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*Research Article*

# Permeation Studies of Indomethacin from Different Emulsions for Nasal Delivery and Their Possible Anti-Inflammatory Effects

**H. Yeşim Karasulu,[1,3] Z. Evren Şanal,[1] Sumru Sözer,[2] Tamer Güneri,[1] and Gökhan Ertan[1]**

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***Abstract.*** The purpose of this research was to develop an emulsion formulation of indomethacin (IND) suitable for nasal delivery. IND was incorporated into the oil phases of oil in water (O/W) and water in oil (W/O) emulsions. For this purpose, different emulsifying agents (Tween 80, Span 80 and Brij 58) were used in two emulsion formulations. When the effects of several synthetic membranes (nylon, cellulose, cellulose nitrate) were compared with the sheep nasal mucosa, the cellulose membrane and sheep nasal mucosa showed similar permeation properties for O/W emulsion ($P > 0.05$). To examine the absorption characteristics of IND, the anti-inflammatory properties of intravenous solution of IND, intranasal O/W emulsions of IND (with or without enhancers) and intranasal solution of IND (IND-Sol) were investigated in rats with carrageenan-induced paw edema. When citric acid was added to the nasal emulsion, the anti-inflammatory activity was similar to that of intravenous solution ($P > 0.05$). Finally, it was concluded that, intranasal administration of IND emulsion with citric acid may be considered as an alternative to intravenous and per oral administrations of IND to overcome their adverse effects.

**KEY WORDS:** anti-inflammatory activity; indomethacin; nasal emulsion; penetration enhancer.

## INTRODUCTION

The systemic bioavailability and efficacy of drugs differ according to their administration route and pharmaceutical form. Nonparenteral drug administration has been shown to be effective for systemic drug delivery (1). The nasal route of drug delivery has advantages over the other alternative systems of non-invasive drug administration (2,3). Nasal administrations may be a promising route for long-term systemic delivery particularly when the drug is ineffective orally due to first-pass metabolism. It is known that small lipophilic molecules are generally well absorbed through the nasal mucosa. The nasal cavity has a relatively large absorptive surface area and the high vascularity of the nasal mucosa ensures that absorbed compounds are rapidly removed. Drugs absorbed into the rich network of blood vessels pass directly into the systemic circulation (4). The plasma profile following intranasal (ins) drug absorption is similar for some drugs to that obtained by bolus intravenous (iv) injection (5,6).

Prolonging residence time in the nasal cavity is possible by increasing the viscosity of the vehicle, using bioadhesives or making other suitable formulation changes such as emulsions, suspensions or liposomes (7). Emulsions generally provide the most suitable pharmaceutical formulations for dissolving lipophilic drugs, by raising the soluble concentration of the drug. Additionally, the oil phase of an emulsion allows the lipophilic drug to stay in the form of a solution. Therefore, emulsions can potentially enhance the bioavailability of poorly water-soluble drug (6). When emulsions are used in nasal formulations, the interaction between the oil droplets and nasal mucosal membrane is likely to play an important role in the enhancement mechanism. Emulsions also provide good biocompatibility (8). Furthermore, morphological studies have shown that emulsions do not cause any significant changes at nasal mucosa.

A number of lipophilic drugs, such as propronalol, naloxone, salmon calcitonin and testosterone have been shown to be completely or almost completely absorbed nasally in animal models (3,9). Indomethacin (IND), a lipophilic model drug in this study, is an anti-inflammatory and analgesic-antipyretic drug used in experiments. It produces erosions and ulcers in the gastrointestinal tract. An alternative route of administration for IND would allow blood drug concentrations to be maintained in the therapeutic range while avoiding gastric irritation. Huang *et al.* (10) showed that IND solutions could easily pass through the nasal mucosa in rats and enter the systemic circulation. In this study, nasal absorption was close to that obtained after per oral (po) dosing. Therefore, new studies should be performed to show the therapeutic activity of IND, which can readily pass through the nasal mucosa using other pharmaceutical dosage forms.

