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IN-VITRO AND IN VIVO EVALUATION OF CERTAIN NON STEROIDAL ANTI-INFLAMMATORY DRUGS IN OPHTHALMIC HYDROGEL FORMS

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ABSTRACT

Monophenylbutazone and flufenamic acid are two non steroidal anti-infalmmatory drugs. They were formulated in carbopol 940 hydrogel. The effect of drug concentration and ageing on the in vitro release characteristics was studied. The release profile agreed with the partition controlled mechanism. Increasing drug concentration accompanied by a decrease in the release rate. Ageing affected the release rate to varing degrees depending on the incorporated drug and its concentration. The prepared hydrogels were evaluated for their healing activities on induced corneal

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ulcers on rabbit's eye. The tested preparations brought about a significant healing effect on the induced ulcers . The healing activity of the monophenylbutazone gel preparations was found to be higher than that in case of the flufenamic acid

INTRODUCTION

Ophthalmic drugs are usually administrated in the form of eyedrops; a dosage form consisting of buffered isotonic aqueous solutions or suspensions of the drug. These medications present the inconvenience of a low bioavailability(only 0.5-2.0% of the applied drug penetrates the cornea), and of pulsed drug delivery (The concentration of the drug available for penetration decreases exponentially, as the medication is diluted by the tears and is eliminated from the eye via the lacrimal drainage system [1-4]. The bioavailability of these medications can be enhanced by increasing the vehicle viscosity up to a gel-like consistency. Owing to the unsatisfactory bioavailabilities obtained with the conventional eye drops, viscous liquids and semisolid preparations were utilized as an alternative therapeutic systems [5-7]. Also, it has been suggested by several authors that the use of ophthalmic vehicles based on natural, semisynthetic or synthetic hydrogels may be

advantageous. The use of such vehicles proved to enhance the ocular bioavailability or the therapeutic efficacy of the applied drugs, prolong the drug duration and reduce the patient noncompliance problems[8-12]. Crosslinked ethyleneoxides, hydroxyethylmethacrylate crosslinked with different agents, crosslinked polyvinylalcohol and polyethylene glycols are just few examples of the widely employed hydrogels[13-16]. However,many of the investigated ophthalmic gels have been formulated with either carbopols, polyalkylene oxide copolymers or cellulose derivatives[11,17-19].

Non steroidal anti-inflammatory drugs have been shown to inhibit protein synthesis on ribosomes thus,inhibiting the formation of different enzymes involved in the healing process of corneal epithelium[20]. Monophenbutazone(mofebutazone); an anti-inflammatory drug, was found to inhibit the lipoxygenase and cycloxygenase enzyme systems.Lipoxygenase products have been identified in inflammed ocular tissues and reported to impair the clarity of the optical media of the eye[21].Flufenamic acid is also an anti-inflammatory drug , similar to mefenamic and tolfenamic acids. The latter was established to inhibit the biosynthesis of prostaglandins and thus moderates the inflammatory process. Morover, fenamates exert an inhibitory effect on prostaglandin receptors[22].

The purpose of the work hereby described is therfore , to formulate each of monophenbutazone and flufenamic acid in an ophthalmic hydrogel vehicle, to study the release characteristics of the medicaments from the prepared vehicles and evaluate the ocular healing activities of these preparations. Carbopol 940 was used at 2% concentration to prepare the employed ophthalmic gel.

MATERIALS AND METHODS

Carbopol 940(Goodrich Chem .Co.Cleveland, OHIO), flufenamic acid (The Nile Co.for Pharmaceuticals and Chem. ind, Cairo, Egypt),and monophenbutazone(Kahira Pharm.and Chem.ind.Co.,Cairo, Egypt). All the other chemicals were analytical grade and were used as received.

A total of 12 nature albino type rabbits was used, body weights ranged from 1.9 to 2.3 kg.

1- Gel Preparation: The gel was prepared adopting a previously mentioned procedures[23] as follows: Carbopol 940 was dissolved in a mixture of cold water and propylene glycol(4:1). Sodium hydroxide solution(0.1 N)was added to neutralize the prepared polymers solution.To the formed gel were added:Sodium edetate(3% as stabilizer) and sodium metabisulphite (5% as antioxidant). The gel

was allowed to stand for 24 hours before the inclusion of drugs. Each drug was incorporated, at 0.1, 0.5 and 1.0% concentrations, into the prepared gel. After inclusion of flufenamic acid, the gel lost its consistency. To retain gel consistency sodium hydroxide was added (to adjust the pH value to about 7). The final products were clear and their colour was yellowish in case of flufenamic acid and colourless with monophenylbutazone. The prepared gels were packaged in small collapsible tubes and stored at room temperature.

