Formulation and Evaluation of Ciprofloxacin Hydrochloride Vaginal Tablet and Gel

*Akpabio, E. I.[1] and Ofoefule, S. I.[2]

1. Department of Pharmaceutics /Pharmaceutical Technology University of Uyo, Uyo, Akwa Ibom State Nigeria.
2. Department of Pharmaceutical Technology and Industrial Pharmacy, University of Nigeria, Nsukka Enugu State Nigeria.

ABSTRACT

Two different dosage forms of ciprofloxacin hydrochloride (CH), namely tablet and gel intended for vaginal drug delivery were formulated. The vaginal tablet was evaluated for weight uniformity, dissolution through an artificial membrane and bioadhesion on pig's vaginal epithelium. In addition CH vaginal gel was evaluated for pH, consistency and its activity against Escherichia coli and Staphylococcus aureus. The weight, thickness, diameter and hardness of CH vaginal tablets were 0.614 ± 0.20g, 5.53 ± 0.01mm, 12.54 ± 0.02 mm and 1.92 ± 0.20 kgf respectively. There was gradual release of CH from the vaginal tablets through an artificial membrane over ninty minutes. The CH vaginal gel had a pH of 6.67 and consistency of 115.40 ± 0.77 mPa.s. CH vaginal gel was more active against Escherichia coli (MIC = 0.03125 mg/mL) than Staphylococcus aureus (MIC = 0.0625 mg/mL). The release kinetics exhibited by the CH vaginal tablet was zero order (r2 = 0.9032).

KEYWORDS: Ciprofloxacin hydrochloride, vaginal tablet, vaginal gel

INTRODUCTION

Vaginal drug delivery has been of interest to scientist to varying degrees during the last century (Woolfson et al.,2000). The vagina as a route of drug delivery, offers a means to administer drugs for local benefit or systemic distribution. For years, antifungal and antibacterial agents have been administered vaginally to treat yeast and bacterial infections, administration of local spermicides and cleansing products is also a current practice (Sitruk-Ware, 2008). The introduction of Intravaginal rings (IVRS) in the 1990s to administer steroid hormones for hormone replacement therapy represented a major advance in both vaginal drug delivery and the drug delivery field as a whole. These devices were designed to sustain the release of steroid hormones for up to one month.

Traditionally, available dosage forms include liquids (solutions, emulsions and suspension, pessaries, tablets, creams and ointments). Gels and vaginal rings have gained increased popularity due to their unique advantages and women's acceptance. Others such as capsules, foams, vaginal films,vaginal tablets, medicated tampons, sponges or diaphragms are also of interest.

Bacterial vaginosis is a non specific infection associated with positive cultures for Gardnerella vaginalis, characterised by increased malodourous vaginal discharge. It is the most common vaginal infection of reproductive age. This condition is treated by using medicine taken orally or intravaginally. Antibiotics such as metronidazole, Clindamycin (tablets and gels) as well as tinidazole have been the drug of choice for the treatment of bacterial vaginosis (Hilier et al.,2008). Ciprofloxacin hydrochloride, a fluoroquinolone antibacterial agent has been found to be active against Gardnerella vaginalis and other susceptible vaginal pathogens (Chein, 2007)

Treatment of bacterial vaginosis has largely been restricted to use of orally or vaginally administered metronidazole, clindamycin and tinidazole. The susceptibility of Gardnerella vaginalis to ciprofloxacin hydrochloride has open a new window of opportunity in the management and treatment of bacterial vaginosis, and other infections of the vagina sensitive to ciprofloxacin hydrochloride.

The design, formulation and evaluation of vaginal tablets and gel of ciprofloxacin hydrochloride for the treatment of vaginal infections caused by susceptible micro-organisms were undertaken in this study.