It is possible to highly improve the nasal absorption of drugs by administering them in combination with a penetration enhancer that promotes the transport of the drug across the nasal membrane (7,11,12). Therefore, many studies have

[1] Department of Pharmaceutical Technology, Faculty of Pharmacy, University of Ege, 35100 Bornova, Izmir, Turkey.
[2] Department of Pharmaceutical Toxicology, Faculty of Pharmacy, University of Ege, 35100 Bornova, Izmir, Turkey.
[3] To whom correspondence should be addressed. (e-mail: yesim.karasulu@ege.edu.tr)

been directed towards identifying an efficacious and safe agent to promote the transport of drugs across the nasal mucosa without adversely affecting the physiology of the nose. Several enhancers such as bile salts, cyclodextrins, surfactants, fatty acids, phosphatidylcholine and water-soluble polymers have been used in nasal drug delivery systems (3,5, 13,14).

The first objective of this study was to develop a lipid emulsion loaded with IND for nasal administration and therefore obtain a longer therapeutic effect when compared to the IND oily solution. The second objective was to increase the permeation of IND through the sheep nasal mucosa using suitable enhancers and the third objective was to compare the anti-inflammatory activity of iv solution of IND with that of ins emulsion in rats with carrageenan-induced paw edema.

**Table I.** The Composition (%), Droplet Size Distribution, Viscosity, Conductivity and Final Drug Loading of Water in Oil Emulsion (W/O) and Oil in Water Emulsion (O/W)

| Parameter | E-W/O | E-O/W |
|---|---|---|
| Soybean oil | 38.0 | 22.0 |
| Tween 80 | – | 22.5 |
| Span 80 | 31.7 | 2.5 |
| Brij 58 | 3.5 | – |
| Ethyl alcohol | 8.8 | 8.5 |
| Isotonic phosphate buffer | 18.0 | 44.5 |
| Droplet size (nm) ± SD[a] | 62.6 ± 2.40 | 185.0 ± 8.90 |
| Drug loading (mg/mL) ± SD[a] | 3.0 ± 0.03 | 2.9 ± 0.08 |
| Viscosity (cp) ± SD[a] | 120.0 ± 5.00 | 88.0 ± 3.00 |
| Conductivity (μS/cm) | 16.9 ± 0.08 | 179.5 ± 0.12 |

[a] Each value represents the mean ± SD ($n = 6$)

## MATERIALS AND METHODS

### Materials

IND was kindly supplied by PharmaTech (Ireland). The emulsifying agents; polyoxyethylene 20 sorbitan monooleate (Tween 80), sorbitan monooleate (Span 80) and polyoxyethylene alkyl ether (Brij 58) were purchased from E. Merck. Co (Schuchardt, Germany). Ethyl alcohol was used as a cosurfactant and purchased from E. Merck. Co (Schuchardt, Germany). The penetration enhancers; polyvinylpyrolidone (PVP), citric acid (CA), sodium taurocholate (NaT), soybean oil as an oily phase and carrageenan were purchased from Sigma Chemical Co. (St. Louis., Mo, USA). All chemicals used were analytical grade.

Nylon membrane (Millipore AB, Solna, Sweden), cellulose membrane (Sigma Chemical Co., St. Louis, Mo, USA), cellulose nitrate membrane (Whatman, Maidstone, England) and sheep nasal mucosa (Pınar Integrated Meat and Flour Inc., Izmir, Turkey) were used as membranes.

### Preparation of Intranasal Emulsions

Oil-in-water (E-O/W) and water-in-oil (E-W/O) emulsions of soybean oil were prepared using different emulsifying agents. Ethyl alcohol was added as cosurfactant. Isotonic phosphate buffer was used as the aqueous phase of emulsions. IND was incorporated into the oil phases of emulsions (3mg/mL) and were stirred at 20500rpm for 5min (Ultra Turrax (CATX620), Staufen Etzenbach, Germany). All emulsions were stable and were in neutral pH during the time course of the experiments. The composition of different emulsions, coded E-W/O and E-O/W were given in Table I. Penetration enhancers (1%) such as polyvinylpyrolidone (PVP), citric acid (CA), and sodium taurocholate (NaT), were added to the emulsion at the last stage.

### Preparation of Nasal Indomethacin Solution

IND was solved in soybean oil at a concentration of 3mg/mL for permeation studies (IND-Sol). All IND solutions were freshly prepared daily.

### Drug Content Studies

For the assay of IND, IND (0.01g) was put into a 10mL volumetric flask and the volume was adjusted with ethyl alcohol. Then 20–100μL of this solution was transferred to another 10mL volumetric flask and adjusted with pH7.4 isotonic phosphate buffer. Each concentration of IND was calculated against a blank at 320nm using spectrophotometer (UV-1208, UV-Vis spectrophotometer, Shimadzu, Kyoto, Japan). A standard curve was plotted and regression variations were calculated.

