

Original Research Article**EFFECT OF VARIOUS PRESERVATIVES ON PECTIN BASE OFLOXACIN****VAGINAL GEL****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.

Key Words: preservatives, microorganism, gel, vaginal gel and susceptibility

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 (Hardeep et al.,2013)

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. (Kumar and Verma , 2010)

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. (Patil , 2011)

Gels often provide a faster release of drug substance, independent of the water solubility of the drug, as compared to creams and ointments (Abdel-Hamid *et al.*, 2006). They are highly biocompatible with a lower risk of inflammation or adverse reactions, easily applied and do not need to be removed (Chang *et al.*,2002).

50

51 Ofloxacin is a synthetic broad spectrum antimicrobial agent for oral and intravenous
52 administration, with an in vitro activity against a broad spectrum of Gram positive and Gram
53 negative aerobic and anaerobic bacteria. It exerts a bactericidal effect on susceptible micro
54 rganisms by inhibiting DNA gyrase, an essential enzyme that is a critical catalyst in the
55 duplication, transcription and repair of the bacterial DNA (Physicians's Desk Reference)

56

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

59 MATERIALS AND METHODS

60 MATERIALS

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

65 Microorganisms

66 Staphylococcus aureus (clinical isolate) Escherichia coli (chemical isolate),

67 METHODS

68 Preparation of Ofloxacin Vaginal Gel:

69 Formula:

70	Ofloxacin	1%
71	Pectin	8.5%
72	Glycerol	7%
73	Preservatives	0.2%
74	Purified water	100%

The above formula was used in the preparation of ofloxacin vaginal gel. Ofloxacin powder (1g) was mixed with 8.5g of pectin and 0.2g of the preservative in a mortar. Glycerol 5.6g was placed in a 250ml 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 preservations.

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 (1.261g/cm^3) and its mass (7g).

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.

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 showed 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 (5g) and beef extract (3g) and dissolve theme in appropriate volume of distilled water (1000ml). 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 15mg of agar into 100ml 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. While in a molten form, the nutrient agar was poured out in equal volumes in different petri – dishes, nearer a flame. It was shaken well to evenly distribute and the plates were covered. It was allowed to stand in horizontal position until the agar solidified.
3. The agar plates were aseptically inoculated with a loopful (0.1ml) 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 37⁰C 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.

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 (0.1mg/ml) (but 1mg/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, 2ml for each.
3. A two-fold serial dilution was carried out aseptically by the antibiotic substance by transferring 2ml 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 2ml 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 0.1ml of the test culture and the tubes were incubated at 37⁰C 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.

Determination of the Minimum Bactericidal Concentration (MBC)

1. The MIC tubes without growth were selected and incubated again at 37⁰C 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).

149 **N/B:** The MIC and MBC were carried out using the Staphlococcus aureus and Escherichia
150 coli.

151

152 **RESULTS AND DISCUSSION**

153 **Gel Properties:**

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

162 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

163

164 Antibiotic Sensitivity

165 Antibiotic sensitivity is a term used to describe the susceptibility of bacterial to antibiotics.

166 Antibiotic sensitivity test is a laboratory method for determining the susceptibility of

167 organism to therapy with antibiotics and the concentration at which the antibiotics can be

168 administered for therapy. Hence the five batches of the pectin-based ofloxacin vaginal gel

169 were tested at different concentrations and the results obtained are shown below in tables 2 to

170 5.

171 Table 2: Zone of Inhibition after 24 hours of incubation with S. aureus.

		Inhibition Zone Diameter (mm)				
Drug	Concentration	Control	Methyl	Propyl	Methyl	Benzoic Acid
mcg/ml			Paraben	Paraben	Praben:Propyl Paraben (1:10)	
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

172

173

174 Table 3: Zone of Inhibition after 48 hours of incubation with S. aureus.

Drug Concentration mcg/ml	Inhibition Zone Diameter (mm)				
	Control	Methyl Paraben	Propyl Paraben	Methyl Praben: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	23mm	28mm	30mm	27mm	29mm

0.078125					
Tube 8	20mm	28mm	26mm	25mm	26mm
0.0390625					

175

176 Table 4: Zone of Inhibition after 24 hours of incubation with E. coli.

Drug Concentration mcg/ml	Inhibition Zone Diameter (mm)				
	Control	Methyl Paraben	Propyl Paraben	Methyl Praben: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	27mm	22mm	22mm	22mm	24mm

0.0390625					
-----------	--	--	--	--	--

177

178 Table 5: Zone of Inhibition after 48 hours of incubation with E. coli.

Drug Concentration mcg/ml	Inhibition Zone Diameter (mm)				
	Control	Methyl Paraben	Propyl Paraben	Methyl Praben:Propyl Paraben (1:10)	Benzoic Acid
Tube 1 5	42mm	37mm	38mm	38mm	39mm
Tube 2 2.5	38mm	33mm	34mm	35mm	37mm
Tube 3 1.25	35mm	30mm	31mm	32mm	35mm
Tube 4 0.625	32mm	27mm	28mm	29mm	32mm
Tube 5 0.3125	30mm	25mm	26mm	26mm	30mm
Tube 6 0.15625	28mm	23mm	24mm	24mm	28mm
Tube 7 0.078125	27mm	22mm	24mm	23mm	27mm
Tube 8 0.0390625	26mm	21mm	22mm	21mm	24mm

179

180

181

182 The zone of clearing varies depending upon its concentrations and its chemical properties.

183 Standard zone of inhibition have been established for each antibiotic. If the zone of inhibition

184 is greater than the standard, the organism is considered to be sensitive to the antibiotic. If the

185 zone of inhibition is less than the standard, the organism is considered to be intermediate or

186 resistant. The zone interpretation using the Kirby-Bauer method are recommended:

187 Resistant = up to 12mm

188 Intermediate = 13-19mm

189 Susceptible = 20mm or more (Grimm et al., 1986).

190 From the result above, it was observed that at some concentrations the zone of inhibition

191 increased after 48 hours of incubation as compared to that of 24 hours, this may be due to the

192 extent of diffusion. Also at some concentrations the zone of inhibition reduces after 48 hours

193 of incubation compared to that of 24 hours incubation.

