DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY, 13(8), 1447-1469 (1987)

IN-VIVO PERFORMANCE OF OPTHALMIC SOLUTIONS OF BETAMETHASONE AND PHENYLEPHRINE HYDROCHLORIDE IN THE EYE OF RABBIT II. EFFECT OF METHYLHYDROXY-ETHYLCELLULOSE .

M.A. Kassem, M.A. Attia, F.S. Habib & A.A. Mohamed

Assiut University, Faculty of Pharmacy, Dept.of Pharmaceutics, Assiut, Egypt.

ABSTRACT

The effect of different concentrations of methyl-hydroxyethylcellulose on the activity of betamethasone (increase of the intraocular pressure, IOP) and phenyl-ephrine hydrochloride (decrease in the IOP and increase pupil diameter) was investigated. The results obtained demonstrate that the parameters of action of betamethasone are influenced by solution viscosity only in the lower range, viz < 4 cP ($D \simeq 0$ sec^{-1}). The parameters of action of phenylephrine hydrochloride are also dependent on the viscosity of the ophthalmic solution. The data reveal that the dependency of drug action on solution viscosity is reflected by the IOP to an extent greater than that by the pupillary response.

INTRODUCTION

Methylcellulose was used in ophthalmology to increase the viscosity of aqueous ophthalmic solutions in 1945[1].

In 1965 other workers[2] introduced polyvinyl alcohol (PVA) to ophthalmology and it was proved that PVA does not blur vision[3].

In 1968 a study[4] on hydroxypropylmethylcellulose which is a derivative of methylcellulose was carried out. The authors found in experiments on human subjects that, this compound has a corneal contact time superior to that obtained with PVA.

The purpose of this work (as a series of experiments on viscolizers) was : i) elucidation of the dependency of the in-vivo perfermance of drug on the viscosity of the ophthalmic preparation, ii) elucidation of optimum conditions for the application of viscolizers in ophthalmology , (iii) to study the effect of methylhydroxyethylcellulose on the activity of ophthalmic solutions of betamethasone and phenylephrine hydrochloride in the rabbit's eye. Non viscous solution was used as a control

EXPERIMENTAL

Materials :

Betamethasone (Schering), phenylephrine hydrochloride (Siegfried), methylhydroxyethylcellulose (Tylose 4000-Hoechst).

Procedure:

Preparation of Ophthalmic Solutions :

Ophthalmic solutions containing 0.05% w/v betamethasone and/or 2.5% w/v phenylephrine hydrochloride were prepared according to the following procedure : Tylose was dissolved in isotonic phosphate buffer solution (pH 6.8). Betamethasone was dissolved in the least amount (1% of the ophthalmic solution) of

polyethylene glycol 400. Solutions of betamethasone and phenylephrine hydrochloride (the latter in buffer) were mixed with isotonic buffer. The concentration ranges of tylose was : 0.125 - 0.5% w/v.

Investigation of the Rheology of Ophthalmic Solutions:

The viscosity of the ophthalmic solutions was determined using Ferranti-Shirley cone and plate rotational viscometer at 33 ± 0.1°.

Presentation of the Flow Behaviour :

An ideal equation giving an indication of the viscosity of the system at negligible rates of shear, and allowing the calculation of the apparent viscosity at any particular shearing rate, together with the characterization of the flow pattern of the system under investigation was looked at among the equations available for the characterization of non-Newtonian flow. Steiger-Trippi's[5] equation seemed to fullfil the above mentioned requirements.

The viscosity was calculated at low limiting levels of shear dictated by the physiology of blinking in the eye of rabbit. Since the blinking rate is known to be very low in rabbit (4 times. hr^{-1})[6] the lower level of shear was a value near to zero ($\simeq 0$) to represent the non-blinking conditions, this was represented by the basic viscosity. The upper limit of shear was a rate similar to that calculated for human eye during blinking[7]; a value of 4500 sec^{-1} was taken.

Measurement of IOP and pupil Diameter :

Albino rabbits , 1.8 - 2.8 Kg receiving green fodder were used as experimental animals. Isotonic

xylocaine solution (1% w/v) was dropped into the rabbit's eyes to anaesthetise the cornea. In all cases topical doses each of 50 µl of ophthalmic solution were applied. The dose was placed in the lower conjunctival sac. Non medicated formulations were applied to the opposite eye which served as control. Each formulation was tested in each of six rabbits kept in a room with standardized illumination. The assigned formulation was applied to the right eye, while the control one was applied to the left eye. Before and after application of both control and test formulations, the pupil diameter and the IOP of both eyes were measured using Haab's pupillometer and Maclocof tonometer, respectively, every one hour.

The parameters of activity of both drugs are: area under (or above) the curve, AUC, maximum response, MR, time of maximum response, TMR, and duration of drug action, DA.

RESULTS AND DISCUSSION

The activity of ophthalmic solutions of betamethasone or phenylephrine hydrochloride in the eye of rabbit was investigated in relation to the concentration of methylhydroxyethylcellulose (Tylose) in the solution. The concentration of the solution was 0.05 and 2.5 percent with regard to betamethasone and phenylerphrine hydrochloride, respectively.