For the assay of drug content in the emulsion, accurately weighed IND emulsion (100mg) was put into a 50mL volumetric flask and the volume was adjusted with ethyl alcohol. The mixture was shaken for 1h at 200 cycles/min (B. Braun Melsungen AG, Melsungen, Germany) and then 100μL of this solution was transferred into a 10mL volumetric flask and adjusted with pH7.4 isotonic phosphate buffer (15, 16). The resulting solutions were then filtered by 0.45μm membrane filters and then assayed as described above.

### *In Vitro* Permeation Studies

The permeation studies were carried out by vertical diffusion cell method (1cm$^2$) thermostatted at 37°C in water bath (Variomag, Seelbach, Germany) in the dark. The apparatus consisted of clamped preconditioned synthetic membrane (nylon; 0.45μm, cellulose; MW: 12.000, cellulose nitrate; 0.45μm) on to glass diffusion cell between donor and receptor compartments. The receptor solution was 10mL of isotonic phosphate buffer at pH7.4. The receptor solutions were magnetically stirred at 600rpm throughout the experiment. The donor compartment was one mL of the emulsion or solution containing IND (3mg/mL). The aliquots withdrawn from the receptor compartment at various intervals for 8h were immediately analyzed for drug concentration in spectrophotometry (320nm) directly and the receptor compartment was refilled with the same volume (10mL) of fresh buffer solutions. Six replicates of each experiment were performed. Synthetic membrane was first hydrated for 30min in the buffer solution (pH7.4 isotonic

phosphate buffer) at 20°C. Sink conditions were maintained in the receptor compartment during *in vitro* permeation studies.

### *Ex Vivo* Permeation Studies of Indomethacin Emulsion and Solution

Sheep nasal mucosa was used for the *ex vivo* permeation studies. The mean weight of the white Karaman sheep aged 4–8months was 35 ± 5kg. The study was approved by the Animal Ethical Committee in University of Ege, Faculty of Pharmacy, 35100 Bornova-Izmir, Turkey (10/14/2002). Vertical diffusion cell apparatus (1cm$^2$) was also used to study the permeation of IND from emulsions with or without penetration enhancers (PVP, CA, NaT, %1) and IND-Sol from sheep nasal mucosa as a model of nasal mucosa. The mucosa was removed carefully from each nostril of the sheep. Delipidized mucosa was prepared with 1mL of chloroform-methanol (2:1, *v/v*) for 60min in order to extract the lipids from mucosa. The delipidized process extracted the lipid content of the whole mucosa, thus no ultraviolet absorbance was observed. The delipidized sheep nasal mucosa was stored at −80°C. Before the permeation studies, the mucosa was hydrated for 24h in the buffer solution (pH7.4 isotonic phosphate buffer) at 4°C and then permeation studies were carried out with the same method as described above. In the *ex vivo* permeation studies, emulsion permeated without IND was used as blank to determine the drug concentration. Six replicates of each experiment were performed.

### Droplet Size Determination of Emulsions

The particle size distribution and average droplet size of emulsions were measured using Zetasizer 3000 HSA (Malvern Instrument, Malvern, UK).

### Conductivity Measurements

Electrical conductivity of the emulsions (O/W or W/O) were measured using a Jenway Conductivity Meter 4071 (Jenway, UK). The measurements were carried out at 25°C.

### Viscosity Measurements

The viscosity of emulsions was measured on a Brookfield viscometer, model DV-III spindle SC4-21, I equipment with Rheocalc V1.1 software, Brookfield Engineering Laboratories Inc. (Massachusetts, USA). A 'Double cylinder' geometry was used. The temperature was controlled and kept constant at room temperature (20°C ± 0.2°C) using a model TC-500 thermostat from Braive Instruments (Liege, Belgium). The volume of sample was 10mL.