2- Release Studies: A dialysis method[24] was used as follows: the gel(1 gm)was placed on a semipermeable cellophane membrane. The loaded membrane was streched over an end of an open glass cylinder(14.14 cm^2) crossectional area)and tied firmly with a cotton thread.The dialysing chamber was then suspended into a 250 ml - beaker containing 50 ml of isotonic phosphate buffer(pH 6.8).The system was adjusted so that the membrane might be just below the surface of the dialysis medium. Samples each of 5 ml were withdrawn from the dialysis medium after 0.5,1,2,3,4,5and 6 hours. The 5 ml aliquots were replaced with fresh isotonic buffer immediately after each sample was removed.The amount of drug released in each sample was spectrophotometrically determined at 285 nm and 287 nm for monophenbutazone

and flufenamic acid respectively. The test was done on the gel immediately after preparation and after one and two months of storage.

Kinetic Studies: The release data were analysed according to zero order, first order kinetics and the diffusion-controlled release mechanism(Higuchi's model).

3- Evaluation of the Healing Activity:

Test Animals: The tested rabbits were divided into two treatment groups,each of 6 rabbits. One group was used for monophenbutazone and the other for flufenamic acid gel preparations. For each rabbit, the left eye served as the test while the right one served as the control.

Induction of Corneal Ulcers: Two drops of xylocaine hydrochloride solution(2% solution in freshly boiled and cooled water) were instilled in each eye. One to two minutes post instillation, 4 ulcers were thermally induced on the corneal surface away from the pupil. The ulcers had a circular shape(about 2 mm in diameter) and reached in depth the corneal epithelium. This was confirmed by the corneal staining with fluorescin. Two drops of sterile chloramphenicol solution(0.5%)were immediately instilled after induction of ulcers.

Treatments:

a- Antibiosis: All rabbits, in the two groups, received 2 drops of chloramphenicol solution (0.5%) every morning through out the observation period (4 weeks) in both eyes.

b- Gel preparations: Smear of the medicated gel preparation(0.5%)was applied thrice daily,to the left eye of each rabbit, through out the observation period. Also, smear of the non-medicated gel preparation(placebo)was similarly applied but to the right eye.

Observations: Observations were made daily,by the same investigator,for both control and test treatments. Fluorescin solution(0.2% as the sodium salt)was instilled in the eye and the ulcers aquired green fluorescnce were counted.

Statistical Analysis: The healing activity of the tested preparations in terms of residual ulcers was subject to statistical analysis according to the student t-test[27].

RESULTS AND DISCUSSION

Carbopol 940; synthetic carboxyvinyl polymer crosslinked with allyl sucrose, thickens at relatively low concentrations i.e 0.5 to 5.0%[28]. This hydrophilic polymer was used in this

Table I: Kinetic Assessment of Release Data

Kinetic Model		Calculated Correlation Coefficient Values					
		Monophenylbutazone			Flufenamic Acid		
		0.1%	0.5%	1.0%	0.1%	0.5%	1.0%
Zero Order Q ⟶ t	A	0.9972	0.9787	0.9904	0.9936	0.9986	0.9874
	B	0.9751	0.9838	0.9959	0.9939	0.9953	0.9902
	C	0.9595	0.9873	0.9872	0.9893	0.9783	0.9941
First Order log Q ⟶ t	A	0.9488	0.9413	0.9627	0.9981	0.9949	0.9817
	B	0.9635	0.9749	0.9432	0.9964	0.9964	0.9729
	C	0.9294	0.9442	0.9486	0.9570	0.9570	0.9696
Diffusion Q ⟶ $\sqrt{t}$	A	0.9673	0.9558	0.9682	0.9959	0.9901	0.9549
	B	0.9808	0.9759	0.9596	0.9924	0.9903	0.9887
	C	0.9862	0.9983	0.9962	0.9633	0.9439	0.9717

A: Immediately after preparation
B: After storage for 1 month
C: After storage for 2 months

study at 2% concentration to prepare the hydrogel. The polymer solution was neutralized with sodium hydroxide to increase the consistency and to produce the gel. Sodium edetate was used as a gel stabilizer[29].