Betamethasone:

The effect of betamethasone on the time course of the interaocular pressure was investigated as a function of the concentration of the polymer in the range of 0 - 0.5 percent. The results are presented in Figure 1. It is obvious from the figure that tylose markedly increases the effect of the drug on the IOP. It is also

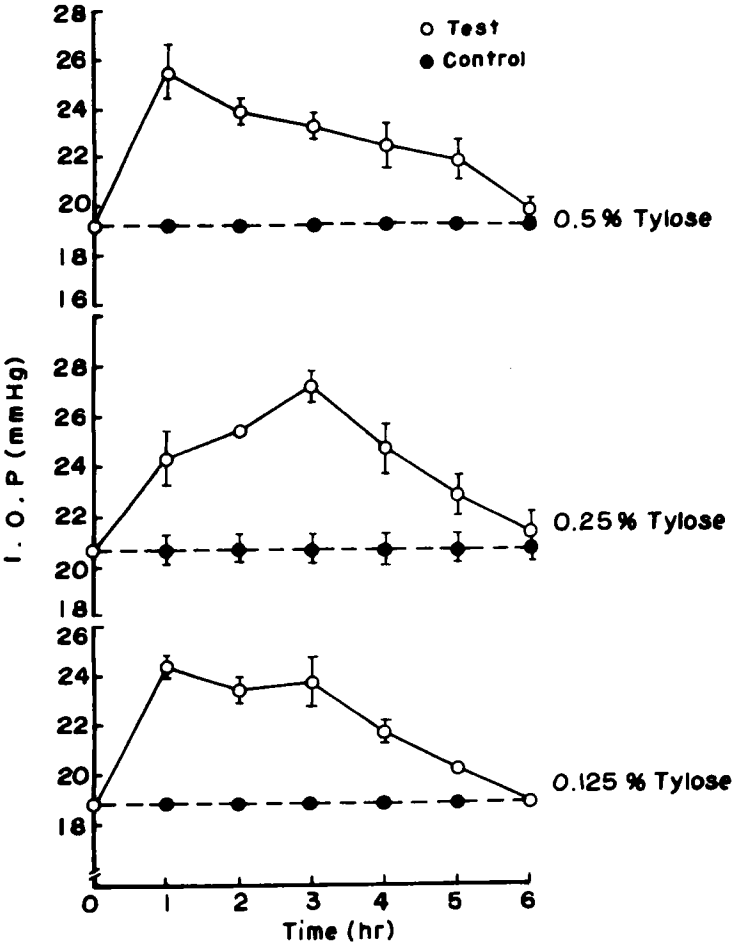

Fig. (1) : Intraocular Pressure (in mm.Hg) of Rabbit's Eye Post-Instillation of 0.05% (w/v) Betamethasone Ophthalmic Solutions Containing Different Concentrations of Methylhydroxyethylcellulose (Tylose).

evident that the polymer exerts its effect already at concentrations as low as 0.125%.

AUC in Relation to Viscosity of Ophthalmic Solution.

The area under the IOP/time curve is presented in Table 1 as a function of viscosity at D ≃ 0 or 4500 sec^{-1}. The data reflect one and the same behaviour which represents a high dependency of the AUC at low viscosities and a very low dependency at higher viscosities.

At D ≃ 0 $sec.^{-1}$, the AUC increases greatly as the viscosity increases to values approaching 4 cP. In this range a I-fold increase in viscosity brings about a 3.5-fold increase in the AUC. Above 4 cP, on the other hand, a 4-fold increase in viscosity brings about an increase in the AUC of only 0.2-fold.

Statistical analysis of the data (table 2)reveals that the differences between the non viscous solution, on one hand, and the viscous solutions, on the other hand, are highly significant.

It is also obvious that the differences between the individual viscous solutions are not statistically significant.

The above mentioned results indicate that, to increase the amount of drug absorbed (expressed as AUC), there may be no need to raise the viscosity of the solution to values higher than 4 cP (D ≃0 $sec.^{-1}$).

MR in Relation to Viscosity of Ophthalmic Solution.

Table (1) depict the dependency of the MR on the viscosity of ophthalmic solution both at D ≃ 0 and

Table (1): Correlation of AUC, MR, TMR and DA to the Viscosity of Ophthalmic Solutions of Betamethasone Containing Methylhydroxy-ethylcellulose (Tylose)

Concen-tration	Viscosity (cP)		Parameters of Activity			
	D ≈ 0 $sec.^{-1}$	D= 4500 $sec.^{-1}$	AUC[1] (mm Hg.hr)	MR (mm Hg)	TMR (hr)	D A (hr)
0 %	1.67	1.46	4.33(0.74)*	2.67(0.44)	1.5(0.22)	2.0 (0.44)
0.125%	3.24	3.1	19.17(1.95)	6.67(0.31)	2 (0.45)	4.83(0.17)
0.25 %	4.81	4.62	19.92(0.71)	7.08(0.95)	2.83(0.40)	5.17(0.17)
0.5 %	15.87	12.62	22.58(2.58)	6.99(0.54)	1 (0)	5.33(0.21)

1). In excess to the corresponding control viz. corresponding solution without drug.

