—Notes—

# Stability Studies on Aqueous and Oily Ophthalmic Solutions of Diclofenac

Munish AHUJA,[a] Avinash Shridhar DHAKE,[b]
Surendra Kumar SHARMA,[a] and Dipak Kanti MAJUMDAR*,[c]

[a]*Department of Pharmaceutical Sciences, Guru Jambheshwar University of Science and Technology, Hisar, Haryana–125 001, India,* [b]*L. B. Rao Institute of Pharmaceutical Education and Research, Khambhat, Anand, Gujarat–388 620, India, and* [c]*Department of Pharmaceutics, Delhi Institute of Pharmaceutical Sciences and Research, Formerly College of Pharmacy, (University of Delhi), Pushp Vihar, Sector-III, New Delhi–110 017, India*

(Received August 10, 2008; Accepted December 25, 2008)

Various aqueous and oily diclofenac ophthalmic formulations were subjected to accelerated and long term stability studies. Degradation of diclofenac was found to follow first-order kinetics. Among the aqueous formulations containing preservative, formulation with PMA, PMN, SA, MP/PP and SMS showed diclofenac content above 90% after 6 months of accelerated and 12 months of room temperature storage. Diclofenac 0.1%, w/v aqueous formulation (pH 7.4), with 5–10% overages, containing SMS, MP/PP or PMN look promising taking both stability and corneal permeability in view. However, for use in cataract surgery formulation without preservative appears ideal. Oily ophthalmic formulations except those in olive and mustard oil, had more than 90% drug content after 6 months of accelerated and 12 months of room temperature storage. Diclofenac (0.2%, w/v) ophthalmic solution in sesame oil with 3% overage and containing benzyl alcohol (0.5%, v/v) as preservative, appears ideal, taking both stability and corneal permeability in view.

**Key words**——diclofenac; first-order kinetics; ophthalmic formulation; stability study

## INTRODUCTION

Diclofenac, 2(2,6-dichloroanilino) phenyl acetic acid, is a potent non-steroidal anti-inflammatory drug (NSAID). It has a poor aqueous solubility, and is commonly used as sodium, potassium and diethylamine salts.[1,2] Sodium salt of diclofenac is used for making aqueous ophthalmic solutions. Diclofenac sodium tends to precipitate from aqueous solutions in a crystalline form even when the concentration is below the limit of saturation. Thus, to improve its solubility, various solubilizers like polyoxyethylene-35-castor oil, hydroxypropyl-$\beta$-cyclodextrin,[3] n-octenylsuccinate starch,[4] and $\alpha$-tocopheryl polyethylene glycol succinate[5] have been employed. In aqueous acidic solutions, diclofenac undergoes cyclization to indolinone.[6] In a study conducted earlier, 1-(2,6-dichlorophenyl)-indolin-2-one, [2-(2,6-dichlorophenyl) amino]-phenyl] methanol and 2-[(2,6-dichlorophenyl) amino]-benzaldehyde were detected as degradation products, in ophthalmic solutions of diclofenac exposed to accelerated testing conditions of 60°C for 9 weeks.[7] Thermal stability of diclofenac sodium and its inclusion complex with $\beta$-cyclodextrin have been characterized in the solid state[8] and in aqueous solutions.[9] It has been observed that formation of inclusion complex of diclofenac with $\beta$-cyclodextrin improves the thermal stability of diclofenac sodium in solid state as well as in aqueous solutions. Further, the studies have shown that the degradation of diclofenac sodium and its inclusion complex in solid state followed first-order kinetics,[8] while in aqueous solution the degradation is of pseudo-first order.[9] Diclofenac is susceptible to photochemical oxidation.[3] In photolytic degradation studies of diclofenac conducted by exposing aqueous solutions to solar radiation, it has been observed that diclofenac undergoes cyclization to carbazole derivatives. The main photodecomposition product was identified as 8-chloro-9H-carbazole-1-yl-acetic acid.[10] The *in vitro* corneal permeation characteristics of diclofenac from formulated aqueous[11] and oily[12] ophthalmic solutions of diclofenac have been investigated earlier. The purpose of the present investigation was to conduct stability studies on the aqueous and oily ophthalmic solutions of diclofenac.

*e-mail: dkmajumdaar@yahoo.com,
dkmajumdar@gmail.com

## EXPERIMENTAL

**Material** Diclofenac sodium (purity 98.58%) was obtained as a gift sample from Dabur Research Foundation (Ghaziabad, India). High performance liquid chromatography (HPLC) grade acetonitrile, glacial acetic acid, triethylamine and water were purchased from Qualigens Fine Chemicals (Mumbai, India). Refined food grade vegetable oils used in the study, *e.g.*, arachis (Amrit Banaspati Co. Ltd., Punjab, India), mustard (National Dairy Development Board, Gujarat, India), soybean (Adani Wilmar Limited, Gujarat, India), sesame (Shanker Udyog, Kanpur, India), kardi (safflower) (Marico Ltd., Mumbai, India), sunflower (Amrit Banaspati Co. Ltd., Punjab, India), olive (SOS Cuetara, Madrid, Spain) and castor oil (S.D. Fine Chem Ltd., Mumbai, India) were purchased from the local market. USP type-I, amber colored, glass ampoules of 2 ml capacity were obtained from Kejariwal Industries (New Delhi, India). All other chemicals purchased were of analytical grade and were used as received.

