Effect of Various Preservatives on Pectin Base of Ofloxacin Vaginal GEL

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Authors’ contributions

This work was carried out in collaboration among all authors. Author EA designed the study and wrote the protocol. Author GE managed the animals, author CJ collected all data, performed the statistical analysis, and wrote the first draft of the manuscript. Authors CJ and TU did the literature search and also wrote part of the manuscript. All authors read and approved the final manuscript.

ABSTRACT

The effect of various preservatives such as Methyl paraben, Propyl paraben, benzoic acid and Methyl paraben, Propyl paraben (1:10) on a pectin – base ofloxacin vaginal gel was tested. Five batches of pectin-based ofloxacin vaginal gel were assessed by antibiotic sensitive test, determination of the minimum inhibitory concentration (MIC) and minimum bacterial concentration (MBC) for both the active drug and the preservative using Staphylococcus aureus and Escherichia coli as the test organism. Physical properties such as colour, texture and PH of the various Pectin-based ofloxacin vaginal gels also were examined.

All the batches of the pectin-based ofloxacin vaginal gel showed susceptibility according to the Kirby-Bauer method of zone interpretations for antibiotics sensitivity test. However for the minimum inhibitory concentration (MIC) determination, the ofloxacin powder and other three batches of the pectin-based vaginal gel (i.e. gel with Propyl paraben, methyl paraben: propyl paraben (1:10) and benzoic acid) showed susceptibility for both organisms according to the interpretation criteria and one batch (i.e. gel with methyl paraben) showed susceptibility to S. aureus and intermediate with E. coli. One of the batches (the control) showed resistance to both organisms. Also ofloxacin powder
showed bactericidal activities on four batches while the control (i.e. without preservative) did not exhibit any antibacterial activity after the MBC determination.

Keywords: Preservatives; microorganism; gel; vaginal gel; susceptibility.

1. INTRODUCTION

Vaginal drug delivery system refers to the system in which drug formulations are directly applied in vaginal cavity for producing local action. It is an important route of drug administration for both systemic and local effect. The vagina has dense network of blood vessels and rich blood supply, which makes the drug absorption so effective [1].

Gels are the semisolid formulations, with water base (hydrogels), or organic liquid base (organogels). Hydrogels also possess a degree of flexibility very similar to natural tissue, due to their significant water content. These pseudohydrogels swell infinitely and the component molecules dissolve from the surface of the matrix. Drug molecules are released through the spaces in the network and also by the dissolution and/or disintegration of the matrix. Mucoadhesive polymers of natural, semisynthetic or synthetic origin are able to form hydrogels. In the simplest case the drug is dispersed in a mucoadhesive polymer, which swells in the presence of water and exhibits bioadhesive properties [2].

Vaginal gels are used for topical delivery of contraceptives and anti-bacterial drugs. The desirable properties of vaginally administered gel against microbicides are acceptability and feasibility. They must be easy to use, non-toxic and non-irritating to the mucus membrane [3].

Gels often provide a faster release of drug substance, independent of the water solubility of the drug, as compared to creams and ointments [4]. They are highly biocompatible with a lower risk of inflammation or adverse reactions, easily applied and do not need to be removed [5,6].

Ofloxacin is a synthetic broad spectrum antimicrobial agent for oral and intravenous administration, with an in vitro activity against a broad spectrum of Gram positive and Gram negative aerobic and anaerobic bacteria. It exerts a bactericidal effect on susceptible microorganisms by inhibiting DNA gyrase, an essential enzyme that is a critical catalyst in the duplication, transcription and repair of the bacterial DNA [7,8].

The objective of this research was to determine the effect of various preservatives on the efficacy of pectin based vaginal gel.

2. MATERIALS

Benzoic acid (BDH Chemical Ltd, Poole, England), methyl paraben (J.T. Baker Chemical, England), Propyl paraben (J.T. Baker Chemical, England), pectin (BDH Chemical Ltd, Poole, England), glycerol (BDH Chemical Ltd, Poole, England), purified water, sterile nutrient agar plates. Other chemicals were of laboratory grade.

2.1 Microorganisms

Staphylococcus aureus (clinical isolate) Escherichia coli (chemical isolate).

