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Pharmaceutical Nanotechnology

Nanosuspension as an ophthalmic delivery system for certain glucocorticoid drugs

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Abstract

Poorly-water-soluble compounds are difficult to develop as drug products using conventional formulation techniques. The use of nanotechnology to formulate poorly-water-soluble drugs as nanosuspensions offers the opportunity to address many of the deficiencies associated with this class of molecules. In the present study, the high pressure homogenization method used to prepare nanosuspensions of three practically insoluble glucocorticoid drugs; hydrocortisone, prednisolone and dexamethasone. The effect of particle size in the micron and nano-size ranges as well as the effect of viscosity of the nanosuspension on the ocular bioavailability was studied by measuring the intraocular pressure of normotensive Albino rabbits using shiØetz tonometer. The results show that compared to solution and micro-crystalline suspensions it is a common feature of the three drugs that the nanosuspensions always enhance the rate and extent of ophthalmic drug absorption as well as the intensity of drug action. In the majority of cases nanosuspensions extend the duration of drug effect to a significant extent. The data presented confirms that nanosuspensions differ from micro-crystalline suspensions and solution as ophthalmic drug delivery systems and that the differences are statistically, highly to very highly significant. The results confirm also the importance of viscosity of nanosuspension especially in increasing the duration of drug action.

Keywords: Nanosuspension; Particle size; Ophthalmology; Glucocorticoids

1. Introduction

Poorly soluble drugs are very often a challenging problem in drug formulation, especially when the drugs are poorly soluble simultaneously in aqueous and non-aqueous media. This leads in most cases to poor bioavailability or poor erratic absorption of these drugs (Merisko-Liversidge et al., 2003; Müller et al., 2001).

Many attempts have been made to increase the saturation solubility of poorly soluble drugs (Liversidge et al., 1992; Sucker, 1998). Recently, drug micro-particle suspensions can be milled by applying a high pressure homogenization process (Müller et al., 1999; Jacobs et al., 2001; Keck and Müller, 2006) leading to a product called nanosuspension. Nanosuspensions are sub-micron colloidal dispersions of pure drug particles in an outer liquid phase (Möschwitzer et al., 2004). An outstanding feature of the nanosuspension is the increase in saturation solubility and consequently an increase in the dissolution rate of the compound (Böhm and Müller, 1999; Müller et al., 2001; Merisko-Liversidge et al., 2003; Rabinow, 2004; Hecq et al., 2005; Kocbek et al., 2006).

Ophthalmic drug delivery, more than any other route of administration, may benefit to a full extent from the characteristics of nano-sized drug particles. The nano-size represents a state of matter characterized by higher solubility (Müller and Böhm, 1998; Müller et al., 1999; Müller and Keck, 2004), higher surface area available for dissolution (Bisrat and Nyström, 1988; Mosharraf and Nyström, 1995), higher dissolution rate (Zhang et al., 2006), higher bioadhesion (Duchêne and Ponchel, 1997; Yoncheva et al., 2005) and corneal penetration. It has been recommended that particles be less than 10 μm to minimize particle irritation to the eye, decrease tearing and drainage of instilled dose and therefore increase the efficacy of an ocular treatment.

Many published articles have indicated the importance of particle size in ophthalmic bioavailability (Hui and Robinson, 1986; Schoenwald and Stewart, 1980); most of these articles

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prove that decreasing the particle size increases the ophthalmic bioavailability. On the other hand, the use of nanosuspensions for improving the ophthalmic bioavailability has been discussed in recent articles (Pignatello et al., 2002a,b).

Hydrocortisone, prednisolone and dexamethasone are three practically water insoluble glucocorticoid drugs. They represent the three classes of short, medium and long acting steroids, respectively. They are widely used topically for the treatment of inflammatory conditions of the conjunctiva and anterior segment of the eye. The present therapy with these drugs, mostly dictates frequent instillation in the conjunctival sac, which, besides leading to poor patient compliance, may result in administration of a large dose which, in turn, may induce glaucoma, cataract formation and damaged optic nerve (Armaly, 1986; Abel and Leopold, 1987).

The present study addresses hydrocortisone, prednisolone and dexamethasone suspensions in the sub-micron range (nano-range) in the form of nanosuspensions using high pressure homogenizer. The nano-size range represents a state of matter different from the conventional micro-crystalline suspensions so far used in practice in order to optimize their biological performance.

2. Materials and methods

2.1. Materials

Hydrocortisone, Dexamethasone, Sigma Chemistry, St. Louis, U.S.A.; Prednisolone, Effe Chemicals; Pluronic F68, BASF, Germany, Hydroxyethyl Cellulose (Natrasol 250 HHR, HERCULES, Aqualon, Netherlands), sodium chloride, disodium hydrogen phosphate anhydrous, sodium dihydrogen phosphate, EDTA, ethanol and methanol: all of analytical grade.