* **The values between parentheses in this table and subsequent ones represent the standard error.**

Table (2): Significance level (value of P) of Differences in the IOP) Between Ophthalmic Solutions of Betamethasone Contining Methylhydroxethyl-cellulose (Tylose)

Pairs of Comparison			Parameters of Activity			
			AUC (mm Hg.hr)	MR (mm Hg)	TMR (hr)	DA (hr)
0 %	with	0.125 %	0.01	0.01	0.1	0.01
0 %	with	0.25 %	0.01	0.01	0.05	0.01
0 %	with	0.5 %	0.01	0.01	0.05	0.01
0.125%	with	0.25 %	0.1	0.1	0.1	0.1
0.125%	with	0.5 %	0.1	0.1	0.1	0.1
0.25 %	with	0.5 %	0.1	0.1	0.01	0.1

4500 sec.$^{-1}$. It is clear that the MR-viscosity profiles are very similar and reflect a high dependency of MR on solution viscosity in the lower viscosity range (< 4 cP, D ≃ 0 sec.$^{-1}$). In the higher viscosity range (> 4 cP),. On the other hand, the MR is almost independent on the viscosity of the ophthalmic solution. In the lower range, a 1-fold increase in viscosity increases the MR 1.5-fold. In the higher range, a four-fold increase in viscosity increases the MR only 0.1-fold.

From the statistical point of view, the differences between the non viscous solution and the viscous ones are highly significant already at a Tylose concentration as low as 0.125%, (table 2). The differences between the individual viscous solutions are not statistically significant.

These findings demonstrate that the intensity of drug effect, expressed as MR, is dependent on solution viscosity in the lower viscosity range viz. 4cP(D≃0 sec.$^{-1}$).

TMR in Relation to Viscosity of Ophthalmic Solution

Table 1 shows that TMR increases first with solution viscosity until the latter approaches a value of 5 cP (D≃ 0 sec.$^{-1}$). Beyond this viscosity, the TMR decreases to a marked extent.

The statistical analysis of the data (table 2) shows that the non viscous solution is significantly different (P = 0.05) only from solutions of a viscosity of ~5 cP (D ≃ 0.0 sec.$^{-1}$) or greater. Also the individual viscous solutions are significantly different (P=0.01) if the viscosities lie in the same range.

DA in Relation to Viscosity of Ophthalmic Solution.

Table 1 illustrates the dependency of the DA in relation to the viscosity of ophthalmic solutions at $D \simeq 0$ and 4500 $sec.^{-1}$.

It is obvious that the duration of drug action is dependent on solution viscosity only in the lower viscosity range viz. below 4 cP ($D \simeq 0$ $sec.^{-1}$). In this range, a 1-fold increase in viscosity increases the duration of action 1.4-fold. Beyond 4 cP, the DA is independent on solution viscosity.

Statistical analysis of the data (table 2) shows that the viscous solutions are significantly different ($P = 0.01$) from the non viscous solution even in the presence of Tylose in a concentration as low as 0.125%.

The individual viscous solutions, on the other hand, are not significantly different from each other.

Summarizing, the findings presented under betamethasone demonstrate that the parameters of drug action are influenced by solution viscosity only in the lower range of viscosity viz. < 4cP ($D \simeq 0$ $sec.^{-1}$). Beyond this range, the viscosity of the solution does not influence the parameters of activity.

The dependency of the different parameters of activity on the viscosity of the solutions decreases in the order of AUC > MR > DA > TMR.

Phenylephrine Hydrochloride .

The effect of methylhydroxyethylcellulose on the time course of the IOP and the pupil diameter was investigated. The results are presented in Figures(2 and 3).

