DEVELOPMENT OF NEW OPHTHALMIC SUSPENSION PREDNISOLONE ACETATE 1%

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ABSTRACT
Formulation of a new Prednisolone acetate (1%) ophthalmic suspension to obtain a better contactibility of the drug at the site of action. The formulation was evaluated and optimized based on physiological, physicochemical and pharmaceutical parameters and prepared aseptically, keeping particle size between 1-3 µm, while viscosity enhancer, preservative, chelating agent were used to increase the transient residence time, antimicrobial preservation respectively. Isotonicity of the formulation was maintained. Buffering agents used were having buffering capacity NMT 0.05%. pH adjusted at which Prednisolone acetate was stable. The finished product subjected to stress conditions to check the physical stability of the formulation. The formulation was packed in LDPE plastic vials, and subjected to accelerated stability studies at 40^{o}C and the kinetic and predictive method was employed for the determination of degradation rate constant and shelf life. The results showed that the development of a new ophthalmic formulation having significantly better contact time by the selection and optimization of viscosity (HPMC) that obtained (21 cps) with better stability studies. The results concluded that prednisolone acetate 1.0 per cent ophthalmic suspension is more effective than prednisolone phosphate 1.0 per cent ophthalmic solution in suppressing corneal inflammation.

Keywords: Ophthalmic suspension, Prednisolone acetate formulation, Formulation development, evaluation studies.

INTRODUCTION

The human eye is a challenging subject for topical administration of drugs. The basis of this can be found in the anatomical arrangement of the surface tissues and in the permeability of the cornea. The protective operation of the eyelids and lacrimal system is such there is rapid removal of material instilled into the eye, unless the material is suitably small in volume and chemically and physiologically compatible with surface tissue (Gennaro and Easton, 1990).

Ophthalmic preparations are sterile liquid, semi-solid or solid preparations intended for administration upon the eyeball and/or to the conjunctiva or for insertion in the conjunctival sac (British Pharmacopoeia, 2007).

These products may be administered topically (in the form of solutions, suspensions, emulsions, lotions (Kramer and Behrens-Baumann, 2002), creams, ointments and gels) or by subconjunctival or intraocular (e.g., intravitreal and intracameral) injection. The sterility of these products, as well as accuracy in the calculation and preparation of doses is of great importance (Anonymous 1990a, b). It is estimated that only one-tenth of a dose penetrates into the eye (Hecht, 1995).

Development of ophthalmic formulation
Effectiveness, tolerance, and stability of the ophthalmic preparation were determined by physiological conditions at the eye, the physico-chemical properties of the drug substance itself and the formulation of the eye preparation (Kramer and Behrens-Baumann, 2002).

The parameters namely sterility (Gennaro and Easton, 1990), clarity "for solution" (www.paddocklabs.com), tonicity, pH, buffer, buffer capacity, preservative (Gennaro and Easton 1990), solubility "for solution", stability, incompatibilities, viscosity (Anonymous 1989), antioxidant (www.paddocklabs.com), particle size "for suspension and ointment" (Gennaro and Easton, 1990), redispersibility "for aqueous suspension" (www.free-patentsonline.com), packaging and storage (Anonymous, 1989) define the bioavailability of the active ingredient. Sometimes the requirements for optimal effectiveness, tolerance, and stability compete with each other and compromises have to be sought.

MATERIALS AND METHODS

Material
Excipients used were purchased from suppliers in compliance with the relevant United States Pharmaco-poeia/National Formulary, British Pharmacopoeia monographs and active prednisolone (as Prednisolone acetate salt) was procured from Crystal Pharma Spain. While the primary packing material like bottles and tips were made up of low density polyethylene and the caps were made of polystyrene. Titanium dioxide was the colorant used in the bottles, tips and caps.

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Table 1: Formulation composition of ophthalmic suspension

S. No.	Ingredients	Function	Percentage range commonly use
1	Prednisolone acetate	Active Ingredient	1% w/v
2	Dimethyl Sulfoxide + PVP K-30	* Solvent	1% w/v
3	Benzalkonium Chloride	Preservative	0.01 – 0.02 % w/v
4	Edetate Disodium	Chelating agent	0.005 – 0.1% w/v[10]
5	Boric Acid	Buffer	Quantity sufficient (Q.S) to buffer capacity NMT 0.5%
6	Sodium Borate	Buffer	
7	Sodium Chloride	Isotonic agent	Quantity sufficient to Osmolality 308 mOsmol/Kg
8	Polysorbate 80	Surfactant	of 0.1 – 3 %[10]
9	HPMC 4000 cps	Viscosity Enhancer	0.45 – 1.0 %
10	HCl / NaOH	pH adjusting agent	Quantity sufficient to pH 5 – 6
11	Purified Water	Vehicle	Quantity sufficient to required volume

Equipments

Manufacturing equipments were Pyrex Glass Beaker, Mechanical Stirrer, Water Bath, Thermometer; 0.2 micron filters (Sartorius) and Vacuum Pump with Filtration Assembly, Stainless Steel Mixer, Homogenizer and Laminar Air flow.