**Preparation of Formulations** Diclofenac sodium was used for preparing aqueous formulations while diclofenac free acid was used for making oily formulations. Diclofenac acid was prepared by acidifying an aqueous solution of diclofenac sodium; the precipitate so obtained was washed with distilled water until it became free from chloride ions. The product so obtained was characterized by spectroscopy.[12]

**Preparation of Aqueous Ophthalmic Solution of Diclofenac** Aqueous ophthalmic solutions of diclofenac were prepared by dissolving various ingredients in 100 ml of Sorenson's phosphate buffer (0.0667 M) as per the composition given in Table 1.

**Preparation of Oily Ophthalmic Solution of Diclofenac** Oily ophthalmic solutions of diclofenac with or without benzyl alcohol (0.5%, v/v) were prepared by dissolving required quantity of diclofenac acid in 100 ml of oily vehicle as per the composition given in Table 2.

Table 1. Composition of Aqueous Ophthalmic Formulations of Diclofenac

| Formulation code | Diclofenac sodium (%, wt/vol) | pH | Tonicity modifier | Preservative (%, wt/vol) | Viscosity modifier |
|---|---|---|---|---|---|
| AF1 | 0.10 | 7.4 | Sodium chloride | — | — |
| AF2 | 0.10 | 7.0 | Sodium chloride | — | — |
| AF3 | 0.05 | 6.5 | Sodium chloride | — | — |
| AF4 | 0.02 | 6.0 | Sodium chloride | — | — |
| AF5 | 0.10 | 8.0 | Sodium chloride | — | — |
| AF6 | 0.05 | 7.4 | Sodium chloride | — | — |
| AF7 | 0.15 | 7.4 | Sodium chloride | — | — |
| AF8 | 0.10 | 7.4 | Sodium chloride | PMA (0.002) | — |
| AF9 | 0.10 | 7.4 | Sodium chloride | PMN (0.002) | — |
| AF10 | 0.10 | 7.4 | Sodium chloride | THM (0.005) | — |
| AF11 | 0.10 | 7.4 | Sodium chloride | BA (0.5) | — |
| AF12 | 0.10 | 7.4 | Sodium chloride | SA (0.2) | — |
| AF13 | 0.10 | 7.4 | Sodium chloride | BAC (0.002) | — |
| AF14 | 0.10 | 7.4 | Sodium chloride | BAC (0.002) + EDTA (0.01) | — |
| AF15 | 0.10 | 7.4 | Sodium chloride | MP (0.02) + PP (0.01) | — |
| AF16 | 0.10 | 7.4 | Sodium chloride | EDTA (0.01) | — |
| AF17 | 0.10 | 7.4 | Sodium chloride | SMS (0.2) | — |
| AF18 | 0.10 | 7.4 | Sodium chloride | — | HPMC (0.1) |
| AF19 | 0.10 | 7.4 | Sodium chloride | — | PVA (1.4) |
| AF20 | 0.10 | 7.4 | Sodium chloride | — | HPC-L (0.1) |
| AF21 | 0.10 | 7.4 | Mannitol | — | — |

PMA: phenyl mercuric acetate, PMN: phenyl mercuric nitrate, BA: benzyl alcohol, SA: sorbic acid, BAC: benzalkonium chloride, EDTA: disodium edetate, MP: methylparaben sodium, PP: propyl paraben sodium, SMS: sodium metabisulphite, HPMC: hydroxypropyl methylcellulose, PVA: polyvinyl alcohol, HPC-L: hydroxypropyl cellulose.

Table 2. Composition of Oily Ophthalmic Formulations of Diclofenac

| Formulation code | Diclofenac acid (%, wt/vol) | Oil | Benzyl alcohol (%, vol/vol) |
|---|---|---|---|
| OF1 | 0.20 | Arachis | — |
| OF2 | 0.20 | Arachis | 0.5 |
| OF3 | 0.50 | Arachis | — |
| OF4 | 0.50 | Arachis | 0.5 |
| OF5 | 0.20 | Castor | — |
| OF6 | 0.20 | Castor | 0.5 |
| OF7 | 0.50 | Castor | — |
| OF8 | 0.50 | Castor | 0.5 |
| OF9 | 1.00 | Castor | — |
| OF10 | 1.00 | Castor | 0.5 |
| OF11 | 0.20 | Sunflower | — |
| OF12 | 0.20 | Sunflower | 0.5 |
| OF13 | 0.50 | Sunflower | — |
| OF14 | 0.50 | Sunflower | 0.5 |
| OF15 | 0.20 | Mustard | — |
| OF16 | 0.20 | Mustard | 0.5 |
| OF17 | 0.20 | Olive | — |
| OF18 | 0.20 | Olive | 0.5 |
| OF19 | 0.20 | Safflower | — |
| OF20 | 0.20 | Safflower | 0.5 |
| OF21 | 0.20 | Soybean | — |
| OF22 | 0.20 | Soybean | 0.5 |
| OF23 | 0.20 | Sesame | — |
| OF24 | 0.20 | Sesame | 0.5 |

**Stability Testing** The amber colored, USP type-I, 2 ml glass ampoules were washed with tap water and distilled water, followed by drying in an oven. The aqueous and oily ophthalmic solutions of diclofenac were filled into dried glass ampoules and heat-sealed. All aqueous ophthalmic solutions were sterilized by moist heat at 15 lbs pressure for 20 min. The accelerated stability testing on ophthalmic formulations was conducted by storage at 40℃ and 75% RH. The long-term stability studies were conducted by storage at room temperature. The samples of ophthalmic formulations kept under accelerated storage conditions were withdrawn at 0 day, 6 weeks, 3 and 6 months and analyzed for diclofenac content by HPLC. The samples stored at room temperature were withdrawn at 0 day, 3, 6 and 12 months, and analyzed for diclofenac contents by HPLC. The samples of aqueous formulations were also tested for pH and appearance, while oily formulations were tested for appearance only.