2.2. Methods

2.2.1. Preparation of the glucocorticoids eye drops (solution)

The eye drops of hydrocortisone, prednisolone or dexamethasone were prepared by dissolving 0.1 g of the drug in few drops of propylene glycol and completing to 100 ml with isotonic phosphate buffer (pH 6.8) containing 0.01% EDTA, 0.35 mg hydroxyethyl cellulose, 0.02% benzalkonium chloride and 0.1% pluronic F68.

2.2.2. Preparation of the glucocorticoids dispersed systems by high pressure homogenization

The drug powder in a concentration of 2.5% was dispersed in a 0.1% surfactant solution (Pluronic F68) by high speed stirrer (ultraturrax T25, Germany) at 13,000 rpm for 3 min. The starting size of each drug pre-suspension was determined using the Laser diffractometer particle size analyzer (Shimadzu, Japan).

The powder pre-suspension was added in the sample compartment of the lab-scale high pressure homogenizer (Emulsiflex C5, Canada) at room temperature. Applied pressures ranged from about 1000 to 1500 bar (15,000–22,000 psi) for up to 10 cycles.

2.2.2.1. Characterization of the prepared dispersed systems.

- *Particle size measurement*
  The particle size of the produced dispersed systems was analyzed by photon correlation spectroscopy (PCS) (Zetasizer 1000 HS, Malvern instruments, UK) yielding the mean particle diameter of the suspension. Additional particle size analysis was performed by Laser diffractometer particle size analyzer (LD) (Shimadzu, Japan). The diameters were calculated using the volume distribution. Diameters 50 and 90% mean that 50% (respectively, 90%) of the particles are below the given size. All samples were measured in suspension after particle preparation without further dilution. All the data presented are the mean values of three independent samples produced under identical production conditions.
- *Determination of the drug content*
  The dispersed systems of hydrocortisone, prednisolone or dexamethasone were assayed spectrophotometrically (Shimadzu UV, Japan) for the drug content at the wavelengthes: 247, 247 and 240 nm, respectively.

2.2.2.2. Preparation of the glucocorticoids eye drops (micro- and nanosuspensions).

An accurate amount of the dispersed systems was taken to prepare the formulae with a concentration of 0.1% of the drug, by dispersion in isotonic phosphate buffer solution of pH 6.8 and containing 0.01% EDTA, 0.02% benzalkonium chloride and 0.35 mg hydroxyethyl cellulose as viscosity imparting agent (3 cP). The surfactant (pluronic F68) was added to adjust the final concentration to be 0.1%.

2.2.3. Preparation of the nanosuspensions of different viscosities

0.1% hydrocortisone and prednisolone nanosuspensions of mean particle diameter 650 and 880 nm, respectively, were prepared in isotonic phosphate buffer of pH 6.8 and containing 0.1% EDTA, 0.02% benzalkonium chloride and 0.1% pluronic F68 using different concentrations of hydroxyethyl cellulose. The viscosity of the prepared nanosuspensions was measured at room temperature at a speed of 60 rpm (Brookfield DV+, UK).

2.2.4. Assessment of ocular bioavailability

The increase in intraocular pressure (IOP) in rabbits was taken as a tool for evaluation of the corticosteroid drug effect (Kassem et al., 1994). Male healthy Albino rabbits weighing 2–2.5 kg with normotensive eyes were considered, 10 rabbits were used for assessing the ophthalmic bioavailability of each preparation. Rabbits were placed in restraining boxes, to which they have been habituated with free access to food and water.

A standardized tonometer (ShiØetz, Reister Germany) was used to determine the intraocular pressure in conscious rabbits after instillation of one drop of tetracaine hydrochloride as local anesthetic. The resting IOP was taken two or three times a day for two days before drug application, so the normal baseline of each animal was established before next treatment. The experiments were carried out in the same laboratory, by the same person using the same instrument. A single 50 μl dose of 0.1% drug solution or suspensions of different particle sizes or different viscosities

were instilled into the lower cul-de-sac of the test eye of the rabbit by an automatic micropipette. The IOP was measured directly before and subsequent to instillation of the eye drops at frequent time intervals up to 12 h ensuring attainment of the preinstillation value. The contralateral eye of the animal was not taken as a control due to a very peculiar phenomenon encountered in such experiments, namely the existence of consensual response in the contralateral control eye which is most often encountered in intraocular pressure studies. Not uncommonly the IOP of the untreated control eye also changes upon treatment (Krakau and Wilke, 1971).

