**Research Article**

# FORMULATION AND DEVELOPMENT OF CELECOXIB OILY EYE DROPS

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**ABSTRACT**

Steroids are used in the treatment of allergic ocular disorders, corneal burns, lacrimal tract inflammation, and other ocular inflammations but their use is limited by their tendency to increase intraocular pressure and to cause cataract upon chronic administration. Steroids also exacerbate ocular infections and diminish corneal/stromal wound healing. Non steroidal anti-inflammatory drugs (NSAIDs) like indomethacin, flurbiprofen, ketorolac, and diclofenac have been found to be viable alternatives to steroids in treating ocular inflammation. Celecoxib is a non-steroidal anti-inflammatory drug (NSAIDS), acts by inhibition of prostaglandins, by inhibiting the activity of the enzyme cyclooxygenase-2 (COX-2). The most convenient way of delivering drug to the eye is topical application of an aqueous solution. Drug from the aqueous solution partitions through the corneal epithelium, stroma, and endothelium into the aqueous humor. One principle demerit of topically applied aqueous drug solution is the loss of drug due to drainage which results in lower ocular availability of drug and a therapeutic effect of shorter duration. One way of overcoming the problem is to apply the drug in the form of an oily solution. Vegetable oils like olive, castor, and sesame oil are used as vehicles for oil-based drops. Considering the formulation factors as discussed, it can be concluded that celecoxib 0.5% (w/v) solution in sesame oil, being below the saturation level; containing benzyl alcohol (0.5%) could be the better choice for formulation.



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## INTRODUCTION

There are most common available ophthalmic formulations such as eye drops and ophthalmic ointments are nearly about 75% of the eye dosage formulation in market. But those preparations when instilled into the eye, they are rapidly drained away from the ocular surface due to blinking tear flow and lacrimal nasal drainage of the eye. Only a small amount of drug is available for its therapeutic effect resulting in regular dosing application to the eye [1]. The eye has two challenging problems

for drug delivery. First is specific to hydrophobic new chemical entity which usually lack suitable vehicles. Second obstacle is generally to all ocular formulations, lesser ocular bioavailability of topically applied drug. Third one is ophthalmic drug applies to anterior segment of the eye only a 5 % actually penetrate cornea reaches to the internal anterior tissue of the eye. Fourth is rapid and efficient drainage of the cornea to the both hydrophilic and lipophilic molecules [2-4]. The various approaches to increase the bioavailability & the duration of therapeutic action can be divided in to two categories. First one use of sustain drug delivery system, second one is maximizing corneal drug absorption & minimizing pre-corneal drug loss through viscosity & penetration enhancers, pro-drugs, colloids [6]. In addition to invasive approaches, topically applied drugs shown to reach the back of eye tissue through trans conjunctival sclera diffusion after multiple instillations [5]. Eye is unique and very precious organ. It is considered as window of the soul. We can enjoy and viewed the whole world only with this organ. There are many eye aliments which affect this organ and one can loss the eye sight also. There are many ophthalmic drug delivery systems are available. These are classified as conventional and newer drug delivery systems [7]. Thus inefficient drug delivery into the eye occurs due to rapid tear turn over, lachrymal drainage and drug dilution by tears [8]. Thus prepared the oily eye drops to remain in the eye for longer time and produce the therapeutic effect.

## EXPERIMENTAL MATERIALS AND METHOD

### Materials used

Materials like celecoxib (Cipla, India), Sodium chloride (Analytical grade), Sodium hydroxide pellet and Methanol (Merck Mumbai), Conc. Hydrochloric acid, Benzyl Alcohol, Acetonitrile and Potassium chloride (Loba chemie pvt. Ltd, Mumbai), Calcium chloride (Glaxo lab ltd, Mumbai), Magnesium chloride (Bur Goyne burbiges and Co., Mumbai), Monobasic sodium phosphate (Merck Specialities Pvt. Ltd, Mumbai), Sodium bicarbonate (West coast lab, Mumbai), Benzyl alcohol (Loba chemie pvt. Ltd, Mumbai).

### Pre-formulation studies

#### *Identification of drug*

#### *General appearance*

Appearance- white powder

State- Solid

Odor- Unpleasant breath odor

Taste- Bitter

#### *Melting point*

Melting point was determined by using melting point apparatus, a small amount of

celecoxib was inserted in a capillary tube and inserted into the apparatus. The time required to melt and convert into liquid form was noted. Melting point is found to be 156-158 $^{\circ}C$

***Fourier Transform Infrared Spectroscopy (FTIR) Spectroscopy***

FTIR spectroscopy has been successfully used for exploring the differences in molecular conformations, crystal packing and hydrogen bonding arrangements for an organic compound. Spectral variations originate due to alteration in bonds that exhibit characteristic vibrational frequencies, leading to frequency shifts and splitting in absorption peaks. The Fourier Transform Infrared (FTIR) spectra is obtained using Fourier Transform Infrared FTIR spectrometer (Shimadzu).The celecoxib was previously ground and mixed thoroughly with potassium bromide. The KBr discs were prepared by compressing the powders at a pressure of 5 tons for 5 min in a hydraulic press and scanned from 4500 to 400 $cm^{-1}$. The Fourier Transform Infrared (FTIR) spectra of the drug showed a characteristic S=O symmetric and asymmetric stretching at 1166.02 $cm^{-1}$ and 1347.34 $cm^{-1}$ respectively. Medium intensity bands at 3337.95 $cm^{-1}$ and 3235.75 $cm^{-1}$ were seen as a doublet, which are attributed to the N–H stretching vibration of $–SO_2NH_2$ group. Vibrational stretching of $-CF_3$ group obtained at 1229.67 $cm^{-1}$ and 1275 $cm^{-1}$. Finally from the above characteristics it was identified that the drug was celecoxib.