Method:

The product was designed as ophthalmic suspension. Efforts were made to produce ophthalmic suspension with reduced particle size to prevent local irritation. During the formulation development it was kept in consideration that the range of excipients will be suitable for large scale industrial manufacturing (table 1). Other parameters like sterility, isotonicity, viscosity, pH, buffers and buffer capacity, redispersibility were also considered to make a quality product.

- Dimethyl Sulfoxide and 1% (w/v) PVP K-30 solution was used in the first step to reduce the particle size of the active ingredient and but it was not the part of finished product.
- Ref.(10): is from "Handbook of Pharmaceutical Excipients" edited by: Rowe, Raymond C.; Sheskey, Paul J.; Owen, Sian C. (2006) Edi: 5th by Wolters kluwer; Alphen aan den Rijn, the Netherlands; is a comprehensive guide to the uses, properties and safety of pharmaceutical excipients and is an essential reference for those involved in the development, production, control or regulation of pharmaceutical preparations. The Information in this book Collects together from various international sources. So the range given in this is according to this excipient book.

Osmolality calculation adjustment

Lacrimal fluid has an osmolality value equivalent to that of a 0.9% sodium chloride solution that is 308 mOsmol / Kg. In the present formulation, the following formula was used where osmolality of each ingredient in formulation was determined and sum of all values was subtracted from 308 and remaining osmolality was adjusted by adding NaCl.

Osmolality of Ingredient = Qty in g/L ÷ mol. wt x (i value) x 1000 = mOsmol / Kg

Study of previous literatures showed that there was no universal approach on the bases of which the osmolality of the formulation could be checked. In the proposed method the attempt was made to develop isotonicity prior to manufacturing of the formulation. Buffering agents were used with buffering capacity not more than 0.05% and the work was done in the range of pH at which Prednisolone acetate was stable.

Maintaining the product sterility during manufacturing

Suspensions and ointments formulation should not be filtered as the drug will be removed by the filter. For these types of formulations, the individual ingredients were sterilized separately and then they are formulated using aseptic techniques (http://pharmlabs.unc.edu).

In the present study, Prednisolone acetate was sterilized during particle size reduction by filtration and the remaining portion was also sterilized by filtration before mixing with active ingredient.

Compatibility with the container/closure system

The compatibility with the container / closure system has been checked by stability testing of the product in the proposed packaging material.

Procedure

1. Prednisolone acetate was dissolved in Dimethyl Sulfoxide (DMSO) and passed the solution through 0.2 micron filters to sterile the solution with continue mixing. 1% (w/v) PVP K-30 solution was passed

through 0.2 micron filter to sterile the solution and transferred it to above solution drop wise. When all solution consumed, resultant mixture was passed through 0.2 micron filter. Filtrate (active material) was washed with purified water previously passed through 0.2 micron filter.

2. In an appropriate amount of purified water, HPMC was slowly dispersed and hydrated.
3. In next step, following ingredients were added in an appropriate amount of purified water in a separate beaker, (in an order allowing each to dissolve completely before adding the next) Edetate Disodium, Boric Acid, Benzalkonium Chloride, Sodium Chloride, Sodium Borate, Polysorbate 80 and finally hydrated HPMC solution was added and pH was adjusted.
4. Solution was passed through 0.2 micron filter and collected in a suitable calibrated vessel for made up the final volume.
5. Under aseptic condition, Filtrate of Active Ingredient was transferred to above solution; volume was adjusted and mixed well. Finally, suspension was filled in previously sterilized LDPE vial under aseptic condition.

RESULTS AND DISCUSSION

The purpose of present study was to develop 1% Prednisolone Acetate ophthalmic suspension in order to obtain an effective response. The selection of excipients (table 1) that were used for the formulation of ophthalmic suspension was done very carefully keeping in mind the compatibility with the active ingredient as well as with the other formulation ingredients and container and closure system.