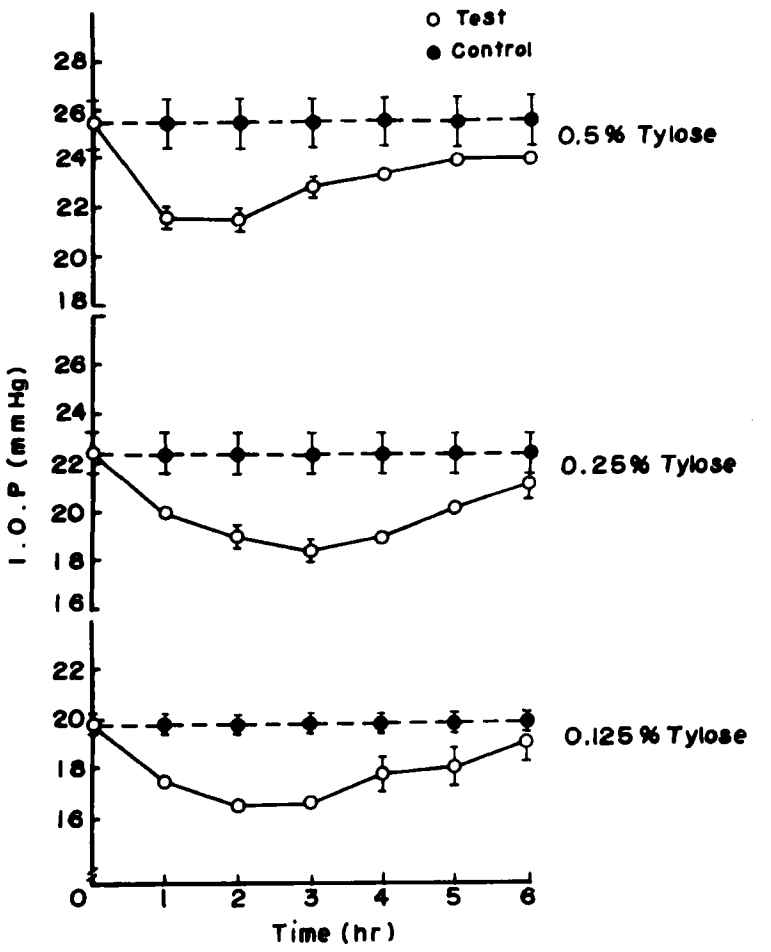

Fig.(2): Intraocular Pressure (in mm.Hg) of Rabbit's Eye Post-Instillation of .25% (w/v) Phenylephrine Hydrochloride Solutions Containing Different Concentrations of Methylhydroxyethylcellulose (Tylose).

It is obvious that Tylose enhances the effect of phenylephrine hydrochloride on the IOP and that the influence is pronounced at polymer concentrations as low as 0.125% (figure 2).

Tylose is also found to enhance the mydriatic effect of the drug in proportion to the concentrations of the polymer.

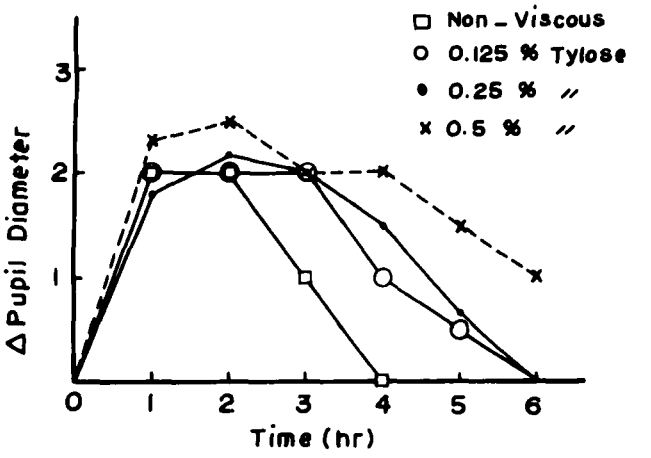

Fig.(3): Pupil Diameter (in mm) of Rabbit's Eye Post-Instillation of 2.5% (w/v) Phenylephrine Hydrochloride Solutions Containing Different Concentrations of Methylhydroxyethylcellulose (Tylose).

Area Above IOP/Time curve (AAC) in Relation to Viscosity of Ophthalmic Solution.

Table 3 shows the dependency of the area above the IOP/time curve on solution viscosity determined at $D \simeq 0$ and 4500 $sec.^{-1}$.

The Table demonstrates that the viscosity of the solution increases, to a great extent, the AAC only in the lower viscosity range, namely, below 5 cP ($D \simeq 0$ sec^{-1}). In this range a 2-fold increase in viscosity brings about 6.3-fold increase in AAC.

Beyond a viscosity of ~ 5 cP, the AAC is independent on solution viscosity.

From the statistical point of view, the differences between the viscous solutions and the non viscous one

Table (3) Correlation of AAC, MR, TMR and DA of (IOP) to Viscosity of Ophthalmic Solutions of Phenylephric Hydrochloride Containing Methylhydroxyethylcellulose (Tylose)

concentration	Viscosity (cP)		Parameters of Activity			
	$D \simeq 0$ $sec.^{-1}$	$D = 4500$ sec^{-1}.	AAC (mm Hg.hr)	MR (mm Hg.)	TMR (hr)	DA (hr)
0 %	1.67	1.46	2.08 (0.39)	1.42 (0.15)	2.33 (0.21)	2.67 (0.21)
0.125%	3.24	3.1	13.42 (2.26)	3.75 (0.46)	2.67 (0.49)	5 (0.52)
0.25 %	4.81	4.62	15.13 (3.68)	4.67 (0.7)	3 (0.26)	5.17 (0.31)
0.5 %	15.87	12.62	15.71 (4.08)	4 (0.48)	1.33 (0.21)	5.5 (0.33)

are consistently significant ($P = 0.01$) already in the presence of tylose in concentrations as low as 0.125% (Table 5). The differences between the individual viscous solutions, on the other hand, are insignificant.

Area Under Pupil Diameter/Time Curve in Relation to Viscosity of Ophthalmic Solution.

Figure 4 and table 4 illustrate the dependency of AUC on the solution viscosity at $D \simeq 0$ and 4500 $sec.^{-1}$. It is obvious that the AUC increases with increasing viscosity of the ophthalmic solution throughout the experimental range of $\sim$ 2 - 16 cP ($D \simeq 0$ $sec.^{-1}$). Throughout this range a 9-fold increase in viscosity brings about 1.2-fold increase in AUC. The rates of increase of the AUC before and beyond 5 cP are different. Unlike the AAC-for the effect on the IOP- the AUC did not reach an asymptotic value at a viscosity as high as 16 cP ($D \simeq 0$ $sec.^{-1}$). For the AAC, the asymptotic value was reached already at a viscosity of $\sim$ 5 cP.