### Data Treatment

The permeation of IND from nasal preparations was investigated. The cumulative amount-time profiles were plotted. A linear profile (steady state) was observed during 2–8h period and the slope of the linear portion of the curve was determined by linear regression. The effective permeability coefficients and flux values at steady state were calculated from the slope according to Eqs. 1 and 2, respectively (17,18,19).

$$P_{\text{eff}} = \frac{V}{AC_{\text{o}}}\frac{\mathrm{d}c}{\mathrm{d}t} \tag{1}$$

$$J = \left(\frac{\mathrm{d}c}{\mathrm{d}t}\right)\frac{V}{A} \tag{2}$$

$V$ Volume of the receiver compartment (mL)
$C_{\text{o}}$ Initial concentration in the donor compartments (mg/mL)
$P_{\text{eff}}$ Effective permeability coefficient (cm/s)
$J$ Flux (mg/cm$^2$s)
$A$ Permeation area (cm$^2$)
$\frac{\mathrm{d}c}{\mathrm{d}t}$ Pseudo steady-state change of concentration over time (mg/mLs)

Results are expressed as the mean ± SD from at least six measurements.

### Suppression of Carrageenan-induced Inflammation

Male Wistar rats, weighing between 280 and 300g (provided by Central Animal Laboratory of Univ. of Ege, the animal study protocol was reviewed and approved by Ethics Committee at the Faculty of Pharmacy of Ege University) were randomly divided into seven groups of six rats for administration. The rats of the first standard group were treated with iv solution of IND (IND was dissolved with 2.5% sodium carbonate solution at a concentration of 3mg/mL and pH of this solution was arranged to 7). The other five experimental groups received different intranasal formulations of IND emulsion with or without enhancer and IND solution (E-O/W, E-O/W+PVP, E-O/W+NaT, E-O/W+CA, IND-Sol; 3mg/kg), respectively. The rats in the last group were treated with intranasal emulsion without IND as a control group (20).

Three hundred microliters (3mg/kg) of different emulsions-loaded IND or IND oily solution were administered to the nasal cavity by means of micropipette into each nostril (50μl six times in 3-min time intervals) or 300μL iv solution of IND was injected into the tail vein of the rats. Three hundred microliters emulsion without IND was applied to the control group rats to evaluate the ins anti-inflammatory activity of emulsion. After 1h, 0.1mL of 1% carrageenan was injected into the left hind foot of each rat under the planter aponeurosis. The contralateral paw was injected with 0.1mL saline and used as other control. Measurements of foot volume were performed by water plethysmometer (LE 7500, Letica Scientific Instruments, Barcelona, Spain) before and 1, 2, 3, 4, 5 and 6h after the injection of carrageenan into

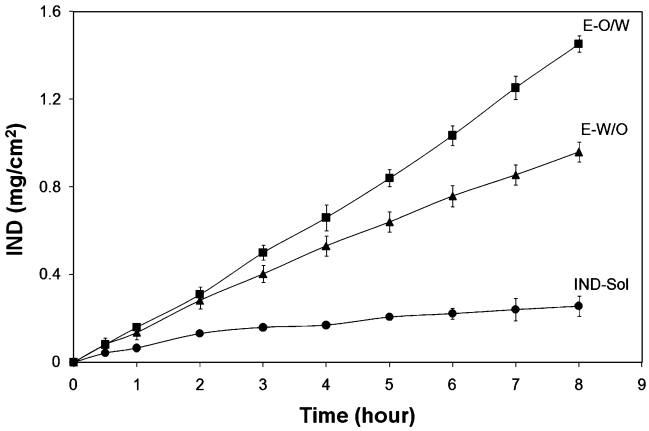


**Fig. 1.** *In vitro* permeation profiles of IND into isotonic phosphate buffer (pH 7.4) at 37°C from nasal preparations using nylon membrane. Each point shows the means of six determinations±SD

the planter region of the left hind paw. The edema rate and inhibition rate of each group were calculated as follows;

$$\text{Edema rate}(E) = \frac{V_t - V_o}{V_o} 100 \quad (3)$$

$$\text{Inhibition rate}(I) = \frac{E_c - E_t}{E_c} 100 \quad (4)$$

Where $V_o$ is the mean paw volume before carrageenan injection (ml), $V_t$ is the mean paw volume after carrageenan injection (mL), $E_c$ is the edema rate of control group and $E_t$ is the edema rate of the treated group.

### Statistical Analysis

Data were expressed as mean ± SD and the differences in the results of *in vitro* and *ex vivo* studies were evaluated using one-way ANOVA followed by Duncan or Dunnett C dependent to Levene test result for homogeneity of variances. Differences were considered statistically significant when $P < 0.05$.