The product was inspected through different aspects that specified their limits. Following tests were carried out by using calibrated instruments to ensure quality of product (table 2). All tests were carried out and documented in an average of triplicate results:

Table 2 indicated that the Physical Appearance of the formulated suspension was as that reported in the standard that was dense, white microfine suspension, having pH 5.0-6.0, Osmolality 255- 315 mOsm/Kg. Particle Size was adjusted between 1-3 micron to obtain macroscopic appearance not more than (NMT) 10 agglomerates/ml. To check the abrasiveness of formed agglomerates, Grit Test was performed that indicated no any type of irritation, absence of dense particulates indicated that there were no deposits; and Viscosity was 15-25 cps. The irritation was checked by instillation of suspension into the eye, the formulation qualified the Local irritation test, whereas the Resuspendability or Physical stability of the formulation was checked by accelerated settling resuspension time that must be “NMT 8 Seconds”; and Real time settling number of inversions for complete resuspension that must be “NMT 10 inversions” had been done, the result indicated that the Resuspendability as well as the physical stability complied the standard. The assay for active ingredient (Prednisolone Acetate) was also performed that was between 0.90% - 1.10% and finally the Sterility test indicated that the sterility limits of the suspension was according to the USP requirements.

Shelf life of Prednisolone acetate suspension

As the stability was the main concern (table 3), product subjected to 40^{o}C and sample was analyzed initially and then after 1 month, 2 months, 3 months and 6 months. The result obtained from accelerated temperature study that provided further support to the shelf life of the product that can be calculated using Arrhenis Equation.

The analytical methods used to determine the stability of a product was capable of separating the intact compound of interest from any decomposition products or from potentially interfering substances (USP 2007).

Fig. 1 showed that there was a linear relation ($r^2 = 0.9809$)

Table 2: Physical parameters of ophthalmic suspension

S. No.	Test	Limits	Results
1	Physical Appearance	Dense, white microfine suspension	Passes
2	pH	5.0 – 6.0	5.62
3	Osmolality	255- 315 mOsm/Kg	307 mOsm/Kg
4	Particle Size	1 - 3 micron	1 - 3 micron
5	Grit Test	Passes if no abrasiveness of formed agglomerates	Passes
6	macroscopic appearance	NMT 10 agglomerates/ml	2 agglomerates/ml
7	Absence of dense particulates	Passes if no deposits	Passes
8	Viscosity	15–25 cps	21 cps
9	Resuspendability / Physical stability	*NMT 8 seconds / NMT 10 inversions	5 sec / 4 inversions
10	Local irritation	Pass if no irritation upon instillation into eye	Passes
11	Sterility	Meet USP requirements	Passes
12	Prednisolone Acetate	0.90% - 1.10 %	1.04%

*Resuspendability / Physical stability (Accelerated settling Resuspension time “NMT 8 Seconds”; Real time settling number of inversions for complete resuspension “NMT 10 inversions”).

between the Sampling interval (hr) when plotted versus remaining quantity of drug (mg) in Prednisolone Acetate ophthalmic suspension (10mg/ml

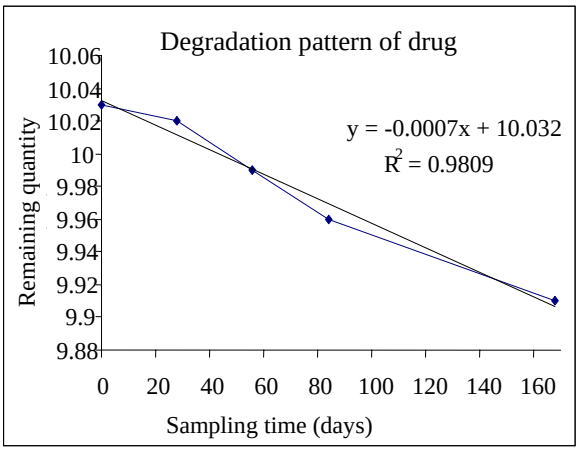

Fig. 1: Estimation of shelf-life Degradation (In days).

1st condition (First order reaction)

$$\text{Log}\left[\frac{a}{a-w}\right] = \frac{kt}{2.303}$$

Shelf Life (t_{90}) = $\dfrac{0.1046}{k}$

Half life ($t_{1/2}$) = $\dfrac{0.693}{k}$

Rate constant of degradation at 95% confidence limit

$K = (2.303)\,[(-1.35771 \times 10^{-6}) \pm (2.02)\,(1.4381 \times 10^{-7})]$
$= 2.303\,[(-1.35771 \times 10^{-6}) \pm (2.90496 \times 10^{-7}]$
$= -3.79582 \times 10^{-6}$ mg/hr

Determination of Shelf life t_{90}

$T_{90\%} = \underline{0.105} = \dfrac{0.105}{K} \qquad 3.79582 \times 10^{-6}$

= 27662 hours = approx. 1152 days and 6hours

or 3.15 years

2nd condition; (Zero Order Rate Kinetic)

