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Original Research Article

FORMULATION AND CHARACTERIZATION OF EUDRAGIT NANOSUSPENSION FOR OCULAR DELIVERY OF INDOMETHACIN

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ABSTRACT

The aim of this study is to formulate a novel ocular Nanosuspension (ONS), an alternative carrier system to traditional colloidal carriers for controlled release (CR) of Indomethacin (IM) .In the present study, ONS is employed to avoid some of major disadvantages of colloidal carriers systems such as instability in cul de sac and short half-life by increasing efficiency of drug encapsulation as well as by CR. A quassi-emulsion solvent evaporation method was used to prepare IM loaded Eudragit RS 100 ONS with the aim of improved ocular bioavailability and distribution. Five different formulations were prepared and evaluated for pH of ONS, particle size, entrapment efficiency, differential scanning Caloritmetry (DSC), in vitro release profile, in vivo release studies and stability studies. An average size range of 50 to 500 nm in diameter was obtained and encapsulation efficiency up to 96.0% was observed for all the formulations. Cumulative percent drug released for all the formulation after 14 h was between 86.24 to 96.18% indicating effective CR property of ONS. The release profile revealed from best formulations followed Non- Fickian diffusion mechanism.

Keywords: Indomethacin, Nanosuspension, Eudragit, Benzalkonium chloride, *In vivo* drug release study, *In vivo* drug study.

INTRODUCTION

Drug delivery in ocular therapeutics is a challenging problem and is a subject of interest to scientists working in the multi-disciplinary areas pertaining to the eye, including chemical, and Biochemical, pharmaceutical, medical, clinical, and toxicological sciences. Recently, increased attention has been focused on two main objectives (Ludwig, 2005; Kaur, 2004).

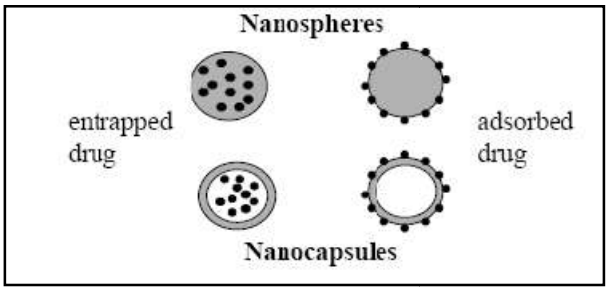
Nanosuspensions

Controlled drug delivery technology represents one of frontier areas of science, which involves multidisciplinary scientific approach contributing to human health care. These delivery systems offer numerous advantages compared to conventional dosage forms, which improved efficacy, reduced toxicity and improved patient compliance and convenience. Such systems often use nanoparticles as carrier for the drug.

This field of pharmaceutical technology has grown and diversified rapidly in recent years. Understanding the derivation of the methods of controlled release and the range of new polymers can be a barrier to involvement from the nanospecialist of the different dosage forms reported nanoparticles attained much importance due to an accumulative in inflamed areas of the body. Nanoparticles for their attractive properties occupy unique position in drug delivery technology.

Nanoparticles and Nanospheres:

Nanoparticles were first developed around 1970. The colloidal carriers on biodegradable and biocompatible polymeric system have largely influenced the controlled and targeted drug delivery. The nanoparticles loaded bioactives could not only deliver drug to specific organs within the body but delivery rate in addition could be controlled or modulated (Ashim, 1993; Worakul and Robinson, 1997). Nanoparticle are solid colloidal particle ranging from 10-1000 nm in which the active principle (drug or biologically active material) is dissolved, entrapped or encapsulated and/or to which the active principle is absorbed or attached.

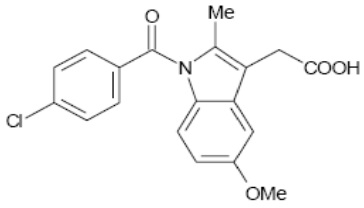


Indomethacin:

IUPAC name: [1-(4-chlorobenzoyl)-5-methoxy-2-methylindol-3-yl] acetic acid

Empirical formula: $C_{19}H_{16}ClNO_4$

Mechanism of Action: Inhibits prostaglandin synthesis by decreasing the activity of the enzyme, cyclooxygenase, which results in decreased formation of Prostaglandin precursors.



MATERIALS AND METHODS

Preformulation studies

Preformulation testing is the first step in the rational development of dosage forms of a drug substance. It can be defined as an investigation of physical and chemical properties of a drug substance alone and when combined with excipients. The overall objective of preformulation testing is to generate information useful to the formulator in developing stable and bioavailable dosage forms which can be mass produced. The FT-IR spectrum of pure Indomethacin, Eudragit RS 100 and physical mixture of Indomethacin, Eudragit RS 100 were analyzed for compatibility study (Vyas and Khar, 2002; Vyas and Khar, 2002).