The change in IOP after instillation of the eye drops was calculated in terms of percentage increase in IOP as follows:

$$\%\ \text{increase in IOP} = \frac{\text{IOP}_{\text{postdosing}} - \text{IOP}_{\text{predosing}}}{\text{IOP}_{\text{predosing}}} \times 100$$

The pharmacodynamic parameters taken into consideration were the maximum percentage increase in IOP (% IOP_{max}), the time of maximum response (T_{max}) and the area under percentage increase in IOP versus time curve (AUC) which was calculated adopting the trapezoidal rule. Also other parameters were considered to assess the duration of drug action such as the time period over which half peak IOP response intensity prevails (HVD), half value duration relative to reference (HVDR) and the mean residence time (MRT).

Statistical analysis of the results was performed using one-way analysis of variance (ANOVA), followed by the least significant difference test (LSD). This statistical analysis was computed with the SPSS® software.

3. Results and discussion

3.1. Ophthalmic bioavailability of the glucocorticoid drugs

3.1.1. Effect of particle size in the micron and nano-size ranges on the ophthalmic bioavailability of the glucocorticoid drugs

In this study, four formulations for each drug were prepared namely, 0.1% drug solution and 0.1% drug suspensions of different mean particle diameters (539 nm, 1.39 μm and 3.94 μm) for hydrocortisone, (211 nm, 1.626 μm and 4.0 μm) for prednisolone and (930 nm, 2.46 μm and 4.89 μm) for dexamethasone. Tables 1–3 show the mean particle diameter and particle size distribution of these preparations. It should be noted, that the LD data are volume based, the PCS mean diameter is the light intensity weighted size. Therefore the PCS mean diameter and the diameter 50% from the LD are not identical. It is evident from the tables that for each drug, two formulations are in the micron-size range (mean values) and one formulation is in the nano-size range.

The mean percentage increase in IOP after instillation of drug solutions or suspensions of different mean particle diameters was computed and the data presented in Figs. 1–3. The figures show that there are marked differences between the mean percentage increase in IOP/time profile of the drug solutions and suspensions and that these differences increase with decreasing the particle diameter of drug. Fig. 1 shows that the maximum percentage increase in IOP for the hydrocortisone solution occurs 1.5 h post dosing and that the drug effect disappears after 5 h while the time for maximum percentage increase in IOP for the investigated suspensions is 1.5–2 h, and the drug effect disappears after 6–8 h. Figs. 2 and 3 show that the maximum percentage increase in IOP for prednisolone and dexamethasone investigated systems occurs 2 h post dosing. The profiles of the investigated prednisolone suspensions show retainment

Table 1
Particle size distribution of 0.1% hydrocortisone suspensions

Mean particle diameter	Particle size distribution
539 nm	90% < 1.00 μm 75% < 0.75 μm 50% < 0.53 μm 25% < 0.38 μm
1.39 μm	90% < 3.26 μm 75% < 2.15 μm 50% < 1.35 μm 25% < 0.87 μm
3.94 μm	90% < 48.1 μm 75% < 7.90 μm 50% < 2.67 μm 25% < 1.37 μm

Table 2
Particle size distribution of 0.1% prednisolone suspensions

Mean particle diameter	Particle size distribution
211 nm	90% < 0.37 μm 75% < 0.21 μm 50% < 0.13 μm 25% < 0.09 μm
1.626 μm	90% < 6.2 μm 75% < 3.3 μm 50% < 1.8 μm 25% < 1.0 μm
4.0 μm	90% < 37.7 μm 75% < 7.80 μm 50% < 4.19 μm 25% < 1.67 μm

Table 3
Particle size distribution of 0.1% dexamethasone suspensions

Mean particle diameter	Particle size distribution
930 nm	90% < 2.78 μm 75% < 1.90 μm 50% < 1.21 μm 25% < 0.68 μm
2.46 μm	90% < 8.14 μm 75% < 5.60 μm 50% < 2.37 μm 25% < 1.22 μm
4.87 μm	90% < 35.2 μm 75% < 7.59 μm 50% < 5.13 μm 25% < 2.27 μm

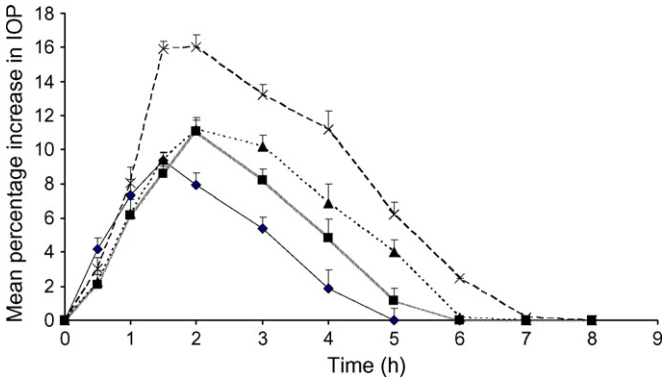

Fig. 1. Effect of drug particle size on mean percentage increase in IOP for normotensive Albino rabbits receiving 50 μl of 0.1% ydrocortisone solution or suspensions of different mean particle diameters particle diameter 539 nm (X), particle diameter 1.394 μm (▲), particle diameter 3.94 μm (■), solution (♦).

