to 1 ratio of piperacillin [log P of 1.21 (Pharma Algorithm) and -0.26 (ChemAxon)] to Tazobactam [log P of -1.8 (Pharma Algorithm) and -1.4 (ChemAxon)]. Gels with drug loading of 1% (0.02 grams of drug in 1.98 grams of gel), 5% (0.1 grams of drug in 1.98 grams of gel) and 10% (0.2 grams of drug in 1.8 grams of gel) were prepared for each base.

Evaluation of visual appearance of gels

The prepared gels were visually inspected for clarity, color and transparency. The prepared gels were also evaluated for the presence of any particles. Smears of gels were prepared on glass slides and observed under the microscope for the presence of any particle or grittiness.

In vitro release studies

To assess the release characteristics of the drug from the different gel formulations, the 1%, 5%, and 10% drug loaded gels of each base were tested in phosphate buffered saline at skin pH of 5 and at 37°C. The Distek Dissolution system model 2100C USP Apparatus 1 with 40 mesh rotating baskets was used for the dissolution test. 2 grams of drug loaded gel formulations containing 0.02 grams (1%), 0.1 grams (5%) and 0.2 grams (10%) of Zosyn, were placed in dialysis membranes with molecular weight cutoff of 6,000 to 8,000 Daltons and a flat width of 40 mm (Spectrum Labs, Rancho Dominguez, CA). Samples were collected at predetermined times over 24 hours for the Methylcellulose and Petrolatum. Owing to its unique characteristics, the release test for the Carbomer gels were run for 52 hours. Samples were tested using UV/VIS spectrometer at 230 and 300 nm for Tazobactam and Piperacillin respectively.

Ex vivo skin permeation studies

The ability of the gel formulations to aid the active drug to penetrate rat skin for effective transdermal delivery were tested using the 1%, 5%, and 10% drug loaded gels of each base. The penetration test was performed using microporation.
Briefly, 2 grams of the 1% and 10% methylcellulose gel formulations were mixed individually with 1 milliliter of the HCL, NaOH, H₂O₂ and KI for two hours at room temperature. Each sample was then tested by UV/Vis analysis at 300 nm for acidic, alkaline, oxidation, and reduction degradation.

**Statistical Analysis**

Results are expressed as the mean ± standard error. Differences were assessed statistically using one-way Analysis of Variance (ANOVA). Comparison of means was performed using Tukey’s post-hoc test. A probability of p<0.05 was considered statistically significant.

**Results and Discussion**

**Zosyn Quantification Standard Curves**

The data on the comparative absorbance per drug concentration at the three wavelengths of 300 nm, 296.3 nm for Piperacillin and 233.3 nm for Tazobactam are shown in table 1a-c respectively. It was determined that the UV/Vis spectrophotometer was more accurate and precise at lower concentrations. Therefore, the standard curves were drawn with absorbance of concentrations below 1%. The accompanying respective standard curves at the three wavelengths 300 nm, 296.3 nm and 233.3 nm are shown in figure 1a-c respectively. The coefficient of determination or R² for the 300 nm, 296.3 nm and 233.3 nm were 0.9997, 0.948 and 0.8069 respectively. Based on the coefficient of determination or R², the 300 nm wavelength with an R² of 0.9997 was selected as the best fit for quantifying Zosyn in the formulations. The high ratio of Piperacillin to Tazobactam in Zosyn (8 parts Piperacillin to 1-part Tazobactam) is attributed to the better quantification of Zosyn using Piperacillin wavelength parameters. It should be noted that a slight deviation of wavelength from 300 nm to 296.3 led to a marked change in the coefficient of determination or R² from 0.9997 to 0.948. This means the assay is highly sensitive to wavelength.

| Concentration (%) | Wavelength of Peak (nm) | Absorbance of Peak | Absorbance of Peak | Absorbance of Peak | Average of Absorbance |
|-------------------|-------------------------|--------------------|--------------------|--------------------|----------------------|
| 50                | 300.0                   | The absorbance ranges much higher than 4.000. | 3.957              | 4.000              | 4.000                | 3.986                |
| 25                | 300.0                   | 4.000              | 4.000              | 3.986              |                      |                      |
| 12.5              | 300.0                   | 4.000              | 4.000              | 3.986              |                      |                      |

