

PREPARATION AND ENHANCED IN-VITRO DIFFUSION PROFILE OF NAPROXEN BY EPAS TECHNIQUE IN HYDROGEL FORMULATION

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The aim of the study was to evaluate the influence of EPAS technique on the *in vitro* diffusion characteristics of Naproxen in three different gel forming agents as poly(vinyl alcohol) (PVA), Carboxymethyl Cellulose (CMC) and Hydroxypropyl methyl cellulose (HPMC). All the formulations were evaluated for their appearance, homogeneity and % drug content and uniformity, pH, Grittiness and *in-vitro* release study through cellulose nitrate membrane in upright Franz diffusion cells. It was found that all the formulated gels were homogenous, smooth in texture and elegant in appearance. The results of the drug content were found in acceptable range indicating uniform distribution of drug throughout the base. The pH of the gel formulations was found in the normal pH range of the skin that would not produce any skin irritation. There was no significant change in pH values and drug content in accelerated stability studies for tested formulations. It is obvious that the EPAS technique adapted has significantly enhanced the dissolution profile of naproxen when compared with pure naproxen in PVA, HPMC and CMC Gel formulation studied. All the gel formulations were subjected to Peppas model kinetic analysis.

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1. Introduction

Naproxen is an NSAID with analgesic and antipyretic properties⁽¹⁻³⁾ used for the treatment of musculoskeletal with non-optimal characteristics to be delivered through the skin.⁽⁴⁾ Oral therapy of NSAIDs is very effective, but the clinical use is often limited because of their potential to cause adverse effects such as irritation and ulceration of the gastro-intestinal mucosa. Cioli et al reported that gastric irritation induced by NSAIDs can be influenced by the route of administration.⁽⁵⁾ These potential side effects may be overcome by the topical administration of the drug.

Different strategies have been used to increase bioavailability of poorly water soluble drugs naproxen topically administered.⁽⁵⁻⁶⁾ These strategies include, but are not limited to, solid dispersion⁽⁸⁾, inclusion complexation⁽⁹⁻¹¹⁾, precipitation techniques⁽¹²⁻¹³⁾, and cryogenic engineering⁽¹⁰⁾. If dissolution profile of these poorly soluble compounds can be enhanced, bioavailability following oral administration may be significantly improved. An attempt has been made, to enhance the topical delivery of naproxen gel by using different types of gel forming agents as delivery vehicles.

The Evaporative Precipitation into Aqueous Solution (EPAS) technique was used to produce a nanoparticle suspension of cyclosporine A and danazol, which showed high dissolution rates. Nanoparticle suspensions produced by the EPAS process can be incorporated into a parenteral dosage form or can be dried to produce solid oral dosage forms.⁽¹²⁻¹³⁾ A heated organic solution of the drug in dichloromethane is sprayed through a fine nozzle into a heated aqueous solution. The rapid evaporation of the organic solvent produces high supersaturation and rapid

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precipitation of the drug in the form of a colloidal suspension that is stabilized by a variety of low molecular weight and polymeric surfactants. The stabilizer adsorbs to the drug surface and prevents particle growth and crystallization during the spray process. ⁽¹²⁻¹³⁾

Therefore, the aim of the experimental plan was to evaluate the influence of EPAS technique on the *in vitro* diffusion characteristics of Naproxen in three different gel forming agents as poly(vinyl alcohol) (PVA), Carboxymethyl Cellulose (CMC) and Hydroxypropylmethyl cellulose (HPMC).

2. Materials and methods

2.1 Materials

Naproxen (Ezo life sciences) (CAS 22204-53-1) was supplied by Sigma Aldrich. Carboxymethyl Cellulose (CMC), Hydroxypropylmethyl cellulose (HPMC), poly(vinyl alcohol) (PVA), methanol, ethyl acetate, sodium chloride, disodium hydrogen phosphate, triethanolamine, cellulose nitrate membrane, isopropyl myristate were supplied by Sigma-Aldrich. All other chemicals and solvents were commercially available products of analytical grade.

2.2 Preparation of nano-suspension by EPAS

Weighed amount of Naproxen was dissolved in 10 ml of ethyl acetate. The resultant solution is sprayed through a fine nozzle into a heated aqueous solution 80-85°C containing stabilizing surfactant, polysorbate 80 and the mixture was continuously stirred magnetically. Ethyl acetate was allowed to evaporate and a nanosuspension has formed. The rapid evaporation of the organic solvent produces high supersaturation and rapid precipitation of the drug in the form of a colloidal suspension. The colloidal suspension is centrifuged and freeze dried.