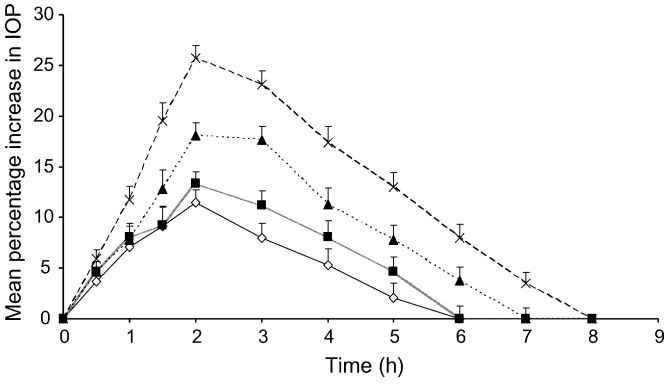

Fig. 2. Effect of drug particle size on mean percentage increase in IOP for normotensive Albino rabbits receiving 50 μl of 0.1% prednisolone solution or suspensions of different mean particle diameters particle diameter 211 nm (X), particle diameter 1.626 μm (▲), particle diameter 4.0 μm (■), solution (◊).

of drug effect up to 7–8 h (Fig. 2), while that of dexamethasone show retainment of effect up to 9–11 h (Fig. 3). It is also evident from the Figs. 1–3 that suspensions compared to the solution, lead to an increase in maximum percentage increase in

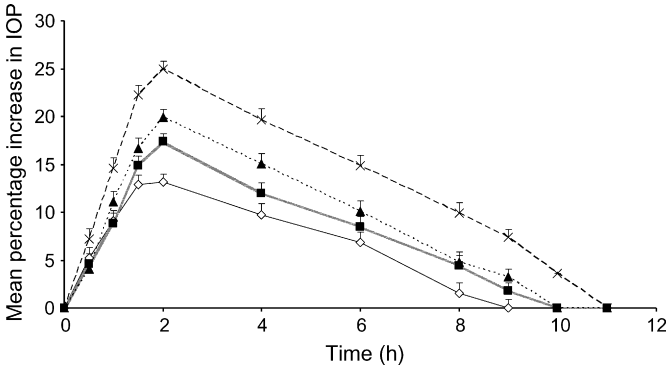

Fig. 3. Effect of drug particle size on mean percentage increase in IOP for Albino rabbits receiving 50 μl of 0.1% dexamethasone solution or suspensions of different mean particle diameters particle diameter 930 nm (X), particle diameter 2.46 μm (▲), particle diameter 4.87 μm (■), solution (◊).

IOP. The greatest increase in peak occurs after instillation of the nanosuspensions.

Tables 4–6 summarize the pharmacodynamic parameters for 0.1% drug solutions and suspensions of different mean particle diameters. It is obvious from the tables that there are marked differences between the pharmacodynamic parameters of the drug solutions and suspensions and that these differences are more prominent in the case of the nanosuspensions.

The suspensions are found to induce a particle size dependent increase in maximum percentage increase in intraocular pressure (Tables 4–6). The percent increase in IOP is the least for the solution; it is slightly higher in the case of micron-sized suspensions. The nanosuspension behaves totally different not only from the solution but also from the micron-sized suspensions. The nanosuspension nearly doubles the percentage IOP_{max} observed for the solution (76–124% increase) (Tables 4–6). These results point to an increase in the intensity of drug action when present in form of nanosuspension.

The results show that the time of maximum response (T_{max}) is reached in the case of solution earlier than in case of the suspensions. For hydrocortisone there is an observed retardation in T_{max} of the drug with decreasing the particle size (Table 4),

Table 4
Mean values of pharmacodynamic parameters for hydrocortisone solution and as well as micro- and nanosuspensions (value ± S.E.)