***Corresponding author: Email: pharmeka2000@yahoo.com Phone: +2348027302734**

MATERIALS AND METHODS

Materials

Ciprofloxacin hydrochloride (99.53% Purity, Batch No. CH 1177-11010) was obtained as a gift sample from (Arch Pharma Labs. Ltd, India). polyethylene glycol 8000 (Sigma-Aldrich, Germany) Polyvinyl Pyrollidone (E. Merck Darmstadt) microcrystalline cellulose, glycerol, talc, magnesium stearate (BDH chemicals limited Poole, England) Sodium alginate (Batch No. AC177775000) was a free gift from (Janssen Pharmaceuticalaan Geel, Belgium) methyl paraben (Nipa Lab; Hamburg, West Germany) cellulose membrane, M. W. Cut-off 6000-8000 (Spectrum Labs; Breda, The Netherlands).

Methods

Formulation of Ciprofloxacin Hydrochloride Vaginal Tablet

The composition of ciprofloxacin hydrochloride tablet is shown in Table 1. A modified wet granulation method was adopted. The tablet was formulated with a blend of Ciprofloxacin hydrochloride (10 g) microcrystalline cellulose (16.75 g). The blend was granulated with a solution of 7.5 % polyvinyl pyrrolidone in sufficient isopropyl alcohol. The wet mass was passed through a 2.00 mm stainless steel sieve and dried in a Genlab hot air oven at 60oC for 2 hours. The dried granules were passed through a 1.00 mm sieve. Polythylene glycol 8000 (11.25 g), talc (0.30 g) and magnesium stearate (0.15g) were added extragranularly to the screened granules and the final mixing was carried out in a powder bottle for 3 minutes. Compression was carried out in a single punch tablet press (Cadmach, Ahmedabad, India), fitted with 12.5 mm flat faced punches at low pressure to a hardness of 2 kg/f.

Table 1: Composition of Ciprofloxacin Hydrochloride Vaginal Tablet

Ingredients(mg per tablet)	
Ciprofloxacin hydrochloride	200
Microcrystalline cellulose	375
Polythelene glycol 8000	225
Polyvinyl pyrolidone (in ethanol)	7.5
Talc	3
Magnesium stearate	6

Evaluation of Vaginal Tablets

Weight Variation Test

Twenty tablets selected at random were weighed using an electronic balance (OHAUS, Galaxy).

Friability Test

The friability test was carried out using 10 tablets. A Roche friabilator (UNID 056830 Campbell Electronic, Mumbai, India) was used. The tablets were selected at random, dedusted and subjected to abrasive shock at 100 rpm for 4 minutes. The friability 'F' was calculated from Equation 1.

$$100\frac{(Wo-Wi)}{Wo} \text{ ----------Eqn(1)}$$

Where, Wo is the initial weight of tablets, and Wi is the weight after abrasive shock.

Diameter and Thickness Test

The diameter and thickness of 10 tablets were measured using a micrometer screw gauge. The result was expressed as mean ± standard deviation.

Dissolution Profile of Ciprofloxacin Vaginal Tablets

The release profile of ciprofloxacin hydrochloride from the vaginal tablets was carried out in Erweka dissolution apparatus (DA – 6D model) using the USP basket method. The dissolution medium was 500 mL phosphate buffer (pH4) maintained at a temperature of 36± 0.5oC and agitation speed of 50 rpm. A sample (10 mL) was withdrawn at 5 minute interval. For each 10 mL of phosphate buffer maintained at the same temperature was added to the dissolution medium. Absorbance of withdrawn samples were measured at 278 nm in a UV/visible spectrophotometer model UV 2100PC (Shanghai Instr. Co. Ltd China). Cumulative percentage drug released was calculated using an equation obtained from the standard plot. The dissolution study was carried out with the tablet placed in an artificial cellulose membrane (figure 4). Results obtained were fitted into zero order, first order, and the Higuchi square root kinetics and Korsemeyer-Peppas model to ascertain drug release mechanism.