2.3 Particle size studies for nanosuspension

Samples morphology was examined under field emission scanning electron microscope (FE-SEM)

2.4. Preparation of Naproxen Gel Formulations:

2.4.1 Preparation of poly(vinyl alcohol) gels:

Weighed amount of PVA powder was sprinkled gently in beaker containing warm water and continuously stirred magnetically until thin hazy dispersion without lumps formed. Remaining amount of water was added on cold and mixing continue until smooth homogenous gel formed.

2.4.2 Preparation of Carboxymethyl Cellulose (CMC) Gels:

The weighed amount of CMC powder were sprinkled gently in 100 ml beakers, containing boiling distilled water and stirred with magnetic stirrer, stirring was continued until a thin hazy dispersion, without lumps, were formed.

2.4.3 Preparation of Hydroxypropylmethyl cellulose (HPMC) Gels:

The weighed amount of HPMC powder were sprinkled gently in 100 ml beakers, containing a portion of hot water at 80°C and stirred magnetically at high speed. Stirring was continued until a thin hazy dispersion, without lumps, was formed the remaining amount of water was added on cold and mixing was continued till smooth homogenous gel is formed.

All gel bases were neutralized and made viscous by the addition of triethanolamine and left overnight in the refrigerator to allow complete gel dispersion. To each of the prepared gel bases, the pure naproxen and nanoparticle of naproxen prepared by EPAS technique is added at 10% w/w concentration was added by mean of gentle levigation with mortar and pestle. ⁽¹⁴⁾

2.5 Physicochemical Evaluation of Gel formulations

The physical appearance, homogeneity, and texture of the prepared gels were tested by visual observations.

2.5.1 Homogeneity

All developed gels were tested for homogeneity by visual inspection after the gels have been set in the container. They were tested for their appearance and presence of any aggregates.

2.5.2 Grittiness

All the formulations were evaluated microscopically for the presence of particles and no appreciable particulate matter was seen under light microscope. Hence obviously the gel preparation fulfils the requirement of freedom from particular matter and from grittiness as desired for any topical preparation.⁽¹⁵⁾

2.5.3 pH Value

2 gm of gel was stirred in distilled water until a uniform suspension was formed. The volume was made up to 50ml and the pH of solution was measured using a glass electrode pH meter at room temperature. Measurement were done on 1, 7, 14 days after the hydrogel preparation.

2.5.4 Drug Content

For assay of the drug in gels, naproxen was extracted from 1 g of each gel formulations with 20 ml of methanol for 30 min. The resultant mixture was filtered through membrane filter (pore size 0.45 µm). The absorbance of the sample was determined spectrophotometrically at 331nm using methanol as a blank.

2.5.5 Accelerated stability studies:

All the gel formulations with stabilized naproxen particles were subjected to a stability testing for three months as per ICH guidelines at a temperature of 40°C ± 2°C.⁽¹⁶⁾ All selected formulations were analyzed for the change in appearance, pH and drug content.

2.6 In vitro diffusion studies

In vitro Permeation studies were conducted using pre-calibrated, upright Franz diffusion cells with an average surface area of 0.5cm² and receiver compartment volume of 2.5mL. Cellulose nitrate membrane (0.1 µm pore diameter) was soaked with isopropyl myristate to simulate the lipophilic properties of stratum corneum and mounted on diffusion cell.

The gel formulation (1.0 g) was gently placed in the donor chamber and then fixed in between donor and receptor compartment of diffusion cell. The receptor chamber was filled with freshly phosphate buffer of pH 6.8. The diffusion cell was maintained at 37°C using a recirculating water bath and the solution in the receptor chambers was stirred continuously at 300 rpm. The cells were allowed to equilibrate in the water bath for 1 h prior to experiment initiation. Care was exercised to remove any bubbles between the underside of the membrane and solution in the receiver compartment.

Using a long needle, samples (0.5 ml) were removed from the receptor compartment at defined time intervals (30, 60, 120, 180, 240, 300 and 360 min). This volume was immediately replaced using blank, pre-warmed buffer. No interference of the other formulation components was observed. The samples withdrawn were UV spectrophotometrically estimated at 331nm. Each formulation is performed in triplicate (n=3).

2.7 In vitro Release kinetic studies

All naproxen gel formulae were subjected to Peppas Model kinetic analysis by fitting the release of naproxen from various gel preparations.