## RESULTS AND DISCUSSION

### Characteristics of the Emulsions

The characteristics of O/W and W/O emulsions such as droplet size, viscosity, conductivity measurements and final drug loading were given in Table I. The O/W emulsions provided higher conductivity values than the W/O emulsions due to the conductivity properties of the aqueous external phase. The mean droplet diameters of O/W and W/O emulsions were determined 185 ± 8.90nm and as 62.6 ± 2.40nm respectively. The formation of emulsions with droplet size within nanometer range, hence called nano-emulsions can be achieved mechanically, which involves high-energy input that is generally achieved by high-shear stirring. The high-energy input leads to deforming forces that are able to break the droplets into smaller ones, provided that the Laplace pressure has been overcome. Additionally, an increase of surfactant content at the interface reduces the Laplace pressure. Therefore, the smaller the droplet size, the more energy and/or surfactant are required (21). It is known that, the droplet size distribution of an emulsion governs emulsion properties such as texture and optical appearance. Furthermore particle size distribution is one of the most important characterizations of emulsion for evaluation of stability. The role of the surfactant (Tween 80, Span 80 and Brij 58) and cosurfactant (Ethyl alcohol) in the emulsion system is to reduce the interfacial tension between oil and water. An essential requirement for the emulsion formation and stability is the attainment of a very low interfacial tension (8,21).

### *In vitro* Permeation Studies

The profiles of permeation of IND from O/W and W/O emulsion formulations through the nylon membrane were shown in Fig. 1. According to the experimental results, the rank order for *in vitro* flux values of IND from these emulsions and IND solution in oil during 8 hours was found to be: E-O/W> E-W/O>IND-Sol (Flux values (mg/cm$^2$ s) = $5.18 \times 10^{-4} \pm 0.88 \times 10^{-4} > 3.13 \times 10^{-4} \pm 0.18 \times 10^{-4} > 0.585 \times 10^{-4} \pm 0.048 \times 10^{-4}$). Additionally, permeation rates of IND from O/W and W/O emulsions were smooth and continuous during 8h compared with IND solution in oil (Fig. 1). As can be seen in that Figure, a linear relationship ($r^2 = 0.999$) was obtained, when the cumulative amount of IND released from E-O/W formulation was plotted against the time.

As seen from Fig. 1, after 8h, the permeation rate of IND was 48.4%, 31.9% and 8.53% for E-O/W, E-W/O formulations and IND-Sol respectively. The flux value of IND during continuous permeability studies has been illustrated by a gradual increase in the receptor compartment as a function of time. The results indicate that IND permeation from E-O/W was 1.5 and 5.6 times greater than E-W/O and IND-Sol respectively. When the oil phase constituted the external phase, as in W/O emulsion, IND permeability from nylon membrane was found to be in small amounts when compared with O/W emulsion permeability. However, the presence of oil droplets containing IND along with external aqueous phase appeared in favor of IND permeability. It might be

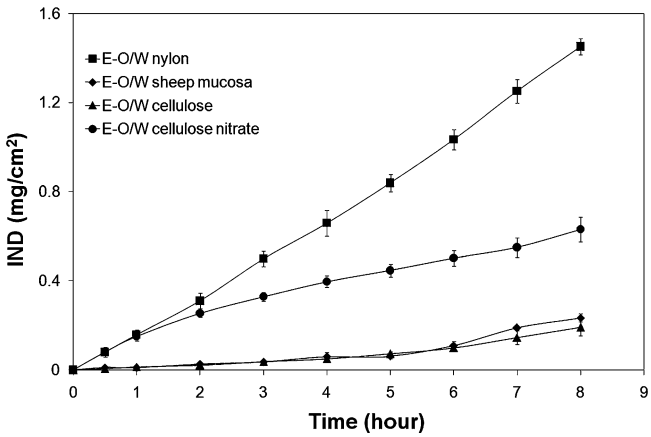


**Fig. 2.** *In vitro* permeation profiles of IND into isotonic phosphate buffer (pH 7.4) at 37°C from E-O/W emulsion formulation using different membranes. Each point shows the means of six determinations±SD