**HPLC Analysis** Assay of the aqueous ophthalmic formulations was carried out by injecting 20 $\mu$l of the filtered (through 0.2 $\mu$ syringe filter) and appropriately diluted solution, spiked with ketorolac tromethamine as internal standard into a chromatographic system equipped with 600 pump controllers (Waters), 2487 dual $\lambda$ absorbance detector (Waters), and 7725i Rheodyne injector. For assay of oily solutions, appropriate dilution of the solution was done with acetone followed by filtration. The resolution of diclofenac was achieved using acetonitrile : water : acetic acid : triethylamine (60 : 38.25 : 1.65 : 0.10, vol/vol) at a flow rate of 1 ml/min, as the mobile phase in an isocratic run through Spherisorb (Waters) C 18, 5 $\mu$ (250×4.6 mm *i.d.*) column. The eluant was monitored for diclofenac at 276 nm. The retention time and the lowest limit of quantification of diclofenac were 5.6 min and 0.4 $\mu$g/ml, respectively.

## RESULTS

The results of accelerated and long-term stability studies conducted on aqueous diclofenac ophthalmic formulations are shown in Tables 3 and 4. It can be observed from the results that the formulations of pH 6.0 (AF4) and 6.5 (AF3) kept under room temperature showed precipitation along with significant loss of drug at the end of twelve months, while the said formulations kept at 40℃ did not show any precipitation. Diclofenac formulations containing benzalkonium chloride (AF13) or benzalkonium chloride with EDTA (AF14) were opalescent and showed some precipitation at the end of six months under accelerated storage conditions but no such precipitates were visible in solutions stored under room temperature at the end of twelve months. However, both the solutions preserved with benzalkonium chloride showed diclofenac content more than 90%. All the formulations showed more than 90% diclofenac content on storage under accelerated conditions for 6 months. Similarly, all the formulations except AF3 and AF4 showed diclofenac content above 90% after 12 months of storage at room temperature. The aqueous ophthalmic formulations were subjected to three freeze-thaw cycles. It was observed that formulations containing diclofenac 0.15%, w/v (AF7), THM (AF10) and BA (AF11) showed some precipitates, which did not dissolve on shaking.

Tables 5 and 6 present the results of accelerated and long-term stability studies conducted on oily diclo-

Table 3. Stability of Diclofenac in Aqueous Ophthalmic Solutions under Accelerated Storage Conditions

| Formulation | Diclofenac content* | | | | pH | | | | Appearance | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 D | 6 W | 3 M | 6 M | 0 D | 6 W | 3 M | 6 M | 0 D | 6 W | 3 M | 6 M |
| AF1 | 100.2±0.4 | 99.8±0.1 | 97.6±0.3 | 94.7±1.3 | 7.4 | 7.4 | 7.5 | 7.3 | Cl | Cl | Cl | Cl |
| AF2 | 99.8±0.3 | 99.9±0.3 | 98.1±0.3 | 93.5±0.9 | 7.0 | 7.1 | 7.1 | 7.2 | Cl | Cl | Cl | Cl |
| AF3 | 100.3±0.5 | 100.0±0.2 | 97.3±0.4 | 91.4±0.8 | 6.5 | 6.5 | 6.4 | 6.4 | Cl | Cl | Cl | Cl |
| AF4 | 100.1±0.4 | 100.0±0.2 | 96.9±0.5 | 91.6±1.1 | 6.0 | 6.1 | 6.0 | 6.1 | Cl | Cl | Cl | Cl |
| AF5 | 99.5±0.5 | 99.5±0.8 | 97.8±0.1 | 92.8±1.2 | 8.0 | 8.0 | 8.1 | 8.1 | Cl | Cl | Cl | Cl |
| AF6 | 99.1±0.7 | 99.3±0.2 | 97.4±0.3 | 92.9±0.7 | 7.4 | 7.4 | 7.4 | 7.3 | Cl | Cl | Cl | Cl |
| AF7 | 100.3±0.8 | 100.0±0.3 | 98.3±0.2 | 93.5±1.4 | 7.4 | 7.5 | 7.5 | 7.5 | Cl | Cl | Cl | Cl |
| AF8 | 100.0±0.2 | 99.7±0.3 | 98.2±0.2 | 94.1±0.5 | 7.4 | 7.4 | 7.5 | 7.5 | Cl | Cl | Cl | Cl |
| AF9 | 100.0±0.2 | 99.5±0.3 | 96.4±0.6 | 94.2±0.9 | 7.4 | 7.5 | 7.4 | 7.5 | Cl | Cl | Cl | Cl |
| AF10 | 100.0±0.3 | 99.5±0.5 | 96.6±0.4 | 92.7±1.6 | 7.4 | 7.4 | 7.3 | 7.4 | Cl | Cl | Cl | Cl |
| AF11 | 99.8±0.2 | 99.8±0.2 | 97.1±0.8 | 90.2±0.8 | 7.4 | 7.4 | 7.3 | 7.3 | Cl | Cl | Cl | Cl |
| AF12 | 99.9±0.3 | 99.3±0.6 | 96.2±0.4 | 91.4±1.4 | 7.4 | 7.4 | 7.3 | 7.3 | Cl | Cl | Cl | Cl |
| AF13† | 100.1±0.3 | 99.5±0.2 | 95.6±0.3 | 90.1±1.1 | 7.4 | 7.5 | 7.5 | 7.4 | Op | Op | Op | Op+Ppt. |
| AF14† | 99.8±0.4 | 99.7±0.6 | 96.4±0.1 | 91.5±0.9 | 7.4 | 7.5 | 7.5 | 7.5 | Op | Op | Op | Op+Ppt. |
| AF15 | 100.4±0.5 | 99.6±0.4 | 98.1±0.7 | 93.1±0.8 | 7.4 | 7.6 | 7.5 | 7.5 | Cl | Cl | Cl | Cl |
| AF16 | 100.2±0.2 | 99.9±0.2 | 95.7±0.5 | 92.2±1.3 | 7.4 | 7.4 | 7.4 | 7.5 | Cl | Cl | Cl | Cl |
| AF17 | 100.1±0.2 | 100.0±0.2 | 96.3±0.4 | 91.6±0.8 | 7.4 | 7.3 | 7.3 | 7.2 | Cl | Cl | Cl | Cl |
| AF18 | 100.0±0.3 | 100.0±0.4 | 96.2±0.4 | 92.3±1.2 | 7.4 | 7.4 | 7.5 | 7.5 | Cl | Cl | Cl | Cl |
| AF19 | 100.0±0.4 | 99.9±0.6 | 97.6±0.5 | 92.4±0.5 | 7.4 | 7.4 | 7.4 | 7.3 | Cl | Cl | Cl | Cl |
| AF20 | 99.8±0.4 | 99.6±0.3 | 95.8±0.3 | 93.1±0.7 | 7.4 | 7.4 | 7.4 | 7.3 | Cl | Cl | Cl | Cl |
| AF21 | 99.8±0.1 | 99.1±0.3 | 97.5±0.6 | 93.2±0.9 | 7.4 | 7.5 | 7.5 | 7.5 | Cl | Cl | Cl | Cl |