Preparation of standard curve:

Preparation of Phosphate Buffer pH 7.4:50.0 ml of 0.2 M potassium di-hydrogen phosphate was placed in a 200 ml volumetric flask, added the specified volume of 39.1 ml of 0.2 M sodium hydroxide and then made up to the volume by water.

Potassium di-hydrogen phosphate, 0.2 M:27.218 g of potassium di-hydrogen phosphate was dissolved in distilled water and diluted to 1000 ml.

Sodium hydroxide solution 0.2 M: 8g of sodium hydroxide was dissolved in distilled water and diluted to 1000ml.

Preparation of Standard Curve of Indomethacin with Phosphate Buffer pH7.4:100 mg of Indomethacin was accurately weighed and dissolved in a small portion of phosphate buffer pH 7.4 in a 100 ml volumetric flask then the volume was made up to 100 ml with phosphate buffer pH 7.4. This was primary stock solution, contained 1000 µg/ml. From this primary stock solution 10 ml was pipetted out and transferred in to a 100 ml volumetric flask and volume was made up to 100 ml with phosphate buffer pH 7.4 which contained the concentration of 100µg/ml. From the second stock solution again 10 ml was pipetted out and diluted up to 100 ml with phosphate buffer pH 7.4 to get concentration of 10 µg/ml.From third stock solution aliquots equivalent to 1-10 µg (1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 ml) were pipette out in to a series of 10 ml volumetric flask and volume was made up to 10 ml with phosphate buffer pH 7.4.

Preparation of nanosuspension:

Nanosuspensionwas prepared by the quassi-emulsion solvent diffusion technique. Nanosuspension was prepared by using different drug to polymer ratio. Quantity of drug in all formulation was kept constant i.e. 100 mg. The different ratio of drug and polymer is as given in table 1. The drug and polymer were co-dissolved at room temperature in ethanol (5 ml) and sonicated for 10 minutes. The solution was slowly injected with syringe into 45 ml water containing Tween 80 (0.02 %w/v) and Benzalkonium chloride (0.1 % w/v) and kept at low temperature in an ice water bath. During injection the mixture was mixed by mechanical stirring (propeller 4000 rpm) for one hour. The solution immediately turned into a pseudo-emulsion of the drug and polymer-ethanol solution in the external aqueous phase. The counter diffusion of ethanol and water out of and into the micro droplets. After completion of stirring the solution dispersion wasSubjected to ultra-sonication for a period of 10 minutes. The gradual evaporation of the organic solvent determined the in situ preparation of the polymer and the drug with the formation of matrix type nanoparticles. Ethanol residues were left to evaporate off under slow magnetic stirring of the Nanosuspension at the room temperature for 8-12 hours. Using this above method 5 formulations of Nanosuspension FN-1, FN-2, FN-3, FN-4 and FN-5 were prepared by varying polymer concentration (Patravale *et al.,* 2004; Moschwitzer and Muller, 2007; Kayser *et al.,* 2003).

Table 1: Formulation of different batches of Indomethacin Nanosuspension

Ingredients	FN-1	FN-2	FN-3	FN-4	FN-5
Indomethacin (% w/w), mg	100	100	100	100	100
Eudragit RS 100 (%w/w), mg	500	400	300	200	100
Tween 80 (% w/v)	0.02	0.02	0.02	0.02	0.02
Benzalkonium chloride (% w/v)	0.1	0.1	0.1	0.1	0.1
Ethanol (ml)	5.0	5.0	5.0	5.0	5.0
Water qs to (ml)	50	50	50	50	50

RESULTS AND DISCUSSION

Preformulation study: The FT-IR spectra of the pure Indomethacin and formulation were recorded to check interaction between drug and polymers. Before FT-IR examination formulation is kept for the stability testing (Zhang, 2007; Zeng *et al.,* 2008). The characteristic peak due to pure Indomethacin has appeared in the spectra without any markable change in the position. It indicates that there was no chemical interaction between Indomethacin and Eudragit RS 100.