Drug release from the gels was studied immediately after preparation and after storage of one and two months. The in vitro release data were kinetically analysed according to zero order, first order and the diffusion-controlled mechanism (Table I). The linear regression analysis of data was

adopted[30]. The low correlation coefficient values obtained in the analysis of log amount released versus time($\log Q \rightarrow t$) excluded the first-order dependency. The high values in the analysis of amount released versus time attest to the zero order kinetic model i.e the drug release profile follows a $Q \longrightarrow t$ linearity. Thus, the release can be described as a parition-controlled process. A result which is in a good agreement with the assumption of Roseman and Higuchi[31] that the rate of diffusion from the surface of a polymeric matrix to the surrounding bulk solution makes a significant contribution to the total diffusional process, and of Haleblian et al.[32] that the rate of solute transfer across the matrix-solution interface may control the release. Hence, it can be concluded that drug solubility in the elution medium, the partition coefficient of the solution-polymer system and the thickness of hydrodynamic diffusion layer are highly significant in determining the magnitude of drug release.Also,from the linear regression analysis of the in vitro release data, a relatively high correlation coefficient values were obtained on applying the $Q \longrightarrow \sqrt{t}$ model. A result which suggest that the diffusion-controlled mechanism may be operative. The release data were plotted according to both $Q \longrightarrow t$ and $Q \longrightarrow \sqrt{t}$ (Figures 1 & 2) relationship. It is obvious that the over-all release profile can be best described as a partition-controlled process. However, the diffusion-controlled or

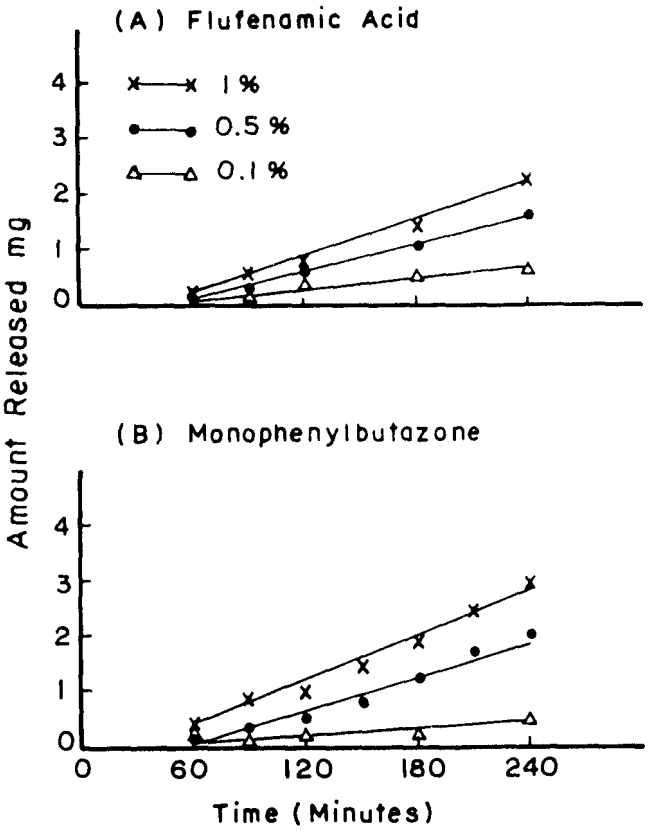

Fig. 1: Release of Flufenamic Acid and Monophenylbutazone From Carbopol Hydrogel.

matrix-controlled mechanism if it is operative, it may be only applicable after an initial release phase. A finding which can not be attributed to the actual release pattern of drug from the matrix but may reflect the use of a diffusion barrier (dialysis membrane).