* Values are mean±SD ($n=3$), D: days, W: weeks, M: months, Op: opalescence, Cl: clear, Ppt: precipitation.

Table 4. Stability of Diclofenac in Aqueous Ophthalmic Solutions under Room Temperature Storage

| Formulation | Diclofenac content* | | | | pH | | | | $K_{calc}$ ($day^{-1}\times10^4$) | $t_{90}$ days | $Int_{calc}$ for 2 years |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 D | 3 M | 6 M | 12 M | 0 D | 3 M | 6 M | 12 M | | | |
| AF1 | 100.2±0.4 | 98.2±0.1 | 97.4±0.9 | 92.1±0.6 | 7.4 | 7.5 | 7.5 | 7.6 | 2.31 | 450 | 106.6 |
| AF2 | 99.8±0.3 | 98.6±0.4 | 96.9±0.4 | 90.4±0.7 | 7.0 | 7.1 | 7.0 | 6.9 | 2.71 | 384 | 109.6 |
| AF3† | 100.3±0.5 | 95.2±0.3 | 91.3±0.8 | 82.1±1.3 | 6.5 | 6.4 | 6.4 | 6.3 | 5.49 | 190 | — |
| AF4† | 100.1±0.4 | 94.2±0.6 | 90.4±1.1 | 83.6±0.8 | 6.0 | 6.0 | 5.9 | 5.8 | 4.94 | 211 | — |
| AF5 | 99.5±0.5 | 99.1±0.2 | 96.8±0.7 | 91.7±0.9 | 8.0 | 7.9 | 7.9 | 7.9 | 2.24 | 465 | 105.7 |
| AF6 | 99.1±0.7 | 98.7±0.4 | 97.3±0.6 | 93.2±0.3 | 7.4 | 7.4 | 7.3 | 7.3 | 1.68 | 618 | 101.7 |
| AF7 | 100.3±0.8 | 98.9±0.2 | 96.7±0.5 | 94.4±0.4 | 7.4 | 7.5 | 7.4 | 7.3 | 1.66 | 626 | 101.6 |
| AF8 | 100.0±0.2 | 99.1±0.4 | 97.6±0.8 | 93.1±1.2 | 7.4 | 7.5 | 7.5 | 7.5 | 1.96 | 532 | 103.8 |
| AF9 | 100.0±0.2 | 99.0±0.3 | 97.2±1.2 | 92.4±1.0 | 7.4 | 7.4 | 7.4 | 7.5 | 2.17 | 480 | 105.4 |
| AF10 | 100.0±0.3 | 98.9±0.4 | 95.8±1.4 | 91.7±1.3 | 7.4 | 7.4 | 7.5 | 7.4 | 2.37 | 438 | 107.0 |
| AF11 | 99.8±0.2 | 98.6±0.2 | 96.3±0.7 | 93.2±1.8 | 7.4 | 7.3 | 7.3 | 7.3 | 1.87 | 555 | 103.1 |
| AF12 | 99.9±0.3 | 97.4±0.6 | 95.9±0.9 | 92.6±0.7 | 7.4 | 7.3 | 7.4 | 7.3 | 2.08 | 500 | 104.7 |
| AF13†† | 100.1±0.3 | 97.1±0.2 | 96.1±1.3 | 91.9±1.1 | 7.4 | 7.4 | 7.3 | 7.4 | 2.34 | 444 | 106.8 |
| AF14†† | 99.8±0.4 | 98.4±0.1 | 96.7±0.6 | 93.1±0.7 | 7.4 | 7.3 | 7.4 | 7.5 | 1.90 | 546 | 103.4 |
| AF15 | 100.4±0.5 | 98.9±0.6 | 95.4±1.0 | 90.6±0.8 | 7.4 | 7.5 | 7.5 | 7.3 | 2.81 | 370 | 110.5 |
| AF16 | 100.2±0.2 | 97.4±0.3 | 96.5±0.7 | 93.3±1.9 | 7.4 | 7.4 | 7.4 | 7.3 | 1.96 | 532 | 103.8 |
| AF17 | 100.1±0.2 | 97.5±0.2 | 95.5±1.1 | 91.7±0.6 | 7.4 | 7.3 | 7.3 | 7.3 | 2.40 | 433 | 107.5 |
| AF18 | 100.0±0.3 | 97.2±0.4 | 95.8±0.8 | 91.3±0.8 | 7.4 | 7.3 | 7.4 | 7.3 | 2.49 | 417 | 108.0 |
| AF19 | 100.0±0.4 | 98.1±0.1 | 96.9±1.2 | 94.2±0.5 | 7.4 | 7.3 | 7.3 | 7.2 | 1.64 | 635 | 101.4 |
| AF20 | 99.8±0.4 | 97.8±0.2 | 95.7±0.6 | 91.1±0.8 | 7.4 | 7.4 | 7.4 | 7.3 | 2.50 | 416 | 108.0 |
| AF21 | 99.8±0.1 | 98.4±0.2 | 95.3±1.1 | 91.7±1.2 | 7.4 | 7.5 | 7.4 | 7.6 | 2.32 | 448 | 106.6 |