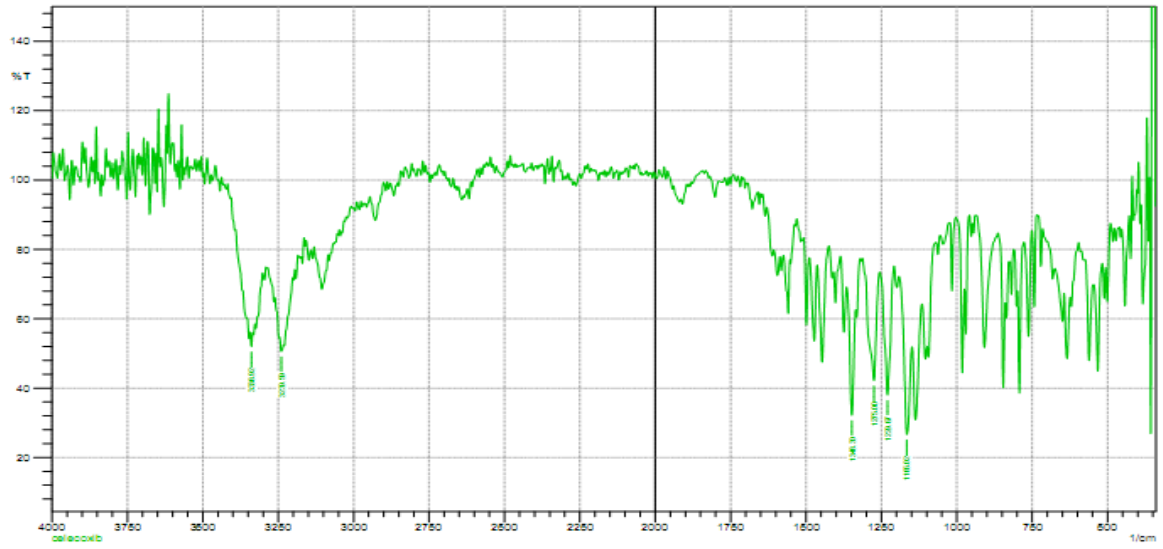


Figure 1: Fourier Transform Infrared (FTIR) Spectroscopy of celecoxib

**Preparation of calibration curve of celecoxib**

***Calibration curve of celecoxib in distilled water at 248 nm***

***Standard stock solution***

100 mg of celecoxib was dissolved in 10 mL of methanol and then the volume was made up to 100 ml with distilled water to form a clear solution.

***Working stock solution***

A series of celecoxib solutions ranging from 2 to 22 µg/ml were prepared from standard stock solution by dilution with distilled water. The low concentrations were scanned in the range of 200-400 nm to get the maximum absorbance at 248 nm and the absorbances of the solutions were measured spectrophotometrically at 248 nm.

Table 1: Calibration curve of celecoxib in distilled water at 248 nm

| Sl. No. | Conc. (ppm) | Absorbance at 248 nm |
|---|---|---|
| 1 | 2 | 0.091 |
| 2 | 6 | 0.291 |
| 3 | 10 | 0.479 |
| 4 | 14 | 0.674 |
| 5 | 18 | 0.87 |
| 6 | 20 | 0.948 |
| 7 | 22 | 1.035 |

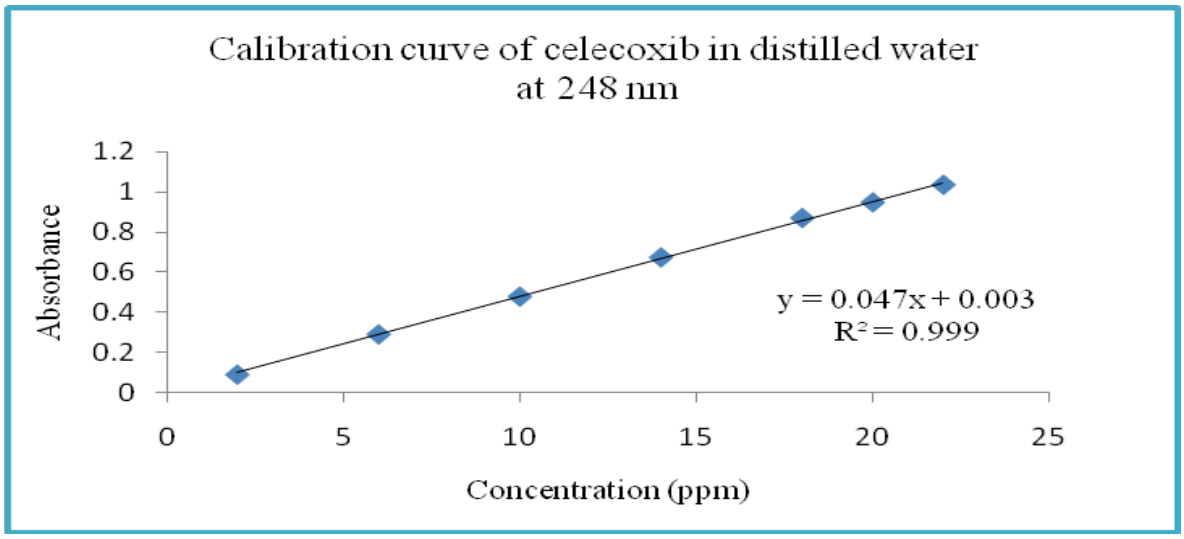


Figure 2: Calibration curve of celecoxib in distilled water at 248 nm

***Calibration curve of celecoxib in bicarbonate ringer of pH 7.2***

***Standard stock solution***

About 100 mg of celecoxib was dissolved in 10 ml of methanol and then the volume was made up to 100 ml with bicarbonate ringer to form a clear solution.

***Working stock solution***

A series of celecoxib solutions ranging from 2 to 20 µg/ml were prepared from standard stock solution by the dilution

with BCR pH 7.2. The low concentration was scanned in the range of 200-400 nm to get the maximum absorbance at 248 nm and the absorbance of the solutions were measured using UV Vis spectrophotometer at 248 nm.

Table 2: Calibration curve of celecoxib in bicarbonate ringer of pH 7.2

| Sl. No. | Conc. (ppm) | Absorbance at 248 nm |
|---|---|---|
| 1 | 2 | 0.131 |
| 2 | 6 | 0.336 |
| 3 | 10 | 0.530 |
| 4 | 14 | 0.728 |
| 5 | 18 | 0.921 |
| 6 | 20 | 1.024 |

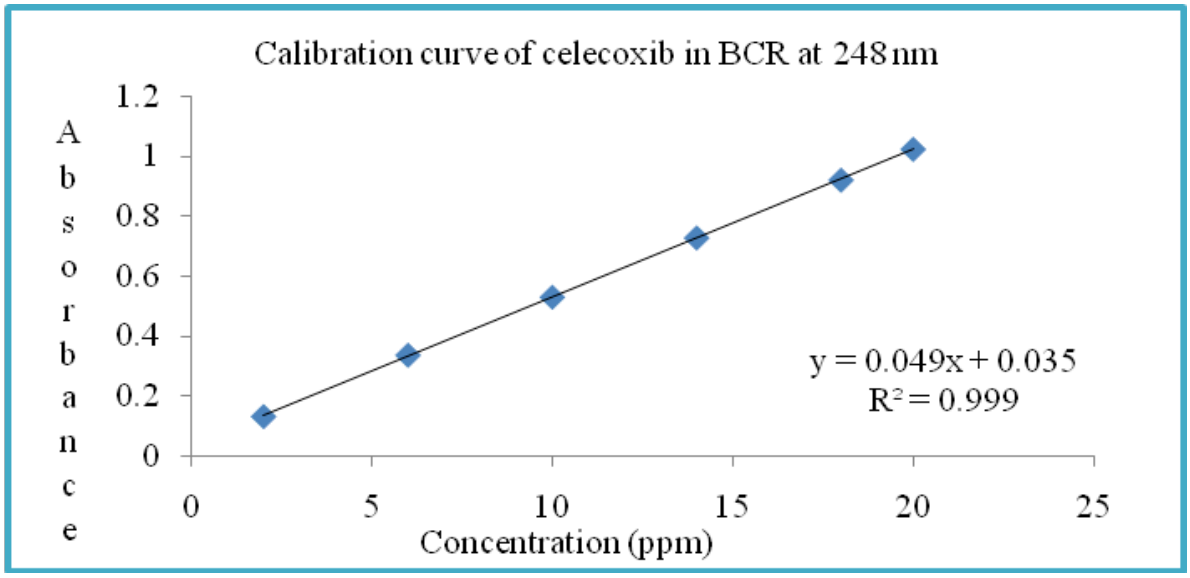


Figure 3: Calibration curve of celecoxib in BCR at 248 nm

***Calibration curve of celecoxib in methanol at 253 nm***

***Standard stock solution***

About 100 mg of celecoxib was dissolved in 100 mL of methanol to form a clear solution.