**Effect of storage and use temperature and skin pH on Zosyn stability test**

The 1% and 10% methylcellulose gel formulations with superior physicochemical and penetration characteristics were chosen for the physical and chemical degradation tests. The gels were visually inspected every day for the 12 weeks. The effect of skin pH at various storage and use temperature on drug stability studies were performed at pH of 5 and at temperatures of 25°C (ambient temperature), at body temperature of 37°C and 45°C. 5-gram test samples of the 1% and 10% methylcellulose gel formulations were stored in stability chambers at the above stated temperatures. The test samples were analyzed using UV/VIS spectrometer at 230 and 300 nm for Tazobactam and Piperacillin respectively.

**Chemical stability studies of Zosyn in conditions similar to human skin and wound surfaces**

The formulations were tested for chemical degradation under acidic/basic and oxidizing/reduction conditions similar to those found on human skin and in wounds with 0.01 Mol/L Hydrochloric acid (HCL), 0.01 Mol/L Sodium hydroxide (NaOH), 0.1% Hydrogen peroxide (H₂O₂), and 1 M Potassium Iodide (KI).
Table 1a: Drug concentrations and the respective absorbance at 300 nm for Zosyn (Piperacillin).

| Concentration (%) | Absorbance of Peak | Absorbance of Peak | Absorbance of Peak | Average of Absorbance |
|-------------------|--------------------|--------------------|--------------------|----------------------|
| 50                | 3.763              | 3.462              | 3.806              | 3.677                |
| 25                | 3.436              | 3.718              | not taken          | 3.577                |
| 12.5              | 3.325              | 3.414              | 3.462              | 3.400                |
| 6.25              | 3.374              | 3.263              | 3.612              | 3.416                |
| 3.125             | 3.311              | 3.263              | 3.047              | 3.207                |
| 1.5625            | 3.047              | 3.047              | 2.941              | 3.012                |
| 1.2               | 2.985              | 2.941              | 2.941              | 2.956                |
| 0.78125           | 2.754              | 2.754              | 2.713              | 2.740                |
| 0.6               | 2.533              | 2.534              | 2.561              | 2.543                |
| 0.390625          | 1.914              | 1.909              | 1.908              | 1.910                |
| 0.3               | 1.476              | 1.479              | 1.475              | 1.477                |
| 0.1953125         | 0.969              | 0.969              | 0.967              | 0.968                |

Table 1b: Drug concentrations and the respective absorbance at 296.3 nm for Zosyn (Piperacillin).

| Concentration (%) | Absorbance of Peak | Absorbance of Peak | Absorbance of Peak | Average of Absorbance |
|-------------------|--------------------|--------------------|--------------------|----------------------|
| 50                | 0.484              | 0.271              | 0.333              | 0.363                |
| 25                | 0.245              | 0.294              | 0.032              | 0.270                |
| 12.5              | 0.032              | -0.030             | 0.032              | 0.011                |
| 6.25              | 0.094              | -0.030             | 0.032              | 0.032                |
| 3.125             | -0.030             | -0.181             | 0.032              | -0.060               |
| 1.5625            | 0.032              | -0.030             | -0.030             | 0.009                |
| 1.2               | 0.032              | -0.030             | -0.030             | -0.009               |
| 0.78125           | -0.118             | -0.092             | 0.032              | -0.059               |
| 0.6               | -0.030             | -0.030             | -0.030             | -0.030               |
| 0.390625          | -0.092             | -0.092             | -0.030             | -0.071               |
| 0.3               | -0.181             | -0.030             | -0.092             | -0.101               |
| 0.1953125         | -0.030             | -0.092             | -0.092             | -0.071               |

Table 1c: Drug concentrations and the respective absorbance at 233.3 nm for Zosyn (Tazobactam).