Adhesion/Erosion Test

The vaginal tablets were subjected to the same experiment as described for pessaries. The percentage bioadhesion and erosion were calculated from the equations 2 and 3 below;

$$\text{Percentage bioadhesion} = 100\frac{\text{(No of tablet after bioadhesion experiment)}}{\text{Initial number of tablets}} \text{ ----- Eqn (2)}$$

$$\text{Percentage erosion} = 100\frac{\text{(Initial weight –final weight)}}{\text{Initial weight}} \text{ --------Eqn (3)}$$

Figure 1:Photograph showing Ciprofloxacin hydrochloride vaginal tablet

Bioadhesive Strength Determination

The Lecomte du Nouy tensiometer (model Nr 3124, A. Kruss Hamburg, Germany) was used for this study. A freshly excised pig vaginal epithelium with dimensions 11.5 cm by 7.5 cm was used for this study. The tissue was pinned unto the polyethylene support of the bioadhesive instrument placed on a metal support.(Ofoefule and Ike-Unor, 2001). The instrument was zeroed, and the bioadhesion of the clean plate determined in degrees, after the addition of 1µl of phosphate buffer pH 4 at the interface and leaving it for 10 minutes (Attama et. al., 2000). A tablet was glued to the plastic plate using a cyanoacrylate adhesive. The tablet was then brought into contact with the everted mucous surface and 1□l of the phosphate buffer pH 4 added at the interface to activate the interaction. A contact time of 10 minutes was allowed for bioadhesive interaction to take place. The force required to remove the tablet was recorded in degrees and appropriate conversion to bioadhesive strength was obtained from the modified equation of Harkins and Jordan (Harkins and Jordanm 1930). Average of five determinations on a fresh vaginal epithelium was taken as the bioadhesive strength

$T = (Mg/2L).F$ -----Eqn (4)

Where T is the tension equivalent to bioadhesive strength, M is the mass required to return the lever to zero position after each bioadhesion experiment, g is the acceleration due to gravity, F is the instrument constant and L is the area of the bioadhesive interface. The tablets were circular and flat faced. The bioadhesive interface, L, is the area of the tablet in contact with the mucus and is equivalent to the area (A) of one side of the tablet.

$A = \pi r^2 = \pi (D^2/4)$ ------- Eqn (5)

Where A is the area of one side of the tablet,

L from Equation 4 becomes: $L = \pi (D^2/4)$ --Eqn (6)

Therefore, $T = [Mg/(2 [D^2/4])]. F$ ---------- Eqn (7)

$$T = (2. mg/\pi D^2). F\text{----------Eqn (8)}$$

Where D is the mean tablet diameter.

Equation 8 was used to calculate the tension equivalent to the bioadhesive strength of the tablets.

Formulation of Ciprofloxacin Hydrochloride Vaginal Gel

The composition of Ciprofloxacin hydrochloride vaginal gel is shown in Table 2. The gel was prepared by triturating a mixture of ciprofloxacin hydrochloride (1 %), sodium alginate (7 %) and methyl paraben (0.2 %) in a mortar.

Glycerol (7 %) was measured and placed in a beaker; the powder mix was added slowly with stirring to the beaker to form a smooth flowing liquid. The distilled water (84 mL) was added all at once and stirred gradually to avoid the incorporation of air bubbles. A portion of the prepared gel was filled into a collapsible tube while the remaining was stored in an airtight specimen bottle for further experiments.

Evaluation of Ciprofloxacin Hydrochloride Vaginal Gel

Determination of the Organoleptic properties of the Ciprofloxacin Hydrochloride Vaginal Gel

The ciprofloxacin hydrochloride vaginal gel was examined visually for colour and the odour was perceived by smelling.

Table 2: Composition of Ciprofloxacin Hydrochloride Vaginal Gel

Ingredients	**Weight (%)**
Ciprofloxacin Hydrochloride	1.0
Sodium alginate	7-10
Polyhydric alcohol	7-10
Preservative	0.2-0.4
Vehicle to	100

Determination of pH of Ciprofloxacin Hydrochloride Vaginal Gel

The vaginal gel (1 g) was dispersed in 30 mL of distilled water and the pH was measured using a pH meter.