3. METHODS

3.1 Preparation of Ofloxacin Vaginal Gel

Formula:

| Component | Percentage |
|-----------|------------|
| Ofloxacin | 1%         |
| Pectin    | 8.5%       |
| Glycerol  | 7%         |
| Preservatives | 0.2% |
| Purified water | 100% |

The above formula was used in the preparation of ofloxacin vaginal gel. Ofloxacin powder (1 g) was mixed with 8.5 g of pectin and 0.2 g of the preservative in a mortar. Glycerol 5.6 g was placed in a 250 ml beaker. Then the mixed ingredients were added in small quantity with stirring into the glycerol until a free flowing liquid was formed (i.e. stirred for three minutes at each inclusion). It was then stirred for 10 minutes for the gel to form completely. The gel was then allowed to stand for some time before packaging for complete hydration. Test for pH, consistency, antibiotic sensitivity, minimum inhibitory concentration (MIC) and minimum bactericidal concentration were carried out on the gel. This procedure was carried out for the four different batches using the different types of preservatives.

A blank product was prepared without a preservative to serve as the control.
N/B: The volume of glycerol used was calculated using its B.P stated density (1.261 g/cm$^3$) and its mass (7 g).

3.2 pH Determination

The pH of the five batches preparations were carried out with the aid of a pH meter. This was carried out by placing the pH meter inside gel with a little bit of stirring, then the value is read.

3.3 Antibiotic Sensitivity Test

The sensitivity test was carried out to determine the concentration at which the antibiotic (ofloxacin) is sensitive to both Staphylococcus aureus and Escherichia coli. The Agar-well diffusion method was used. The following procedures were carried out;

1. Two-fold dilution was carried out aseptically for each product to reduce both the concentration of the antibiotics and the preservative using water. This was diluted eight times each to give different concentrations, after preparing the stock solution the calculations are shown below.
2. The agar plates were used as the culture media as was prepared as follows; firstly the nutrient agar prepared by weighing appropriate quantity of peptone (5 g) and beef extract (3 g) and dissolve them in appropriate volume of distilled water (1000 ml). It was then shaken to ensure mixing uniformly and heated gently for complete dissolution of the ingredients. It was allowed to cool at room temperature. Secondly, the nutrient agar was prepared by weighing 15 mg of agar into 100 ml of the nutrient broth in the Erlenmeyer flask. It was then plugged with non-absorbent cotton-wool and placed in a boiling water bath to heat gently and mixed until the agar completely dissolved. The agar plates were prepared by measuring the nutrient agar into a conical flask and sterilized.
3. The agar plates were aseptically inoculated with a loopful (0.1 ml) suspension of the test organism, using a sterile glass spreader (a plate for each organism).
4. The sterile flamed cork-borer was used to bore holes on the seeded agar plates. The removed agar rings were discarded into a disinfectant solution.
5. The wells were aseptically filled with solution of the ofloxacin gel (i.e. the different concentrations) using a Pasteur pipette.
6. The plates were inverted and incubated at 37°C for 18-24 hours.
7. The plates were observed and the diameter of inhibition zone around each well was measured.
8. The results were recorded.

3.4 Determination of Minimum Inhibitory Concentration (MIC) by the Tube-dilution Method

1. The stock solution was prepared for each of the pectin-based gel preparation and also for ofloxacin powder (0.1 mg/ml) (but 1 mg/ml stock solution was used for control).
2. The empty sterile test-tubes were labelled (1,2,3,4,5,6,7,8,9) and the sterile nutrient broth were transferred to each of the test tube, 2 ml for each.
3. A two-fold serial dilution was carried out aseptically by the antibiotic substance by transferring 2 ml of antibiotic/preservative broth into test-tube 1 using sterile pipette and was shaken well to mix. The calculations are shown below.
4. Step 2 was repeated for other test – tubes by transferring 2 ml from one test – tube to the other. For the last test-tube 2ml was discarded into a beaker of bactericidal disinfectant.
5. Each of the dilutions was inoculated with 0.1 ml of the test culture and the tubes were incubated at 37°C for 24 hours.
6. The tubes were examined visually and the results were recorded as turbidity in a tabular form.
7. From the results, the MIC of both the antibiotics and the preservative were determined.