Rate constant of degradation at 95% confidence limit

$K = -3.11658 \times 10^{-5} \pm (2.02)(3.1094 \times 10^{-6})$
$= -3.11658 \times 10^{-5} \pm 6.28099 \times 10^{-6}$
$= -3.174468 \times 10^{-5}$ mg/hr

Initial strength of Prednisolone acetate at 95% confidence limit

$D_O = 10.03 \pm (2.02)\,(3.9480 \times 10^{-3})$
$D_O = 10.03 \pm 7.9749 \times 10^{-3}$
$D_O = 10.02$ mg

Determination of Shelf life $t_{90\%}$

$T_{90\%} = \dfrac{0.1046\, D_O}{K_O} = \dfrac{0.1046 \times 10.02}{3.174468 \times 10^{-5}}$

= 27988 hours = approx. 1166 days and 2 hours

Or 3.19 years

The rate of degradation constant and shelf life (table 4) was determined by using of kinetic and predictive method. The product was tested by both via a zero order and a first order reaction mechanism and the shelf life was determined at which the product was required to loose 10% of the content as per label claim. The justification for 36 month expiry date for Prednisolone acetate ophthalmic suspension was reflected by the linear degradation pattern of drug during 0-6 months that was from 10.03mg to 9.91mg (fig. 1).

From the shelf life study (1st and 2nd conditions), it was observed that the Prednisolone acetate is stable for 3 years. On the bases of this study, an average shelf life of 3 years has been recommended for the formulation when packed in low density polyethylene plastic vial.

It has been reported that Generic preparations of Prednisolone acetate was significantly less potent because of markedly larger particle sizes, approximately 10 µm in diameter. The larger particles decrease effective exposure of drug to ocular surfaces, reducing absorption and potency (Arif and Foster, 2008). It has also been reported that three suspensions of 0.1% Dexamethasone were prepared with mean particle sizes of 5.75, 11.5, and 22.0 micron. A statistically significant rank-order correlation was observed between increasing drug levels and decreasing particle size (Schoenwald and Stewart, 1980). In the present study, a formulation was developed with particle size 1-3 µm to increase effective exposure of drug to ocular surface and ultimately feasible for absorption.

Further it has been reported that a major problem being faced in ocular therapeutics is the attainment of an optimal concentration at the site of action. Poor bioavailability of drugs from ocular dosage forms is mainly due to transient residence time (Kaur and Kanwar, 2002). It is also reported that aqueous ophthalmic drug solutions typically exhibit low bioavailability due to various loss processes such as drainage, tear turnover, nonproductive absorption, and protein binding. In Suspensions, bioavailability may improve by increasing residence time and corneal permeability rate constant, therefore dissolution rate of the drug and its intrinsic solubility must be considered (Hui and Robinson, 1986). In the proposed study, attention was paid onto the increased residence time by the selection and optimizing the quantity of viscosity increasing agent (HPMC) and desired viscosity was obtained (21 cps) by using Fisher Brand Calibrated Viscometer Tube Cannon-Fenske Routine (Model # 40076E).

Sjostrom *et al.* (1993) had reported that submicron particles of sparingly water soluble drugs would be prepared by precipitation in oil-in-water emulsions. In which the steroid was dissolved in an organic solvent, which was emulsified in water in the presence of

Table 3: Inter-day variability (results based on average of three triplicate reading on 40°C)

S.No.	Sampling Interval in Hours	Peak area (standard)	Peak area (sample)	Assay (%)	± SD	± RSD
1	0	2235.55860	2265.37207	100.3		
2	672 (28days)	2235.54896	2263.10371	100.2		
3	1344 (56 days)	2562.88745	2586.70899	99.9	0.487	0.00487
4	2016 (84days)	2235.37122	2249.37334	99.6		
5	4032 (168days)	2243.32277	2246.04250	99.1		

