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Formulation and Evaluation of Ciprofloxacin Hydrochloride Vaginal Pessaries

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ABSTRACT

Ciprofloxacin hydrochloride pessaries were formulated and evaluated for weight uniformity, bioadhesion on pig rectum, drainage through pig rectum and dissolution profile through an artificial membrane as well as determination of their melting points range. The ciprofloxacin pessaries were torpedo shaped with no visible cracks or depressions. The coefficient of weight variations ranged from 0.006 to 0.183%. Pessaries that contained 0.2% Primogel®, 0.01% Ac-Di-Sol® and 0.1% Sterotex® had percentage erosion of 29.73% ± 15.3, 36.46 ± 8.1 and 35.75 ± 1.8% respectively, while those that contained no additive, 0.1% Primogel® 0.2% Sterotex®, 0.01% Carbopol 971 and the pessary containing a mixture of PEG4000 and PEG8000 (ratio 1:1) had percentage erosions of 22.15 ± 3.60, 22.92 ± 7.30, 22.15 ± 3.60, 21.00 ± 3.90 and 18.51 ± 3.40% respectively. Results of bioadhesion of the pessaries to pig rectum showed 100% bioadhesion except the control batch which had bioadhesion of 66.7%. Pessaries containing no additive exhibited the highest drainage (66.9%) and the least percentage of pessary retained on the pig rectum, while the pessaries containing 0.01% Ac-Di-Sol® exhibited the least drainage (11.5%) and the highest percentage of the pessary (88.5%) retained on the pig rectum. All the pessaries melted within the temperature range of 38 to 420C. Fastest release through an artificial membrane occurred from pessaries containing 0.2%. Primogel® and slowest from pessaries that contained no additive. The mechanisms and kinetics of drug release were also evaluated. Results indicated that formulations containing 80% PEG 4000, 1% Primogel® as well as 0.1% Sterotex followed zero order kinetics via non-Fickian mechanism. Formulations containing 0.01% Ac-Di-Sol® followed Higuchi and zero order kinetics (r2 = 0.978, 0.9961) with diffusion controlled mechanism (n = 0.50). The formulation containing 0.1% Carbopol 971® exhibited, Higuchi kinetics with Fickian diffusion mechanism (n = 0.52). (r2 = 0.9032) .

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 action. 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, 2005). 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 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 also been found to be active against Gardnerella vaginalis (Chein, 2007).

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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 opened 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 ciprofloxacin hydrochloride vaginal pessaries, for the treatment of vaginal infections caused by susceptible micro-organisms were undertaken in this study.

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 4000 was procured from Carl Roth GMBH, Karlsruhe Germany, polyethylene glycol 8000 (Sigma-Aldrich, Germany), Ac-Di-Sol® (FMC, Philadelphia), Sterotex® (Abitech, USA) Primojel® (DMV, International. Vieghel, the Netherlands). Carbopol 971 (BF, Goodrich Co. USA), cellulose membrane, M. W. Cut-off 6000-8000 (Spectrum Labs; Breda, The Netherlands).

Preparation of Ciprofloxacin Pessaries

The compositions of the different formulations of ciprofloxacin pessaries are shown in Table 1. Ciprofloxacin pessaries containing 0.2g of ciprofloxacin, polyethylene glycol 4000, 1.6g and distilled water 0.2g were prepared by the fusion method (Carter, 2008), using a metal mould with twelve cavities each with a capacity of 2g. Polyethylene glycol 4000 was melted over a water bath at a temperature range of 60-63oC. On cooling to 400C a solution of 0.2g Ciprofloxacin hydrochloride in distilled water was added to the base and stirred slowly to avoid the incorporation of air bubbles. The mixture was poured into the mould and refrigerated for three hours. Batches of pessaries containing varying concentrations of Primojel®, Ac-Di-Sol®, Sterotex®, Cabopol 971® as excipients and Polyethylene glycol 8000, PEG 4000 were similarly prepared.