3.5 Determination of the Minimum Bactericidal Concentration (MBC)

1. The MIC tubes without growth were selected and incubated again at 37°C for 24 hours.
2. The tubes were examined again for evidence growth.
3. The lowest concentration of the antibiotic/preservative that allow less than 0.1% of the logically inoculums to survive is said to be the Minimum Bactericidal Concentration (MBC).

N/B: The MIC and MBC were carried out using the Staphylococcus aureus and Escherichia coli.

4. RESULTS AND DISCUSSION

4.1 Gel Properties

The physicochemical properties of the five types of the pectin-based ofloxacin vaginal gels were represented in Table 1. The gels all has the same colour and texture which and brown and smooth respectively. However, they all showed different pH values even though they were within a close range of 2.79 – 2.83. The different pH values exhibited by these products showed that a preservative has great influence on a product. The preparation with propyl paraben as the preservative and that which had methyl paraben: propyl paraben (1:10) as the preservative had the same pH value of 2.81 and this because the ratio of propyl paraben was more than that of methyl paraben in the later preparation.

4.2 Antibiotic Sensitivity

Antibiotic sensitivity is a term used to describe the susceptibility of bacterial to antibiotics. Antibiotic sensitivity test is a laboratory method for determining the susceptibility of organism to therapy with antibiotics and the concentration at which the antibiotics can be administered for therapy. Hence the five batches of the pectin-based ofloxacin vaginal gel were tested at different concentrations and the results obtained are shown below in Tables 2 to 5.

Table 1. The physicochemical properties of the pectin-based ofloxacin vaginal gels

| Types                        | Colour  | Texture | pH value |
|------------------------------|---------|---------|----------|
| Control                      | Brown   | Smooth  | 2.32     |
| Gel with benzoic acid        | Brown   | Smooth  | 2.79     |
| Gel with methyl-paraben      | Brown   | Smooth  | 2.83     |
| Gel with propyl-paraben      | Brown   | Smooth  | 2.81     |
| Gel with methyl paraben: propyl paraben (1:10) | Brown | Smooth   | 2.81     |

Table 2. Zone of Inhibition after 24 hours of incubation with Staphylococcus aureus

| Drug concentration mcg/ml | Control | Methyl paraben | Propyl paraben | Methyl paraben:propyl paraben (1:10) | Benzoic acid |
|---------------------------|---------|----------------|----------------|----------------------------------------|--------------|
| Tube 1 5                  | 48mm    | 39mm           | 38mm           | 39mm                                   | 36mm         |
| Tube 2 2.5                | 45mm    | 38mm           | 37mm           | 37mm                                   | 33mm         |
| Tube 3 1.25               | 41mm    | 37mm           | 36mm           | 36mm                                   | 32mm         |
| Tube 4 0.625              | 34mm    | 36mm           | 35mm           | 33mm                                   | 31mm         |
| Tube 5 0.3125             | 31mm    | 32mm           | 34mm           | 31mm                                   | 38mm         |
| Tube 6 0.15625            | 26mm    | 31mm           | 32mm           | 30mm                                   | 37mm         |
| Tube 7 0.078125           | 23mm    | 30mm           | 30mm           | 26mm                                   | 26mm         |
| Tube 8 0.0390625          | 21mm    | 25mm           | 28mm           | 24mm                                   | 25mm         |
Table 3. Zone of Inhibition after 48 hours of incubation with *Staphylococcus aureus*

| Drug concentration mcg/ml | Control | Methyl paraben | Propyl paraben | Methyl paraben:propyl paraben (1:10) | Benzoic acid |
|---------------------------|---------|----------------|----------------|----------------------------------------|-------------|
| Tube 1 5                  | 50mm    | 40mm           | 38mm           | 42mm                                   | 39mm        |
| Tube 2 2.5                | 47mm    | 39mm           | 37mm           | 39mm                                   | 37mm        |
| Tube 3 1.25               | 42mm    | 35mm           | 36mm           | 37mm                                   | 35mm        |
| Tube 4 0.625              | 34mm    | 34mm           | 34mm           | 36mm                                   | 33mm        |
| Tube 5 0.3125             | 32mm    | 33mm           | 33mm           | 33mm                                   | 32mm        |
| Tube 6 0.15625            | 29mm    | 31mm           | 32mm           | 30mm                                   | 31mm        |
| Tube 7 0.078125           | 23mm    | 28mm           | 30mm           | 27mm                                   | 29mm        |
| Tube 8 0.0390625          | 20mm    | 28mm           | 26mm           | 25mm                                   | 26mm        |