This kinetic model is generally used to analyse the release of hydrogel semisolid dosage forms, when the release mechanism is not well known or when more than one type of release phenomena could be involved. A more stringent test was used to distinguish between the mechanism of drug release. In order to ascertain the mechanism of drug release, the release data was fitted into the general equation⁽¹⁷⁾

$$M_t / M_\infty = K t^n$$

Where M_t is the amount of drug released at t and M_∞ is amount of drug released at infinite time, K is the constant incorporating characteristics of the polymer network system and the drug and n is

the diffusional exponent indicative of the transport mechanism. The release exponent takes various values depending upon different geometries. ⁽¹⁸⁾

Table 1. Variation of *n* values with mechanism of diffusion.

n	Mechanism	dMt/dt Dependence
<0.45	Fickian diffusion	$t^{-0.5}$
0.45< <i>n</i> >0.89	Anomalous diffusion or Non-Fickian diffusion	t^{n-1}
0.89-1.0	Case II transport or zero order	Zero order
<i>n</i> >1.0	Super case II transport	t^{n-1}

3. Result and discussion

3.1 UV Analysis

The scanning of the naproxen in the UV Spectrophotometer was performed. The maximum absorption value for the naproxen was found to be 331 nm. The linear regression equation obtained by least square regression method was $y = 0.0108 X - 0.028$ with a correlation coefficient if 0.9984, where *y* is the absorbance and *x* is the concentration of the pure naproxen solution. The method was validated for several parameters like linearity, accuracy (recovery), precision, and specificity as per International Conference on Harmonization (ICH) guidelines. The limits of detection (LOD) and of quantification (LOQ) were 1.53 μ g/mL and 5.11 mg/mL, respectively

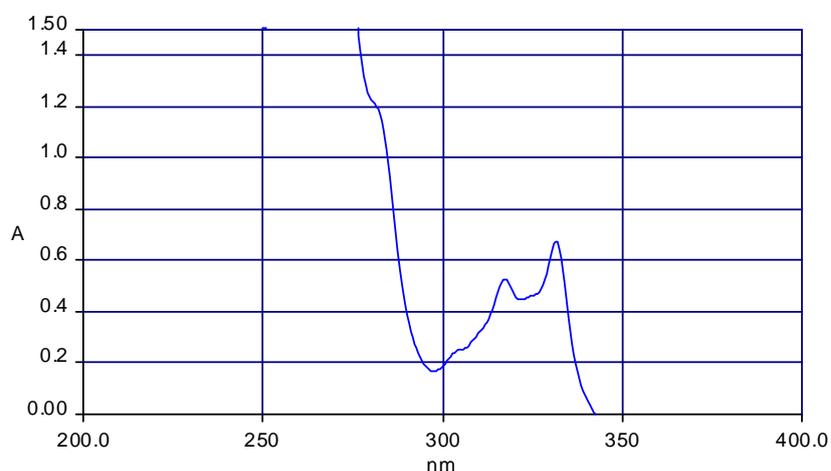


Fig. 1: UV Spectrum of naproxen in methanol

3.2 Characterization of Formulations

The prepared gel formulations shared a smooth and homogeneous appearance. All preparations were easily spreadable with free grittiness, good homogeneity with absence of lumps, and fair gel formulation properties. The pH values ranged from 6.72 to 6.74, which are considered acceptable to avoid the risk of irritation after skin application. ⁽¹⁹⁾ Drug content of all tested formulations was found within the acceptable limit.

Table 2. Physicochemical characteristics of prepared naproxen hydrogel

Formulation	Description	Grittiness	Physical Appearance	Homogeneity	pH	Drug content
PVA-1	Pure naproxen in PVA gel	No particles	Transparent	Good	6.72±0.22	98.26±0.25
PVA-2	Nanoparticle of naproxen in PVA gel	No particles	Transparent	Good	6.83±0.12	97.62±0.52
HPMC-1	Pure naproxen in HPMC gel	No particles	Transparent	Good	6.91±0.18	96.17±0.26
HPLC-2	Nanoparticle of naproxen in HPMC gel	No particles	Transparent	Good	6.87±0.43	98.58±0.31
CMC-1	Pure naproxen in CMC gel	No particles	Transparent	Good	6.82±0.56	98.75±0.14
CMC-2	Nanoparticle of naproxen in CMC gel	No particles	Transparent	Good	6.94±0.11	99.36±0.17