* Values are mean±SD ($n=3$), D: days, M: months, $K_{calc}$: calculated first-order degradation rate constant, $t_{90}$: time to reach 90% of initial drug concentration, $Int_{calc}$: calculated initial drug concentration for shelf life ($t_{90}$) of 2 years, † precipitation third months onward, †† opalescence throughout.

Table 5. Stability of Diclofenac in Oily Ophthalmic Solutions under Accelerated Storage Conditions

| Formulation | Diclofenac content* | | | | Appearance (Color) | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 D | 6 W | 3 M | 6 M | 0 D | 6 W | 3 M | M |
| OF1 | 99.6±0.2 | 99.0±0.2 | 97.4±0.1 | 93.2±0.9 | LY | LY | LY | LY |
| OF2 | 100.0±0.2 | 99.2±0.2 | 97.6±0.3 | 94.8±1.1 | LY | LY | LY | LY |
| OF3 | 100.1±0.1 | 99.9±0.3 | 98.2±0.3 | 92.5±0.7 | LY | LY | LY | LY |
| OF4 | 99.9±0.1 | 99.4±0.5 | 97.9±0.5 | 92.6±1.4 | LY | LY | LY | LY |
| OF5 | 100.5±0.6 | 99.7±0.6 | 99.2±0.2 | 93.5±0.8 | LY | LY | LY | LY |
| OF6 | 100.6±0.2 | 100.1±0.1 | 98.3±0.4 | 95.1±0.6 | LY | LY | LY | LY |
| OF7 | 99.4±0.3 | 99.2±0.4 | 97.6±0.3 | 92.7±1.4 | LY | LY | LY | LY |
| OF8 | 99.5±0.4 | 99.3±0.3 | 97.1±0.1 | 93.8±0.9 | LY | LY | LY | LY |
| OF9 | 100.4±0.3 | 99.8±0.5 | 98.5±0.1 | 91.4±0.6 | LY | LY | LY | LY |
| OF10 | 99.8±0.2 | 99.6±0.2 | 98.1±0.6 | 92.6±1.7 | LY | LY | LY | LY |
| OF11 | 99.1±0.2 | 99.3±0.2 | 97.9±0.2 | 93.3±0.9 | LY | LY | LY | LY |
| OF12 | 99.0±0.2 | 98.8±0.4 | 97.2±0.5 | 91.5±0.8 | LY | LY | LY | LY |
| OF13 | 99.5±0.4 | 99.2±0.2 | 97.1±0.7 | 90.6±1.3 | LY | LY | LY | LY |
| OF14 | 99.5±0.5 | 99.3±0.5 | 97.3±0.4 | 90.8±0.5 | LY | LY | LY | LY |
| OF15 | 99.6±0.3 | 99.4±0.3 | 96.5±0.7 | 85.8±1.4 | Y | Y | Y | Y |
| OF16 | 99.6±0.4 | 99.6±0.3 | 97.2±0.3 | 87.5±0.8 | Y | Y | Y | Y |
| OF17 | 98.9±0.4 | 98.8±0.2 | 96.8±0.7 | 88.0±0.7 | Y | Y | Y | Y |
| OF18 | 99.5±0.3 | 99.1±0.1 | 97.6±0.4 | 90.4±1.0 | Y | Y | Y | Y |
| OF19 | 100.1±0.2 | 99.9±0.3 | 97.9±0.6 | 92.1±0.8 | LY | LY | LY | LY |
| OF20 | 99.5±0.5 | 99.5±0.3 | 96.4±0.7 | 91.4±0.6 | LY | LY | LY | LY |
| OF21 | 99.5±0.3 | 99.0±0.1 | 96.3±0.8 | 92.1±1.5 | LY | LY | LY | LY |
| OF22 | 99.5±0.4 | 99.2±0.2 | 96.1±0.6 | 92.4±0.9 | LY | LY | LY | LY |
| OF23 | 100.4±0.3 | 100.2±0.2 | 96.9±0.6 | 91.9±0.7 | LY | LY | LY | LY |
| OF24 | 100.1±0.2 | 99.9±0.1 | 97.2±0.4 | 93.4±0.6 | LY | LY | LY | LY |