Hydrocortisone preparations	% IOP_{max}	T_{max} (h)	$AUC_{0-8\,h}$ (% increase in IOP. h)	HVD (h)	HVDR (h)	MRT (h)
(A) Hydrocortisone solution	9.77 ± 0.37***	1.35 ± 0.11†	23.27 ± 1.08***	2.36 ± 0.28*	2.36 ± 0.28***	2.40 ± 0.08***
(B) Hydrocortisone nanosuspension of mean particle diameter 539 nm	17.22 ± 1.32	1.60 ± 0.10	56.89 ± 4.60	3.13 ± 0.29	4.69 ± 0.26	3.47 ± 0.15
(C) Hydrocortisone microsuspension of mean particle diameter 1.39 μm	11.19 ± 0.93***	1.80 ± 0.08†	39.74 ± 4.11**	3.58 ± 0.25†	3.95 ± 0.32†	3.10 ± 0.14*
(D) Hydrocortisone microsuspension of mean particle diameter 3.94 μm	11.04 ± 0.71***	1.90 ± 0.07*	30.85 ± 2.52***	3.00 ± 0.28†	3.23 ± 0.28**	2.85 ± 0.11**

Statistical differences between the nanosuspensions and the other investigated systems.
* $p = 0.05$ significant.
** $p = 0.01$ highly significant.
*** $p = 0.001$ very highly significant.
† Insignificant.

Table 5
Mean values of pharmacodynamic parameters for prednisolone solution and as well as micro- and nanosuspensions (value ± S.E.)

Prednisolone preparations	% IOP_{max}	T_{max} (h)	$AUC_{0-8\,h}$ (% increase in IOP. h)	HVD (h)	HVDR (h)	MRT (h)
(A) Prednisolone solution	11.49 ± 0.83***	1.80 ± 0.08†	34.60 ± 2.64***	3.11 ± 0.31*	3.11 ± 0.31***	2.88 ± 0.08***
(B) Prednisolone nanosuspension of mean particle diameter 211 nm	25.70 ± 1.24	1.85 ± 0.07	103.18 ± 7.21	3.87 ± 0.28	5.82 ± 0.27	3.71 ± 0.07
(C) Prednisolone microsuspension of mean particle diameter 1.626 μm	18.38 ± 0.43***	2.00 ± 0.13†	66.44 ± 2.84***	3.20 ± 0.19†	4.85 ± 0.29*	3.50 ± 0.08†
(D) Prednisolone microsuspension of mean particle diameter 4.0 μm	13.30 ± 1.03***	1.90 ± 0.06†	44.95 ± 2.92***	3.60 ± 0.16†	4.17 ± 0.18***	3.06 ± 0.09***

* $p = 0.05$ significant.
*** $p = 0.001$ very highly significant.
† Insignificant.

while in the case of prednisolone and dexamethasone, T_{max} is generally slightly affected by the particle size (Tables 5 and 6).

It is obvious that the area under percentage increase in IOP versus time curve (AUC) values for all the suspensions are higher than that for the drug solutions (Tables 4–6). This effect is most pronounced in the case of the nanosuspension; there is an inverse relationship between the particle size of the suspension and the AUC. These results are in good agreement with the previously reported results (Hui and Robinson, 1986; Schoenwald and Stewart, 1980) which found that the bioavailability of the drug increase with decreasing the particle size. The results indicate a pronounced augmentation of the bioavailability of the drug when prepared in a nanosuspension form.

The half value duration (HVD) expression was proposed (Meier et al., 1974) to describe duration of drug action in extended release dosage forms. Meier and Coworkers assumed that the conventional, i.e. non-controlled release product is effective in the neighborhood of the maximum concentration. The time during which the plasma concentration is at least half of the effective maximum concentration is considered as a pharmacokinetic parameter which is correlated to the efficacy range. In this work we adopt the expression of Meier "HVD" replacing, drug concentration by drug effect. The results show that the HVD-values are greater for the suspensions as compared to the solution. However within hydrocortisone suspensions, there is little correlation between the particle size and HVD-values (Table 4). These results are not astonishing, since the maximum effect corresponding to the maximum concentration of Meier differed by more than 20%, which according to Meier, leads to misleading results. In the case of prednisolone and dexamethasone (Tables 5 and 6) the greatest augmentation in HVD-values occurs after the instillation of their nanosuspension.

To overcome the problem of the great differences (exceeding 20%) in peak heights, another approach is adopted. This approach is based on taking the half value (HV) of maximum response for drug solution as a basis for comparison. The duration for drug suspensions is then compared at this half value of maximum response for the solution (taken as reference) i.e. the duration is compared for one and the same level of drug effect. By calculating the mean of the half value of maximum response for the drug solution, it was found to be 4.78, 5.75 and 6.90% IOP for hydrocortisone, prednisolone and dexamethasone, respectively. At this value the duration of effect for all the investigated systems is calculated. It is evident from the Tables 4–6 that the HVDR-values are greater for drug suspensions as compared to the solution and that the duration is inversely proportional to the

Table 6
Mean values of pharmacodynamic parameters for dexamethasone solution and as well as micro- and nanosuspensions (value ± S.E.)