Evaluation of Ciprofloxacin Pessaries Weight Variation

Eight pessaries were selected at random and weighed individually on an electronic scale (OHAUS, Galaxy). The average weight of the pessaries was determined, and the weight of individual pessaries was compared with the average weight

Figure 1: Photograph showing Ciprofloxacin hydrochloride pessaries

Adhesion/Erosion Experiment

The apparatus for this experiment is shown in Fig 2. It is made up of a separating funnel clamped on a retort stand with a metal support used to position a plastic support at an angle of 250 (Ofoefule et. al., 2001). Freshly isolated and excised Pig rectum (11.5cm by 7.5 cm) was pinned on the plastic support. A collecting dish was placed directly under the plastic support to collect detached pessaries. The Pig intestine was flushed with 50 mL of normal saline and the experiment was set at 10 drops of the phosphate buffer (pH 4) per minute.The pessary was allowed to equilibrate on the intestine for 5 minutes,

and the duration of the experiment was 60 minutes. The procedure was repeated for all the pessary batches.The percent bioadhesion and erosion were calculated for each of the batches, from Equation 1and 2 respectively.

Percentage bioadhesion=

$$\frac{\text{No of pessary after bioadhesion experiment}}{\text{Initial number of tablets}} \times 100 \text{--Eqn(1)}$$

$$\text{Percentage erosion} = \frac{\text{Initial weight} - \text{final weight}}{\text{Initial weight}} \times 100 \text{ ---------. Eqn(2)}$$

Dissolution Profiles of Ciprofloxacin Pessaries

Dissolution studies of the pessaries were conducted with Erweka dissolution apparatus using USP method. The dissolution medium was 500 mL of phosphate buffer (pH 4) maintained at a temperature of 36± 0.5oC. The agitation rate of the basket was 50 rpm. In each of the dissolution profile study a pessary was wrapped in an artificial cellulose membrane as shown in Figure, 3.

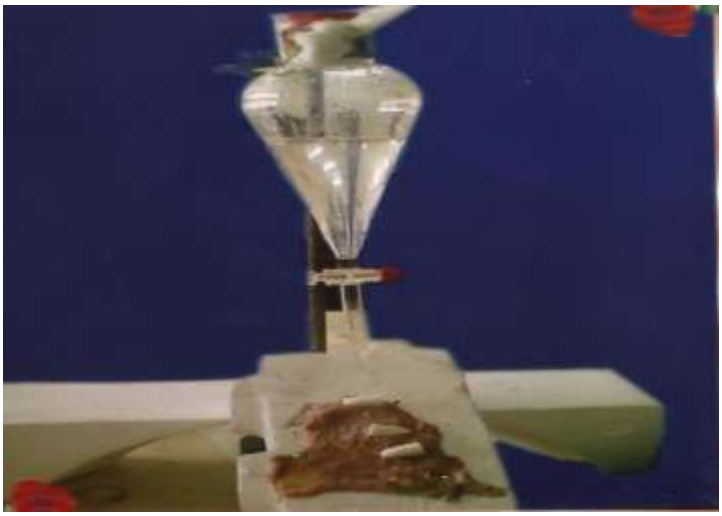

Figure 2: Photograph showing the Erosion/Bloadhesion test for Ciprofloxacin Pessary

Ten millilitres (10 mL) samples were withdrawn up to 1hr 25min.For each 10 mL withdrawn, 10 mL of phosphate buffer maintained at the same temperature was added to the dissolution medium. The drug content was determined spectrophotometrically at 278 nm using Unico Spectrophotometer model UV 2100 PC (Shanghai instrument Co. Ltd., China) . Absorbances of withdrawn samples were converted to concentration from the Beer's calibration curve of ciprofloxacin hydrochloride.