* Values are mean±SD ($n$=3), D: days, W: weeks, M: months, LY: light yellow, Y: yellow.

fenac ophthalmic formulations. The results of stability study reveal that the oily diclofenac ophthalmic solutions formulated in mustard and olive oils, showed a significant loss of drug under accelerated and room temperature storage. However, no change in appearance of any of the oily formulations was observed. The rest of the formulations showed more than 90% diclofenac content both under accelerated conditions for 6 months as well as room temperature storage conditions for 12 months. Formulation containing 1 % (w/v) diclofenac in castor oil and benzyl alcohol (0.5% v/v) (OF 10) showed least degradation on storage at room temperature.

## DISCUSSION

Diclofenac ophthalmic solution is commercially available as 0.1% (w/v) aqueous solution having pH between 7.0 and 7.3.[11] To study the effect of pH/concentration on permeability and stability, diclofenac formulations of different pH were formulated depending on its aqueous solubility at different pH. The aqueous solubility of diclofenac sodium was determined by shaking excess drug with fixed volume of 0.0667 M phosphate buffer of pH 6.0, 6.5, 7.0 or 7.4 in a reciprocating shaker at 37℃ for 24 h and analyzing the drug dissolved by HPLC. The aqueous solubility of diclofenac sodium at pH 6.0, 6.5, 7.0 and 7.4 was found to be 0.02%, 0.06%, 0.11% and 0.15% (w/v) respectively. As expected, the aqueous solubility of diclofenac, a weak acid (pKa 4.2),[1] increased with increase of pH of the solution due to increased ionization. Thus, aqueous ophthalmic solutions of diclofenac sodium 0.02% (w/v) of pH 6.0 (AF4), 0.05% (w/v) of pH 6.5 (AF3) and 0.1% (w/v) of pH 7.0 (AF2) were made in 0.0667 M phosphate buffers. The saturation solubility of diclofenac sodium at pH 7.4 and 37℃ is 0.15% (w/v). Since diclofenac 0.1% (w/v) concentration is below the saturation solubility of drug at pH 7.4 and 37℃, the said concentration was selected for formulation of aqueous ophthalmic solutions having pH 7.4 (AF1). Accordingly diclofenac 0.1% (w/v) aqueous formulations with or

Table 6. Stability of Diclofenac in Oily Ophthalmic Solutions under Room Temperature Storage

| Formulation | Diclofenac content* | | | | $K_{calc}$ ($day^{-1} \times 10^4$) | $t_{90}$ days | $Int_{calc}$ for 2 years |
|---|---|---|---|---|---|---|---|
| | 0 D | 3 M | 6 M | 12 M | | | |
| OF1 | 99.6±0.2 | 98.2±0.2 | 96.6±0.8 | 94.3±1.0 | 1.50 | 694 | 100.4 |
| OF2 | 100.0±0.2 | 98.7±0.2 | 96.2±0.5 | 91.9±0.9 | 2.31 | 449 | 106.5 |
| OF3 | 100.1±0.1 | 99.1±0.5 | 95.8±0.9 | 92.6±0.7 | 2.13 | 487 | 105.2 |
| OF4 | 99.9±0.1 | 98.2±0.4 | 95.4±0.9 | 92.1±1.2 | 2.23 | 467 | 105.9 |
| OF5 | 100.5±0.6 | 99.5±0.6 | 97.6±1.1 | 92.3±0.8 | 2.33 | 446 | 106.7 |
| OF6 | 100.6±0.2 | 99.4±0.1 | 96.4±0.7 | 93.4±1.3 | 2.03 | 511 | 104.4 |
| OF7 | 99.4±0.3 | 98.5±0.3 | 96.2±1.2 | 92.5±0.6 | 1.97 | 528 | 104.0 |
| OF8 | 99.5±0.4 | 99.1±0.3 | 96.8±0.9 | 91.7±0.8 | 2.24 | 465 | 105.9 |
| OF9 | 100.4±0.3 | 98.9±0.4 | 95.9±1.0 | 92.6±1.1 | 2.22 | 469 | 105.8 |
| OF10 | 99.8±0.2 | 98.6±0.5 | 97.2±0.5 | 95.0±1.2 | 1.35 | 770 | 99.31 |
| OF11 | 99.1±0.2 | 98.3±0.6 | 96.8±1.2 | 92.7±0.8 | 1.83 | 568 | 102.8 |
| OF12 | 99.0±0.2 | 98.5±0.3 | 96.9±1.3 | 93.8±0.9 | 1.48 | 703 | 100.2 |
| OF13 | 99.5±0.4 | 98.7±0.4 | 95.3±0.8 | 91.6±1.3 | 2.27 | 459 | 106.2 |
| OF14 | 99.5±0.5 | 98.4±0.4 | 96.1±0.7 | 91.5±0.9 | 2.30 | 453 | 106.4 |
| OF15 | 99.6±0.3 | 98.2±0.5 | 95.1±0.7 | 89.2±1.6 | 3.02 | 344 | 112.2 |
| OF16 | 99.6±0.4 | 98.3±0.7 | 96.7±1.2 | 90.1±0.7 | 2.75 | 379 | 110.0 |
| OF17 | 98.9±0.4 | 98.4±0.6 | 96.3±0.4 | 89.9±0.8 | 2.61 | 398 | 108.9 |
| OF18 | 99.5±0.3 | 98.3±0.5 | 95.4±0.3 | 91.3±0.6 | 2.36 | 441 | 106.9 |
| OF19 | 100.1±0.2 | 98.7±0.3 | 96.0±0.8 | 93.6±1.3 | 1.84 | 565 | 102.9 |
| OF20 | 99.5±0.5 | 98.1±0.5 | 95.8±1.4 | 92.0±1.4 | 2.15 | 484 | 105.3 |
| OF21 | 99.5±0.3 | 97.8±0.6 | 95.3±0.9 | 91.7±1.0 | 2.24 | 465 | 105.9 |
| OF22 | 99.5±0.4 | 97.9±0.4 | 96.5±0.5 | 92.4±1.1 | 2.02 | 513 | 104.3 |
| OF23 | 100.4±0.3 | 98.5±0.8 | 95.9±1.3 | 93.1±0.9 | 2.07 | 503 | 104.7 |
| OF24 | 100.1±0.2 | 98.4±0.3 | 96.3±1.1 | 93.4±1.2 | 1.90 | 548 | 103.4 |