***Working stock solution***

A series of celecoxib solutions ranging from 2 to 14 µg/ml were prepared from standard stock solution by the dilution with methanol. The low concentration was scanned in the range of 200-400 nm to get the maximum absorbance at 248 nm and the absorbances of the solutions were measured spectrophotometrically at 248 nm.

Table 3: Calibration curve of celecoxib in methanol at 253 nm

| Sl. No. | Conc.(ppm) | Absorbance at 248 nm |
|---|---|---|
| 1 | 2 | 0.132 |
| 2 | 4 | 0.278 |
| 3 | 6 | 0.437 |
| 4 | 8 | 0.589 |
| 5 | 10 | 0.754 |
| 6 | 12 | 0.910 |
| 7 | 14 | 1.061 |

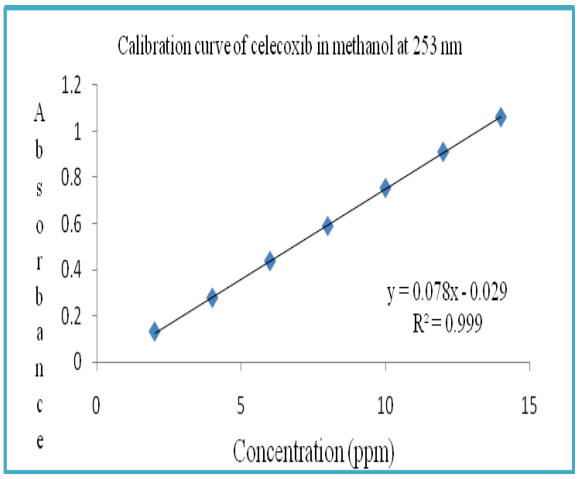


Figure 4: Calibration curve of celecoxib in Methanol at 253 nm

***Calibration curve of celecoxib in acetonitrile and methanol (1:1) by high performance liquid chromatography (HPLC):***

***Standard stock solution***

About 100 mg of celecoxib was dissolved in 100 ml of acetonitrile and methanol at 1:1 ratio to form a clear solution.

***Working stock solution***

A series of celecoxib solutions ranging from 5 to 100 µg/ml were prepared from standard stock solution by the dilution with acetonitrile and methanol (1:1) ratio. The low concentration was scanned in the range of 200-400 nm to get the maximum absorbance at 248 nm and the retention time and peak area of the solutions were measured chromatographically at 248 nm.

Table 4: Calibration curve of celecoxib in acetonitrile and methanol (1:1) by HPLC:

| Sl. No. | Conc. (ppm) | Peak area |
|---|---|---|
| 1 | 5 | 9.114 |
| 2 | 10 | 17.649 |
| 3 | 20 | 35.867 |
| 4 | 40 | 67.558 |
| 5 | 60 | 105.578 |
| 6 | 80 | 138.683 |
| 7 | 100 | 175.379 |

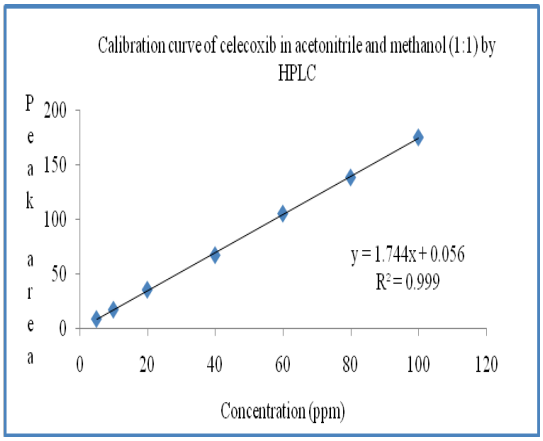


Figure 5: Calibration curve of celecoxib in acetonitrile and methanol (1:1) by high

performance liquid chromatography (HPLC)

**Solubility of celecoxib in distilled water**

Excess amount of celecoxib was added to 10 ml of the distilled water taken in a closed vial and shaken in a thermostat water bath shaker for 24 hrs at 25 $^0$C ± 0.5 $^0$C. Finally the solutions were filtered by Whatman filter paper (grade 41, HiMedia) and after a suitable dilution of the filtrate the drug concentration was determined spectrophotometrically at 248 nm.

Table 5: Solubility of celecoxib in distilled water

| Solvent | pH | Solubility (mg/ml) | Solubility (% w/v) |
|---|---|---|---|
| Distilled Water | 6.8 | 0.0068 | 0.00068 |

**Solubility of celecoxib in different oils**

An excess amount of celecoxib was added into different oils at 50 $^0$C to prepare a saturated solution. The solution of celecoxib in different oils were then cooled and left overnight at 4 $^0$C in refrigerator. The solution was subsequently centrifuged (cooling centrifuge) at 4 $^0$C at 5000 rpm (Remi Equipments Ltd., Mumbai, India). One milliliter of the clear supernatant was dissolved in 10 ml of acetonitrile: methanol (1:1) and analyzed for celecoxib content by reverse-phase HPLC.

Table 6: Solubility of celecoxib in different oils

| Sl. No. | Oils | Solubility (mg/ml) | Solubility (% w/v) |
|---|---|---|---|
| 1 | Arachis | 11.739 | 1.173 |
| 2 | Castor | 11.542 | 1.154 |
| 3 | Mustard | 14.169 | 1.416 |
| 4 | Olive | 9.888 | 0.988 |
| 5 | Sesame | 5.111 | 0.511 |
| 6 | Sunflower | 3.902 | 0.390 |

**Preparation of test solutions**

***Preparation of celecoxib oily eye drops without preservative (13)***

The required amounts of celecoxib was dissolved in different oily vehicles and stirred for half an hour to give celecoxib (1% w/v) solution in arachis, castor and mustard. Similarly celecoxib (0.5% w/v) solutions in arachis, castor, mustard, olive, sesame, and celecoxib (0.3% w/v) in sunflower and sesame were also prepared without addition of preservative.

Table 7: Preparation of celecoxib oily eye drops without preservative

| Sl. No. | Oils | Celecoxib (% w/v) |
|---|---|---|
| 1 | Arachis | 1% |
| 2 | Arachis | 0.5% |
| 3 | Castor | 1% |
| 4 | Castor | 0.5% |
| 5 | Mustard | 1% |
| 6 | Mustard | 0.5% |
| 7 | Olive | 0.5% |
| 8 | Sesame | 0.5% |
| 9 | Sesame | 0.3% |
| 10 | Sunflower | 0.3% |

***Preparation of celecoxib oily eye drops with preservative***

The required amounts of celecoxib was dissolved in different oily vehicles and stirred for half an hour, during the stirring 0.5% of benzyl alcohol was added as preservative to give celecoxib (1% w/v) solution in arachis, castor and mustard. Similarly celecoxib (0.5% w/v) solutions in arachis, castor, mustard, olive, sesame, and celecoxib (0.3% w/v) in sunflower and sesame were also prepared.