Table (4): Correlation of AUC, MR, TMR and DH of Pupil Diameter to Viscosity of Ophthalmic Solutions of Phenyl_ephrine Hydrochloride Containing Methylhydroxyethylcellulose (Tylose)

concentration	Viscosity D = 0 $sec.^{-1}$	Viscosity D =4500 $sec.^{-1}$	AUC (mm.hr)	MR (mm)	TMR (hr)	DA (hr.)
0 %	1.67	1.46	5 (0)	2 (0)	1 (0)	3 (0)
0.125 %	3.24	3.1	7.5 (0.22)	2 (0)	1 (0)	4.5 (0.22)
0.25 %	4.81	4.62	8.17 (0.54)	2.17 (0.17)	1.17 (0.17)	4.67 (0.22)
0.5 %	15.87	12.62	10.83 (0.42)	2.5 (0.22)	1.33 (0.22)	6 (0.0)

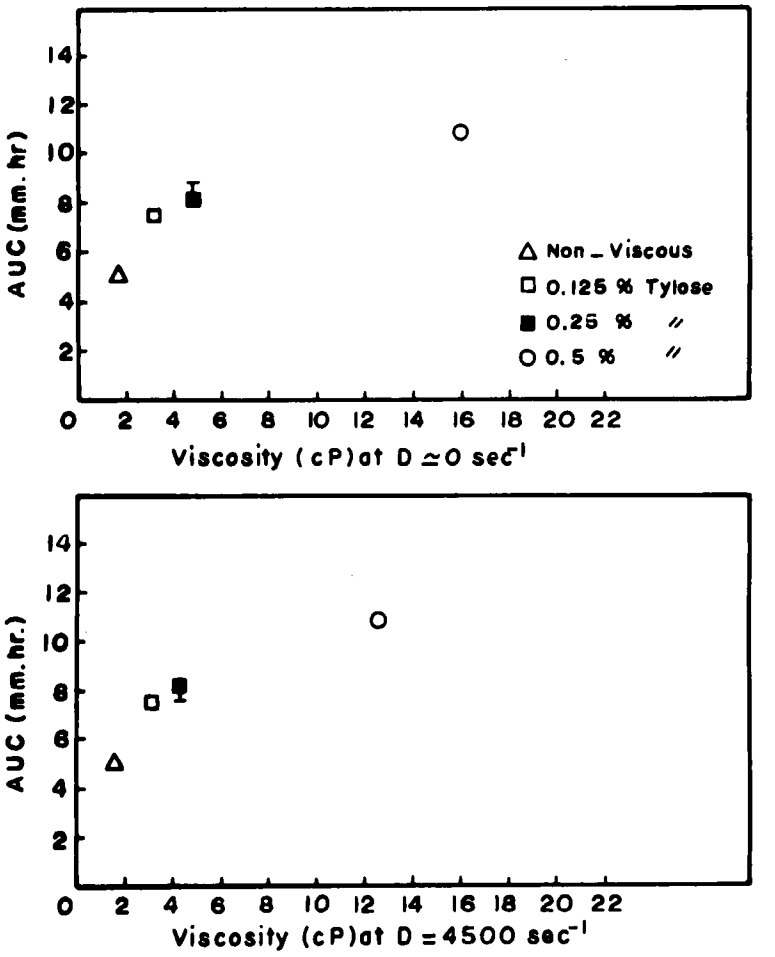

Fig.(4): Correlation of the AUC (Pupil Diameter) to the Viscosity of Ophthalmic Solutions of Phenylephrine Hydrochloride Containing Methylhydroxyethylcellulose (Tylose).

Statistical analysis of the data reveals that the viscous solutions are significantly ($P = 0.01$) different from the non viscous solution, already in the presence of 0.125% tylose. The differences between the individual viscous solutions are significant ($P = 0.01$) only for solutions containing 0.125 or 0.25 and 0.5% of the

Table (5): Significance level (value of P) of Differences in the IOP parameters Between Ophthalmic Solutions of Phenylephrine Hydrochloride Containing Methylhydroxyethylcellulose(Tylose)

Pairs of Comparison	Parameters of Activity			
	AAC (mm Hg.hr)	M.R. (mm Hg.)	TMR (hr)	DA (hr)
0 % with 0.125 %	0.01	0.01	0.1	0.01
0 % with 0.25 %	0.01	0.01	0.1	0.01
0 % with 0.5 %	0.01	0.01	0.01	0.01
0.125 % with 0.25 %	0.1	0.1	0.1	0.1
0.125 % with 0.5 %	0.1	0.1	0.05	0.05
0.25 % with 0.5 %	0.1	0.1	0.01	0.01

polymer. There is no statistically significant difference between 0.125 and 0.25% solutions of the polymer (table 6).