Drainage Experiment

The method of (Onyechi et al.,2009) was adopted, with slight modifications. A metallic stand (Figure 4) was constructed which allowed the Pig intestine to be mounted and maintained vertically in the stand. The intestine was attached to the metal support with cotton thread which was used to prevent the tissue from collapsing. The stand was suspended vertically in a high humidity environment (Figure 4) and allowed to equilibrate in a hot air oven at a temperature of 39oC. The pessary under test was weighed and inserted at the upper end of the mounted Pig's intestine and supported by office pins to prevent it from falling off. A Petri dish of known weight was placed beneath the tissue assembly to collect the molten and detached pessary mass. The whole set up was placed in a glass jar containing 300 mL of normal saline and left in the oven for an average time interval of 680min.

After complete drainage the Petri dish and its content were dried and weighed. The percentage of pessary drained out was calculated using the formula below:

% Pessary drained out = (Wi-Wf/Wi) 100 --- Eqn (3)

Where Wi is initial weight of pessary, Wf is the final weight of pessary

Percentage of pessary retained =
(initial weight of pessary– weight of pessary drained) 100
Initial weight of pessary
----Eqn (4)

Melting Point Determination

The various Batches of pessaries (1-8) were placed in capllary tubes with dimensions 1 cm diameter and 11 cm in length fabricated in the glass blowing section of the Chemistry Department, University of Uyo, Uyo. The capillary tubes containing the different batches of pessaries were placed inside a thermostated water bath (Grant, England). The melting point was taken as the temperature range at which the pessary just sintered and passed into solution.

RESULTS AND DISCUSSION

Physical Properties of Ciprofloxacin Hydrochloride Pessary

The average weight of the pessaries was 2.32g and the weight variation for every batch was less than ± 7.5%.

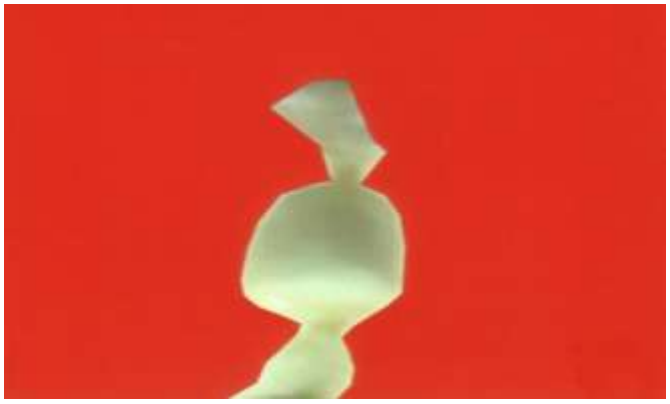

Figure 3: Photograph showing the dissolution test for Ciprofloxacin Hydrochloride Pessary

Figure 4: Photograph showing the drainage test for Ciprofloxacin Pessary

Erosion/Bioadhesion Test of Ciprofloxacin Pessaries

The result of the erosion/bioadhesion test of ciprofloxacin hydrochloride pessaries is shown in Table 4. From the result, batch 4 exhibited the highest percentage erosion. This was followed closely by batch 5. Batch 8 showed the least percentage erosion. All the batches except batch I exhibited percentage bioadhesion of 100%.

The high value of percentage erosion for the pessary containing Ac-Di-Sol® could be due to the super disintegrant property of this compound, resulting from its good water uptake, rapid swelling properties and efficient fluid channeling on account of its long fiber length (Ofoefule, 2002). The least value of percentage erosion exhibited by Batch 8 pessaries could be attributed to the non-inclusion of other excipient that could have resulted in alteration in the hardness and dissolution time of the pessary which would affect the rate of erosion.

Table 1: Composition of Ciprofloxacin Hydrochloride Pessary

Ingredient (%)	B_1	B_2	B_3	B_4	B_5	B_6	B_7	B_8
Ciprofloxacin Hydrochloride	10	10	10	10	10	10	10	10
Polyethylene glycol 4000	80	80	80	80	80	80	80	80
Primogel®		0.1	0.2					
Ac-Di-Sol®				0.01				
Sterotex®					0.1	0.2		
Carbopol®							0.01	
Polyethlene glycol 8000								40