| Concentration (%) | Absorbance of Peak | Absorbance of Peak | Absorbance of Peak | Average of Absorbance |
|-------------------|--------------------|--------------------|--------------------|----------------------|
| 50                | 3.957              | 3.957              | 3.763              | 3.892                |
| 3.125             | 3.612              | 3.763              | 3.675              | 3.683                |
| 1.5625            | 3.263              | 3.374              | 3.436              | 3.358                |
| 1.2               | 3.101              | 3.101              | 3.141              | 3.114                |
| 0.78125           | 2.417              | 2.412              | 2.412              | 2.414                |
| 0.6               | 1.873              | 1.869              | 1.864              | 1.869                |
| 0.390625          | 1.239              | 1.239              | 1.234              | 1.237                |
| 0.3               | 0.942              | 0.941              | 0.938              | 0.940                |
| 0.1953125         | 0.601              | 0.600              | 0.600              | 0.600                |

Figure 1: Standard curve of Zosyn at 300.0 nm (1a), 296.3 nm (1b), and 233.3 nm (1c) respectively. The 300.0 nm and 296.3 nm are wavelengths for Piperacillin and the 233.3 nm wavelength is for Tazobactam.
Figure 1a: Piperacillin UV/VIS Standard Curve.

Figure 1b: Piperacillin UV/VIS Standard Curve at 296.3 nm

Figure 1c: Piperacillin UV/VIS Standard Curve at 233.3 nm

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**Cellulose selection viscosity studies**

The results of the comparative study of viscosities of gels with increasing concentrations of Methylcellulose (MC), Hydroxypropyl Cellulose (HPC) and Hydroxypropyl Methylcellulose (HPMC) are shown in figures 2a and b. In figure 2a, the viscosity of Hydroxypropyl methylcellulose increased significantly after 2% gel concentration and increased exponentially after 3% concentration, whilst the viscosities of Hydroxypropyl cellulose and Methylcellulose remained significantly very low up to the 4% concentration studied. The result in figure 2b shows the differences in viscosities at the 1% to 4% concentrations. The figures 2a and b shows that Methylcellulose had lower viscosities than Hydroxypropyl cellulose at these concentrations.

**Figure 2:** Comparative viscosity studies of cellulose polymers at different gel concentration. Figure 1a shows the comparative viscosities of Hydroxypropyl methylcellulose (HPMC), Hydroxypropyl cellulose (HPC) and Methylcellulose (MC). The Hydroxypropyl cellulose and Methylcellulose gels showed not much change in viscosity with increasing gel concentration as compared to the pronounced change with the Hydroxypropyl methylcellulose. Figure 1b is the comparative viscosities of Hydroxypropyl cellulose and Methylcellulose with increasing gel concentration.
Viscosity plays a major role in topical drug formulation. Generally, drug release and consistency of gel formulations are dependent on the viscosity of the formulations [22] and decrease with increasing viscosity. Consistency is particularly important for effective spread of the formulation on surfaces of wound and skin. The ability of formulation to release the drug substance for permeation also depends to large extent on the viscosity. The higher the viscosity, the greater the difficulty in the movement of the drug molecules through the gel. This difficulty limits the quantity of drug substance made available on the surface of the skin for permeation through the stratum corneum. This is particularly important for drug substance required to pass through the skin in transdermal delivery. For the three polymers studied, methylcellulose had the lowest viscosities over the recommended gel concentrations and was chosen as the cellulose polymer for further studies.

Methylcellulose has extra advantage of being a cellulose polymer with unsubstituted methyl groups. This lack of substitution has the effect of lower affinity for hydration, which is appropriate for drugs that are subject to hydrolysis such as Piperacillin, the major component of Zosyn. The lack of substitution also results in thinner gels with the highest tolerance for added drugs and salts with wide pH ranges.

**In vitro drug release from the gel formulations**

Drug dissolution/release test is one of the key tests of dosage form development, quality control and performance [23]. In order to assure these key parameters and safety of novel delivery systems, there is the need to assess the pattern of drug dissolution/release from the formulation and other attributes relating to *in vivo* performance [24]. This is because the dissolution/release process is prerequisite for drug pharmacological activity.