* Values are mean±SD ($n=3$), D: days, M: months, $K_{calc}$: calculated first-order degradation rate constant, $t_{90}$: time to reach 90% of initial drug concentration, $Int_{calc}$: calculated initial drug concentration for shelf life ($t_{90}$) of 2 years.

without additives were made in 0.0667 M phosphate buffer (pH 7.4).

Diclofenac is a weak acid (pKa 4.2).[1] The *in vitro* corneal permeation studies conducted earlier revealed a higher apparent corneal permeability of diclofenac from aqueous solutions of pH 6.0 (0.02%, w/v) and 6.5 (0.05%, w/v) as compared to the formulation of pH 7.4 (0.1%, w/v), which appears to be due to decreased ionization of drug at lower pH.[11] However, due to the lower drug concentration, lesser cumulative permeation and lag time of 30 min was observed from the diclofenac formulation (0.02%, w/v) of pH 6.0 (AF4). In the stability study, formulations of pH 6.0 and 6.5, kept at room temperature, showed precipitation of diclofenac, which seems to be due to presence of drug at a concentration nearing super-saturation at the low pH of the formulations at room temperature. However, the formulations kept at higher temperature did not show precipitation, because at high temperature solubility of the drug is increased. Formulations of concentration smaller than 0.02% (w/v) and 0.05% (w/v) at pH 6.0 and 6.5 would have provided much diminished cumulative permeation and/or a greater lag time. Besides, reducing the pH of the formulation will increase the irritation potential of diclofenac, which is inherently irritating. Considering the same concentrations below 0.02% (w/v) and 0.05% (w/v) at pH 6.0 and 6.5 were not tested. It has been reported earlier that diclofenac precipitates from solutions even when the concentration is below the limit of saturation.[13] Commercially available eye drops employ surfactants to keep diclofenac in solution, which increases the irritation potential of the formulation of inherently irritant diclofenac.[14] In an earlier study, $\alpha$-tocopheryl polyethylene glycol succinate, a solubilizer used in commercial eye drops has been traced, to be responsible for severe corneal toxicity.[15] However, none of the diclofenac 0.1% (w/v) aqueous formulations with or without additives showed any precipitation

after one year storage at room temperature. Diclofenac is incompatible with benzalkonium chloride. Addition of benzalkonium chloride to diclofenac solution produces opalescence due to the formation of less water-soluble ion-pair between anionic diclofenac and cationic benzalkonium chloride. It appears that at high temperature, the opalescence or colloid is precipitated. Freeze-thaw cycling of aqueous formulations showed precipitation in the formulations containing diclofenac 0.15%, w/v (AF7), THM (AF10) and BA (AF11). As 0.15% (w/v) is the saturation concentration of diclofenac at pH 7.4 and 37℃, on freezing, the solution will attain super-saturation concentration at zero degree Celsius, and the same could account for the precipitation in 0.15% (w/v) diclofenac formulation. But the rest of the formulations, except AF10 and AF11, containing 0.1% (w/v) diclofenac in 0.0667 M phosphate buffer (pH 7.4) with or without additives did not show any precipitation, on freeze-thaw cycling, indicating that diclofenac at 0.1% (w/v) concentration, in these formulations, remains below the super-saturation level at 0℃, resulting in improved stability. Hence these formulations would be able to withstand cold climatic conditions.