Evaluation of nanosuspension:

1: Drug entrapment efficiency: The drug content in five batches of Indomethacin nanoparticles was studied. The amount of drug bound per 1 ml of Nanosuspension was determined in each batch. The maximum entrapment was found in FN-3(96.0%) and lowest entrapment in FN-5 (83.57%). Sonication of the solution after addition of drug in polymer solution also plays important role in drug entrapment efficiency, as sonication leads to uniform distribution of the drug (Kayser, 2001; Agnihotri and Vavia, 2009; Pignatello *et al.,* 2002). Uniform distribution of drug will also give consistent entrapment efficiency of the same batch with less deviation.

pH of Nanosuspension: pH values for all the formulations were within acceptable range 5.8-6.5 and hence would not cause any irritation upon administration of the formulation. It was also observed that increase in Eudragit RS 100 polymer causes a slight increase in pH for formulations (Jacobs *et al.,* 2001; Langguth *et al.,* 2005; Grau *et al.,* 2000).

Stability Study: Stability study was carried out for the formulation FN3 by exposing it to various temperature 5-8^{o}C, at room temperature and 40 $\pm$ 2^{o}C for 3 months. The sample was analyzed for drug content at regular interval of three months and it was evident that there was no remarkable change in the drug content of Nanosuspension. Results show that formulation FN3 was stable at mentioned temperatures.

***In vitro* drug release study:** The release study was conducted for all the five formulations. Most of the formulations were found to have a linear release and the formulations were found to provide approximately 84.59% release within a period of 14 hours. Cumulative percent drug released for FN-2, FN-3 after 14 hours was 92.14%, 96.18% and for FN-1, FN-4 and FN-5 after 14 hours was 89.14%, 88.24% and 84.26%, respectively. Thein vitro release of all the five batches of Nanosuspension showed an interesting bi-phasic release with an initial burst effect. In the first hour, drug released was 14.97%, 14.06%, 15.24%, 14.91% and 13.87 % for FN-1, FN-2, FN-3, FN-4 and FN-5, respectively. Afterwards the drug release followed a steady pattern approximating zero order release. The burst release in the first hour can be attributed to the drug loaded on the surface of nanoparticles. The kinetic values obtained for different formulations are indicated in following tables. The correlation coefficients for formulations FN-1 to FN-5 of first order plot were found to be 0.9877, 0.9961, 0.9919, 0.9854 and 0.9514, respectively. The correlation coefficients of formulations FN-1 to FN-5 of Higuchi matrix plot were found to be 0.9953, 0.9824, 0.9916, 0.9912 and 0.9819. It was observed that FN-2,

FN-3 followed Higuchi matrix suggesting drug release by diffusion process.

Kinetic modeling: The various kinetic models were applied to in vitro release data for prediction of the drug release kinetic mechanism (Jacobs *et al.,* 2000; Moschwitzer and Achleitner, 2004). The release constants were calculated from the slope of appropriate plots, and the regression coefficient (r^2) was determined. It was found that the in vitrodrug release of Nanosuspension was best explained by zero order kinetics as the plots shows highest linearity. The correlation coefficient (r^2) was in the range of 0.9854 to 0.9978 for various formulations as shown in Table 17. For formulation FN_3 correlation coefficient (r^2) is found to be 0.9919, indicating that the drug release was nearly independent of concentration, followed by Higuchi's (r^2 = 0.9916 with K_H = 24.5834). In the current study, drug release kinetic according to korsmeyer-peppa's model is also followed. The values of release rate exponent (n), calculated as per the equation proposed by peppa's, and all the slope values ranges from 0.5061 to 0.5204 revealed the fact that the drug release follows a Non Fickian Diffusion.

Sterility Testing: Ultra-Violet radiation was used to sterilize the formulation and sterility testing was carried out under aseptic conditions. It was found visually that the Alternate thioglycolate, Soybean casein digest media; Fluid thioglycolate media containing sterilized formulation was free from turbidity. This confirmed the absence of aerobic organism, anaerobic organism and fungi. From this it confirms the sterility of formulation; therefore, the sterilized formulation was considered suitable for in vivo studies.

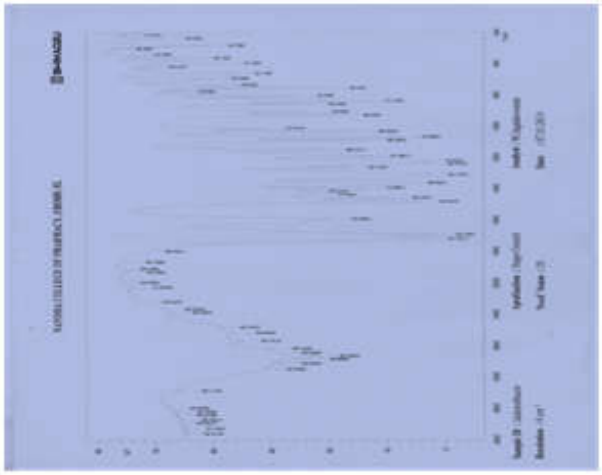
Rabbit eye irritation: The prepared Nanosuspension of Indomethacin showed satisfactory Ocular tolerance. No ocular damage or abnormal clinical sings were visible. Only a few sings of increased lacrimation were noted.

