Journal of Applied Pharmaceutical Science Vol. 3 (04), pp. 139-142, April, 2013
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2013.3425
ISSN 2231-3354

In-Vitro Dissolution Study and Shelf Life Calculation of Developed Sol-To-Gel Ocular Drug Delivery System of Brimonidine for Conjunctivitis during Accelerated Stability Study

Manu Babbar*, Kanchan Kohli, Anil Bhandari
Faculty of Pharmaceutical Sciences, Jodhpur National University, Jodhpur (Rajasthan)-342001, India.

ARTICLE INFO

Article history:
Received on: 11/10/2012
Revised on: 29/11/2012
Accepted on: 15/01/2013
Available online: 27/04/2013

Key words:
Brimonidine, Conjunctivitis, *In-Vitro* Drug Release, Shelf Life.

ABSTRACT

The present research work deals with the *in- vitro* dissolution study of developed sol to gel ocular drug delivery system of brimonidine for conjunctivitis during accelerated stability study. The formulation of brimonidine was developed and optimized formulation coded as X4Y2D containing optimized amount of sodium alginate and HPMC K100LvP was evaluated for the physico-chemical characterization and drug release initially and during accelerated stability study. The drug release was determined using fabricated continuous flow through cell apparatus. The difference between initial drug release and after three month drug release was 2.606% and the calculation was done for shelf life and found 2 years.

INTRODUCTION

Ophthalmic drug delivery is one of the most interesting and challenging endeavors facing the pharmaceutical scientist. The anatomy, physiology and biochemistry of the eye render this organ exquisitely impervious to foreign substances. The challenge to the formulator is to circumvent the protective barriers of the eye without causing permanent tissue damage and at the same time delivering drug at a uniform rate to give a constant effective therapeutic level of drug concentration.

One of the major problems encountered with solutions is the rapid and extensive elimination of drugs from the precorneal lachrymal fluid by solution drainage, lachrymation, and nonproductive absorption by the conjunctiva, which may cause undesirable side effects (Lee et al., 1979). Initial attempts to overcome the poor bioavailability of topically instilled drugs typically involved the use of ointments based on mixtures of white petrolatum and mineral oils and suspensions (Chrai, 1973; Felt, 1999). Ointments ensure superior drug bioavailability by increasing the contact time with the eye, minimizing the dilution by tears, and resisting nasolachrymal drainage. Because these vehicles have the major disadvantage of providing blurred vision, they are nowadays mainly used for either nighttime administration or for treatment on the outside and edges of the eyelids (Greaves and Wilson 1993). Use of suspensions as ophthalmic delivery systems relies on the assumption that particles may persist in the conjunctival sac.

The efficiency of suspensions has shown high variability, which occurred as a result of inadequate dosing, probably mainly due to the lack of patients compliance in adequately shaking the suspension before administration. These disadvantages have led to other approaches being investigated (Mazor et al., 1979; Gangrade et al., 1996). Various approaches that have been attempted to increase the bioavailability and the duration of therapeutic action of ocular drugs can be divided into two categories. The first is based on the use of the drug delivery systems, which provide the controlled and continuous delivery of ophthalmic drugs. The second involves, maximizing corneal drug absorption and minimizing precorneal drug loss. The typical pulse entry type drug release behavior observed with ocular aqueous solutions (eye drops), suspensions, and ointments can be replaced by a more controlled, sustained, and continuous drug delivery, using a controlled release ocular drug delivery system. These systems can achieve therapeutic action with a smaller dose and a fewer systemic and ocular side effects (Olejnik, 1993; Bangham et al., 1965).

* *Corresponding Author*

MATERIAL AND METHOLOGY

Before starting the formulation development calculation of dose, adjustment of isotonicity, Isotonicity calculation for polymers was done.

Preparation of medicated sol to gel formulation

All the formulae selected on the basis of physical and rhelogical examination viz. X3D, X4D, X3Y1D, X4Y1D, X2Y2D, X3Y2D, X4Y2D, X2Y3D, X3Y3D and X4Y3D were used for medicated sol to gel formulation development. The preparation of the medicated sol to gel formulation was carried out by the incorporation of drug in the placebo (previously prepared without using API) formulation in which the volume was not made up. The drug brimonidine was dissolved in acetate buffer of pH 4.8 and it was added to the placebo formulations. Then the final volume was made up with purified distilled water. The pH was adjusted to 6.0 with 0.1 M sodium hydroxide and 0.1 M hydrochloric acid solution. The developed formulations were filled in 5-ml capacity amber glass vials, closed with gray butyl rubber closures and sealed with aluminium caps. The formulations, in their final pack, were subjected to terminal sterilization by autoclaving at 121^{o}C and 15 psig for 20 min. The formulae for the medicated formulations are given in table no 1.