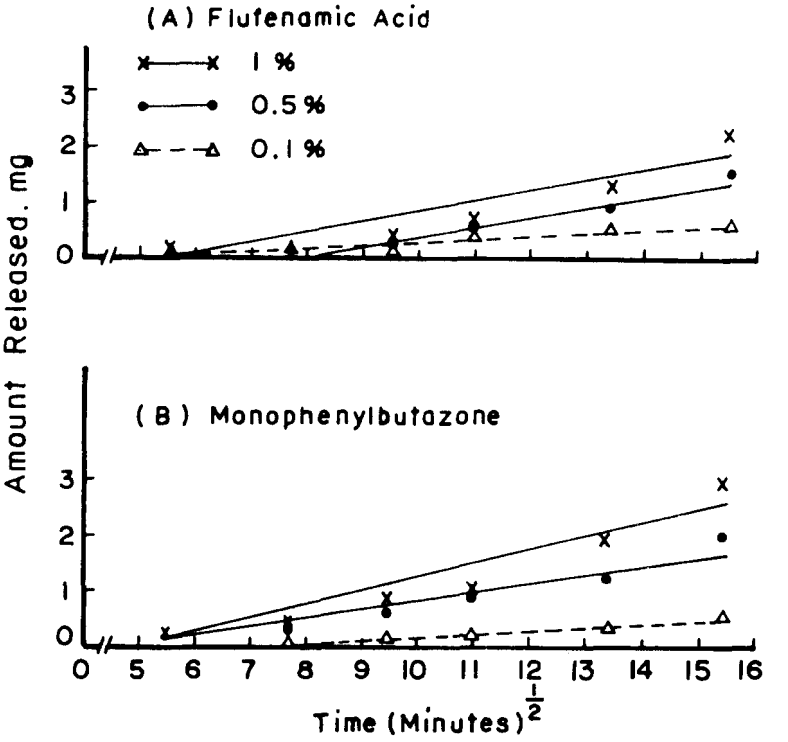

Fig. 2: Release of Flufenamic Acid and Monophenylbutazone From Carbopol Hydrogel.

The drug release rate constant (K)and the drug diffusion coefficient (D) were calculated from the $Q \longrightarrow t$ and $Q \longrightarrow \sqrt{t}$ relationships respectively. The two parameters were calculated for both drugs as function of drug concentration and ageing. The release rate constant (K) was calculated as the slope of the linear relation $Q \longrightarrow t$. The diffusion coefficient (D) was calculated using the equation:

$$D = \left(-\frac{b}{2C_o A}\right)^2 \pi$$

Where

b: Slope of the linear relation $Q \longrightarrow \sqrt{t}$.

A: Area of diffusion (14.14 cm^2).

C_o: Initial drug concentration.

The calculated values appear in Table II. It is obvious that the release rate of drug from the prepared gels decreased by increasing the initial drug concentration. An effect which may be attributed to the limited capacity of the dialysis membrane towards the diffusing drug in addition to the limited movement of the drug molecules within the gel preparation due to the increased concentration. Ageing was found to be accompanied by an increase in the drug release rate. The effect was more pronounced at the 0.1% drug loading and after the second month of storage as well as with the monophenylbutazone preparations. In this respect, 0.5% drug loading gave the most stable release profile. The increased in the release rate with ageing may be attributed to a reduction in the gel consistency.

The anti-inflammatory effect of the gel peparations was assessed by measuring their healing activities on the induced corneal ulcers. The number of residual ulcers (after healing) over an observation period of 4 weeks after control (placebo)and test treatments were counted for each

Table II: Effect of Initial Concentration and Ageing on the Release Pattern of Drug from Carbopol 940 gel.

Initial Drug Concentration (%)		Monophenylbutazone $Kx10^3$, $mg.min^{-1}.mg^{-1}$	Monophenylbutazone $Dx10^7$, $Cm^2.Sec^{-1}$	Flufenamic Acid $Kx10^3$, $mg.min^{-1}.mg^{-1}$	Flufenamic Acid $Dx10^6$, $Cm^2.Sec^{-1}$
0.1	A	3.1	1.3	2.7	3.8
	B	5.3	13.7	4.2	5.4
	C	12.6	19.4	7.9	33.0
0.5	A	1.6	0.3	3.1	1.7
	B	1.6	3.6	2.1	1.4
	C	7.3	4.8	2.2	2.5
1.0	A	1.1	0.1	1.6	0.6
	B	3.1	1.9	1.2	0.7
	C	6.5	4.3	3.0	4.6

A, B, &C : As in Table I

K: Zero order release constant.

D: Diffusion coefficient.

individual rabbit. Statistical analysis of the differences between the control and test treatments was done according to t-test (Table III). It is evident that the treatment involving flufenamic acid brings about a singificant difference after the elapse of two weeks treatment period.How-

Table III-a: Significance of Differences Between Test(Monophenylbutazone) and Control Treatment.