Degradation of diclofenac followed first-order kinetics. The $K_{calc}$ and $t_{90}$ values of all the formulations at room temperature are shown in Table 4. The $K_{calc}$/$t_{90}$ values suggest that most of the formulations will not provide 2 years shelf life ($t_{90}$) of the product and might need some overages resulting in higher initial drug concentration, and the same has been shown in the last column of Table 4. Among the formulations containing preservative, formulation with PMA (AF8), PMN (AF9), SA (AF12), MP/PP (AF15) and SMS (AF17) provided reasonably good stability. Out of these, formulation with SMS (AF17) provided maximum apparent corneal permeability (Papp) followed by MP/PP (AF15) and PMN (AF9).[11] These formulations appear to be eye-friendly, contrary to the marketed formulations which contain surfactants. Thus, taking both stability and corneal permeability in view, these formulations look promising. These formulations would require 5–10% overages to ensure a shelf life of 2 years at room temperature. However, for use in cataract surgery, one might look for a formulation without preservative, and for the said purpose AF1 *i.e.*, formulation containing 0.1%, w/v diclofenac sodium (pH 7.4) and sodium chloride as tonicity modifier appears ideal.

Oily solutions of poorly water-soluble drugs have earlier been reported to prolong the pre-corneal residence and promote the ocular bioavailability of drugs. So formulations of diclofenac in different food grade refined vegetable oils were formulated. *In vitro* corneal permeability studies, conducted earlier, showed enhanced permeability of diclofenac from ophthalmic solution in sesame oil containing benzyl alcohol (0.5%, v/v) (OF24) while formulation in castor oil (OF10) provided least permeability.[12] The degradation of diclofenac from oily drops also followed first order kinetics. The formulations, except OF10, however, might need overage to ensure a 2-year shelf life at room temperature, resulting in higher initial drug concentration, which is shown in the last column of Table 6. During earlier studies, saturation solubility of diclofenac acid in various oils was determined at 4℃, and concentration below the saturation solubility of diclofenac acid at 4℃, was employed for formulating the oily ophthalmic solutions of diclofenac.[12] So these oily diclofenac ophthalmic solutions would withstand cold climatic conditions. Diclofenac (0.2%, w/v) ophthalmic solution in sesame oil containing benzyl alcohol (0.5%, v/v) appears ideal taking both stability and corneal permeability in view. The formulation would require 3% overage to ensure a shelf life of 2 years at room temperature.

## CONCLUSION

The stability studies conducted on aqueous ophthalmic solutions of diclofenac indicated that diclofenac (0.1%, w/v) formulation of pH 7.4, with 5–10% overage, containing sodium chloride as tonicity modifier, preserved using SMS, MP/PP or PMN could provide a shelf life ($t_{90}$) of 2 years, and the formulations appear promising from corneal permeation point of view. However, for use in cataract surgery, one might look for a formulation without preservative, and for the said purpose, formulation containing 0.1%, w/v diclofenac sodium (pH 7.4) and sodium chloride, as tonicity modifier appears ideal. Similarly, among the oily formulations, diclofenac (0.2%, w/v) ophthalmic solution in sesame oil, with 3% overage, containing benzyl alcohol (0.5%, v/v) appears ideal from both stability and corneal permeability point of view. However, further studies are needed to comment more in this respect.

**Acknowledgements** Authors are grateful to Dabur Research Foundation (Ghaziabad, India) for gift sample of diclofenac sodium.

## REFERENCES

1) Lund W., ''The Pharmaceutical Codex: Principles and Practice of Pharmaceutics,'' The Pharmaceutical Press, London, 1994, pp. 835–838.
2) Ahuja M., Dhake A. S., Sharma S. K., Majumdar D. K., *AAPS J.*, **10**(2), 229–241 (2008).
3) Reer O., Bock T. K., Muller B. W., *J. Pharm. Sci.*, **83**, 1345–1349 (1994).
4) Baydoun L., Muller-Goymann C. C., *Eur. J. Pharm. Biopharm.*, **56**, 73–79 (2003).
5) Desai S., Bawa R. (inventors). Alcon Laboratories Inc. (assignee). Topical ophthalmic acidic drug formulations. US patent 5 558 876. September 24, 1996.
6) Larsen C., Bundgaard H., *Arch. Pharm. Chem. Sci. Ed.*, **8**, 100–108 (1980).
7) Galmier M. J., Bouchon B., Madelmont J. C, Mercier F., Pilotaz F., Lartigue C., *J. Pharm. Biomed. Anal.*, **38**, 790–796 (2005).
8) Cwiertnia B., Hladon T., Stobiecki M., *J. Pharm. Pharmacol.*, **51**, 1213–1218 (1999).
9) Chadha R., Kashid N., Jain D. V., *Pharmazie*, **58**, 631–635 (2003).
10) Agüera A., Pérez Estrada L. A, Ferrer I., Thurman E. M., Malato S., Fernández-Alba A. R., *J. Mass Spectrom.*, **40**, 908–915 (2005).
11) Ahuja M., Dhake A. S., Majumdar D. K., *Yakugaku Zasshi*, **126**, 1369–1375 (2006).
12) Ahuja M., Sharma S. K., Majumdar D. K., *Yakugaku Zasshi*, **127**, 1739–1745 (2007).
13) Baekensfeld T., Muller B. W., Kolter K., *Int. J. Pharm.*, **74**, 85–93 (1991).
14) Guidera A. C., Luchs J. I., Udell I. J., *Ophthalmology*, **108**, 936–944 (2001).
15) Hargrave S. L., Jang J. C., Fini M. E., Gelender H., Cather C., Guidera A., Udell I., Fisher S., Jester J. V., Bowman R. W, McCulley J. P., Cavanagh H. D., *Ophthalmology,* **109**, 343–350 (2002).