Table 8: Preparation of celecoxib oily eye drops with preservative

| Sl. No. | Oils | Celecoxib (% w/v) | Preservatives (B.A) |
|---|---|---|---|
| 1 | Arachis | 1% | 0.5% |
| 2 | Arachis | 0.5% | 0.5% |
| 3 | Castor | 1% | 0.5% |
| 4 | Castor | 0.5% | 0.5% |
| 5 | Mustard | 1% | 0.5% |
| 6 | Mustard | 0.5% | 0.5% |
| 7 | Olive | 0.5% | 0.5% |
| 8 | Sesame | 0.5% | 0.5% |
| 9 | Sesame | 0.3% | 0.5% |
| 10 | Sunflower | 0.3% | 0.5% |

**Evaluation of oily eye drops (14-17)**

***Physical appearance***

All formulations were observed for physical appearance like color and precipitate and concluded that eight solutions are colorless and no precipitants

are formed. But in case of mustard 1% and 0.5 % ophthalmic solutions are turbid and precipitates are formed and the solution is not clear.

Table 9: Physical appearance of the drug

| Sl. No. | Oils | % Drug | Visual assessment | Precipitants |
|---|---|---|---|---|
| **1** | Arachis | 1% | yellow | Not found |
| **2** | Arachis | 0.5% | yellow | Not found |
| **3** | Castor | 1% | Dark yellow | Not found |
| **4** | Castor | 0.5% | Dark yellow | Not found |
| **5** | Mustard | 1% | Dark yellow | Found |
| **6** | Mustard | 0.5% | Dark yellow | Found |
| **7** | Olive | 0.5% | Yellowish white | Not found |
| **8** | Sesame | 0.5% | yellow | Not found |
| **9** | Sesame | 0.3% | yellow | Not found |
| **10** | Sunflower | 0.3% | Yellowish white | Not found |

***Drug content***

Assay of the formulations were carried out by diluting with acetonitrile: methanol (1:1) and 20 µl of the diluted solution injected into a chromatographic system equipped with 600 pump controllers, 2487 dual absorbance detector, and 7725i Rheodyne injector. The resolution of celecoxib was achieved using acetonitrile : methanol (1:1) at a flow rate of 1.5 ml/min as the mobile phase in an isocratic run through a Spherisorb C 18, 5 m (250×4.6 mm i.d.) column. The eluent was monitored for celecoxib at 248 nm. The retention time and the lowest limit of quantification of celecoxib were 5.6 min and 1.5 mg/ml, respectively.

***Drug permeation study (9-12, 19)***

Freshly excised goat cornea was fixed between clamped donor and receptor compartments of an all-glass modified Franz diffusion cell in such a way that its epithelial surface faced the donor compartment. The corneal area available for diffusion was 0.67 $cm^2$. The receptor compartment was filled with 10 mL of freshly prepared bicarbonate ringer solution pH 7.2, and all air bubbles were expelled from the compartment. An aliquot (1 mL) of test solution was placed on the cornea, and the opening of the donor cell was sealed with a glass cover slip, while receptor fluid was kept at 37°C with constant stirring using a Teflon-coated magnetic stirrer bead. The permeation study was continued for 120

minutes; samples were withdrawn from the receptor and analyzed for celecoxib content by measuring absorbance at 248 nm in a spectrophotometer (1800 Shimadzu, Kyoto, Japan). Results were expressed as amount permeated and percent permeation or in vitro ocular availability. The permeation (%) or in vitro ocular availability was calculated as follows.

%Permeation = [Amount of Drug permeated in receptor / (Initial amount of drug in donor)] ×100

Table 10: Permeation of celecoxib oily eye drops without preservative

| Sl. No. | Oils | % Drug | Amt permeated (mg) | %Permeation | %Hydration |
|---|---|---|---|---|---|
| 1 | Arachis | 1% | 0.077±0.001 | 0.775±0.016 | 73.065±0.07 |
| 2 | Arachis | 0.5% | 0.036±0.007 | 0.722±0.043 | 77.841±0.062 |
| 3 | Castor | 1% | 0.04±0.003 | 0.404±0.031 | 76.566±0.042 |
| 4 | Castor | 0.5% | 0.036±0.001 | 0.721±0.029 | 79.468±0.097 |
| 5 | Mustard | 1% | 0.099+0.005 | 0.998±0.005 | 76.086±0.064 |
| 6 | Mustard | 0.5% | 0.048±0.005 | 0.975±0.014 | 75.409±0.008 |
| 7 | Olive | 0.5% | 0.047±0.009 | 0.946±0.099 | 79.834±0.036 |
| 8 | Sesame | 0.5% | 0.074±0.011 | 1.489±0.037 | 71.717±0.029 |
| 9 | Sesame | 0.3% | 0.059±0.009 | 1.981±0.019 | 75.966±0.09 |
| 10 | Sunflower | 0.3% | 0.051±0.008 | 1.727±0.074 | 74.728±0.019 |

Table 11: Permeation of celecoxib formulation with 0.5% benzyl alcohol as preservative

| Sl. No. | Oils | % Drug | Amt permeated (mg) | %Permeation | %Hydration |
|---|---|---|---|---|---|
| 1 | Arachis | 1% | 0.089 | 0.895 | 74.647 |
| 2 | Arachis | 0.5% | 0.058 | 1.179 | 74.931 |
| 3 | Castor | 1% | 0.069 | 0.693 | 75.240 |
| 4 | Castor | 0.5% | 0.064 | 1.297 | 75.401 |
| 5 | Mustard | 1% | 0.108 | 1.083 | 75.510 |
| 6 | Mustard | 0.5% | 0.066 | 1.322 | 77.306 |
| 7 | Olive | 0.5% | 0.071 | 1.420 | 77.061 |
| 8 | Sesame | 0.5% | 0.072 | 1.448 | 75.365 |
| 9 | Sesame | 0.3% | 0.054 | 1.809 | 77.692 |
| 10 | Sunflower | 0.3% | 0.051 | 1.727 | 77.525 |

***Corneal hydration***

At the end of the experiment, each cornea was weighed accurately and soaked in 1ml of methanol, dried for overnight at 90 °C in the hot air oven and reweighed. From the difference in weight corneal hydration was calculated as follow:

% Corneal Hydration= [(weight of wet cornea - weight of dry cornea)/ weight of wet cornea] × 100

***Viscosity determinations of oil bases (18)***

Viscosity of oils bases in the formulation with and without combinations and excipients proposed to be used in the formulation development of celecoxib eye drops was determined using Brookfield programmable viscometer (Model – DVIII).