The *in vitro* release studies were performed to predict the release characteristics of the drug from the different gel formulations with 1%, 5%, and 10% drug loading. For a faster and more precise quantification of drug released using the standard curve for the different drug loading samples, the results were analyzed as percentage concentration of drug released from the gels. However, the cumulative quantities in milligram of drug at 24 hours were calculated. Figure 3 shows the release of Zosyn from the Methylcellulose, Carbomer and Petrolatum. As expected, the release of the drug from the different gel formulations increased with increasing drug loading (Figure 3a) and the differences between the drug loading concentrations were significant (p<0.05). This may be attributed to the increasing amount of drug molecules with increasing concentrations. In all the formulations, the release even from the 1% drug loading with only 0.02 grams of drug in 2-gram gel specimen was detectable at all the time points.

The cumulative concentration of drug released from the gels of the individual polymers are shown in figures 3b-d. The Methylcellulose gel (Figure 3b) showed a more efficient drug release with as much as 20% (4 mg out of drug loading of 20 mg), 25.3% (25.3 mg out of drug loading of 100 mg) and 40.9% (81.8 mg out of drug loading of 200 mg) cumulative drug concentrations released from the 1%, 5% and 10% drug-loaded gels respectively over the 24 hours sampling time. The Carbomer gel formulation (Figure 3c) showed a much less efficient drug release as compared to the Methylcellulose gel. However, there was a significant cumulative concentration of 13.14% (2.628 mg out of drug loading of 20 mg), 22.7% (22.7 mg out of drug loading of 100 mg) and 27.6% (55.2 mg out of drug loading of 200 mg) of drug released from the 1%, 5% and 10% drug-loaded gel respectively over the 24 hours period. The cumulative drug concentration released from the 10% Carbomer gel was significantly higher than the 5% Methylcellulose drug-loaded gel at all the sampling time point until the 24th hour. This may be as a result of the difference in drug loading. This shows that a modification of the Carbomer formulation can make it comparatively as efficient as the Methylcellulose formulation. The difference in drug release between the Methylcellulose and Carbomer gel formulations may be a result of higher viscosity and thickness of the three-dimensional Carbomer gels.

The pattern of drug release was most inefficient from the petrolatum formulations (Figure 3d). Though there was relatively higher concentration of drug released with increasing concentration, there was no significant difference in drug release amongst the 1%, 5%, and 10% drug-loaded formulations. The total cumulative concentration of drug released from the 1%, 5% and 10% drug-loaded formulations were 0.87% (0.174 mg out of drug loading of 20 mg), 2.19% (2.19 mg out of drug loading of 100 mg) and 3.96% (7.92 mg out of drug loading of 200 mg) respectively. These results were significantly lower than the Methylcellulose and Carbomer formulations (Figure 3e). This may be because of the hydrophobic nature of petrolatum that prevents it from allowing the aqueous buffer to penetrate the gel to dissolve the drug molecules for release. This finding is important since the dissolution process is the rate-limiting step in the overall release process. Therefore, a special precaution may be needed in drug release studies involving petrolatum because of its complex and unpredictable characteristics. It is known that at the molecular level, it is comprised of heterogeneous mixture of a variety of hydrocarbon species, and depending on the supplier, the grades and therefore, the physico-chemical characteristics may vary.

**Figure 3:** *In Vitro* drug release from the 1%, 5% and 10% Methylcellulose (MC), Carbomer (CB) and Petrolatum Gels for
the 24 hours study period. 3a: comparative drug releases from the nine formulations of the three polymers; the comparative of the formulations are shown in 3b: Methylcellulose; 3c: Carbomer, and 3d: Petrolatum. Figure 3e is the comparative cumulative percent concentration of drug released from the 10% drug loaded gels of Methylcellulose and Carbomer.

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The results of the study showed that there was a continuous burst release of drug from the Methylcellulose and Carbomer gels up to the 14th hour of sampling (Figure 3b and c). This may be a result of increasing wetting of the gel matrices with increasing time exposure to the study buffer. This finding may be good for an extended release of drug from these formulations.