194 The control showed a greater zone of inhibition compared to those with preservatives. This

195 could be due to antagonistic effect between the antibiotics and the preservative.

196 **Antibiotic Susceptibility Testing**

197 The basic quantitative measures of the in vitro activity of antibiotics are Minimum inhibitory

198 concentration (MIC) and minimum bactericidal concentration (MBC). Antibiotics are

199 categorized as bacteriostatic if they reversibly inhibit the growth of bacteria or bactericidal if

200 they kill the susceptible bacteria. In general, the use of bactericidal antibiotic is preferred but

201 many factors may dictate the use of a bacteriostatic antibiotic. When a bacteriostatic

202 antibiotic is used the duration of the therapy must be sufficient to allow cellular and humoral

203 defence mechanism to eradicate the bacteria.

204 For an antibiotic to be effective the MIC or MBC must be able to be achieved at the site of

205 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.

Bacteriostatic Activity

Bacteriostatic activity stops the organism from multiplying but does not kill it. All antimicrobial are bacteriostatic at some concentrations (example Fluoroquinolones) while 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:

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)

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

227 concentrated or in situations where high dosage of drug can be used. This category also
 228 provide a buffer zone which prevents small uncontrolled technical factors from causing major
 229 discrepancies in interpretation. A report of “R” indicates that the pathogen is not likely to be
 230 inhibited if the antimicrobial compound in the blood reaches the concentrations usually
 231 achievable; other therapy should be selected.

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

238

239 Table 7: MIC with S. 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	+	+	+	+	+

240

241 Table 8: MIC with E. 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	-	-	-	-	-
Tube 4 6.25	-	-	-	-	-
Tube 5 3.125	-	-	-	-	-
Tube 6	+	-	-	-	-

1.5625					
Tube 7 0.78125	+	+	+	+	+
Tube 8 0.39063	+	+	+	+	+
Tube 9 0.19532	+	+	+	+	+

242

243 Table 9: MIC for control using S. 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)	+

244

245

246

247 Table 10: MIC for control using E. coli.

248

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)	+

249

250 Conclusion

251 From the result above ofloxacin powder shows MIC at 0.78125mcg/ml with S. aureus and
 252 1.5625mcg/ml with E. coli whereas the control has MBC at 7.825mcg/ml with S. aureus and
 253 15.625mcg/ml with E. coli. While gel with methyl paraben showed 1.5625mcg/ml with S.
 254 aureus and 3.125mcg/ml with E. coli, gel with propyl paraben showed 1.5625mcg/ml with S.
 255 aureus and 3.125mcg/ml with E. coli, gel with methyl paraben:propyl paraben (1:10) showed
 256 0.78125mcg/ml with S. aureus and 1.5625mcg/ml with E. coli, gel with benzoic acid showed
 257 0.78125mcg/ml with S. aureus and 1.5625mcg/ml with E. coli.

258 This result shows that ofloxacin powder has same MIC values with gel with methyl
 259 paraben:propyl paraben (1:10) and gel with benzoic acid for S. aureus. Also ofloxacin has
 260 the same MIC values with gel with propyl paraben, gel with methyl pparaben: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.

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 37⁰C. At the end of incubation the tube

REFERENCE

Abdel-Hamid,S.M.,Abdel-Hady,S.E.,El-Shamy,A.A.andEl-Dessouky,H.F.,Formulation of an antispasmodic drug as a topical local anesthetic, Int. J. Pharm.,2006 : 326,107.

Chang, J.Y.; Oh, Y.K.; Choi, H.G.; Kim, Y.B.; Kim, C.K.: Development of mucoadhesive and thermosensitive gel containing clotrimazole for prolonged antifungal activity, Int. J. Pharm. 2002;241: 155-163.

Chatterjee A, Bhowmik BB and Thakur YS; Formulation, *in vitro* and *in vivo* pharmacokinetics of anti HIV vaginal bioadhesive gel, Journal of Young Pharmacists, 3(2), 2011, 83-89

Hardeep Singh Dhaliwal*, Dhruba Sankar Goswami, Nidhi uppal, Mona Seth, Swati Kashyap, Kapil Sharma, Preparation and characterization of bioadhesive vaginal gel of Propranolol hydrochloride. *Indian J.of Res.Pharm. Biotech.* 2013; 1(6): 869 – 874

296 Kumar L, Verma R, Advantages of intra-vaginal drug delivery system: an overview, International
297 Journal of Pharmaceutical Research and Development, 2(6), 2010, 15-23.
298
299 Nayak SB, Rout PK, Nayak UK and Bhowmik BB, Development and characterization of bioadhesive
300 gel of microencapsulated metronidazole for vaginal use, Iranian Journal of Pharmacy Research, 9(3),
301 2010, 209-219.
302
303 Patil SA, Rane BR, Bakliwal SR and Pawar SP, Pragmatic hydrogels, International Journal of
304 Research in Ayurveda and Pharmacy, 2(3), 2011 758-766.
305
306 Physicians's Desk Reference, 51th Edition, Medical Economics Company, Montvale, NJ,
307 USA, 1997, p.1577 -1582.
308
309