Table 12: Viscosity of different oil bases

| Sl. No. | Oils | Viscosity in centipoises |
|---|---|---|
| 1 | Arachis | 50 cps |
| 2 | Castor | 900 cps |
| 3 | Mustard | 700 cps |
| 4 | Olive | 810 cps |
| 5 | Sesame | 391 cps |
| 6 | Sunflower | 600 cps |

***Measurement of partition coefficient***

Equal volumes of formulations (1%w/v, 0.5%w/v, 0.3%w/v) with or without the additives (control) and phosphate buffer pH 7.4 were shaken for 45 minutes at room temperature by hand. The experiment was done with duplicate samples of each formulation. The concentration of drug in each phase i.e. oil and phosphate buffer was analyzed by HPLC Spectrophotometer at 248nm and the partition coefficient was calculated

Table 13: Partition Coefficient of different oils

| Sl. No. | Oils | Peak area | Solubility in oil (mg/ml) | Amt. in Oil mg/ml | PC |
|---|---|---|---|---|---|
| 1 | Arachis | 225.955 | 11.739 | 10.589 | 9.208 |
| 2 | Arachis BA | 220.065 | 11.739 | 10.619 | 9.484 |
| 3 | Castor | 134.359 | 11.54 | 10.862 | 15.974 |
| 4 | Castor BA | 139.903 | 11.542 | 10.834 | 15.293 |
| 5 | Mustard | 616.635 | 14.169 | 11.015 | 3.492 |
| 6 | Mustard BA | 616.615 | 14.169 | 11.015 | 3.492 |
| 7 | Olive | 154.837 | 9.888 | 9.103 | 11.595 |
| 8 | Olive BA | 186.719 | 9.888 | 8.939 | 9.424 |

| | | | | | |
|---|---|---|---|---|---|
| 9 | Sesame | 134.845 | 5.111 | 4.428 | 6.488 |
| 10 | Sesame BA | 250.073 | 5.111 | 3.837 | 3.012 |
| 11 | Sunflower | 239.178 | 3.902 | 2.684 | 2.204 |
| 12 | Sunflower BA | 227.431 | 3.902 | 2.745 | 2.371 |

***Stability Studies***

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 oily ophthalmic solution of celecoxib were filled into dried glass ampoules and heat sealed. The accelerated stability testing on ophthalmic formulations was conducted by storage at 45 $^{o}$ C and 75% RH. The long-term stability studies were conducted by storage at room temperature. The samples of ophthalmic formulations were kept under accelerated storage conditions were withdrawn at 0 day, 7 days, 15days and 30 days then analyzed for celecoxib content by HPLC. The samples stored at room temperature were withdrawn at 0 day, 7 days, 15 days, 30 days and 45 days then analyzed for celecoxib contents by HPLC. The samples of oil formulations were also tested for appearance (color, and precipitants).

Table 14: Composition of formulations and estimated drug content

| **Formulation** | **Oil** | **% drug added** | **Benzyl Alcohol (% V/V)** | **% Drug content as estimated** |
|---|---|---|---|---|
| F1 | Arachis | 1 | - | 99.9±0.1 |
| F2 | Arachis | 1 | 0.5 | 100.1±0.1 |
| F3 | Arachis | 0.5 | - | 99.6±0.2 |
| F4 | Arachis | 0.5 | 0.5 | 100.0±0.2 |
| F5 | Castor | 1 | - | 99.8±0.2 |
| F6 | Castor | 1 | 0.5 | 100.4+0.3 |
| F7 | Castor | 0.5 | - | 99.4±0.3 |
| F8 | Castor | 0.5 | 0.5 | 99.5±.4 |
| F9 | Mustard | 1 | - | 99.8±.3 |
| F10 | Mustard | 1 | 0.5 | 99.9±0.6 |
| F11 | Mustard | 0.5 | - | 99.6±0.3 |

| | | | | |
|---|---|---|---|---|
| F12 | Mustard | 0.5 | 0.5 | 99.6+0.4 |
| F13 | Olive | 0.5 | - | 98.9±0.4 |
| F14 | Olive | 0.5 | 0.5 | 99.5±0.3 |
| F15 | Sesame | 0.5 | - | 100.1±0.3 |
| F16 | Sesame | 0.5 | 0.5 | 100.4±0.2 |
| F17 | Sesame | 0.3 | - | 99.6±0.3 |
| F18 | Sesame | 0.3 | 0.5 | 99.9±0.6 |
| F19 | Sunflower | 0.3 | - | 99.5±0.4 |

Table 15: Stability of celecoxib in oily ophthalmic solutions under accelerated storage conditions (40 $^{o}$C/75% RH)

| **Formulations** | **Celecoxib content** | | | |
|---|---|---|---|---|
| | **Initial** | **7 days** | **15 days** | **30 days** |
| F1 | 99.9±0.1 | 99.4±0.5 | 97.9±0.5 | 92.5±0.7 |
| F2 | 100.1±0.1 | 99.9±0.3 | 98.2±0.3 | 92.6±1.4 |
| F3 | 99.6±0.2 | 99.0±0.2 | 97.4±0.1 | 93.2±0.9 |
| F4 | 100.0±0.2 | 99.2±0.2 | 97.6±0.3 | 94.8±1.1 |
| F5 | 99.8±0.2 | 99.6±0.2 | 98.1±0.6 | 91.1±0.6 |
| F6 | 100.4+0.3 | 99.8+0.5 | 97.5±0.7 | 92.6±0.7 |
| F7 | 99.4±0.3 | 99.2±0.4 | 97.2±0.4 | 92.7±0.7 |
| F8 | 99.5±.4 | 99.3±0.3 | 97.6±0.3 | 93.8±0.9 |
| F9 | 99.8±.3 | 98.9±0.2 | 97.2±0.3 | 99.8±0.4 |
| F10 | 99.9±0.6 | 99.6±0.1 | 98.1±0.1 | 91.5±0.2 |
| F11 | 99.6±0.3 | 99.4±0.3 | 96.5±0.7 | 85.8±0.4 |
| F12 | 99.6+0.4 | 99.6+0.3 | 97.2+0.3 | 87.5±0.8 |
| F13 | 98.9±0.4 | 98.8±0.2 | 96.8±0.7 | 88.0±0.7 |
| F14 | 99.5±0.3 | 99.1±0.1 | 97.6±0.4 | 90.4±0.1 |
| F15 | 100.1±0.3 | 99.9±0.1 | 96.9±0.6 | 91.9±0.7 |
| F16 | 100.4±0.2 | 100.2±0.2 | 97.2±0.4 | 93.4±0.6 |
| F17 | 99.6±0.3 | 99.1±0.3 | 96.8±0.5 | 92.5±0.8 |
| F18 | 99.9±0.6 | 99.4±0.1 | 99.1±0.6 | 98.5±0.3 |
| F19 | 99.5±0.4 | 99.2±0.2 | 97.1±0.7 | 90.6±0.3 |
| F20 | 99.5±0.5 | 99.3±0.5 | 97.3±0.4 | 90.8±0.5 |