Dexamethasone preparations	% IOP_{max}	T_{max} (h)	$AUC_{0-11\,h}$ (% increase in IOP. h)	HVD (h)	HVDR (h)	MRT (h)
(A) Dexamethasone solution	13.87 ± 1.05***	1.55 ± 0.09†	66.02 ± 5.85***	4.82 ± 0.33*	4.82 ± 0.33***	4.33 ± 0.11*
(B) Dexamethasone nanosuspension of mean particle diameter 930 nm	24.97 ± 1.27	1.75 ± 0.08	148.05 ± 7.69	6.10 ± 0.29	8.39 ± 0.17	4.95 ± 0.30
(C) Dexamethasone microsuspension of mean particle diameter 2.46 μm	19.95 ± 0.43***	1.90 ± 0.06†	101.49 ± 7.43***	5.00 ± 0.49*	6.56 ± 0.47***	4.78 ± 0.15†
(D) Dexamethasone microsuspension of mean particle diameter 4.89 μm	17.35 ± 0.81***	1.80 ± 0.08†	88.92 ± 8.59***	4.69 ± 0.42*	6.05 ± 0.50***	4.83 ± 0.14†

* $p = 0.05$ significant.
*** $p = 0.001$ very highly significant.
† Insignificant.

Table 7
Particle size distribution of 0.1% hydrocortisone and prednisolone nanosuspensions

Drug	Mean particle diameter (nm)	Particle size distribution
Hydrocortisone	650	90% < 2.77 μm 75% < 1.95 μm 50% < 0.94 μm 25% < 0.11 μm
Prednisolone	880	90% < 4.3 μm 75% < 2.3 μm 50% < 1.1 μm 25% < 0.4 μm

particle size of the suspension. The greatest increase in HVDR is observed for the nanosuspension. This reveals that preparation of the drug in the form of nanosuspension leads to an increase in duration of drug action.

The mean residence time (MRT) (Riegelman and Collier, 1980; Yamaoka et al., 1978) can also be considered as the expected value of the distribution of the residence time of the molecules administered with one dose. In this case, the AUC adjusted concentration/time curve serves as density function of the residence time (Brockmeier, 1982). In this part, the expression MRT is borrowed from the pharmacokinetics and utilized to describe the pharmacodynamics of the ophthalmic preparations through replacing drug concentration by drug effect (IOP). It is evident from Tables 4–6 that the MRT-values increase with decreasing the particle size of the suspension and that the nanosuspension shows the highest MRT values. This indicates a pronounced augmentation of the duration of drug action when the drug is prepared in the form of nanosuspension.

Statistical analysis (Tables 4–6) of the pharmacodynamic parameters for the three drugs show that the differences between the nanosuspensions and all the other investigated systems in most cases are very highly significant ($p < 0.001$), while the differences is not significant ($p > 0.05$) in some cases of T_{max} and parameters showing the duration of drug action.

3.1.2. *Effect of viscosity of vehicle on the ophthalmic bioavailability of hydrocortisone and prednisolone nanosuspensions*

Table 7 shows the particle size distribution of hydrocortisone and prednisolone nanosuspensions of different viscosities. The mean values of the percentage increase in intraocular pressure (% IOP) for Albino rabbits after inistillation of 50 μl of 0.1% hydrocortisone nanosuspensions of mean particle diameter 650 nm and of different viscosities (7.08, 10.2, 14.5, 22.0 and 27.5 cP) and prednisolone nanosuspensions of mean particle diameter 880 nm and of different viscosities (6.0, 8.28, 15.1, 24.0 and 33.5 cP) as function of time are shown in Figs. 4 and 5. It is evident from the figures that there are marked differences in the mean of the percent increase in IOP/time profile for all the investigated systems. In the case of hydrocortisone (Fig. 4) it could be seen that the time of maximum percent increase in intraocular pressure (% IOP_{max}) is 1.5 h for the nanosuspensions of viscosity 7.08 cP and 10.2 cP; this time increases with increasing