Key: Batch 1 = 80% PEG 4000. Batch 2 = 80% PEG 4000 + 0.1% Primogel®. Batch 3 = 80% PEG 4000 + 0.2% Primogel®
Batch 4 = 80% PEG 4000 + 0.01% Ac-Di-Sol®. Batch 5 = 80% PEG 4000 + 0.1% Sterotex®. Batch 6 = 80% PEG 4000 + 0.2% Sterotex®
Batch 7 = 80% PEG 4000 + 0.01% Carbopol 971®. Batch 8 = 40% PEG 4000 + 40% PEG 8000

Table 2: Physical properties of Ciprofloxacin Hydrochloride Pessary

Parameter	**Batches**							
Mean + SD	1	2	3	4	5	6	7	8
Weight Variation (g)	2.34± 0.18318	2.42± 0.17246	2.21± 0.12599	2.50± 0.0061354	2.26± 0.10729	2.09± 0.21001	2.51± 0.05656	2.25± 0.16539
Melting Point Range (^oC)	38-40	38-42	38-42	38-42	38-40	38-40	38-40	38-40

Key: Batch 1 = 80% PEG 4000. Batch 2 = 80% PEG 4000 + 0.1% Primogel®. Batch 3 = 80% PEG 4000 + 0.2% Primogel®
Batch 4 = 80% PEG 4000 + 0.01% Ac-Di-Sol®. Batch 5 = 80% PEG 4000 + 0.1% Sterotex®. Batch 6 = 80% PEG 4000 + 0.2% Sterotex®
Batch 7 = 80% PEG 4000 + 0.01% Carbopol 971®. Batch 8 = 40% PEG 4000 + 40% PEG 8000

Table 3: Result of Drainage Experiment with Ciprofloxacin Hydrochloride Pessary

Batch	**Mean % Drainage**	**Mean % Retained**	**Mean drainage Time (min)**
1	66.9	33.1 ± 3.81	680
2	25.2	74.8 ± 14.6	680
3	55.0	45.0 ± 22.7	680
4	11.5	88.5 ± 6.65	680
5	32.7	67.3 ± 4.48	680
6	19.8	80.3 ± 20.8	680
7	19.4	80.6 ± 12.6	680
8	26.4	73.6 ± 18.4	680

Key: Batch 1 = 80% PEG 4000 Batch 2 = PEG 4000 + 0.1 Primogel®. Batch 3 = 80% PEG 4000 + 0.2% Primogel®
Batch 4 = 80% PEG 4000 + 0.01% Ac-Di-Sol®. Batch 5 = 80% PEG 4000 + 0.1% Sterotex®. Batch 6 = 80% PEG 4000 + 0.2% Sterotex®
Batch 7 = 80% PEG 4000 + 0.01% Carbopol 971® . Batch 8 = 40% PEG 4000 + 40% PEG 8000

Table 4: Erosion/Bioadhesion Test of Ciprofloxacin Pessaries.

Batch	**Erosion (%) Mean ± SD**	**Bioadhesion (%)**
1	22.15 ± 3.6	66.7
2	22.92 ± 7.3	100
3	29.73 ± 15.3	100
4	36.46 ± 8.1	100
5	35.75 ± 1.8	100
6	22.15 ± 3.6	100
7	21.00 ± 3.9	100
8	18.51 ± 3.4	100

Key: Batch 1 = 80% PEG 4000. Batch 2 = 80% PEG 4000 + 0.1% Primogel®. Batch 3 = 80% PEG 4000 + 0.2% Primogel®
Batch 4 = 80% PEG 4000 + 0.01% Ac-Di-Sol®. Batch 5 = 80% PEG 4000 + 0.1% Sterotex®. Batch 6 = 80% PEG 4000 + 0.2% Sterotex®
Batch 7 = 80% PEG 4000 + 0.01% Carbopol 971®. Batch 8 = 40% PEG 4000 + 40% PEG