Table 16: Stability of celecoxib in oily ophthalmic solutions under room temperature (25 $^{o}$C/60% RH)

| Formulations | Celecoxib content | | | | |
|---|---|---|---|---|---|
| | Initial | 7 days | 15 days | 30 days | 45 days |
| F1 | 99.1±0.1 | 99.4±0.5 | 97.9±0.5 | 91.5±0.7 | 85.2±0.3 |
| F2 | 99.8±0.1 | 99.9±0.3 | 98.2±0.3 | 91.6±1.4 | 87.0±0.1 |
| F3 | 99.01±0.2 | 99.0±0.2 | 97.4±0.1 | 92.2±0.9 | 84.9±0.6 |
| F4 | 99.5±0.2 | 99.2±0.2 | 97.6±0.3 | 93.8±1.1 | 89.1±0.1 |
| F5 | 99.06±0.2 | 99.6±0.2 | 98.1±0.6 | 90.1±0.6 | 81.8±0.3 |
| F6 | 100.1±0.3 | 99.8±0.5 | 97.5±0.7 | 91.6±0.7 | 85.5±0.7 |
| F7 | 99.04±0.3 | 99.2±0.4 | 97.2±0.4 | 91.7±0.7 | 84.7±0.3 |
| F8 | 99.5±0.4 | 99.3±0.3 | 97.6±0.3 | 92.8±0.9 | 87.1±0.1 |
| F9 | 99.2±0.3 | 98.9±0.2 | 97.2±0.3 | 98.8±0.4 | 88.4±0.7 |
| F10 | 99.3±0.6 | 99.6±0.1 | 98.1±0.1 | 90.5±0.2 | 81.6±0.3 |
| F11 | 99.6±0.3 | 99.4±0.3 | 96.5±0.7 | 84.8±0.4 | 79.9±0.2 |
| F12 | 99.6±0.4 | 99.6±0.3 | 97.2±0.3 | 86.5±0.8 | 81.1±0.4 |
| F13 | 98.9±0.4 | 98.8±0.2 | 96.8±0.7 | 87.0±0.7 | 81.7±0.6 |
| F14 | 99.5±0.3 | 99.1±0.1 | 97.6±0.4 | 89.4±0.1 | 82.4±0.8 |
| F15 | 100.1±0.3 | 99.9±0.1 | 96.9±0.6 | 90.9±0.7 | 83.1±0.6 |
| F16 | 99.9±0.2 | 100.2±0.2 | 97.2±0.4 | 92.4±0.6 | 83.9±0.9 |
| F17 | 99.6±0.3 | 99.1±0.3 | 96.8±0.5 | 91.5±0.8 | 91.1±0.8 |
| F18 | 99.9±0.6 | 99.4±0.1 | 99.1±0.6 | 97.5±0.3 | 97.0±0.4 |
| F19 | 99.5±0.4 | 99.2±0.2 | 97.1±0.7 | 89.6±0.3 | 81.6±0.2 |
| F20 | 99.1±0.5 | 99.3±0.5 | 97.3±0.4 | 90.3±0.5 | 82.5±0.4 |

## RESULTS AND DISCUSSION

### Solubility and partition coefficient

Due to poor solubility of celecoxib in distilled water (0.00068 % w/v); different oils have been taken as vehicle. Solubility was measured at 4 $^{o}$C. celecoxib was found to have maximum solubility (%w/v) in Mustard (1.41) followed by arachis (1.17) and castor oil (1.15). In the rest of the oils like olive, sesame, and Sunflower oil, the solubility was between 0.39 to 0.98%. The partition coefficient of celecoxib between oil and phosphate buffer (pH 7.4) was also found to be maximum with castor oil, followed by olive oil, arachis oil, while the

minimum partition coefficient was observed with sunflower.

Table 17: Solubility of celecoxib in different oils

| Sl. No. | Oils | Solubility (mg/ml) | Solubility (% w/v) |
|---|---|---|---|
| 1 | Arachis | 11.739 | 1.173 |
| 2 | Castor | 11.542 | 1.154 |
| 3 | Mustard | 14.169 | 1.416 |
| 4 | Olive | 9.888 | 0.988 |
| 5 | Sesame | 5.111 | 0.511 |
| 6 | Sunflower | 3.902 | 0.390 |

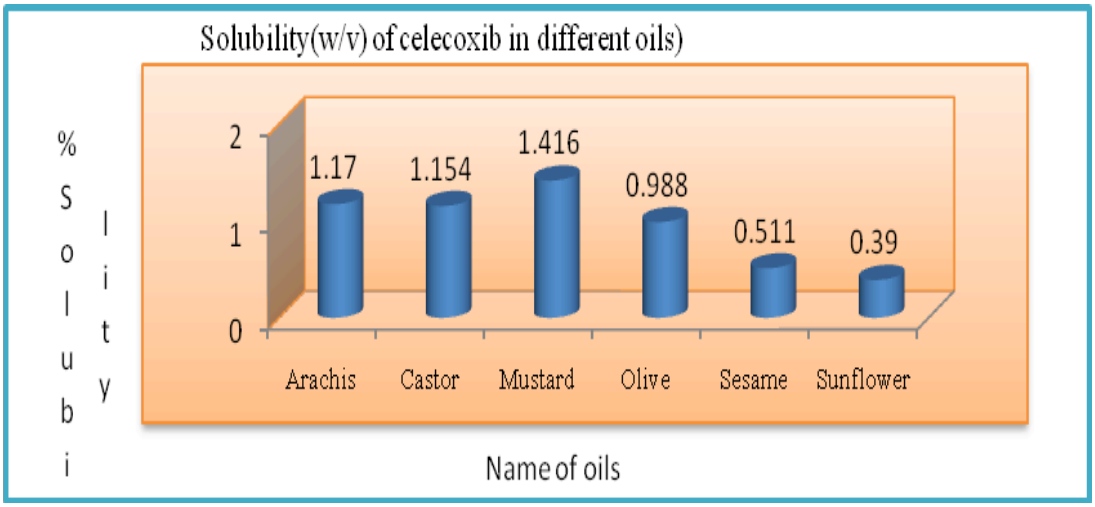


Figure 6: Solubility (w/v) of celecoxib in different oils

**Physical Appearance**

All formulations were observed for physical appearance like color and precipitate and concluded that eight solutions are colorless and no precipitants are formed. But in case of mustard 1% and 0.5% ophthalmic solutions are turbid and precipitates are formed and the solution is not clear.