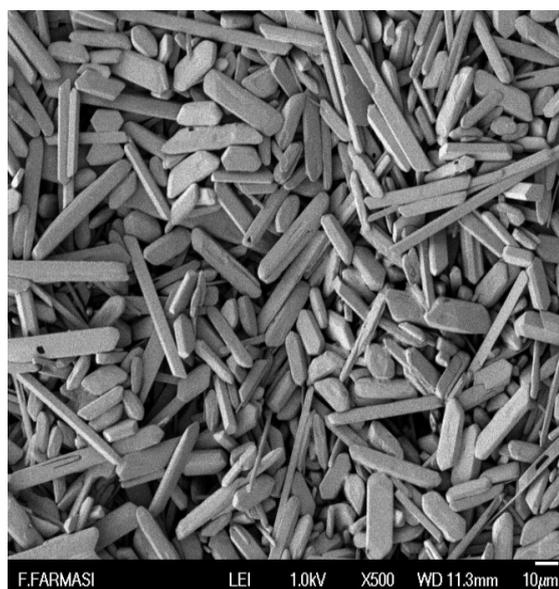


Fig. 2. Scanning electron micrograph of pure naproxen

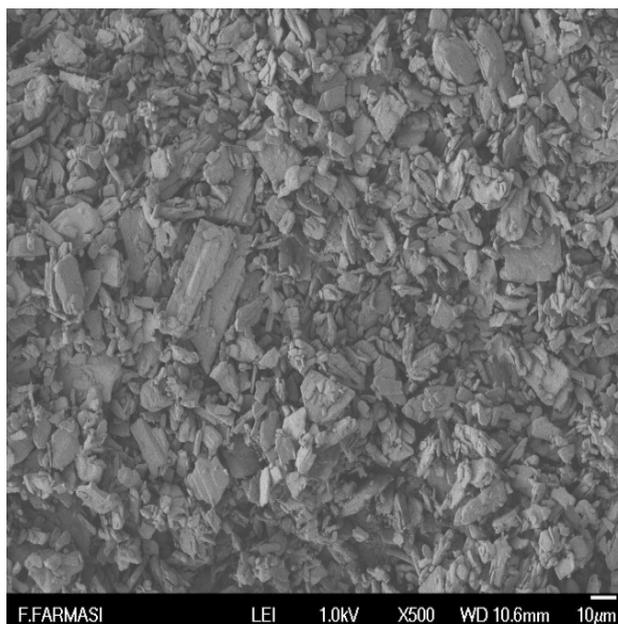


Fig. 3. Scanning electron micrograph of nanoparticle of naproxen

SEM micrographs of the pure naproxen and stabilized naproxen particles precipitated by EPAS technique are shown in Figure 2 and 3. Surface morphology differences of the stabilized naproxen particles precipitated, the particles were more distinctly smaller size and shape compared to pure naproxen (figure 1). Smaller size of naproxen particles prepared by EPAS technique (Figure 3) is due to absorption of surfactant, polysorbate 80 on the drug surface and prevents particle growth as reported by Chen X et al. ⁽¹²⁾

IN Vitro Release Studies:

Dissolution profiles of 10% naproxen gel formulations prepared with 2, 4, and 6% polymer PVA, HPMC and CMC are shown in Figure. 3. The in-vitro study showed a variation in the release rate of the naproxen dependent on the gelling agent used for the formulation, suggesting that the choice of the gelling agent is important for achieving a desired drug release profile.

As 99% release of naproxen was shown after 6 hours from gel formulation prepared with HPMC with naproxen nanoparticles. On the contrary, the gel formulation prepared with pure naproxen in all three polymers showed a comparative smaller release rate in the dissolution medium tested.

The results clearly indicated that the diffusion profile of pure naproxen and nanoparticle of naproxen were better from HPMC gel base when compared to that from the other two bases.

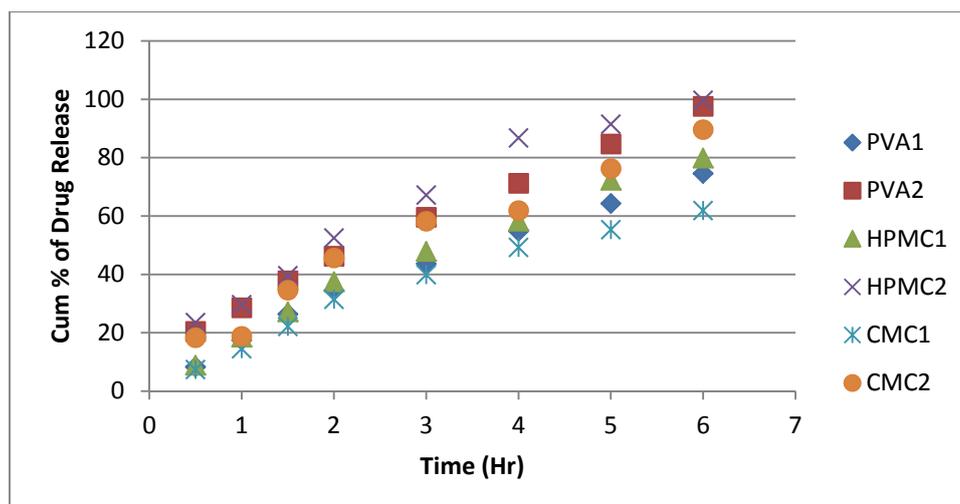


Fig. 4. Cumulative percentage drug release from prepared gel formulation at different time intervals