Maximum Response (IOP) in Relation to Viscosity of Ophthalmic Solution.

Table 3 demonstrates the dependency of the maximum response to phenylephrine hydrochloride- in terms of IOP- in relation to viscosity at $D \simeq 0$ and 4500 $sec.^{-1}$.

It is evident that the maximum response increase with increasing viscosity in the range below 5 cP ($D \simeq 0 sec.^{-1}$). In this range a 2-fold increase in viscosity brings about a 2.3-fold increase in MR.

Beyond a viscosity of ~5 cP, on the other hand, the MR becomes independent on the viscosity of the ophthalmic solution even if it increases to 16 cP.

The statistical analysis of the data (table 5) shows that the differences between the viscous solutions and the non viscous one are consistently highly significant. The differences between the individual viscous solutions, on the other hand, are insignificant.

These findings reveal that 0.125% solution of the polymer may be as effective as solutions of higher polymer concentration with regard to enhancing the intensity of drug action.

Maximum Pupillary Response in Relation to Viscosity of Ophthalmic Solution.

Figure 5 demonstrates the dependency of the maximum pupillary response to phenylephrine hydrochloride in relation to solution viscosity at $D \simeq 0$ and 4500 $sec.^{-1}$. It is obvious that there is no pronounced dependency throughout the investigated viscosity range, viz $\sim$ 2 - 16 cP ($D \simeq 0$ $sec.^{-1}$). A measurable increase in the maximum response takes place only if the viscosity reaches about 16 cP. The differences between the different solutions are statistically significant ($P = 0.01$) only for 0.5% solution of the polymer compared to 0.125% solution or the non viscous solution (table 6).

The findings mentioned under (Phenylephrine hydrochloride) again demonstrate that the intensity of drug action is not reflected in one and the same way be the pupil diameter and the I.O.P.

Time of Maximum Response(IOP) in Relation to Viscosity of Ophthalmic Solution.

Table 3 shows the dependency of the time of maximum response- in terms of IOP-on the viscosity of ophthalmic solution at $D \simeq 0$ or 4500 $sec.^{-1}$. The data demonstrate a

Table (6): Significance level (value of P) of Differences in the Pupil Diameter Between Ophthalmic Solutions of Phenylephrine Hydrochloride Containing Methylhydroxyethylcellulose(Tylose)

Pairs of Comparison	AUC (mm.hr)	MR (mm)	TMR (hr)	DA (hr)
0 % with 0.125 %	0.01	0.1	0.1	0.01
0 % with 0.25 %	0.01	0.1	0.1	0.01
0 % with 0.5 %	0.01	0.01	0.1	0.01
0.125% with 0.25 %	0.1	0.1	0.1	0.1
0.125% with 0. 5 %	0.01	0.01	0.1	0.01
0.25 % with 0.5 %	0.01	0.1	0.1	0.01

slight increase in the time of maximum response as the viscosity increases, in the lower viscosity range, to values of ~ 5 cP ($D \simeq 0$ sec.$^{-1}$). The increase of TMR throughout this range is, however, statistically insignificant (table 5) . As the viscosity further increases to values approaching 16 cP ($D \simeq 0$ sec.$^{-1}$) the TMR decreases to a marked extent to reach values less than those observed for solutions of viscosities lying in the range ~ 2-5 cP.

The differences between the TMR of the solution of 16 cP on one hand, and all other solutions, on the other hand, are consistently statistically significant (table 5).

These findings demonstrate that Tylose does not significantly influence the TMR unless the viscosity of the ophthalmic solutions reaches values approaching 16 cP.