Physico-chemical Characterization

The optimized ophthalmic sol to gel formulations coded as X4Y2D in table 1 was evaluated for the following required characteristics for an ophthalmic solution and was compared with a marketed ophthalmic solution of brimonidine.

A. Clarity,
B. pH
C. Refractive index
D. Surface tension
E. Viscosity, The all results of physiological characterization are tabulated and shown in table 5.

In- Vitro Release Studies of the Medicated Ophthalmic Sol to Gel Preparations

In vitro release studies were performed to study the release of brimonidine from the medicated ophthalmic gels. In vitro release studies were done using fabricated continuous flow through cell apparatus designed by Ali and Sharma. All the medicated formulations of codes X3, X4, X3Y1, X4Y1, X2Y2, X3Y2, X4Y2, X2Y3, X3Y3, and X4Y3 were subjected in to vitro release studies. Graphs were plotted between cumulative % of drug release Vs time and Log % drug remaining Vs time for the better assessment of release pattern. Graphs plotted for cumulative % of drug release Vs time were curve indicating a first order release. However, it was found that the graph for formulation X3Y3D and X4Y2D showed missed character of both curve and straight line showing that release was approaching towards zero order. Graphs plotted between Log % of drug remaining Vs time were almost straight lines showing first order release. Finally formulation X4Y2D was selected for further studies.

Table. 1: Formulation for medicated ophthalmic sol to gel formulations.

INGREDIENTS	FORMULATION CODES									
	X3D	X4D	X3Y1D	X4Y1D	X2Y2D	X3Y2D	X4Y2D	X2Y3D	X3Y3D	X4Y3D
Brimonidine (mg)	67.5	67.5	67.5	67.5	67.5	67.5	67.5	67.5	67.5	67.5
Acetate buffer pH 4.8 (mL)	3.4.	3.4	3.4	3.4	3.4	3.4	3.4	3.4	3.4	3.4
Sodium alginate (mg)	125	150	125	150	100	125	150	100	125	150
HPMC K100LvP (mg)	-	-	250	250	375	375	375	500	500	500
Benzalkonium Chloride (%)	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Purified Distilled water q.s to (mL)	25	25	25	25	25	25	25	25	25	25

Table. 2: *In-vitro* release of brimonidine from medicated sol to gel formulation (code X4Y2D) initially.

Time (in hrs)	Mean absorbance (n=3)	Amount of drug released (mg)	Amount of drug remaining in gel (mg)	Cumulative % of drug released from gel (mg)	Cumulative % of drug remaining in gel	Log % of drug remaining	K at various time intervals	
							K_0 (moles litre^{-1} hr^{-1})	K_1 (hr^{-1})
0.5	0.072	0.0654	0.8346	7.27	92.73	1.967	14.54	0.1510
1.0	0.107	0.1245	0.7755	13.83	86.17	1.935	13.83	0.1489
2.0	0.244	0.3309	0.5691	36.77	63.23	1.800	18.385	0.2292
3.0	0.295	0.4092	0.4908	45.47	54.53	1.736	15.157	0.2022
4.0	0.354	0.4966	0.4034	55.18	44.82	1.651	13.795	0.2006
6.0	0.444	0.6324	0.2676	70.27	29.73	1.473	11.711	0.2022
8.0	0.491	0.7032	0.1968	78.14	21.86	1.339	9.767	0.1901
10.0	0.535	0.7716	0.1284	85.74	14.26	1.154	8.573	0.1948
12.0	0.573	0.8221	0.0779	91.35	8.65	0.936	7.612	0.2040
X = Mean, S.D = Standard deviation and C_v = Coefficient of variation							X=12.5963	X=0.1914
Dilution factor = 2 n = Results are the mean of three readings							S.D=3.4753	S.D=0.02558
K = Release rate constant, K_0 = Zero order and K_1 = First order rate constant							C_v=29.5901	C_v =13.5168

Table 3: *In vitro* release of brimonidine from medicated sol to gel formulation (code X4Y2D) after one month .

Time (in hrs)	Mean absorbance (n=3)	Amount of drug released (mg)	Amount of drugremaining in gel (mg)	Cumulative % of drug released from gel	Cumulative % of drug remaining in gel	Log % of drug remaining	K at various time intervals K_0 (moles litre^{-1} hr^{-1})	K_1 (hr^{-1})
0.5	0.069	0.0606	0.8394	6.74	93.26	1.969	13.48	0.1395
1.0	0.097	0.1094	0.7906	12.16	87.84	1.943	12.16	0.1297
2.0	0.174	0.2235	0.6765	24.84	75.16	1.875	12.42	0.1428
3.0	0.246	0.3324	0.5676	36.94	63.06	1.795	12.313	0.1537
4.0	0.296	0.4112	0.4888	45.70	54.30	1.734	11.425	0.1526
6.0	0.374	0.5299	0.3701	58.88	41.12	1.614	9.813	0.1481
8.0	0.462	0.6539	0.2461	72.66	27.33	1.436	9.0825	0.1621
10.0	0.522	0.7448	0.1552	82.76	17.24	1.236	8.276	0.1758
12.0	0.564	0.8056	0.0944	89.52	10.48	1.020	7.46	0.1879
X = Mean, S.D = Standard deviation and C_v = Coefficient of variation							X =10.7137	X =0.1547
Dilution factor = 2 n = Results are the mean of three readings							S.D=2.1125	S.D=0.0182
K = Release rate constant, K_0 = Zero order and K_1 = First order rate constant							C_v=19.7180	C_v=11.7631