Table 4. Zone of Inhibition after 24 hours of incubation with *Escherichia coli*

| Drug concentration mcg/ml | Control | Methyl paraben | Propyl paraben | Methyl paraben:propyl paraben (1:10) | Benzoic acid |
|---------------------------|---------|----------------|----------------|----------------------------------------|-------------|
| Tube 1 5                  | 41mm    | 36mm           | 36mm           | 36mm                                   | 38mm        |
| Tube 2 2.5                | 38mm    | 33mm           | 34mm           | 34mm                                   | 36mm        |
| Tube 3 1.25               | 35mm    | 32mm           | 32mm           | 32mm                                   | 33mm        |
| Tube 4 0.625              | 34mm    | 29mm           | 29mm           | 30mm                                   | 32mm        |
| Tube 5 0.3125             | 31mm    | 27mm           | 28mm           | 29mm                                   | 30mm        |
| Tube 6 0.15625            | 29mm    | 25mm           | 26mm           | 27mm                                   | 27mm        |
| Tube 7 0.078125           | 27mm    | 24mm           | 25mm           | 24mm                                   | 25mm        |
| Tube 8 0.0390625          | 27mm    | 22mm           | 22mm           | 22mm                                   | 24mm        |

The zone of clearing varies depending upon its concentrations and its chemical properties. Standard zone of inhibition have been established for each antibiotic. If the zone of inhibition is greater than the standard, the organism is considered to be sensitive to the antibiotic. If the zone of inhibition is less than the standard, the organism is considered to be intermediate or resistant. The zone interpretation using the Kirby-Bauer method are recommended:

- **Resistant** = up to 12mm
- **Intermediate** = 13-19mm
- **Susceptible** = 20mm or more (Grimm et al. 1986).
Table 5. Zone of inhibition after 48 hours of incubation with *Escherichia coli*

| Drug concentration mcg/ml | Control | Methyl paraben | Propyl paraben | Methyl paraben:propyl paraben (1:10) | Benzoic acid |
|---------------------------|---------|----------------|----------------|----------------------------------------|--------------|
| Tube 1                    | 42mm    | 37mm           | 38mm           | 38mm                                   | 39mm         |
| 5                         |         |                 |                |                                        |              |
| Tube 2                    | 38mm    | 33mm           | 34mm           | 35mm                                   | 37mm         |
| 2.5                       |         |                 |                |                                        |              |
| Tube 3                    | 35mm    | 30mm           | 31mm           | 32mm                                   | 35mm         |
| 1.25                      |         |                 |                |                                        |              |
| Tube 4                    | 32mm    | 27mm           | 28mm           | 29mm                                   | 32mm         |
| 0.625                     |         |                 |                |                                        |              |
| Tube 5                    | 30mm    | 25mm           | 26mm           | 26mm                                   | 30mm         |
| 0.3125                    |         |                 |                |                                        |              |
| Tube 6                    | 28mm    | 23mm           | 24mm           | 24mm                                   | 28mm         |
| 0.15625                   |         |                 |                |                                        |              |
| Tube 7                    | 27mm    | 22mm           | 24mm           | 23mm                                   | 27mm         |
| 0.078125                  |         |                 |                |                                        |              |
| Tube 8                    | 26mm    | 21mm           | 22mm           | 21mm                                   | 24mm         |
| 0.0390625                 |         |                 |                |                                        |              |

From the result above, it was observed that at some concentrations the zone of inhibition increased after 48 hours of incubation as compared to that of 24 hours, this may be due to the extent of diffusion. Also at some concentrations the zone of inhibition reduces after 48 hours of incubation compared to that of 24 hours incubation.