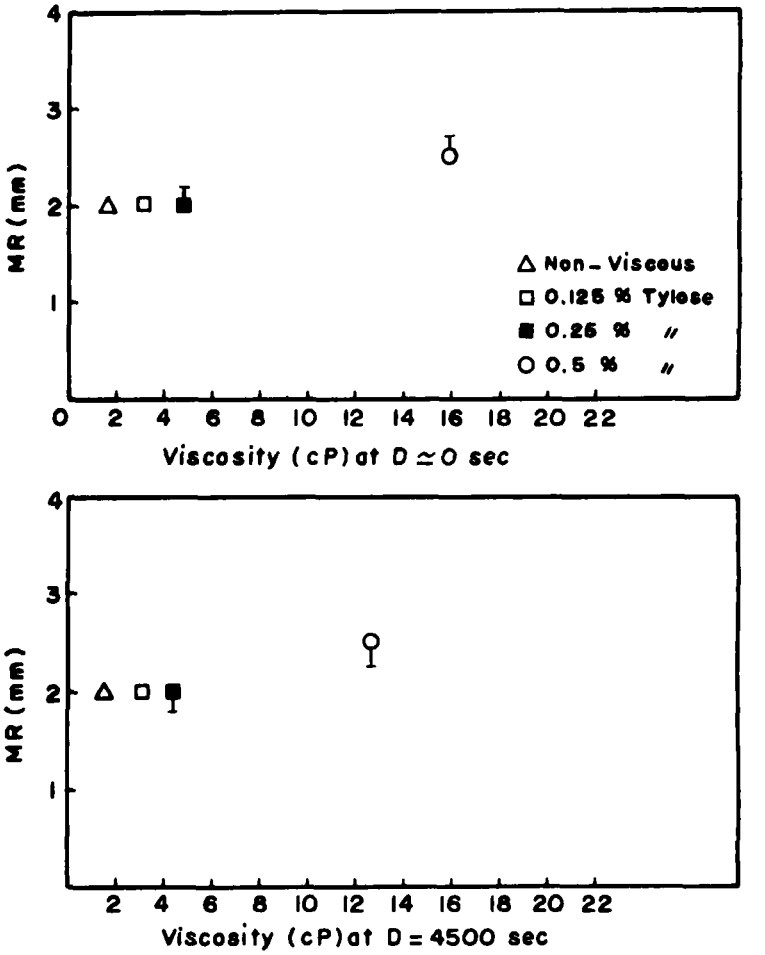

Fig.(5) : Correlation of the MR(Pupil Diameter) to the Viscosity of Ophthalmic Solutions of Phenylephrine Hydrochloride Containing Methylhydroxyethylcellulose (Tylose).

This behaviour recalls that observed for the TMR to betamethasone.

Time of Maximum Pupillary Response in Relation to Viscosity of Ophthalmic Solution.

Figure 6 shows the dependency of the time of maximum pupillary response on the viscosity of the ophthalmic solution.

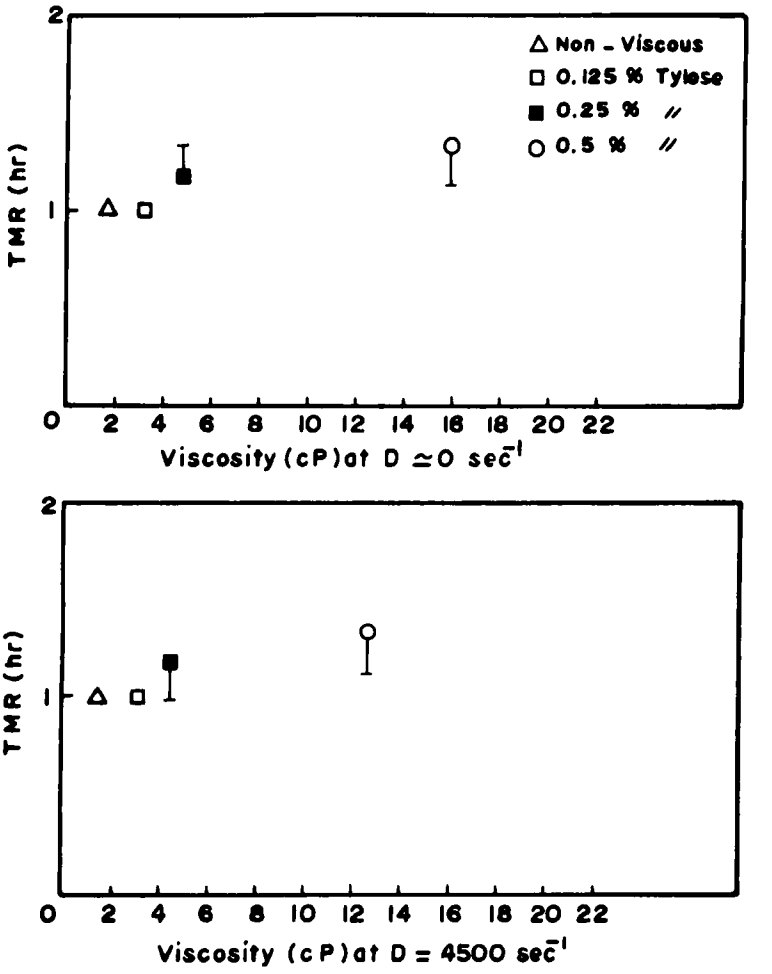

Fig. (6): Correlation of the TMR (Pupil Diameter) to the Viscosity of Ophthalmic Solutions of Phenylephrine Hydrochloride Containing Methylhydroxyethylcellulose (Tylose).

It is evident that the TMR does not depend on the viscosity of the ophthalmic solution to any marked extent. The differences between the different solutions are statistically insignificant (table 6).

Duration of Drug Action (IOP) in Relation to Viscosity of Ophthalmic Solution.

The duration of the action of phenylephrine hydrochloride on the IOP in relation to the viscosity of the ophthalmic solution is presented in Table 3 . It is obvious that the duration of action is highly dependent on solution viscosity in the lower range of viscosity i.e. values below ~4 cP ($D \simeq 0$ sec.$^{-1}$). At viscosities higher than ~ 4cP, the duration of drug action becomes almost independent on solution viscosity.

Statistical analysis of the data (table 5) reveals that the non viscous solution is significantly different ($P=0.01$) from all viscous solutions. The individual viscous solutions, on the other hand, do not significantly differ from one another.

Duration of Pupillary Response in Relation to Viscosity OF Ophthalmic Solution.

The dependency of the duration of the pupillary response to the drug on the viscosity of the ophthalmic solution (at $D \simeq 0$ or 4500 sec.$^{-1}$) is presented in Figure 7.