The pattern of released was different from the Petrolatum formulation (Figure 3d). The figure 3d shows that it took up to the 18th hour of sampling to see a bump in drug release from all the formulations. It can be inferred that it took a long time to get the release buffer to have even a limited amount of mixing in the Petrolatum to release drugs. Therefore, buffer diffusion and mixing in Petrolatum may be a rate-limiting factor for drug release in the formulations like the one in this study. However, for an effective drug release performance of a gel formulation for topical and transdermal drug delivery system, the buffer solvent should be able to freely penetrate the matrix of the gel to dissolve the drug. The dissolved drug substance subsequently diffuses out along the concentration gradient. The hydrophobic petrolatum was, therefore, not efficient in the release of Zosyn in the study.

**Microporation skin permeation studies**

The *ex vivo* drug permeation of Zosyn through the rat skin model by the microporation technique studies were done with the 1%, 5% and 10% drug-loaded formulations of the three polymers over 24-hour period at skin pH of 5. The results of the studies are reported in figure 4. Figures 4a-c show the cumulative percent concentration of drug detected in the receptor compartment of the Franz Diffusion cells from the 1%, 5% and 10% drug-loaded formulations of the Methylcellulose, Carbomer and Petrolatum polymers. As the figures 4a-c show, the concentration of drug permeated increased with increasing drug loading in all the polymer gel formulations. The cumulative permeation from the Methylcellulose (Figure 4a) were 4.08% (816 micrograms), 6.16% (6,160 micrograms) and 12.72% (25,440 micrograms) drug concentration from the 1%, 5% and 10% drug-loaded formulations respectively. The permeation of drug from the Carbomer and Petrolatum were comparatively lower than the Methylcellulose polymer. There were 2.72% (544 micrograms), 3.08% (3,080 micrograms) and 6.08% (12,160 micrograms) cumulative concentration of drug permeated from the 1%, and 5% and 10% Carbomer gels respectively, whilst those of the Petrolatum were 2.08% (416 micrograms), 3.89% (3,890 micrograms) and 6.97% (13,940 micrograms) drug from the 1%, 5% and 10% respectively.

The results of the skin permeation by the microporation technique showed a completely different pattern from that of the drug dissolution/release studies. Whilst the release was consistently inefficient with the Petrolatum gel, the pattern of skin permeation from all the polymer gel formulations were consistently and positively high and increased with increasing drug loading. Figure 4d shows the comparative cumulative permeation of drug from the 10% drug-loaded formulations of the three polymers. The results show that the drug permeation from the Petrolatum was high with no significant difference between the Petrolatum and Carbomer formulations. This may mean that the Diffusion Apparatus 1 with the volume of buffer used may not be the most appropriate testing equipment for the Petrolatum formulation. On the other hand, the high skin permeation from all the gel may be because of the characteristics of gels as good topical drug delivery systems. They are known to spread well and have good bioadhesion properties necessary for longer contact time with the stratum corneum needed for good penetration. Moreover, Petrolatum being occlusive in nature has high emollient and moisturizing properties that required for good skin penetrations.

The comparative permeation of Zosyn at each sampling point from the 10% drug-loaded Methylcellulose, Carbomer and Petrolatum formulations is shown in figure 4e. The pattern of drug permeation from all the polymers showed a peak around the 8th hour of testing. The Maximum Permeated Concentrations (Cmax) were 3.11% (6,220 micrograms), 2.13% (4,260 micrograms) and 2.37% (4,740 micrograms) for the Methylcellulose, Carbomer and Petrolatum respectively. From this peak, the drug permeation decreased gradually with time with relatively high permeation after the Cmax. The concentrations permeated on the 24th hour were 0.454% (908 micrograms), 0.095% (190 micrograms) and 0.101% (202 micrograms) for the Methylcellulose, Carbomer and Petrolatum formulations respectively. This pattern of permeation may be attributed to the possible gradual closure of the microspores in the stratum corneum created by the Microporation technique. The results show that the pores were not completely closed for the 24 hours. Earlier work by Kalluri et al., [25], showed that microspores created under non-occlusive conditions completely close after 18 hours. They identified the Trans-Epidermal Water Loss (TEWL) as the main mechanism responsible for pore closure. The longer time it took for the microspores to completely close in this study may be as a result of the moisturizing nature of the gel formulations. The Methylcellulose and Carbomer formulations were constant source of hydrating water from the gels, whilst the occlusive nature of the Petrolatum moisturized the skin. The continuous saturation of the stratum corneum with water may be responsible for the inability of the skin to regain its barrier function and, therefore, the prolong ability of drug molecules to permeate.