Measurement of the Consistency of Ciprofloxacin Vaginal Gel

The viscosity of the vaginal gel was measured at 37oC using a penetrometer (setamatic). The time lapse between each reading was 10 secs. The result is shown in Table 5.

Microbiological Test on Ciprofloxacin Hydrochloride Vaginal Gel

Preliminary Sensitivity Test

The antibacterial activity of the vaginal gel was evaluated using the spread plate method (Collins and Lyne, 1979) with Staphylococcus aureus (NcTc 6571), and Escherichia coli (NcTc 10418) as the test organisms. A 0.1mL volume of standardized bacterial suspensions were aseptically spread plated on sterile nutrient agar (NA) plates, incorporated with 1 mL of 50, 40, 30, 20 and 10 mg/mL of the gel preparation respectively. The contents of the plates were solubilized with Tween 20, and incubated at 39oC for 24hours for growth. After incubation the presence of growth denotes non-sensitivity of the organisms to the gel; or no potency; while the absence of growth denotes sensitivity of the test organisms to the gel, hence high potency.

Antibacterial Sensitivity Evaluation of Ciprofloxacin Hydrochloride Vaginal Gel

The antibacterial potency of the ciprofloxacin hydrochloride (1%) gel was evaluated using the agar well diffusion method (Collins and Lyne, 1979). A 50 mg quantity of the vaginal gel was used. The gel was solubilized with Tween 80. Serial dilutions of 0.5, 0.25, 0.125, 0.0625, 0.03125, and 0.0156 mg/mL were made. A 0.1 mL volume of standardized bacterial suspension were aseptically spread on different sterile nutrient agar plates. With a sterile cork-borer (4 mm in diameter), wells were aseptically bored on the previously seeded plates, after which 0.2 mL of the respective gel dilutions were introduced into the wells. The plates were then held at 4oC for 1 hour followed by incubation at 37oC for 24 hours. At the end of incubation, diameters of zones of inhibition were measured.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the Ciprofloxacin Hydrochloride Vaginal Gel

The MIC of the vaginal gel for each of the test organism was determined according to the macrodilution broth method (Tilton and Howard, 1987) and(Andrews, 2001). Serial dilutions of the vaginal gel (0.5, 0.25. 0.125, 0.0625, 0.03125 and 0.0156mg/ml) were made. To each of the test tubes concentrations of the anti bacterial gel, was added 0.1ml standardized bacterial suspensions and incubated at 37oC for 24 hours. Uninoculated nutrient broth served as control. The MICs of the gel for each of the bacteria was taken as the least concentration of the gel that inhibited the growth of the test organisms as measured by turbidity.

The MBC of the gel was determined from the 24 hour macrodilution broth MIC tubes with no visible growth (turbidity). The non-turbid MIC tubes were further incubated for 48 hours. At the end of incubation, the appearance of growth (turbidity) in the non – turbid MIC tubes denoted the MBC of the gel against the organism (NCCLS, 2000).

RESULTS AND DISCUSSION

Physical Properties of Ciprofloxacin Hydrochloride Vaginal Tablet

The data of physical parameters like thickness, hardness, diameter, weight variation, friability are presented on Table 3. All the parameters except friability lie within acceptable limits (Loyd et al.,2007). The average weight of the tablets was 0.614g and the weight variation in the batch was less than ± 5%. The friability of the tablets was 8.05%. This value though high was not considered to be deleterious to the dosage form. Vaginal preparations are usually enclosed in foils and other primary pages prior to packaging in a secondary container. Such products are not usually exposed to vigorous handling processes.