Table 4: Statistical analysis/determination of degradation rate constant

S.No.	Sampling Interval in Hours (x)	Prednisolone acetate remaining (mg) Conc = mg/ml	Log Conc. of Prednisolone acetate remaining (y)
1	0	10.03	1.0013
2	672	10.02	1.0009
3	1344	9.99	0.9996
4	2016	9.96	0.9983
5	4032	9.91	0.9961

surfactant that gave a water continuous emulsion. As the organic solvent was evaporated, the steroid precipitated. In the present study, a new technique was developed where Prednisolone acetate was dissolved in Dimethyl Sulfoxide and passed the solution through 0.2 micron filter to sterile the solution under aseptic condition and maintained the constant temperature of mixture at 20°C. Speed of mixing adjusted with mechanical stirrer. Start Mixing at 250 rpm. Transfer 1% (w/v) Polyvinyl-pyrrolidone K-30 solution in water that previously passed through 0.2 micron filter, into the above mixture at the rate of 2.5 ml/min. When all the PVP solution consumed with continue stirring, passed the mixture through 0.2 micron filter. Washed filtrate that containing active material, with sterile distilled water. The end product was inspected by Biocular Microscope "Manufacturer Swift" Model # M3300-D, equipped with a micrometer eyepiece. Ninety nine (99) percent particles sizes were in between 1-3 microns.

Howard *et al.* (1987) has been reported that Particle-size of prednisolone acetate was determined by using a resistance particle counter. Where as in the present study Particle-size of prednisolone acetate was determined using Biocular Microscope "Manufacturer Swift" Model # M3300-D, equipped with a micrometer eyepiece.

Howard *et al.* (1977) reported that significant differences in dissolution rates were noted for the generic formulation. Particle size affected dissolution rate but did not relate all observed variations in the dissolution. Formulation differences, specifically the presence of hydroxypropyl methylcellulose, in suspensions seemed to be important in dissolution. Presently hydroxypropyl methylcellulose was used as suspending agent.

Yanagawa *et al.* (1987) suggested that a lipid microsphere ophthalmic preparation of various lipophilic drug including steroids might be useful as a drug delivery system for ophthalmic therapy. Kupferman *et al.* (1981) studied that high-viscous Prednisolone acetate was formulated with carboxypolymethylene gel at concentrations of 0.125% and 1.0%. The ability of these gel preparations to suppress inflammation in the cornea was assessed and compared with the anti-inflammatory capabilities of conventional commercially available prednisolone acetate ophthalmic suspensions. When administered hourly, the gel formulations produced no greater anti-inflammatory effect than the conventional suspensions. Davies (2000) reported that reformulation of ophthalmic suspensions as solutions had many advantages. This might be achieved by complexation using cyclodextrins. Solubilization using cyclodextrins can overcome many of the formulation problems. Howard and Kupferman (1974) determined the relative ability of two of the most widely used ophthalmic Prednisolone formulations that suppressed inflammation in the cornea with an intact epithelium. The results documented that prednisolone acetate 1.0 per cent ophthalmic suspension is more effective than prednisolone phosphate 1.0 per cent ophthalmic solution in suppressing corneal inflammation.

In the present study Prednisolone acetate was formulated as suspension dosage form, though in solution dosage form certain formulation problems are eradicated or could be prevented even then suspension is a preferred dosage form as far as bioavailability is concerned (table 4).

Kwon *et al.* (1996) reported that in ophthalmic suspensions, the mean dose and the uniformity of amounts administered in single drop depends upon the redispersibility of drug particles by shaking. According to the German pharmacopoeia and other authentication it is one of the important parameter for suspension and other liquid dosage forms to have the capability of

redispersibility after particles' sedimentation on storage. However, still no corresponding test method has been specified. In the present study redispersibility was assured by accelerated settling and real time settling. Accelerated settling studies were performed by subjecting 5 gm of sample in a separate 16 x 125 mm flat-bottom glass tube to centrifugation for 30 minutes at 3100 rpm. The resuspendability of the settled material was tested by measuring the number of wrist shaking required to re-suspend the sediment (NMT 8 seconds). Real time settling studies were performed by allowing 5 gm of sample in 16 x 125 mm flat-bottom glass tubes to undergo natural settling (under gravity) for seven days. The resuspendability of the settled material was tested by measuring the number of inversions required to re-suspend the sediment completely (NMT 10 inversions).

Hanna *et al.* (1977) investigated the anti-inflammatory properties of topical ocular dimethyl sulfoxide (DMSO) by using a standard experimental model of an acute inflammatory ocular inflammation. In the proposed study dimethyl sulfoxide was used for micronizing the prednisolone acetate and it was removed by filtration and the filtrate was washed with purified water to remove any traces of dimethyl sulfoxide. However if minute traces of dimethyl sulfoxide are found then it does not have any adverse effect.

CONCLUSION

Present study thus can be applied on all lipophilic drugs which are required to be formulated in ophthalmic suspension dosage forms.

Comparison of the present technique with other one based on Economy and Drug delivery. The technique used in the present study was cost effective because most of the sterilization steps were done with filtration process. The size of the suspending particles was in the ranges of 1-3 µm that increase the patient convenience as well as the release of drug from dosage form.

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