**Figure 4:** *In vitro* drug permeation in rat skin by microporation technique in Franz Diffusion cell apparatus. The study was done...
on the 1%, 5% and 10% Methylcellulose (MC), Carbomer (CB) and Petrolatum Gels for the 24 hours study period. The comparative cumulative concentration of drug permeated from the formulations are shown in 4a - Methylcellulose, 4b - Carbomer, and 4c - Petrolatum. Figure 4d is the comparative cumulative percent concentration of drug permeated from the 10% drug loaded gels of Methylcellulose, Carbomer and Petrolatum. Figure 4e is the comparative Zosyn permeation at the each of the time points from the 10% drug-loaded Methylcellulose, Carbomer and Petrolatum formulations.

Figure 4a: Cumulative Skin Penetration of Zosyn in Methylcellulose Gel formulations.

Figure 4b: Cumulative Skin Penetration of Zosyn in Carbomer formulations.
Figure 4c: Skin Penetration of Zosyn in Petrolatum formulations.

Figure 4d: Comparative Cumulative Permeation from the 10% Drug Loading Methylcellulose (MC), Carbomer (CB), and Petrolatum (PT) Gel Formulations at skin pH of 5.

Figure 4e: Comparative Zosyn Permeation at Each Time Point from the 10% Drug Loading Methylcellulose (MC), Carbomer (CB), and Petrolatum (PT) Gel Formulations at skin pH of 5.
The selection of appropriate vehicle is important in effective transdermal delivery. An appropriate vehicle can optimize the formulation to have appropriate permeation of the drug through the skin particularly in a microporation technique that subsequently ensures effective penetration through the epidermal pores created [13]. In addition, the use of semi-solid formulations such as gels as a delivery system can provide a faster release of drug substances and increase the residence time on skin and good hydration for optimal penetration through the micropores. The results of this study confirm the report that Methylcellulose is effective in enhancing drug permeation through human skin [16,17].

Evaluation of visual appearance of gels

All the nine gel formulations (1%, 5% and 10%) of Methylcellulose, Carbomer and Petrolatum were found to be transparent and uniform in consistency. The formulations did not show any appreciable change in clarity, color or odor. The gels therefore, met the requirement of physical stability.

The formulations were evaluated microscopically for the presence of particulate matter. No appreciable particulate was seen under microscope. The formulations, therefore, fulfilled the requirement of freedom from particulate matter and grittiness, as desired for topical preparation.

Effect of storage and use temperature and skin pH on Zosyn stability studies

Shelf life and long-term stability are crucial issues in evaluating the potential of drug delivery systems. For topical delivery systems such as gels, contact with body surface temperature, skin and wound pH as well as changes in exposed atmospheric conditions are important in determining the stability of the incorporated drug substances, as the chemical stability of the drug substance affects the safety and efficacy of the product [26]. The ICH and FDA guidance state the requirement of stability testing to determine the changes in quality of a pharmaceutically active ingredient and drug product with time under the influence of various environmental and where necessary chemical factors. Such studies are important in selecting the appropriate formulation and packaging as well as the proper storage or shelf-life and use conditions.

The purpose of these sets of studies, therefore, was to evaluate the long-term storage stability and possible degradation of the drug substances at body surface temperature and skin pH of 5. The first sets of studies were done at ambient temperature of 25°C, body temperature of 37°C and possible extreme environmental temperature of 45°C. The second sets of studies were done at the same temperature, but the pH of the gels was adjusted from 7.0 to the skin pH of 5.0. The Methylcellulose gel formulation with superior drug permeation and release characteristics was used for the studies. It has been reported that a concentration of 0.1% (1 mg/ml) is the minimum for stability studies. This concentration makes it possible to get even the minor decomposition products in the range of detection [27]. It is also suggested that the studies should be done at the concentration expected in the final formulations. The formulations with 1% and 10% drug loading with extreme end characteristics were selected for the studies.