**Drug Content:** Drug content was estimated using HPLC and found to be in the range of 99.4±0.3 % to 100.4±0.2

**Permeation Study, effect of benzyl alcohol and percent hydration**

The results reveal the amount of celecoxib permeated with drops formulated as formulation 1% was mustard > arachis > castor, formulation 0.5% was in

sesame>mustard>olive>arachis>castor and formulation 0.3% in sesame>sunflower. The lesser corneal permeability of celecoxib from castor oil drops could be attributed to the higher partitioning of celecoxib in castor oil. Similarly, higher permeability of the drug from sesame and mustard oil drops could be due to the lower partition coefficient of drug between the oil and aqueous phase. The normal cornea has a hydration level of 75-80%. All the formulations were within the normal range of hydration except arachis (1%), sesame (0.5%) and sunflower (0.3%) were showing low hydration (71%-73%) indicating corneal dehydration. Benzyl alcohol, a commonly used preservative, was added to oil formulations at 0.5% (v/v) concentration. The addition of benzyl alcohol to oil drops resulted in increased permeation of celecoxib from all the formulations compared with the formulations without the preservative, which may be due to its surfactant property. Partition experiments indicated higher partitioning of drug from the oil to aqueous phase in the presence of benzyl alcohol in all the formulations except in sesame oil. There was increased corneal hydration associated with the use of benzyl alcohol, in most of the formulations. All the formulations were showed normal corneal hydration. Increased celecoxib concentration in arachis, castor, and mustard oils from 0.5% to 1.0 % resulted in a significant increase in % permeation. Further, the use of higher drug concentrations was associated with higher corneal hydration levels, indicating corneal damage. Among all the formulations, celecoxib 0.3% (w/v) drops in sesame oil containing 0.5% (v/v) benzyl alcohol showed maximum permeation with corneal hydration in normal range. The saturation solubility of celecoxib in sesame oil at 4°C is 0.511 % (w/v). Hence celecoxib 0.5% (w/v) solution in sesame oil, being below the saturation level, will not precipitate at 4 °C and the chances of crystallization of celecoxib from the solution due to climatic change leading to physical instability appear to be remote. The most convenient way of delivering drug to the eye is topical application of an aqueous solution. Drug from the aqueous solution partitions through the corneal epithelium, stroma and endothelium into the aqueous humor. One principle demerit of topically applied aqueous drug solution is the loss of drug due to drainage which results in lower ocular availability of drug and a therapeutic effect of shorter duration. One way of overcoming the problem is to apply the drug in the form of an oily solution. Vegetable oils like olive, castor,

and sesame oil are used as vehicles for oil- based drops.

Table 18: % permeation, % hydration and partition coefficient study of the formulations

| Oils | Celecoxib (%w/v) | Without Benzyl alcohol | | | | With benzyl alcohol | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Amt. permeated (mg) | % Permeation | % Hydration | Partition coefficient | Amt. permeated (mg) | % Permeation | % Hydration | PC |
| Arachis | 1% | 0.077 | 0.775 | 73.065 | 0.902 | 0.089 | 0.895 | 74.647 | 9.208 |
| Arachis | 0.5% | 0.036 | 0.722 | 77.841 | 0.902 | 0.058 | 1.179 | 74.931 | 9.484 |
| Castor | 1% | 0.04 | 0.404 | 76.566 | 0.941 | 0.069 | 0.693 | 75.240 | 15.974 |
| Castor | 0.5% | 0.036 | 0.721 | 79.468 | 0.941 | 0.064 | 1.297 | 75.401 | 15.293 |
| Mustard | 1% | 0.099 | 0.998 | 76.086 | 0.777 | 0.108 | 1.083 | 75.510 | 3.492 |
| Mustard | 0.5% | 0.048 | 0.975 | 75.409 | 0.777 | 0.066 | 1.322 | 77.306 | 3.492 |
| Olive | 0.5% | 0.047 | 0.946 | 79.834 | 0.920 | 0.071 | 1.420 | 77.061 | 11.595 |
| Sesame | 0.5% | 0.074 | 1.489 | 71.717 | 0.866 | 0.072 | 1.448 | 75.365 | 9.424 |
| Sesame | 0.3% | 0.059 | 1.981 | 75.966 | 0.866 | 0.054 | 1.809 | 77.692 | 6.488 |
| Sunflower | 0.3% | 0.051 | 1.727 | 74.728 | 0.687 | 0.051 | 1.727 | 77.525 | 3.012 |

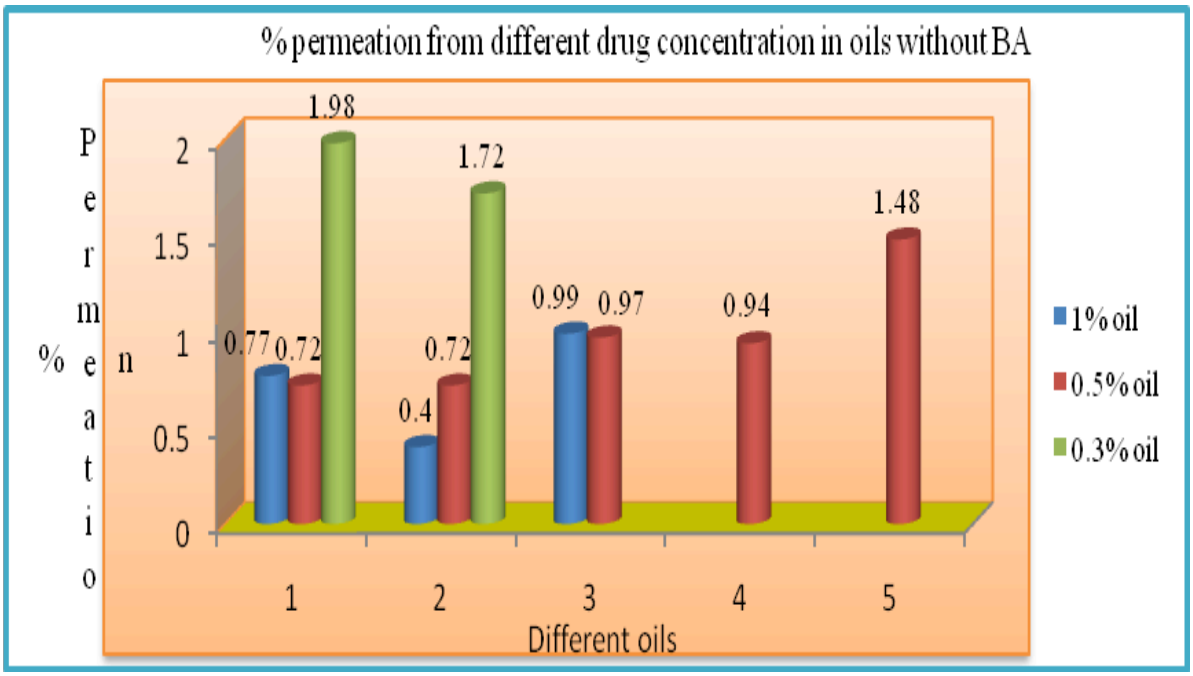


Figure 7: Percent permeation from different drug concentration in oils without benzyl alcohol