Weeks	Type of Treatment						t		
	Test			Control			Calc.	Tab.	
	Mean No.	S.E.	S^2	Mean No.	S.E.	S^2		P=0.05	P=0.01
1	3.16	0.307	0.472	4.00	0.000	0.000	2.73*	2.228	3.169
2	2.16	0.307	0.472	3.50	0.223	0.250	3.53*		
3	1.50	0.223	0.250	3.16	0.166	0.138	5.97*		
4	0.50	0.223	0.250	3.00	0.000	0.000	11.21*		

Table III-b: Significance of Differences Between Test(Flufenamic Acid) and Control Treatment.

Weeks	Type of Treatment						t		
	Test			Control			Calc.	Tab.	
	Mean No.	S.E.	S^2	Mean No.	S.E.	S^2		P=0.05	P-0.01
1	3.83	0.167	0.138	4.00	0.000	0.000	1.02	2.228	3.169
2	2.50	0.224	0.250	2.50	0.224	0.250	3.16*		
3	1.66	0.211	0.220	3.16	0.167	0.138	5.58*		
4	1.16	0.307	0.472	3.00	0.000	0.000	5.99*		

d.F =10

* = Significant difference.

ever, treatment with monophenylbutazone exhibited a significant difference after the first week of treatment.Figures 3 and 4 depict the comparison of control and test treatment with the hydrogel preparations. The response to the control treatment was very slow. With monophenylbutazone