Drainage Experiment with Ciprofloxacin Hydrochloride Pessaries

The result of the drainage experiment is shown in Table 3. From this result, the pessary containing 0.01% Ac-Di-Sol® (Batch 4) had the least percentage of pessary drained and the highest percentage of pessary retained on the tissue. This was followed by batch 7 which contained 0.01% Carbopol 971® with values of 19.4% as mean percentage drainage and 80.6% as mean percentage retained. Batch 1 containing 0.1% Primogel® exhibited the least percentage retained and the highest mean percentage drainage. According to (Onyechi et al ., 2009) drainage technique is used in bioadhesion studies as an aid to develop sustained release bioadhesive formulation. Ac-Di-Sol® is a super disintegrant, and possesses outstanding water wicking capabilities and its cross-linked chemical structure creates an insoluble hydrophilic absorbent material which results in good swelling properties (Gissinger et al.,1980). Its super disintegrant properties was responsible for the spreading of the pessary on the tissue thus resulting in very low percentage drainage. Carbopols usually exhibit good bioadhesive properties largely because they contain carboxy groups which ensure good hydration and strong interaction, which may require greater force to detach the polymer film from a mucus surface. This property provides an explanation for the increased percent retention of pessary containing Carbopol 971 on the tissues (Ofoefule et al., 2001).

Melting Point Determination

The result of the melting point determination is show on Table2. From the result, the melting point of all the batches of pessaries fell within the range 38 to 42oC respectively. This makes room for easy handling since the melting point range is above room temperature. And since PEGs pessaries are usually formulated to dissolve rather than melt at body temperature, this provides more prolonged release than other bases like theobroma oil (Kellaway et al., 1975).

Some Release Parameter of Ciprofloxacin Pessaries

The release rate of ciprofloxacin hydrochloride from the pessaries was analysed on the basis of time taken for 50% and 70% of the drug to be released (T50 and T70 as shown on Table 5. Batch 1 exhibited the slowest rate of release this could possibly result from the hydrophobic nature of polyethylene 4000 since there was no additive, which could have retarded the rate of release of ciprofloxacin hydrochloride from the pessary. Batch 3 exhibited the fastest release rate, releasing 50% and 70% of the drug at 6.6 and 9.5 minutes respectively. This is because, this batch contains primojel which is a super disintegrant and thus enhanced fast disintegration and hence fast dissolution of the pessary, thereby enhancing the fast release of the drug. (Gissinger,et al., 1980).

Kinetics and Mechanism of Release for Ciprofloxacin Hydrochloride Pessary

The data obtained were fitted into different kinetic models viz: zero order, first order Higuchi and Korsemeyer Equations. From Table 6 , all the batches exhibited mixed order release kinetics with zero order, first order and Higuchi kinetics operating at varying degrees. Higuchi kinetics describes the release of drugs from an insoluble matrix as a square of time. The zero order rates describe systems where rate of drug release is independent of concentration. In order to confirm the exact mechanism of drug release from these pessaries the data obtained were fitted into Korsemeyer Equation (Kalam et al., 2007). The n values from the Korsemeyer model indicate Fickian diffusion mechanism for batches 4-7 and anomalous release mechanism for all batches as shown in Table 6. Batches 4-7 contained swelling polymers (Ac-Di-Sol and Carbopol 971) which when in contact with fluid swell, creating a favourable environment for diffusion of dissolved drug out of the pessary matrix. This accounted for the Fickian diffusion exhibited by batches 4-7

Table 5: Some Release parameters of Ciprofloxacin Pessaries

Batch	T_{50} (Min)	T_{70} (Min)	C_{nax} (%)
1	27	45	100
2	11.2	13	100
3	6.6	9.5	100
4	11.5	20	100
5	7.2	95	100
6	8.0	20	100
7	9.5	22.5	100
8	9.5	19.5	100

Key: Batch 1 = 80% PEG 4000. Batch 2 = 80% PEG 4000 + 0.1% Primogel®. Batch 3 = 80% PEG 4000 + 0.2% Primogel®
Batch 4 = PEG 4000 + 0.01% Ac-Di-Sol®. Batch 5 = 80% PEG 4000 + 0.1% Sterotex®. Batch 6 = 80% PEG 4000 + 0.2% Sterotex®
Batch 7 = 80% PEG 4000 + 0.1% Carbopol 971®. Batch 8 = 40% PEG 4000 + 40% PEG 8000