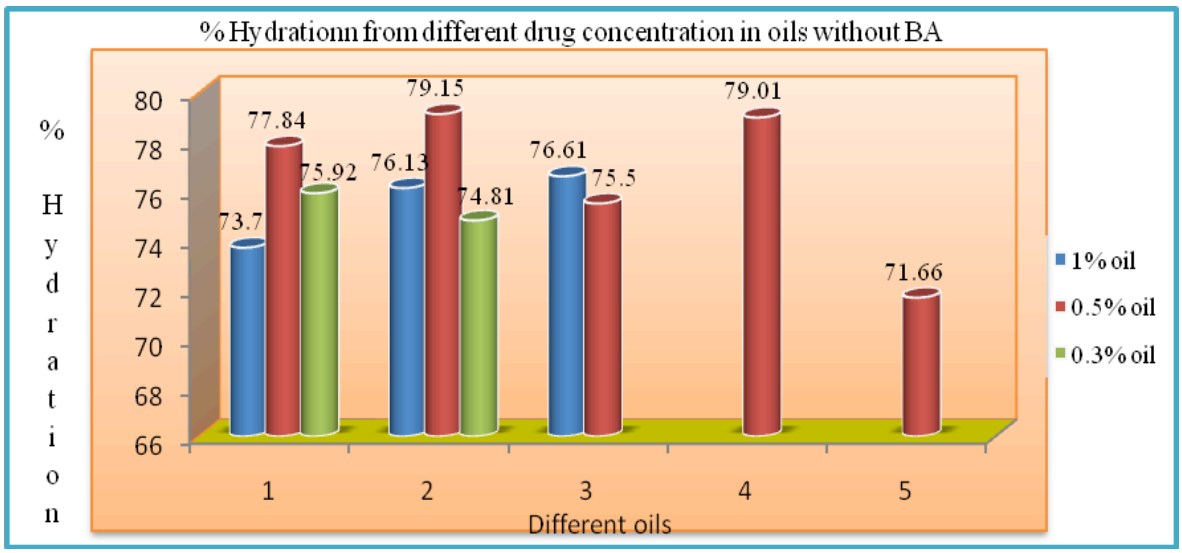


Figure 8: Percent hydration from different drug concentration in oils without benzyl alcohol

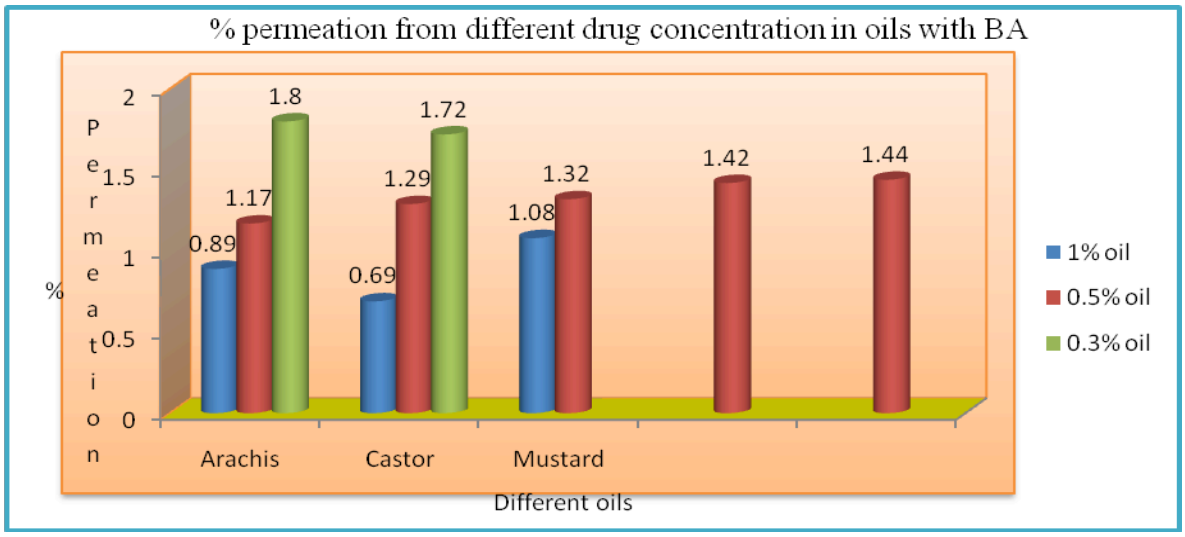


Figure 9: Percent permeation from different drug concentration in oils with benzyl alcohol

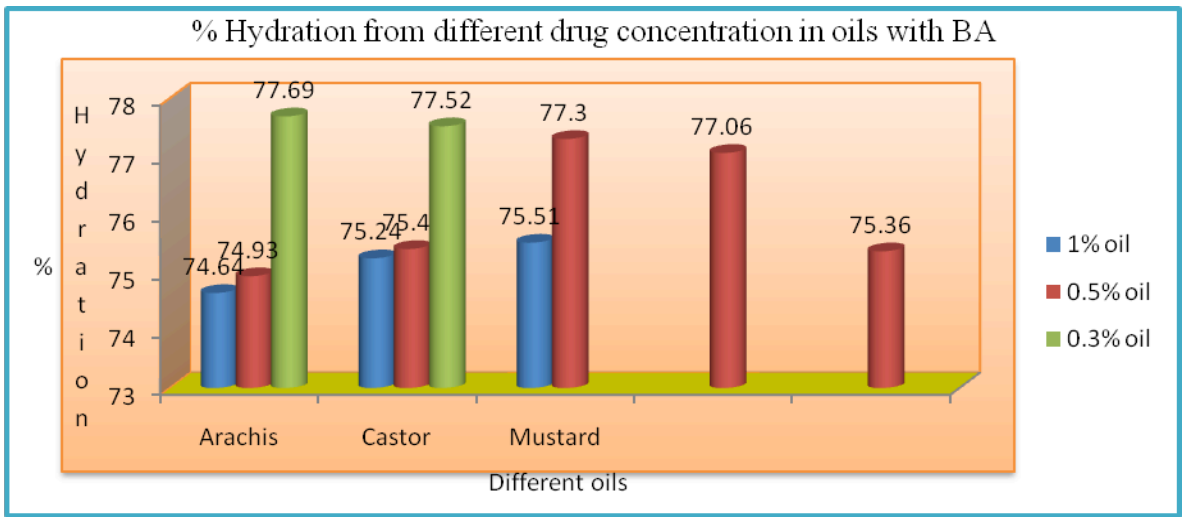


Figure 10: Percent hydration from different drug concentration in oils with benzyl alcohol

**Stability Studies**

From the stability study it was revealed that all the formulations were stable and drug content was in the range 98.9% - 100.4% but precipitate was found in mustard (1%) formulation.

## CONCLUSIONS

Steroids are used in the treatment of allergic ocular disorders, corneal burns, uveal tract inflammation, and other ocular inflammations but their use is limited by their tendency to increase intraocular pressure and to cause cataract upon chronic administration. Steroids also exacerbate ocular infections and diminish corneal/stromal wound healing. Anti-inflammatory drugs (NSAIDs) like indomethacin, flurbiprofen, ketorolac, and diclofenac have been found to be viable alternatives to steroids in treating ocular inflammation. Celecoxib is a non-steroidal anti-inflammatory drug (NSAIDS), acts by inhibition of prostaglandins, by inhibiting the activity of the enzyme cyclooxygenase-2 (COX-2). The most convenient way of delivering drug to the eye is topical application of an aqueous solution. Drug from the aqueous solution partitions through the corneal epithelium, stroma and endothelium to the aqueous humor. One principle demerit of topically applied aqueous drug solution is the loss of drug due to drainage which results in lower ocular availability of drug and a therapeutic effect of shorter duration. One way of overcoming the problem is to apply the drug in the form of an oily solution. Vegetable oils like olive, castor, and sesame oil are used as vehicles for oil-based drops. Considering the formulation factors as discussed above it can be concluded that celecoxib 0.5% (w/v) solution in sesame oil, being below the saturation level; containing benzyl alcohol (0.5%) could be the better choice.

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