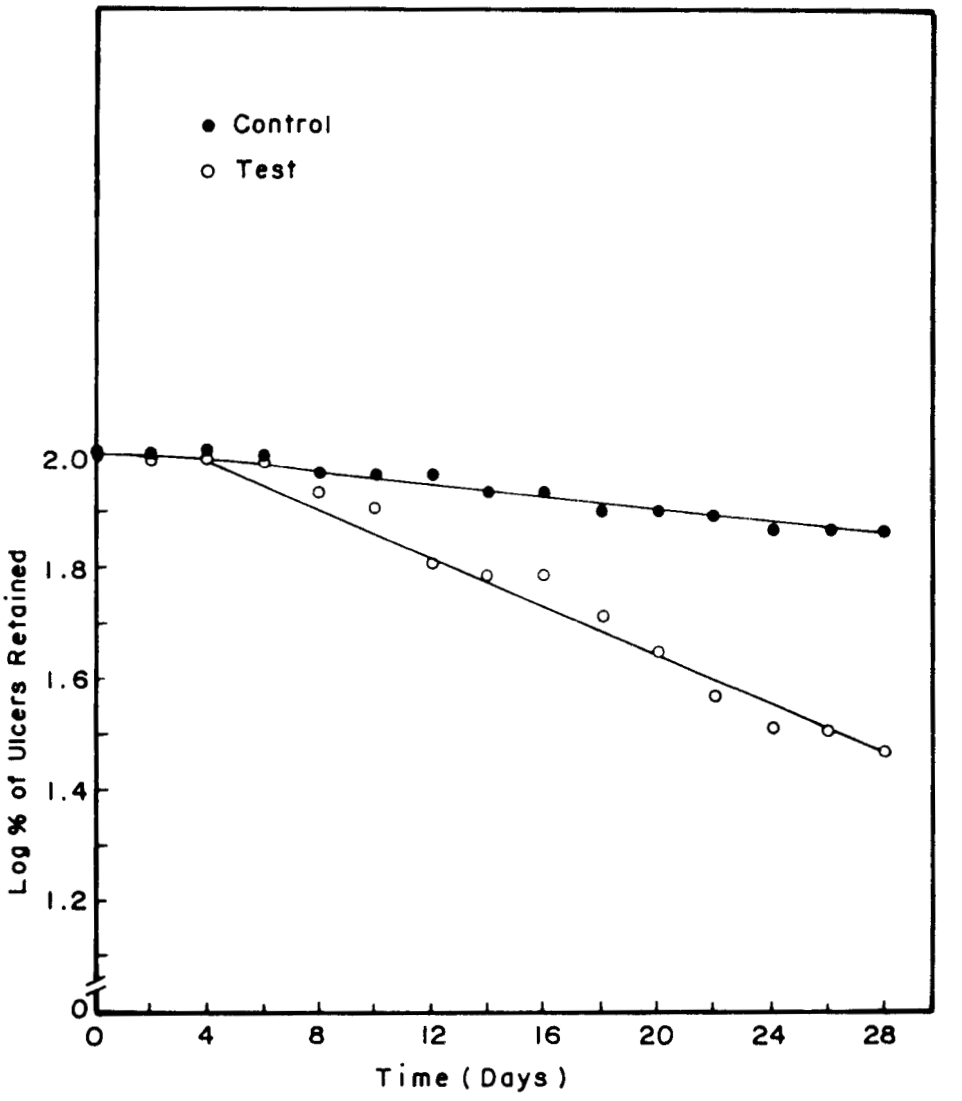

Fig. 3 : Effect of Flufenamic acid on the Healing Rate of Inflammatory Areas (Ulcers)in Rabbits Eye.

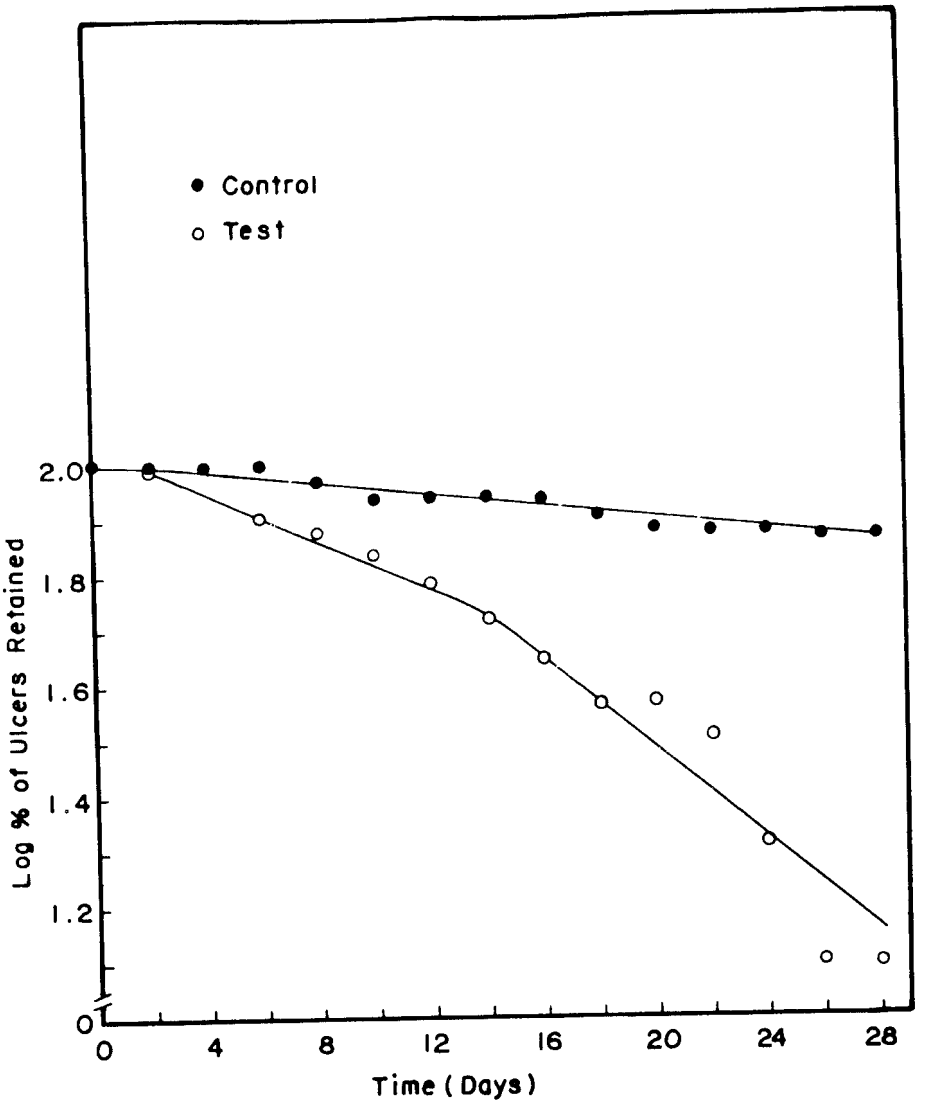

Fig. 4 : Effect of Monophenylbutazone on the Healing Rate of Inflammatory Areas (Ulcers) in Rabbits Eye.

the healing process involved 2 distinct phases proceded by a lag phase extending 3-4 days. The healing rate was higher in the second phase than the first one.With flufenamic acid the healing process followed a nearly constant rate-single phase profile. The healing rate of monophenylbutazone gel preparations was found to be higher. The higher activity of the monophenylbutazone preparations may be attributed to the higher release rate of the drug from the gel.