The results of the studies are reported in figure 5. At 25°C, both the 1% and 10% drug-loaded methylcellulose gels showed high drug stability of 96.25% and 98.5% respectively. Subsequently the stability of the 1% formulation went down with increasing temperature to 26.25% at 37°C and 14.3% at 45°C. The differences in stability at these temperatures and that of the 10% drug-loaded gels were significant (p<0.05). The stability of the 10% gel formulation was maintained at 87.6% at 37°C and 91.4% at the 45°C for the twelve weeks of storage. There was no statistically significant difference in drug stability at these temperatures for this formulation. It can be implied from the data that increased drug loading in the Methylcellulose gel conferred a significant drug stability. The low dose of drug did not receive any protection from the gel formulation. The results show that the drug substances are exposed to degradation mechanisms in the gel formulation. These mechanisms may include the drug-base interactions that may be accelerated by temperature. Furthermore, the high-water content of Methylcellulose gels can lead to significant degradation of the piperacillin, which is subject to hydrolysis. The greater degradation in the formulation with low dose may be because of the relatively large amount of drug degraded as compared to the initial drug loaded.

The solid-state stability of the 1% and 10% drug loaded Methylcellulose gels at storage and use temperatures of 25°C, 37°C and 45°C and at skin pH of 5. The sample were stored in stability chambers at the different temperature for 12 week and analyzed for Piperacillin using UV/Vis spectrophotometer at 300 nm. The studies were repeated by adjusting the pH of the gels from 7.0 to 5.0.

The second set of studies was at these temperatures but with the gel pH adjusted to 5.0. There was a slight but insignificant (p=0.28) reduction in the viscosity. However, Methylcellulose gels unlike that of Carboxymethylcellulose ones are not sensitive to pH changes because of the lack of the carboxylate groups.

At the pH of 5.0, the stability of the 10% drug-loaded gel decreased from the 98.8%, 87.6% and 91.4% at 25°C, 37°C and 45°C respectively to 92.6%, 85.7% and 85.4% at 25°C,
37°C and 45°C respectively. These figures represent a decrease of stability of only 6.2%, 1.9% and 6.0% at the 25°C, 37°C and 45°C respectively. These changes in stability were insignificant 0.57.

The pattern of the decrease in stability at skin pH with storage temperature was also found with the 1% drug-loaded gels. The stability of drug from this gel decreased from 96.25%, 26.25% and 14.25% at 25°C, 37°C and 45°C respectively to 91.7%, 20.8% and 8.7% at the 25°C, 37°C and 45°C respectively. The change in stability were insignificant (p=0.32). The decrease in stability with temperature of this formulation at the pH of 5 was 4.55%, 5.45% and 5.55% at the 25°C, 37°C and 45°C respectively.

Chemical stability studies of Zosyn in conditions akin to skin surface

Drug substances administered on skin surface and in surface wounds are subjected to a number of chemical interactions in these environments. For instance, the outermost and exposed layer of the skin and site of topical drug administration is composed of molecules derived from skin cells, body surface microbiota and the environment [28]. In addition, the surface of the skin is divided into moist, dry, and sebaceous microenvironments that can also be influenced by beauty and hygiene products [29]. Furthermore, many biochemical and physiological processes involved in wound healing results in complex wound chemical environment that have effect on the stability of drug compounds [30]. The goal of these studies of Zosyn, therefore, was to determine the possible degradation of the drug substance under the main chemical pathways of acidic (HCL)/alkalinity (NaOH) and oxidation (H2O2)/reduction (KI) conditions embodied by the numerous chemical microenvironments on skin surface and wounds. These conditions cover a wide range of pH from highly acidic of about pH of 2 to highly basic of about pH of 10. This pH range covers pH 5 of the skin during administration to the different pH likely to be encountered in wounds. The Methylcellulose gel formulation with superior release and permeability characteristics was selected for the studies. The lowest 1% and highest 10% drug loadings were selected to determine the relative effect of drug content on the percent degraded. The results of the studies are indicated in table 2 and figure 6a for acidity and alkalinity, and figure 6b for oxidation and reduction stability.