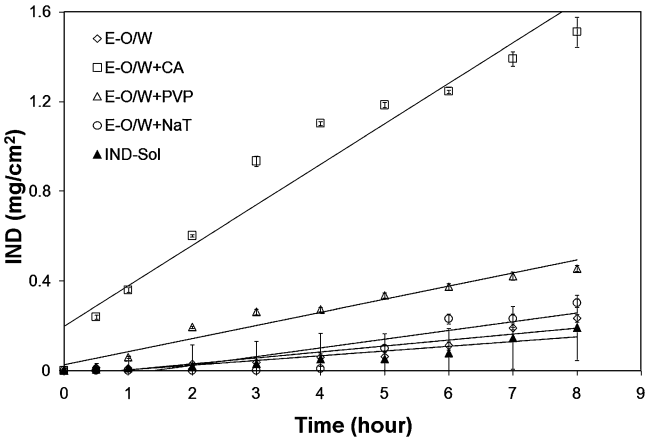


**Fig. 3.** Comparison of the *ex vivo* permeation profiles of emulsion formulation with or without penetration enhancers and IND-Sol from sheep nasal mucosa. Each point shows the means of six determinations ± SD

stated that emulsions could act as drug reservoirs where loaded-drug is released from the internal phase to the external phase and finally onto the skin or mucosa (22,23). On the other hand, when emulsions are used as nasal formulations, the interaction between the oil droplets and the nasal mucosal membrane is likely to play an important role in the enhancement mechanism (5).

In the study of Ugwoke *et al.* (24), it was shown that, the 0.45μ nylon membrane filter separating the drug from the release medium did not impede drug release appreciably. Thus, in this study, the permeation rate of IND from E-O/W through the other synthetic membranes using vertical diffusion cell method was also investigated as described above and the most appropriate synthetic membrane which resembles the sheep nasal mucosa for permeation of IND was proposed.

It was shown that E-O/W has performed the best permeation rate from nylon membrane during 8h (Fig. 2). The released amount of IND was found to be 6.3% for cellulose membrane, 21% for cellulose nitrate membrane and 48.4% for nylon membrane. During 8h, permeation rate of IND from nylon membrane (1.452 ± 0.236mg) was found to be 7.6 and 2.3 times greater than the values observed with cellulose (0.191 ± 0.035mg) and cellulose nitrate membranes (0.631 ± 0.054mg), respectively (Fig. 2).

### *Ex Vivo* Permeation Studies

*Ex vivo* permeation studies from nasal preparations have shown that the cellulose membrane and sheep nasal mucosa showed similar permeation properties when measured every hour during 8h for E-O/W ($P > 0.05$; Fig. 2). Cellulose membrane may be suggested as a model membrane for IND permeation instead of sheep nasal mucosa.

IND showed a better penetration from E-O/W ($0.781 \times 10^{-4} \pm 0.047 \times 10^{-4}$) than from IND-Sol ($0.623 \times 10^{-4} \pm 0.035 \times 10^{4}$) through the sheep nasal mucosa after 8h. Furthermore, the penetration enhancers were added to the E-O/W to increase the permeation rate of IND. The effects of penetration enhancers on the permeation of IND from E-O/W across sheep nasal mucosa were investigated by comparing the permeation rate of IND in the presence and absence of enhancers as *ex vivo*. Figure 3 shows the permeation profiles of IND from IND-Sol and E-O/W emulsion formulations including penetration enhancers such as PVP, CA and NaT. According to the *ex vivo* permeation studies, the permeation rate of IND from sheep nasal mucosa was greatly enhanced when the PVP and CA were incorporated in the O/W emulsion. However, IND permeation rate has slightly changed when NaT was used. Longenecker *et al.* (25) showed that when the bile salt derivative was used at a concentration of 1%, ins insulin absorption was enhanced in sheep. In this study after 8h, the permeation rates of IND from emulsions containing PVP, CA or NaT were found to be 1.9, 6.45 and 1.3 times greater than that of the emulsion without enhancers respectively. CA showed the highest enhancing effect on the permeation rate of IND from sheep nasal mucosa when compared with NaT and PVP. Todo *et al.* (26,27) showed that CA decreased the intracellular ATP level and the intracellular pH. According to the results, it was considered that one of the action mechanisms of acylcarnitines and organic acids is that the intracellular acidosis increases the calcium level through the decrease in ATP levels, opening the tight junction, which is followed by enhanced transport through the paracellular pathway. Thus, CA appears to be a safe and potent enhancer (21,28). In addition the IND permeation from E-O/W containing CA (1.510 ± 0.067mg) was significantly higher than that obtained from IND-Sol (0.191 ± 0.140). The order of the enhancement effect was E-O/W+CA > E-O/W+PVP > E-O/W+NaT > E-O/W> IND-Sol (Table II) for 8h. Furthermore,