The control showed a greater zone of inhibition compared to those with preservatives. This could be due to antagonistic effect between the antibiotics and the preservative.

### 4.3 Antibiotic Susceptibility Testing

The basic quantitative measures of the in vitro activity of antibiotics are Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). Antibiotics are categorized as bacteriostatic if they reversibly inhibit the growth of bacteria or bactericidal if they kill the susceptible bacteria. In general, the use of bactericidal antibiotic is preferred but many factors may dictate the use of a bacteriostatic antibiotic. When a bacteriostatic antibiotic is used the duration of the therapy must be sufficient to allow cellular and humoral defence mechanism to eradicate the bacteria.

For an antibiotic to be effective the MIC or MBC must be able to be achieved at the site of infection. The pharmacological absorption and distribution will influence the dose, route and frequency of the antibiotic in order to achieve an effective dose at the site of infection. Various factors can affect the concentration at which the MIC or MBC is obtained, these include the excipients (such as the base, preservatives, fragrance etc), environmental conditions etc.

### 4.4 Bacteriostatic Activity

Bacteriostatic activity stops the organism from multiplying but does not kill it. All antimicrobial are bacteriostatic at some concentrations (example Fluoroquinolones) wile some antimicrobials are bacteriostatic at all concentrations (example tetracyclines and sulphonamides). The standard method for determining the bacteriostatic effect of certain antibiotics is by MIC determination. Quantitative methods are used to determine antimicrobial MIC values. These MIC values provide estimates of the susceptibility of bacteria to antimicrobial compounds. The MIC values should be determined using a standardized procedure which is based on a dilution method. The MIC values should be interpreted according to the following criteria as shown in Table 6.

A report of “S” indicates that the pathogen is likely to be inhibited if the antimicrobial compound in the blood reaches the concentration usually achievable. A report of “I” indicates that the result should be considered equivocal, and if the microorganism is not fully
susceptible to alternative, clinically feasible drugs, the test should be repeated. This category implies possible clinical applicability in body sites where drug is physiologically concentrated or in situations where high dosage of drug can be used. This category also provide a buffer zone which prevents small uncontrolled technical factors from causing major discrepancies in interpretation. A report of “R” indicates that the pathogen is not likely to be inhibited if the antimicrobial compound in the blood reaches the concentrations usually achievable; other therapy should be selected.

Ofloxacin exhibits in vitro MIC values of 2mcg/ml or less against most strain of microorganism. MIC determination was carried out using tube dilution method as described in section 2.2.5, for ofloxacin powder and the five batches the pectin-based ofloxacin vaginal gel (with different preservative respectively i.e. methyl paraben, propyl paraben, methyl paraben: propyl paraben (1:10), benzoic acid and one without a preservative as control). The result obtained after the MIC determination is shown in Tables 7 to 10.

Table 6. Interpretation criteria

| MIC (mcg/ml) | Interpretation |
|--------------|----------------|
| Less than or equal to 2 | Susceptible (S) |
| 4 | Intermediate (I) |
| Greater than or equal to 8 | Resistant (R) |

Table 7. MIC with *Staphylococcus aureus*

| Drug concentration (mcg/ml) | Methyl paraben | Propyl paraben | Methyl paraben:propyl paraben (1:10) | Benzoic acid | Ofloxacin powder |
|-----------------------------|----------------|----------------|----------------------------------------|--------------|-----------------|
| Tube 1 50                   | -              | -              | -                                      | -            | -               |
| Tube 2 25                   | -              | -              | -                                      | -            | -               |
| Tube 3 12.5                 | -              | -              | -                                      | -            | -               |
| Tube 4 6.25                 | -              | -              | -                                      | -            | -               |
| Tube 5 3.125                | -              | -              | -                                      | -            | -               |
| Tube 6 1.5625               | -              | -              | -                                      | -            | -               |
| Tube 7 0.78125              | +              | +              | -                                      | -            | -               |
| Tube 8 0.39063              | +              | +              | +                                      | +            | +               |
| Tube 9 0.19532              | +              | +              | +                                      | +            | +               |