Table 6: Kinetics and mechanism of Release for Ciprofloxacin Hydrochloride Pessaries

Batch	Zero-order	First-order	Higuchi	Korsmeyer	n
1	0.9889	0.7928	0.9900	0.9946	0.65
2	0.9276	0.8903	0.9127	0.921	0.98
3	0.9226	0.921	0.9657	0.9576	0.74
4	0.9961	0.7775	0.9878	0.9825	0.50
5	0.7625	0.7292	0.775	0.7849	0.58
6	0.9612	0.6877	0.9576	0.9599	0.61
7	0.9097	0.646	0.9098	0.9219	0.52
8	0.9730	0.8425	0.9796	0.9757	0.64

Key: Batch 1 = 80% PEG 4000. Batch 2 = 80% PEG 4000 + 0.1% Primogel® **.** Batch 3 = 80% PEG 4000 + 0.2% Primogel® Batch 4 = PEG 4000 + 0.01% Ac-Di-Sol®. Batch 5 = 80% PEG 4000 + 0.1% Sterotex®. Batch 6 = 80% PEG 4000 + 0.2% Sterotex® Batch 7 = 80% PEG 4000 + 0.1% Carbopol 971®. Batch 8 = 40% PEG 4000 + 40% PEG 8000

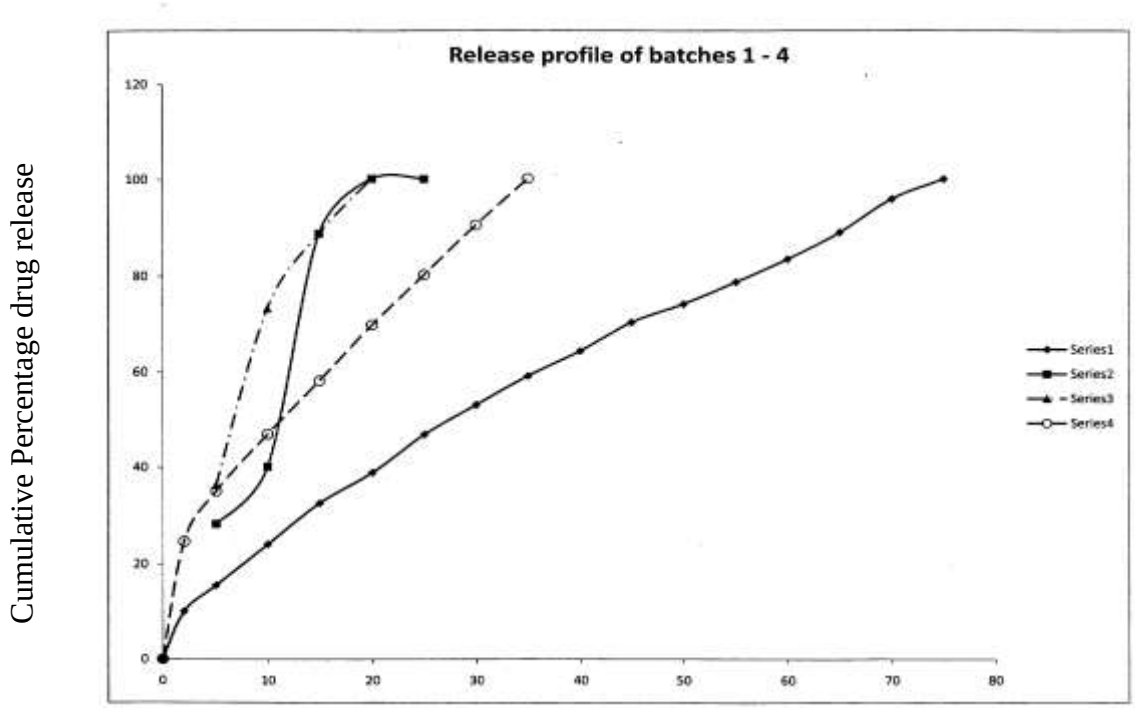

Figure 5: Release profile of Ciprofloxacin Hydrochloride Pessaries in Phosphate buffer (pH 4) (Batches 1-4)
♦ **Batch 1** ■ **Batch 2** ▲**Batch 3** ○**Batch 4**