Table 3: Physical properties of Ciprofloxacin Hydrochloride Vaginal Tablet

Parameter (Mean + SD)				
Weight Variation (g)	**Thickness (mm)**	**Diameter (mm)**	**Hardness kg/f**	**Friability %**
0.614 ± 0.20	5.53 ± 0.0011	12.54 ± 0.0163	1.92 ± 0.2000	8.05 ± 0.2000

Weight Variation: n = 20, Hardness n = 5, Friability n = 10, Diameter n = 10, thickness n = 10

Table 4: Erosion/Bioadhesion Test of Ciprofloxacin Vaginal Tablet

Sample	**Initial weight (g)**	**Final weight(g)**	**Percent erosion**	**Bioadhesion (%)**
1	0.63	0.92	-29	100
2	0.62	0.87	-25	100
3	0.62	0.85	-22	100

Table 5: Physicochemical Properties of Ciprofloxacin Vaginal Gel

Parameters	
Colour	Light yellow
Odour	Characteristic
Ph	6.67
Viscosity	115.4± 0.77 mPa.s

Table 6a: Minimum Inhibitory Test (24 Hours)

Test organisms	**Concentration (mg/ml)**					
	0.500	0.2500	0.1250	0.0625	0.03125	0.0156
Escherichia coli	-	-	-	-	-	+
Staphylococcus aureus	-	-	-	-	+	+

+ (Growth); MIC: *E. coli* = 0.03125mg/ml
- (No growth) *Staph. aureus* = 0.0625mg/ml

Table 6b: Minimum Bactericidal Concentration (48 hours)

Test organisms	**Concentration (mg/ml)**					
	0.500	0.2500	0.1250	0.0625	0.03125	0.0156
Escherichia coli	-	-	-	-	+	+
Staphylococcus aureus	-	-	-	+	+	+

+ (Growth); MIC: *E. coli* = 0.0625mg/ml
- (No growth) *Staph. aureus* = 0.125mg/ml

Erosion /Bioadhesion of Ciprofloxacin hydrochloride Vaginal Tablet

The result of the erosion/bioadhesion test of CH vaginal tablet is shown in Table 4, from the result the vaginal tablet showed 100% bioadhesion but swelled when in contact with the buffer solution (pH4). It exhibited a bioadhesive strength of 101.1Nm. This indicates that it could adhere to the mucous surface for a period of time long enough to release its medicament (Harkins and Jordanm, 1930).

Table 6c: Agar-Well Diffusion

Organism			
Staph. aureus		*Escherichia coli*	
Concentration (mg/ml)	Inhibition zone diameter (mm)	Concentration (mg/ml)	Inhibition zone diameter (mm)
0.5	21.0	0.5	37.0
0.25	15.0	0.25	35.0
0.125	12.0	0.125	33.0
0.0625	-	0.0625	25.0
0.03125	-	0.03125	21.0
0.0156	-	0.0156	19.0