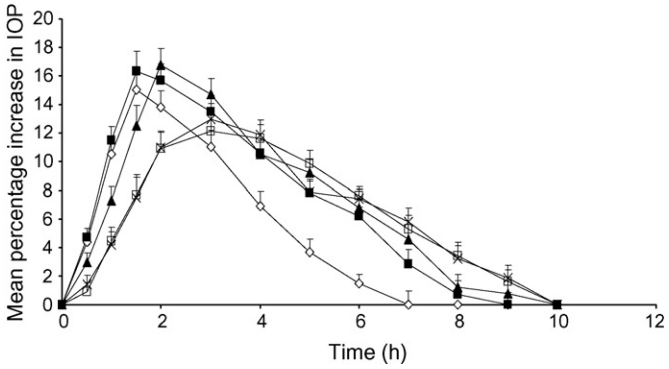

Fig. 4. Effect of viscosity on mean percentage increase in IOP for normotensive Albino rabbits receiving 50 μl of 0.1% hydrocortisone nanosuspension of mean particle diameter 650 nm viscosity 7.08 cP (◊), viscosity 10.2 cP (■), viscosity 14.5 cP (▲), viscosity 22.0 cP (X), viscosity 27.5 cP (□).

viscosity to reach 3 h for nanosuspensions of higher viscosity. On the other hand, the profiles of the investigated nanosuspensions show retainment of effect up to 6–9 h depending on the viscosity. For prednisolone (Fig. 5), the time of maximum percent increase in intraocular pressure (% IOP_{max}) is 2 h post dosing for the nanosuspensions of viscosities 6.0 and 8.28 cP and this time increases with increasing viscosity till it reaches 3 h for the other nanosuspensions. It could be observed that the maximum percent increase in intraocular pressure occurs after instillation of the nanosuspensions of viscosity 24.0 cP; increasing viscosity further to 33.5 cP leads to a decrease in maximum percent increase in intraoccular pressure. On the other hand, the profiles of the investigated nanosuspensions show retainment of effect for 7–11 h depending on the viscosity.

These results may shed light on the increase of duration of drug action with increasing the viscosity and these results are in agreement with many results considering the effect of viscosity on drug action using the rabbit's eye.

Tables 8 and 9 summarizes the pharmacodynamic parameters for 0.1% hydrocortisone and prednisolone nanosuspensions of different viscosities. In the case of hydrocortisone it is obvious that the intensity of drug action increases with increasing

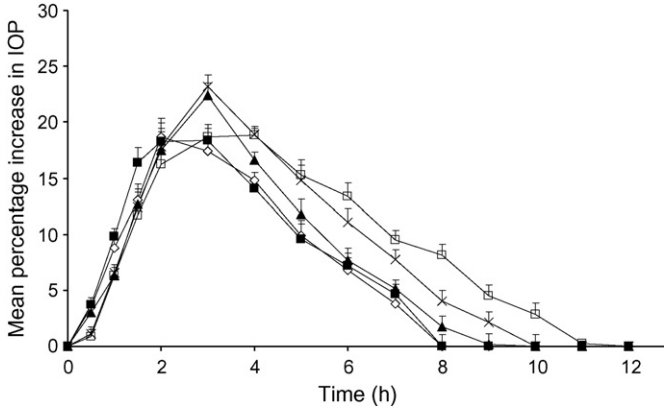

Fig. 5. Effect of viscosity on mean percentage increase in IOP for normotensive Albino rabbits receiving 50 μl of 0.1% prednisolone nanosuspension of mean particle diameter 880 nm viscosity 6.0 cP (◊), viscosity 8.28 (■), viscosity 15.1 cP (▲), viscosity 24.0 cP (X), viscosity 33.5 cP (□).

Table 8
Mean values of pharmacodynamic parameters for hydrocortisone nanosuspensions of mean particle diameter 650 nm and of different viscosities (value ± S.E.)

Hydrocortisone nanosuspensions	% IOP_{max}	T_{max} (h)	$AUC_{0–10\,h}$ (% increase in IOP. h)	HVD (h)	HVDR (h)	MRT (h)
(A) Nanosuspension of viscosity 7.08 cP	15.20 ± 0.97	1.45 ± 0.08	48.49 ± 3.86	3.16 ± 0.14	3.16 ± 0.14	2.93 ± 0.14
(B) Nanosuspension of viscosity 10.2 cP	16.62 ± 0.92†	1.50 ± 0.07†	69.11 ± 7.41*	3.89 ± 0.28*	4.63 ± 0.40**	3.58 ± 0.16**
(C) Nanosuspension of viscosity 14.5 cP	16.82 ± 0.99†	1.75 ± 0.08†	71.86 ± 4.88**	4.01 ± 0.22*	4.96 ± 0.27**	4.09 ± 0.12**
(D) Nanosuspension of viscosity 22.0 cP	12.96 ± 1.13†	2.30 ± 0.15***	67.15 ± 6.93*	5.37 ± 0.29***	4.40 ± 0.53*	4.57 ± 0.13***
(E) Nanosuspension of viscosity 27.5 cP	12.07 ± 1.13*	2.25 ± 0.17***	65.32 ± 6.75†	5.40 ± 0.31***	4.45 ± 0.39*	4.53 ± 0.17**

Statistical differences between the nanosuspension of viscosity 7.08 cP and the other investigated systems.
* $p = 0.05$ significant.
** $p = 0.01$ highly significant.
*** $p = 0.001$ very highly significant.
† Insignificant.