**Table II.** Flux, $P_{eff}$ and $r^2$ of IND Through Different Membranes from Different Type of Emulsions With/Without Penetration Enhancers and from IND-Sol

| Parameter | Flux ($10^{-4}$ mg/cm$^2$ s)±SD | $r^2$±SD | $P_{eff}$ ($10^{-4}$ cm/s)±SD |
|---|---|---|---|
| E-O/W (nylon) | 5.18±0.88 | 0.998±0.009 | 1.720±0.029 |
| E-O/W (cellulose) | 0.64±0.13 | 0.924±0.015 | 0.214±0.043 |
| E-O/W (cellulose nitrate) | 2.97±0.13 | 0.983±0.004 | 0.989±0.042 |
| E-O/W (sheep mucosa) | 0.78±0.05 | 0.852±0.042 | 0.260±0.015 |
| E-W/O (nylon) | 3.13±0.18 | 0.998±0.012 | 1.040±0.052 |
| IND-Sol (nylon) | 0.59±0.05 | 0.983±0.035 | 0.195±0.024 |
| IND-Sol (sheep mucosa) | 0.62±0.04 | 0.861±0.004 | 0.207±0.032 |
| E-O/W $_{+}$CA (sheep mucosa) | 4.70±0.17 | 0.903±0.021 | 1.560±0.057 |
| E-O/W $_{+}$PVP (sheep mucosa) | 1.56±0.05 | 0.944±0.001 | 0.521±0.016 |
| E-O/W $_{+}$NaT (sheep mucosa) | 1.19±0.10 | 0.836±0.014 | 0.398±0.031 |

Each value represents the mean±SD ($n$=6; 2–8 h)

**Table III.** The Anti-inflammatory Effect of Carrageenan-Induced Paw Edema by Nasally and Intravenously Administration of IND Formulations in Rats ($n$=6)

| Formulation | Edema Rate (Inhibition rate) | | | | | |
|---|---|---|---|---|---|---|
| | 1 h | 2 h | 3 h | 4 h | 5 h | 6 h |
| Control | 50.7±3.62 | 61±5.83 | 75.9±5.02 | 91.5±2.07 | 76.1±2.12 | 71.3±5.77 |
| E-O/W (ins) | 45.7±4.47 (9.8) | 53±3.98 (13.1) | 46.8±4.03 (38.3) | 40.8±4.39 (55.3) | 28.4±3.84 (62.6) | 19.1±3.35 (73.2) |
| IV solution | 28.6±5.61 (43.5) | 30.1±5.26 (50.68) | 33.9±4.11 (55.3) | 20.9±3.24 (77) | 13.2±4.27 (82.5) | 12.4±2.21 (82.6) |
| E-O/W $_{+}$CA (ins) | 34.6±3.18 (31.6) | 43.7±5.46 (28.2) | 31.4±2.56 (58.5) | 18.2±5.04 (80.1) | 13.3±3.62 (82.4) | 11.9±5.97 (83.2) |
| E-O/W $_{+}$PVP (ins) | 48.2±3.11 (4.94) | 55.0±1.89 (9.75) | 43.5±3.15 (42.6) | 36.6±4.14 (59.9) | 31.3±4.65 (58.8) | 22.5±11.94 (68.4) |
| E-O/W $_{+}$NaT (ins) | 36.0±5.75 (28.9) | 39.5±4.35 (35.2) | 28.6±6.67 (62.3) | 28.4±8.86 (68.9) | 27.1±8.24 (64.3) | 24.1±5.54 (66.2) |
| IND-Sol (ins) | 45.9±3.88 (9.46) | 56.6±2.45 (7.77) | 54.1±3.75 (28.7) | 48±2.84 (47.5) | 39.5±3.73 (48.1) | 31.7±4.95 (55.5) |

Values represent the mean±SD of six animals for each groups. Each value in parenthesis indicates the percentage inhibition rate ($I$).

it is known that, emulsions are more viscous systems than solutions and they increase the residence time of the drug in the nasal cavity (6,8). On this account, emulsions are preferred to the solution for nasal application because of retaining the drug at the site of action.

Approximate linear permeation profiles (2–8h) were obtained for emulsion formulations (Fig. 1–3). Effective permeability coefficients and flux values were calculated from the linear parts of the concentration time profiles (Table II).