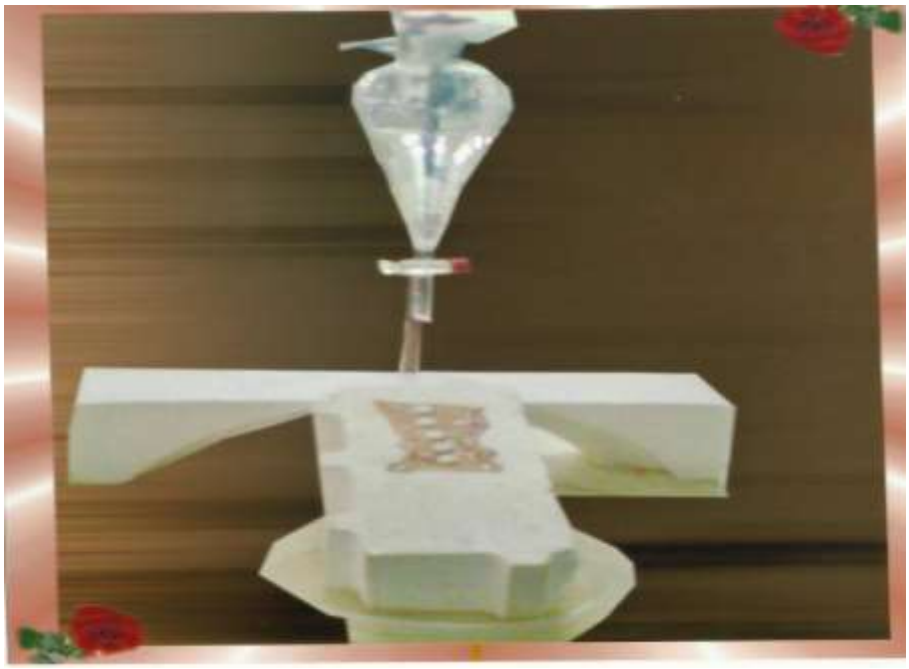

Figure 2: Photograph showing the erosion/bioadhesion test for Ciprofloxacin Hydrochloride Vaginal Tablet

Some Release Parameters of Ciprofloxacin Hydrochloride Vaginal Tablets

The release rate of ciprofloxacin hydrochloride from the vaginal tablet was analysed on the basis of time taken for 50% and 70% of the drug to be released (T50 and T70). The result obtained showed that 50% of the drug was released in 1.3 minutes while 70% was released within 5.0 minutes. Traditional delivery systems are characterized by immediate and uncontrolled drug release kinetics (Kalam et al.,2007). The vaginal tablet released the drug at a relatively controlled rate as depicted by the results.

Kinetics and Mechanism of Release for Ciprofloxacin Hydrochloride Vaginal Tablets

The dissolution data was fitted into zero-order, first order, Higuchi and Korsemeyer models. The regression values for zero order, first order, Higuchi and Korsemeyer models were 0.9032, 0.691, 0.8807 and 0.8164 respectively. From the regression values, the release of CH followed zero order release kinetics. In order to confirm the exact mechanism of drug release from the tablets the data was fitted into the Korsemeyer equation (Korsemeyer et al.,1983). The 'n' value obtained was 1.0 which implies that the predominant mechanism of release is anomalous (non-Fickian transport). This implies that, drug release followed both diffusion and erosion controlled mechanisms(Chime et al.,2013).

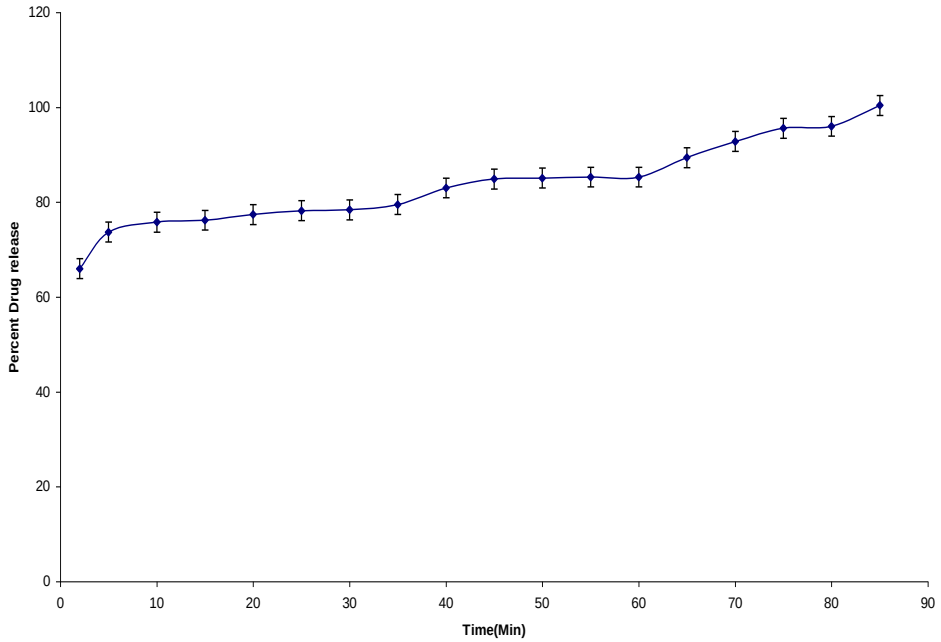

Figure 3: Release profile of Ciprofloxacin Hydrochloride Vaginal Tablet in Phosphate buffer (pH 4)

Figure 4: Photograph showing the dissolution of Ciprofloxacin Vaginal Tablet in a dialysis membrane.