It is obvious that the duration of drug action is markedly dependent on the viscosity of the ophthalmic solution in the viscosity range below 4 cP ($D \simeq 0$ sec.$^{-1}$) In this range a 1-fold increase in viscosity brings about a 0.5-fold increase in the duration of drug action.

The dependency of the duration of drug action on solution viscosity is found to decrease in the viscosity range above ~ 4 cP(~4-16 cP). In the latter range , a 4-fold increase in viscosity brings about an increase in the DA of only 0.3-fold.

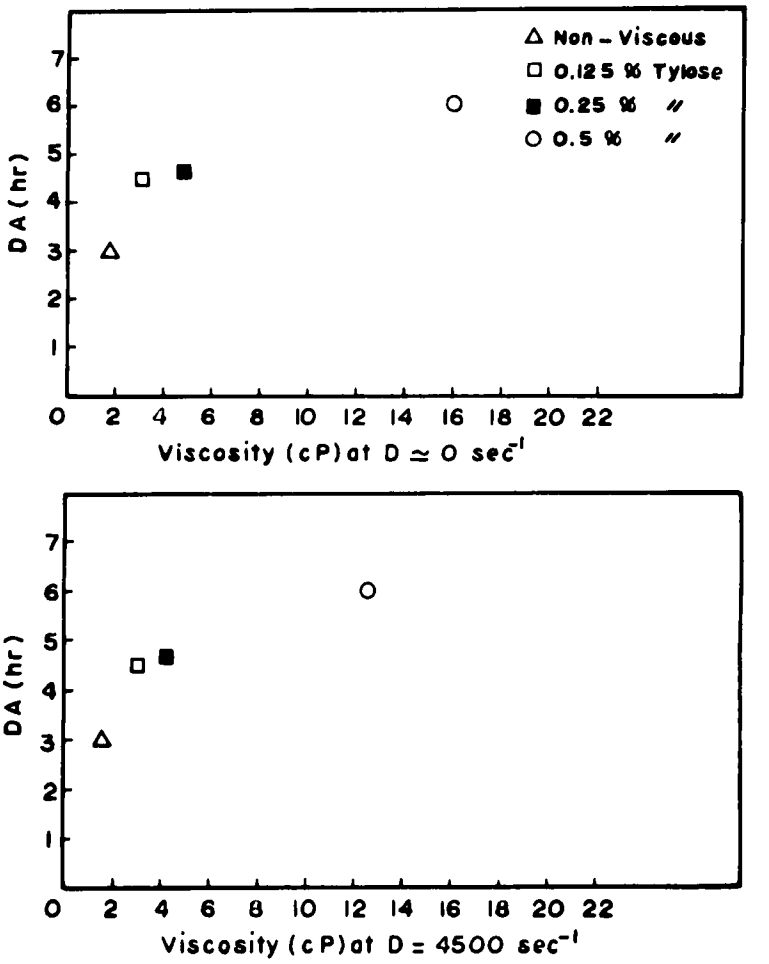

Fig. (7): Correlation of the DA(Pupil Diameter) to the Viscosity of Ophthalmic Solutions of Phenylephrine Hydrochloride Containing Methylhydroxyethylcellulose (Tylose).

Statistical analysis of the data (table 6)reveals that the viscous solutions are significantly (P= 0.01) different from the non viscous solution.

For the individual viscous solutions, the differences are insignificant for viscosities of ~ 1.7 and

~3.2 cP and significant (P = 0.01) for viscosities of ~3.2 and 15 cP or ~ 5 and 16 cP.

The above mentioned findings demonstrate that the parameters of action of phenylephrine hydrochloride are dependent on the viscosity of the ophthalmic solution in the order : area above (under) the curve > maximum response > duration of drug action.

The data also reveal that the dependency of drug action on solution viscosity is reflected by the IOP to an extent greater than that by the pupillary response.

Comparisone of the data of betamethasone and phenylephrine hydrochloride reveal that betamethasone action is dependent on the viscosity of the ophthalmic solution to an extent greater than that of phenylephrine hydrochloride action.

This work will be completed by the use of polyvinyl-alcohol (as viscolizer) and a general discussion on the use of viscolizers and on the usefulness of a combination of betamethasone and phenylephrine hydrochloride in terms of minimizing of the side effect of the anti-inflammatory drug betamethasone will be carried out.

REFERENCES

1. K. C. Swan, Arch Ophthalmol., 33, 378 (1945).
2. N. Krishna and B. Mitchell, Am. J. Ophthalmol. 59, 860-864 (1965).
3. N. Krishna and F. Brow, ibid., 57, 99(1964).
4. M.L. Linn and L.T. Jones, ibid., 65, 76-78 (1968).

5. K. Steiger-Trippi and A.M. Ory, Pharm. Acta Helv., 39, 205 (1961); through Ph.D. Thesis A.G. Matta, Cairo Univer. (1971).

6. C. Melis-Decerf and M. Van Ooleghem. J. Pharm. Pharmac., 31, 12 (1979).

7. R.M. Khalil, M. Pharm. Thesis, Cairo Univer. (1981).