### Suppression of Carrageenan-induced Inflammation

The intraplantar injection of carrageenan caused a time-dependent paw edema in the rat although saline injection caused no swelling (data not shown). Carrageenan injection into the rat paw provokes a local, acute inflammatory reaction that makes it a suitable method for evaluating the effects of anti-inflammatory agents. Induction of acute inflammation in control rats resulted in a prominent increase in paw volume that began after 1 hour following carregeenan injection and reached a peak in 4h (91.5%; Table III). The IND formulations with a dose of 3mg/kg induced a significant anti-inflammatory effect beginning at 2h after carrageenan injection. It was clearly shown in Fig. 4. It has been reported that the carrageenan-induced edema can be divided into two phases. The first phase occurs 1h after carrageenan injection. It is caused by the release of cytoplasmic enzymes and serotonin from mast cells and the increase of prostaglandin in the inflammatory area. The second phase occurs 3–5h after carrageenan injection. In this phase, the macrophages in carrageenan-insulted dermal tissue release Interleuken-1 (IL-1) to induce the accumulation of polymorphic nuclear cells (PMNs) into the inflammatory area. These cells then release the lysomal enzymes and active oxygen to destroy connective tissues and induce paw swelling (20,29). A statistically significant inhibition of the edema was observed following iv administration of IND solution at the fourth hour (77%) when compared with the control group (Fig. 4). On the other hand, nasal IND O/W emulsion with and without CA, PVP, NaT and IND-Sol (3mg/kg) inhibited the edema by 80.1%, 59.9%, 68.9%, 55.3% and 39.6% at the fourth hour of carrageenan administration respectively when compared with control (Table III). The results showed that IND O/W emulsion with CA exhibited similar anti-inflammatory activity comparable to that of standard iv solution of IND ($P > 0.05$). On the other hand, IND-Sol showed the lowest edema inhibition because of decreasing the residence time of the drug in the nasal cavity when inhibition times of the formulations were compared (Table III).

A previous study (10) suggested that IND easily passes through both gastrointestinal tract and nasal mucosa to the systemic circulation because of its high lipophylic property. The AUC were calculated as 71.9 ± 3.1, 84.9 ± 3.6, 67.4 ± 3.1, after po, iv and ins administrations respectively. This also showed that the time to peak ($t_{max}$) of ins IND solution was 0.08h, approached to that after iv route. Therefore, in this study it was suggested that, the incorporation of IND in emulsion may result in a considerable increase in nasal permeation. It is known that, emulsions due to their viscosity are preferred over solutions for nasal application. In addition both hydrophilic and lipophilic drugs can also incorporate in the emulsion phases as solution form. The results of permeation study indicated that only the E-O/W+CA formulation was also shown better permeation rate from that of IND-Sol and permeation of IND from E-O/W+CA was higher than that of the permeation from IND-Sol. When this formulation was administered in intranasal form, the anti-inflammatory activity approached to that of the iv solution ($P > 0.05$) and simultaneously exceeded the that of the IND-Sol ($P < 0.05$).

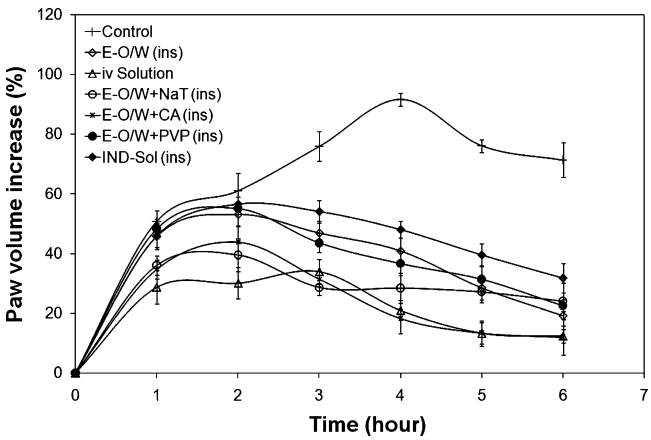


**Fig. 4.** Comparison of inhibition of carrageenan-induced paw edema by nasally and intravenously administration of IND formulations in rats (n=6 for each group). Results are the mean ± SD

## CONCLUSIONS

Given the results of this study, it is clear that emulsion formulation loaded with IND is potentially useful for permeation of IND in nasal delivery. It is possible to conclude that, ins administration of IND may be considered as an alternative to iv and po administration of IND to overcome its disadvantages. Thus, the present study suggests that, nasal administration may be considered as an alternative non-invasive method for IND delivery to achieve rapid onset of its pharmacological effect.

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