The effect of pure naproxen and stabilized naproxen particles from gel formulations prepared with three selected gel forming agents are shown in Figure 4. It is obvious that the EPAS technique adapted has significantly enhanced dissolution properties when compared to pure naproxen in PVA, HPMC and CMC gel formulations studied. This difference should be attributed to the ESPA technique and to the nanoparticle of naproxen incorporated gel formulation (PVA-2, HPMC-2 & CMC-2), which is expressed in the observed increase dissolution profile compared to that of pure naproxen formulation (PVA-1, HPMC-1 & CMC-1).

The rank order of the various gel formulations based upon their maximum drug release is HPMC-2 > PVA-2 > CMC-2 > HPMC-1 > PVA-1 > CMC-1. Based on the physicochemical properties and drug release, the formulations HPMC-2 and PVA-2 were found to be the best among the tested formulations.

In contrast to many solubilisation technologies, which achieve dissolution enhancement by converting drug habits from crystalline to amorphous, ⁽¹⁰⁾ controlled precipitation by EPAS technique significantly enhances dissolution of poorly soluble compounds by stabilizing drug particle that are crystalline.

True L. Rogers et al (20) reported that the crystalline morphologies of the stabilized drug particles from controlled precipitation are similar to those of the pure drug. From these findings, it can be concluded that controlled naproxen precipitation by ESPA technique adds indispensable value to the particle engineering technology area since particle size, morphology, and crystallinity can be independently controlled.

All the release kinetic parameters of naproxen from the studied formulations are given in Table 3. The release exponent (n) values varied from 0.6723 to 0.9116 which indicates that drug release from the prepared gel mostly follows a non Fickian transport mechanism pattern but HPMC-2 formulation shows case II transport or zero order drug release pattern. The correlation coefficient values ranged from 0.9594 to 0.9957.

During the stability studies the gel appearance was transparent and no significant variation in pH were observed. The texture of the gel remained smooth throughout the stability studies with no grittiness. The drug content of the gel was found in the range of 99.85%- 97.12% at 40°C which is within the normal (95% - 105%) permitted range of variation. The pH values ranged from 6.6 to 6.84, which are considered acceptable to avoid the risk of irritation after application of gel formulation. ⁽¹⁹⁾

Table 3. Variation of *n* values with mechanism of diffusion of Peppas plot

Equation of the Model	PVA-1	PVA-2	HPMC-1	HPMC-2	CMC-1	CMC-2
r	0.9886	0.9957	0.9903	0.9822	0.985	0.9594
n	0.6822	0.7998	0.6723	0.9116	0.8091	0.7981
Mechanism	Non Fickian transport	Non Fickian transport	Non Fickian transport	Case II transport or zero order	Non Fickian transport	Non Fickian transport

Table 4. Stability study of various naproxen hydrogel gel formulation

Formulation	Months	Physical Appearance	pH	Drug content
PVA-2	0	Transparent	6.84±0.52	99.25±0.24
	1	Transparent	6.81±0.18	98.74±0.19
	2	Transparent	6.78±0.12	98.12±0.28
	3	Transparent	6.77±0.14	97.39±0.37
HPMC-2	0	Transparent	6.88±0.11	98.93±0.26
	1	Transparent	6.82±0.37	98.14±0.12
	2	Transparent	6.79±0.16	97.45±0.37
	3	Transparent	6.74±0.21	96.82±0.17
CMC-2	0	Transparent	6.85±0.46	99.85±0.24
	1	Transparent	6.78±0.24	98.32±0.16
	2	Transparent	6.72±0.13	97.64±0.41
	3	Transparent	6.66±0.49	97.12±0.33

4. Conclusions

This study demonstrated that incorporating stabilized naproxen particles in three polymers enhances drug penetration through the membrane in-vitro. Gel formulation containing stabilized naproxen particles by EPAS technique may offer promise as an anti-inflammatory dosage form, ensuring more effective therapy, but additional safety tests and in-vivo animal experiments should be performed before the formulation is used in humans.

In conclusion, Evaporative Precipitation into aqueous solution (EPAS) technique offers many significant advantages for dissolution profile of poorly soluble drugs. Future studies include investigating the morphologies of stabilized naproxen and the mechanisms by which these novel morphologies facilitate enhanced dissolution of poorly soluble drugs.

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