Table. 4: *In vitro* release of brimonidine from medicated sol to gel formulation (code X4Y2D) after 3 month.

Time (in hrs)	Mean absorbance (n=3)	Amount of drug released (mg)	Amount of drug remaining in gel (mg)	Cumulative % of drug released from gel	Cumulative % of drug remaining in gel	Log % of drug remaining	K at various time intervals K_0 (moles litre^{-1} hr^{-1})	K_1(hr^{-1})
0.5	0.057	0.0476	0.8524	5.288	94.712	1.976	10.576	0.1090
1.0	0.092	0.0949	0.8051	10.544	89.456	1.951	10.554	0.1115
2.0	0.156	0.1976	0.7024	21.955	78.045	1.892	10.977	0.1240
3.0	0.224	0.2923	0.6077	32.477	67.523	1.829	10.826	0.1309
4.0	0.264	0.3612	0.5388	40.133	59.867	1.777	10.033	0.1283
6.0	0.314	0.4366	0.4634	48.511	51.489	1.711	8.085	0.1107
8.0	0.432	0.618	0.282	68.666	31.334	1.496	8.583	0.1451
10.0	0.491	0.7067	0.1933	78.522	21.478	1.331	7.852	0.1541
12.0	0.542	0.774	0.126	88.744	14.00	1.145	7.167	0.1639
X = Mean, S.D = Standard deviation and C_v = Coefficient of variation							X=9.4096	X= 0.1308
Dilution factor = 2 n = Results are the mean of three readings							S.D=1.4776	S.D=0.01983
K = Release rate constant, K_0 = Zero order and K_1 = First order rate constant							C_v=15.7034	C_v=15.1600

Table . 5: Characteristics of ophthalmic sol to gel formulations of brimonidine.

Formulation code	Clarity	pH	Refractive index	Surface tension	Viscosity (cps)
X3Y3D	Clear	6.0	1.356	43	205
X4Y2D	Clear	6.0	1.343	37	191
Marketed brimonidine solution	Clear	6.2	1.359	42	—

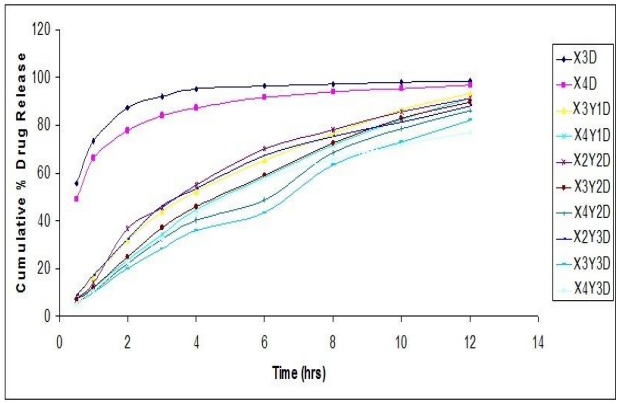

Fig. 1: Cumulative % of drug release Vs time for different medicated ophthalmic sol to gel formulations (initially).

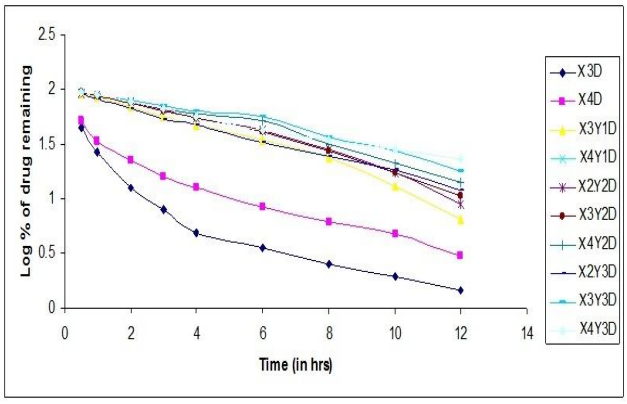

Fig 2: Log % of drug released Vs time for different medicated ophthalmic sol to gel formulations (initially).