Table 9
Mean values of the pharmacodynamic parameters for prednisolone nanosuspensions of mean particle diameter 880 nm and of different viscosities (value ± S.E.)

Prednisolone nanosuspensions	% IOP_{max}	T_{max} (h)	$AUC_{0–12\,h}$ (% increase in IOP. h)	HVD (h)	HVDR (h)	MRT (h)
(A) Nanosuspension of viscosity 6.0 cP	19.69 ± 1.41	2.05 ± 0.12	79.41 ± 7.06	3.81 ± 0.25	3.81 ± 0.25	3.84 ± 0.09
(B) Nanosuspension of viscosity 8.28 cP	19.01 ± 1.41†	2.00 ± 0.12†	82.69 ± 7.07†	3.93 ± 0.27†	6.19 ± 0.38***	3.84 ± 0.08†
(C) Nanosuspension of viscosity 15.1 cP	22.18 ± 1.06†	2.45 ± 0.19†	89.63 ± 7.08†	3.86 ± 0.37†	6.22 ± 0.40***	4.14 ± 0.07**
(D) Nanosuspension of viscosity 24.0 cP	23.26 ± 1.54*	2.60 ± 0.16*	103.98 ± 10.75*	4.17 ± 0.39†	7.36 ± 0.37***	4.54 ± 0.12***
(E) Nanosuspension of viscosity 33.5 cP	19.15 ± 0.93†	3.00 ± 0.26***	113.95 ± 6.55**	5.60 ± 0.26***	8.78 ± 0.29**	5.21 ± 0.13***

Statistical differences between the nanosuspension of viscosity 6.0 cP and the other investigated systems.
* $p = 0.05$ significant.
** $p = 0.01$ highly significant.
*** $p = 0.001$ very highly significant.
† Insignificant.

viscosity up to 14.5 cP. Also the time of maximum drug action increases with increasing viscosity up to 22.0 cP. The onset of drug action is nearly equal in case of viscosities 22.0 and 27.5 cP. On the other hand, the bioavailability of hydrocortisone nanosuspensions increases with increasing viscosity up to 14.5 cP as indicated from the values of $AUC_{0–10\,h}$. In the same time duration of action increases with increasing viscosity up to 14.5–22.0 cP as indicated from the values of HVD, HVDR and MRT.

For prednisolone, it is obvious from Table 9 that increasing the viscosity of prednisolone nanosuspensions up to 33.5 cP leads to a retardation in the onset of drug action as indicated by the values of T_{max} and to an increase in the drug bioavailability as indicated by the values of $AUC_{0–12h}$. The duration of drug action increases with increasing viscosity to 33.5 cP as indicated from the values of HVD, HVDR and MRT. The greatest increase in the intensity of drug action occurs after instillation of the nanosuspension of the viscosity 24.0 cP as indicated from the values of percentage IOP_{max}.

Statistical analysis of the pharmacodynamic parameters for hydrocortisone and prednisolone nanosuspensions of different viscosities show that the effect of viscosity is more prominent in the case of duration of drug action, since the differences in most cases are very highly significant ($p < 0.001$).

In conclusion, compared to solution and micro-crystalline suspensions it is a common feature of the three nanosuspensions that they exhibit a higher intensity of drug action (expressed as IOP_{max}) and higher extent of drug absorption (expressed as AUC). Also the parameters describing duration of drug action namely HVD, HVDR and MRT are for nanosuspensions in the majority of cases higher compared to the solution and microsuspensions. This indicates that the nanosuspensions enhance always the rate and extent of ophthalmic drug absorption as well as the intensity of drug action, this is in accordance with the earlier studies which have concluded that nanoparticles improve the ophthalmic drug bioavailability (Pignatello et al., 2002a,b). In the majority of cases nanosuspensions extend the duration of drug effect to a significant extent. The data presented confirm that nanosuspensions differ from micro-crystalline suspensions and solution as ophthalmic drug delivery systems and that the differences are statistically, very highly to highly significant. The results confirm also the importance of viscosity of nanosuspension especially in increasing the duration of drug action.

Nanosuspensions are effective in ophthalmic drug delivery allowing lower doses and less frequent instillation. The present therapy with conventional eye drops (solution and microsuspensions) dictates frequent instillation, and leads to poor patient compliance which may result in administration of a large dose inducing glaucoma, cataract formation and damage of optic nerve. Nanosuspensions, besides being more convenient to the patient, would provide an alternative therapy with fewer side effects.

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