| Drug Stability | Degradation Chemical Reagent | HCL | NaOH | H₂O₂ | KI |
|----------------|-----------------------------|-----|------|------|----|
|                  | Percent Drug Loading       | 1%  | 10%  | 1%   | 10%| 1%  | 10% |
|                  | Percent Drug Un-degraded   | 96.25 | 98.3 | 34.25 | 38.3| 67.38 | 66.5 | 78.5 | 98.7 |
|                  | Percent Drug Degraded      | 3.75 | 1.7  | 65.75 | 61.7| 32.62 | 33.5 | 21.5 | 1.3 |

Table 2: Chemical Stability Studies of Zosyn in conditions akin to skin surface.

Chemical stability of Zosyn in the 1% and 10% drug-loaded Methylcellulose gel formulations showing the percent drug degraded and un-degraded (percent remaining) in acidic (Hydrochloric acid -HCL), alkaline (Sodium hydroxide - NaOH), oxidizing (Hydrogen peroxide - H₂O₂) and reducing (Potassium iodide - KI) media.
Figure 6: Chemical stability of the 1% and 10% drug loaded Methylcellulose gels in acidic/alkaline media (Ga) and oxidizing and reduction media (6b). These studies were to determine the chemical stability of the Zosyn in the formulations in conditions similar to that found topical administration on the skin and wounds.

The results showed that both the 1% (96.25% un-degraded drug) and 10% (98.3% un-degraded drug) Methylcellulose formulations exhibited high stability of Zosyn in the HCL than in NaOH, KI and H\textsubscript{2}O\textsubscript{2}. In the KI study, there was a significant difference (p< 0.05) between the 1% and 10% formulations with the 10% exhibiting a higher stability of 98.7% as against the 78.5%. The high stability of 10% drug loading in KI was comparable to the stability in HCL. There was no significant difference in degradation between the 1% and 10% formulations in HCL, NaOH and H\textsubscript{2}O\textsubscript{2} (p> 0.05). The degradation was highest in NaOH (65.75% degraded and stability of 34.25%) for the 1% formulation and 61.7% degraded and stability of 38.3% for the 10% formulation), followed by H\textsubscript{2}O\textsubscript{2} (32.6% degraded and 67.4% stability for the 1% formulation, and 33.5% degraded and 66.5% stability for the 10% formulation). This is an indication that Zosyn in Methylcellulose gel is subjected to higher degradation in a basic medium by an oxidation pathway exemplified by the NaOH and H\textsubscript{2}O\textsubscript{2}. The drug compound was highly stable in acidic medium and highly resistant to reduction degradation pathway of the potassium iodide.

Overall, the Methylcellulose gel formulation with 10% drug loading was found to be an efficient platform for the delivery...
of Zosyn for topical and transdermal delivery. The 10% drug loading formulation had the best release profile and more efficient permeation of skin tissue by microporation for effective transdermal delivery. Furthermore, it exhibited a high storage and use stability as well as a high resistance to acidic and reduction degradation pathways similar to conditions on skin surface and in wounds.

Gel based formulations have been found to have many advantages over gauze in wound dressing because of their homogeneous adhesion to the affected parts, easy removal without damage to renewed skin and faster rate of reconstruction of the injured skin [14,31]. Furthermore, they provide the necessary moist wound environment and removal of wound exudate that are necessary for faster wound healing and produce smoother scarring [31]. For topical and transdermal delivery, gel formulations provide the needed hydration of the stratum corneum for efficient permeation of drug molecules and offer protection from secondary infections.

Conclusion

The study showed that the Methylcellulose gel formulation had better physico-chemical characteristics than the Carbomer and Petrolatum gels and can be an efficient platform for the delivery of Zosyn for topical and transdermal delivery. The data clearly shows the 5% Methylcellulose gel has appropriate viscosity and hydration characteristics and spread necessary for the topical administration.

Declaration of Interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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