Packaging, Sterilization and Interaction Studies

The optimized ophthalmic sols to gel formulations were packed in plastic bottles and were evaluated for resistance to autoclaving and closure efficacy. There was no change in shape and texture and the package did not become sticky after sterilization at 121˚C and 15 psig (per centimeter inch gauze) for 20 minutes. Also there was no leakage in the container. Hence, packaging system was found satisfactory for the packaging of optimized formulation.

Test for sterility as per Indian Pharmacopoeia 1996 was performed on the formulation as a measure to check the effectiveness of the sterilization method employed i.e. autoclaving at 121˚C and 15 psig for 20 minutes. These sterile preparations were tested for their sterility by Indian Pharmacopoeia 1996. Interaction studies were carried out to study whether the sterilization cause any kind of interaction among different ingredients of the formulation. U.V. scanning revealed no interaction, as there was no change in the λmax of the formulation before and after sterilization. Assay was also performed by U.V spectroscopy and found the same amount do drug content before and after sterilization. I.R. studies were also done on both sterilized and non sterilized optimized formulations. In both cases the spectrum exhibited maximas at the same wave number as of the reference spectrum of brimonidine (1486 cm^{-1}, 1593 cm^{-1}, 1653 cm^{-1}, 1734 cm^{-1}, 1086 cm^{-1}). TLC studies also revealed no interaction among different ingredients of the optimized formulation, since the similar Rf values were obtained for both unsterilized and sterilized formulation. Hence, no interaction was found to occur due to sterilization by autoclaving.

RESULTS

Prepared Sol-to-Gel formulation observed for in-vitro drug release and it was observed that initially formulations X4Y2D has 91.35% drug release may be due to good proportion of sodium alginate and HPMC K100 LvP, after that X4Y2D was charged for accelerated stability study as per ICH guideline. After one month the drug release was found 89.52 % and with spend of three month the drug release remained 88.744%. Accelerated stability studies were carried out to establish a time period i.e. shelf life over which the formulation can be used safely. Stability studies were carried out according to the International Conference on Harmonization (ICH) guidelines and be preparing the Arrhenius plot for the determination of shelf life of the formulation X4Y2D. The degradation of brimonidine was found less to be 2.606% in three month (90 days) study. Calculation was done for 6 month study and the degradation was found to be less than 5% so, according to the ICH guidelines, a shelf life of 2 years was arbitrarily assigned to the formulation X4Y2D.

CONCLUSION

For the accurate determination of shelf life of the formulation, study was carried out at elevated temperatures and an Arrhenius plot (chemwiki.ucdavis.edu, 2012) was prepared. On calculation a shelf life of 2.216 years was found. Hence formulation was easily assigned a shelf life of 2 years.

REFERENCES

Bangham, A.D., Standish, M.M., Watkins, J.C., 1965. Diffusion of univalent ions across the lamellae of swollen phospholipids. J. Mol. Biol. 13, 238-252.

Chemwiki.ucdavis.edu/Physical.../Arrhenius.../Arrhenius_Plot accessed on 26/06/2012.

Chrai, S.S., Robinson, J.R., 1973. Lacrimal and Instilled Fluid Dynamics in Rabbit Eyes. J. Pharm. Sci. 62, 1112–1121.

Felt, O., Baeyens, V., 1999. Mucosal drug delivery: Ocular, in "Encyclopedia of controlled drug delivery", Vol. 2, John Wiley and Sons, Inc., 605-626.

Gangrade, N.K., Gaddipati, N.B., Ganesan, M.G., Reddy, I.K., 1996. Ocular Therapeutics and Drug Delivery, Technomic Publishing, Lanchester, 377–403.

Greaves, J.L., Wilson, C.G., 1993. Treatment of diseases of the eye with mucoadhesive delivery systems. Adv. Drug Deliv. Rev. 11, 349-383.

Lee, V.H.L., Robinson, J.R, 1979. Mechanistic and quantitative evaluation of precorneal pilocarpine disposition in albino rabbits. J. Pharm. Sci. 68, 673–684.

Mazor, Z., Ticho, U., Rehany, U., Rose, L., 1979. Piloplex, a new long-acting pilocarpine polymer salt. B: comparative study of the visual effects of pilocarpine and Piloplex eye drops. Br. J. Ophthalmol. 63, 58–61

Olejnik, O, 1993. Ophthalmic Drug Delivery Systems, in: A.K. Mitra (Ed.), Novel Control Drug Delivery Systems, Marcel Dekker, New York, 117–197.

How to cite this article:

Manu babbar, Kanchan Kohli, Anil Bhandari, *In-Vitro* Dissolution Study and Shelf Life Calculation of Developed Sol-To-Gel Ocular Drug Delivery System of Brimonidine for Conjunctivitis During Accelerated Stability Study. J App Pharm Sci, 2013; 3 (04): 139-142.