PHYSICOCHEMICAL PROPERTIES OF CIPROFLOXACIN VAGINAL GEL

Organoleptic Properties of Ciprofloxacin Vaginal Gel

The ciprofloxacin vaginal gel was light yellow in colour with a characteristic odour.

It had a pH of 6.67 and a consistency of 115.4 mPa.s. The pH is slightly acidic and it could be applied successful in the female genital tract for the treatment of infections caused by susceptible microorganism. Beyond this pH, CH, a weekly acidic drug precipitates. The consistency which was above 100 mPas will prevent the leakage of the gel from the female genital track thereby preventing staining of underwears (Blaey andPolderman,1980).

Microbiology Test of Ciprofloxacin Hydrochloride Gel (Tube Dilution Test)

Minimum Inhibitory Test (24 Hours) and Minimal Bactericidal Concentration (MBC) (48hrs)

The result of the minimum inhibitory test is presented on Table 6a. From the result the MIC for Escherichia coli was 0.03125 mg/mL, while that of Staphylococcus aureus was 0.0625 mg/L. This indicates higher activity of the gel against infections caused by E. coli but caused by Staph. aureus.

Minimum Bactericidal Concentration (48 Hours)

The results showing the MBC of ciprofloxacin hydrochloride vaginal gel is presented in Table 6b. From the results the MBC of E. coli was 0.0625 mg/mL, while that of Staph aureus was 0.125 mg/mL.

Minimum inhibitory concentrations MICs are considered to be a valueable tool for determining the susceptibility (Andrews,2001) of organisms to antimicrobials and are therefore used to judge the performance of all other methods of susceptibility testing. The range of antibiotic concentrations used for determining MICs is universally accepted to be in doubling dilution step up and down from 1 mg/L as required (Collins and Lyne,1979). According to literature (Andrews, 2001) the suggested MIC values for standard ciprofloxacin powder is given as 0.004-128 mg/mL, for Staph aureus and E. coli respectively. The MIC values in the current research fell within acceptable limits, and therefore shows that the formulated ciprofloxacin gel has significant activity against E. coli and Staphylococcus aureus. The MIC values give indication of the susceptibility or the resistance of a bacteria strain to a given antibiotic. A value of < 1mcg/ml is being interpreted as susceptible, a value of 2mcg/ml mean intermediate activity, and a value of > 4 is being interpreted as resistant. From the results in Table-10a, Escherichia coli and Staph aureus are both susceptible to ciprofloxacin hydrochloride. The minimum bacterial concentration (MBC) generally does not exceed the MIC by more than a factor of 2 (10). The result shown conforms to this standard as the difference between the MIC and MBC in this case is less than 2. (MBC for E. coli and Staph aureus are 0.0625 mg/mL and .125 mg/mL respectively).

Agar-Well Diffusion

The antibacterial sensitivity test results are shown in Table 6c. For the diffusion technique, the expected inhibition zone diameters for both E. coli and Staph. aureus are 30-40 mm and 22-30 mm respectively (Andrews,2001).

From the result, the inhibition zone diameter increased with increase in concentration of the test drug, and the results fell within the accepted limits, with maximum inhibition (37.00 mm) against E. coli and minimum (12.0 mm) against Staphylococcus aureus. Ciprofloxacin hydrochlorides showed marked activity against E. coli based on the zone of inhibition and MIC values (Andrews,2001). This is in accordance to what was stated in the literature (NCCLS,2000).