Table 8. MIC with *Escherichia coli*

| Drug concentration (mcg/ml) | Methyl paraben | Propyl paraben | Methyl paraben:propyl paraben (1:10) | Benzoic acid | Ofloxacin powder |
|-----------------------------|----------------|----------------|----------------------------------------|--------------|-----------------|
| Tube 1 50                   | -              | -              | -                                      | -            | -               |
| Tube 2 25                   | -              | -              | -                                      | -            | -               |
| Tube 3 12.5                 | -              | -              | -                                      | -            | -               |
| Drug concentration (mcg/ml) | Methyl paraben | Propyl paraben | Methyl paraben:propyl paraben (1:10) | Benzoic acid | Ofloxacin powder |
|-----------------------------|----------------|---------------|-------------------------------------|--------------|-----------------|
| Tube 4 6.25                 | -              | -             | -                                   | -            | -               |
| Tube 5 3.125                | -              | -             | -                                   | -            | -               |
| Tube 6 1.5625               | +              | -             | -                                   | -            | -               |
| Tube 7 0.78125              | +              | +             | +                                   | +            | +               |
| Tube 8 0.39063              | +              | +             | +                                   | +            | +               |
| Tube 9 0.19532              | +              | +             | +                                   | +            | +               |

**Table 9. MIC for control using *Staphylococcus aureus***

| Drug concentration (mcg/ml) | Control |
|-----------------------------|---------|
| Tube 1 (500)                | -       |
| Tube 2 (250)                | -       |
| Tube 3 (125)                | -       |
| Tube 4 (62.5)               | -       |
| Tube 5 (31.25)              | -       |
| Tube 6 (15.25)              | -       |
| Tube 7 (7.8125)             | -       |
| Tube 8 (3.90625)            | +       |
| Tube 9 (1.95313)            | +       |

**Table 10. MIC for control using *Escherichia coli***

| Drug concentration (mcg/ml) | Control |
|-----------------------------|---------|
| Tube 1 (500)                | -       |
| Tube 2 (250)                | -       |
| Tube 3 (125)                | -       |
| Tube 4 (62.5)               | -       |
| Tube 5 (31.25)              | -       |
| Tube 6 (15.25)              | -       |
| Tube 7 (7.8125)             | +       |
| Tube 8 (3.90625)            | +       |
| Tube 9 (1.95313)            | +       |

**5. CONCLUSION**

From the result above ofloxacin powder shows MIC at 0.78125 mcg/ml with *S. aureus* and 1.5625 mcg/ml with *E. coli* whereas the control has MBC at 7.825 mcg/ml with *S. aureus* and 15.625 mcg/ml with *E. coli*. While gel with methyl paraben showed 1.5625 mcg/ml with *S. aureus* and 3.125 mcg/ml with *E. coli*, gel with propyl paraben showed 1.5625 mcg/ml with *S. aureus* and 3.125 mcg/ml with *E. coli*, gel with methyl paraben:propyl paraben (1:10) showed 0.78125 mcg/ml with *S. aureus* and 1.5625 mcg/ml with *E. coli*. This result shows that ofloxacin powder has same MIC values with gel with methyl paraben:propyl paraben (1:10) and gel with benzoic acid for *S. aureus*. Also ofloxacin has the same MIC values with gel with propyl paraben, gel with methyl paraben:propyl paraben (1:10) and gel with benzoic acid for *E. coli*. Also it has been observed that the MIC value for *S. aureus* are lower than that of *E. coli*. This could be due to the nature of the microorganism. The control...
showed resistant based on this result and this could be because of its lack of a preservative.

5.1 Bactericidal Activity

Bactericidal activity kills bacteria that are multiplying. Some antimicrobials are capable of bactericidal activity and may occur if concentration of “Cidal” antibiotics is high enough. The rate and extent of bactericidal activity may be concentration dependent (example aminoglycosides), concentration and time dependant (example fluoroquinolones), and time dependant (example betalactams).

MBC determination is a standard method of determining the bactericidal activity of an antibiotic. Therefore the MBC for ofloxacin powder and the five batches of the pectin-based ofloxacin vaginal gel was carried out by sub-culturing the tubes that did not show any visible growth after 24hours incubation. These tubes where then incubated for another 24hours at 37 °C. At the end of incubation the tube.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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