REFERENCES

1- T.F. Patton and J.R. Robinson, J. Pharm.Sci.,65,1295 (1976).

2- R. Gurny, Pharm. Acta Helv., 56, 130(1981) .

3- R.E. Stratford, D.C. Yang, M.A. Reddel and V.H.L.Lee, Curr. Eye Res., 2, 377 (1983).

4- R.W. Wood, V.H.L.Lee, J. Kreuter and J.R.Robinson,Int. J. Pharmaceut., 23, 175 (1985).

5- M.F. Saettone, B. Giannaccini, A. Tenggi,P. Savigni and N. Tellini, J. Pharm. Pharmacol., 34, 464(1982).

6- O. Olejnik, J. Stevens, C.G.Wilson and J.G.Hardy, J. Pharm. Pharmacol., 34, 28 (1982).

7- M.F. Saettone and E. Chiellini, Proc. Int. Symp.Control. Rel.Bioact.Mater.,12, 302 (1985).

8- S.M. Blaugh and A.T.Canada, Amer J. Hosp.Pharm.,22,662 (1965).

9- M.L. Linn and L.T. Jones, Amer. J.Ophthal., 65, 76(1968).

10- S.S.Chrai and J.R. Robinson, J. Pharm.Sci., 63, 1218 (1974).

11- M.F.Saettone,B.Giannaccini,P.Savigni and A. Wirth,J.Pharm. Pharmacol., 32, 519 (1980).

12- R.D. Schoenwald and J.J. Boltralik, Invest.Ophthal.Vis. Sci., 18, 61 (1979).

13- N. Graham and McNeil, Biomaterials, 5, 27 (1984).

14- P. Lee, Proc.Int. Sym. Control.Rel.Bioact.Mat.,10, 136 (1983).

15- P. Gyselinck, E. Schacht,R.Vanseveren and P.Braeckman, Acta Pharm.Techn.,29, 9 (1983).

16- C. Reinhart and N. Peppas, Int. Pharm.Techn., 2,9(1981).

17- S.C.Miller and M.D.Donovan, Int.J. Pharmaceut., 12,147 (1982).

18- T. Boye, R. Gurny and P. Buri, Proc. Int. Symp.Biopharmaceut. and Pharmacok. (1984).

19- R.D. Schoenwald, R.L. Ward,L.M.Desantis and R.E.Roehrs, J. Pharm.Sci.,67, 1280 (1978).

20- K.E. Schulte,V.M.Bass and R.I.Mrongovius, Eur.J. Drug Metabol. Pharmacok.,5, 206(1980).

21- K.E.Schulte, R.I. Mrongovius and V.M.Bass, Eur.J. Med. Chem., 17, 279 (1982).

22- I.B. Linden, J. Parantainen and H. Vapaatalo, Scand. J. Rheum., 5, 129 (1976).

23- L. Turakka and M. Sieppi, Acta Pharm. Suec.,22, 157(1985).

24- M.A.Attia, Ph.D. Thesis, First Medical Institute ,Moscow (1978).

25- J.B. Schwartz, A.P. Simonelli and W.I.Higuchi,J. Pharm.Sci. 57, 274 (1968).

26- T.Higuchi, J. Pharm.Sci., 52, 1145 (1963).

27- L. Saunder and R. Fleming" Mathematics and Statistics", Pharmaceutical Press, London(1966),P. 150.

28- B.W.Barry and M.C. Meyer,Int.J. Pharmaceut., 2, 27(1979).

29- B.W.Barry"Dermatological Formulations,Drugs and Pharmaceutical Sciences" , Vol 18, Marcel Dekker Inc. New York 1983, p., 303.

30- A. Goldstein,"Biostatistics",Macmillan Co.,New York(1965).

31- T.J. Roseman and W.I. Higuchi,J. Pharm.Sci.,59, 353(1970).

32- J.Haleblian, R. Runkel,N.Mueller,J. Christopherson and K.Ng, J. Pharm. Sci., 60, 541 (1971).