CONCLUSION

The physical properties of ciprofloxacin vaginal tablets were within the acceptable limits except friability (8.05 %). The Ciprofloxacin vaginal gel was light yellow in colour with a characteristic odour. It was slightly acidic with a consistency of 115.4 mPa.s. The MIC of the vaginal gel for E. coli and S. aureus were 0.03125mg/ml and 0.0625 mg/mL respectively, while the MBC value were 0.625 mg/mL and 0.125 mg/mL for E. coli and S. aureus respectively. The zone of inhibition for both E. coli and S. aureus were between 19.0-37.0 mm for E. coli and 12.0-21.0 mm for S. aureus. It is evident from this study that ciprofloxacin hydrochloride has more activity against E. coli than S. aureus. The results of the present study has shown that formulation of ciprofloxacin hydrochloride into vaginal tablets and vaginal gel could be utilized to maintain adequate vaginal hygiene in women and for the treatment of vaginal infections caused by susceptible micro-organisms.

REFERENCES

Andrews, J. M. (2001). Determination of minimum inhibitory concentrations. J Antimicrobial Chemotherapy. 48: Suppl., SI, 5-16.

Attama, A. A., Adikwu, M. U. and Okoli, N. D. (2000). Studies on bioadhesive granules: granules formulated with Prosopis africana (Prosopis) Gum. Chem. Pharm. Bull. 48(5): 734-737.

Blaey, C.J and Polderman, J. (1980) Rationales in the design of rectal and vaginal delivery forms of drugs In: Drug design vol.1X, Academic press London. Pp 237-266.

Chein, Y. W. (2007). Novel drug delivery systems. 2nd ed. Marcel Dekker, Inc. New York, pp. 529-583.

Chime Salome A. Onunkwo Godswill C. and Onyishi Ikechukwu I. (2013) Kinetics and Mechanisms of Drug release from swellable and non swellable matrices a Review. Research Journal of Pharmaceutical, Biological and chemical Sciences. 4 :97-103

Collins, C. H. and Lyne, A. (1979). Microbiological methods 4th ed. Butterworth, London.

Harkins, W. D. and Jordann H. F. (1930). A method for determination of surface and interfacial tension from the maximum pull on the ring. J. Amer Chem. Soc. 52:1721.

Hilier, S., Melissa, C. S. and William, C. S. (2008). Bacterial Vaginosis. In: K. K. Holmes et al; Sexually Transmitted Disease, 4th ed., McGraw Hill, New York, pp. 737-768.

Kalam, M. A. Humayunn, M., Par Vez, N., Yadav, S., Garg, A., Amin, K. S. and Sultana, Ali, A. (2007) Release kinetics of modified pharmaceutical dosage forms: a review. Continental J. Pharm. Sciences. 1:30-35.

Korsemeryer, R. W., Gurny, R., Doelker, E. M., Buri, P. and Peppas, N. A. (1983). Mechanism of solute release from porous hydrophilic polymers. Int. J. Pharm., 15: 25-35.

Loyd, V. A., Nicholas, G. P. and Howard C. A. (2007). Tablets: In Ansel's Pharmaceutical Dosage forms and Drug Delivery System. Wolter Kluwer and Lippincott Williams & Wilkings. Philadelphia. p. 227-259

National Committee for Clinical Laboratory Standards (2000). Standard methods for dilution, antimicrobial susceptibility tests for bacteria that grow aerobically. 5th ed., Approved Standard, Document M7-A5 NCCLS, Villannova, Pa.

Ofoefule, S. I. and Ike-Unor, U. O. (2001). Invitro evaluation of bioadhesive polymers and tablet delivery-systems for the administration of dequialinium chloride to the vaginal epithelium. J. Phytomedicine and Therapeutics, 6 (2): 98-105.

Sitruk – Ware, R. (2005). Vaginal delivery of contraceptives. Expert Opinion Drug Delivery. 2(4): 729-736.

Tilton, R. C. and Howard, B. J. (1987). Antimicrobial susceptibility testing. In: B. J. Howard (eds). Clinical and Pathogenic Microbiology. C. V. Mosby, Missouri, USA.

Woolfson, A., Malcom R. K. and Gallagher R. (2000). Drug delivery by the intravaginal route. Crit Rev. Ther. Drug Carrier System 17(5): 509-55.