CONCLUSION

The physical properties of ciprofloxacin pessaries were within the acceptable limits. All batches of the pessaries except Batch1 exhibited 100% bioadhesion to the Pig's rectum. Batch 4 containing 0.01% AC-Di-Sol showed the highest percent erosion, followed by Batch 6 containing 0.01% Sterotex while Batch 8 containing 40% Carbopol 971 exhibited the least percentage erosion. Batch 4 exhibited the least percentage drainage, with the highest percentage of pessary retained on the tissue, while Batch1 had the highest value of percentage drainage and hence the lowest value for pessary retained on the tissue. The melting point range for all the batches of pessaries lied between 38-42^{o}C

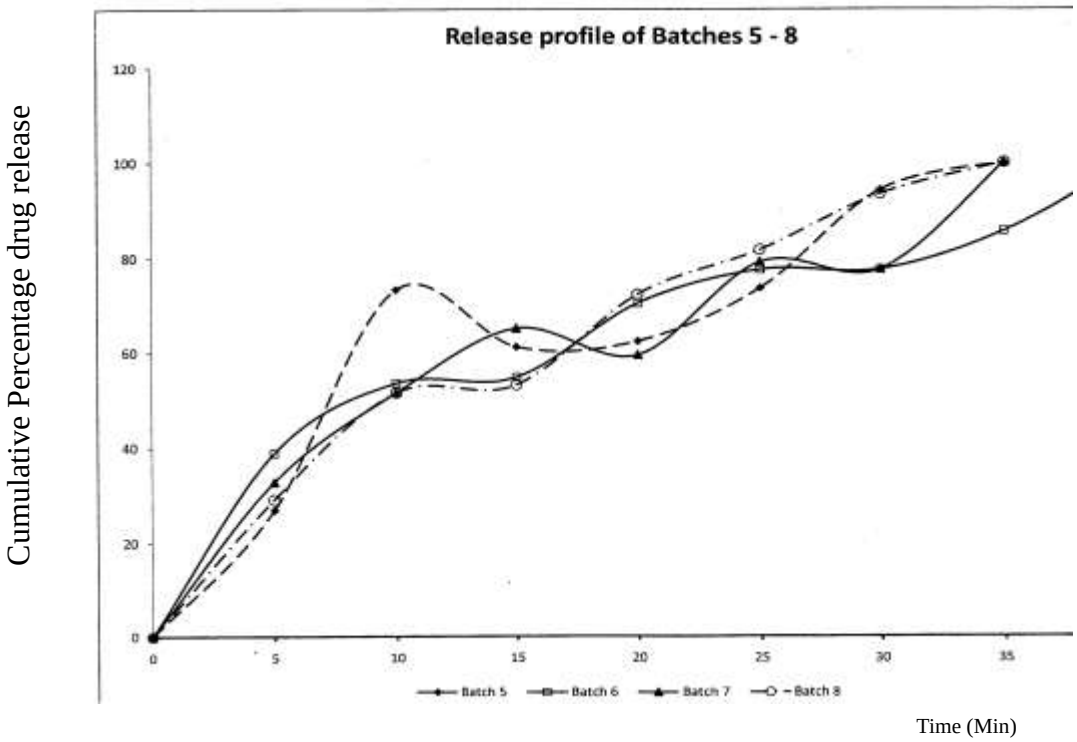

Figure 6: Release profile of Ciprofloxacin Hydrochloride Pessaries in Phosphate buffer (pH 4) (Batches 5-8)

Batch 1 containing no additive showed the fastest rate of release at t50 and t70 while Batch 3 containing 0.2% Primojel exhibited the slowest release rate at the same time interval.

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