***In vivo* drug studies:** Formulation FN3 optimal particle size and satisfactory in vitro release was selected for in vivo drug studies. Drug concentration was determined by HPLC method. The drug concentration was determined in each formulation by calculating the peak areas of formulation FN3 and controlled sample as shown in following figure with HPLC graphs. The in vivo study was carried out using best formulation & standard sample. The maximum concentration was found to be 73.80 µg/ml where as standard controlled sample showing 13.96µg/ml, which indicate sustained action of formulation as compare to standard sample

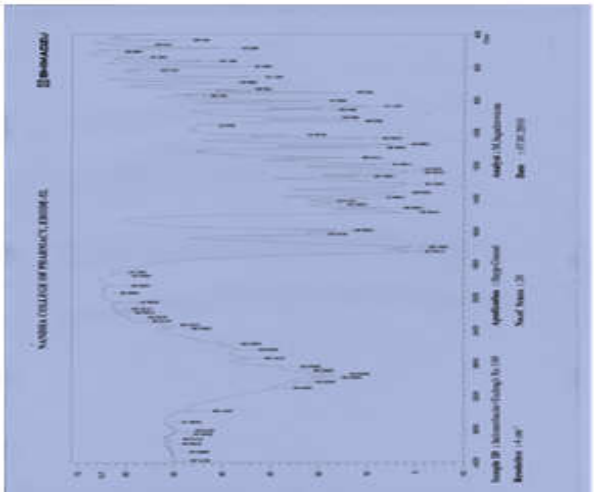
Table 2: Interpretation of FTIR spectra

Transition	IR Range (cm^{-1})	Absorption wave number		
		Indomethacin	Physical mixture	Formulation
C=O stretching	1660-1680	1668.06	1688.81	1677
C-H alkane	2850-2960	2862.36	2882.48	2879
$-CH_3$ stretching	1000-1300	1161.62	1059.90	1296

IR Spectrum of Indomethacin



IR Spectrum of Indomethacin + Eudragit RS 100



IR Spectrum of Eudragit RS 100

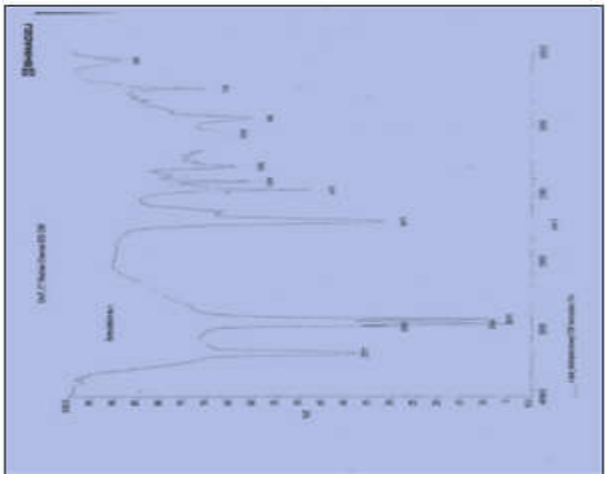


Table 3: Standard curve of Indomethacin in phosphate buffer pH 7.4

Concentration in µg/ml	Absorbance at 320 nm
0	0
1	0.066
2	0.124
3	0.199
4	0.243
5	0.301
6	0.373
7	0.444
8	0.499
9	0.566
10	0.595

Table 4: Drug Entrapment Efficiency for Different Formulation

Formulation	Absorbance	Concentration (µg/ml)	Drug Content %
FN-1	0.748	12.6	90.0
FN-2	0.721	12.8	91.42
FN-3	0.711	13.3	96.0
FN-4	0.682	12.1	86.42
FN-5	0.659	11.7	83.57

Table 5: Viscosity of Indomethacin Loaded Nanosuspension

Angular velocity (rpm)	Velocity in cps				
	FN-1	FN-2	FN-3	FN-4	FN-5
10	73.3	67.2	61	57.2	42
20	66.3	56.8	48.4	43.1	33.2
30	54.2	43.9	34.2	27.2	22.3
40	36.3	32.5	26.9	23.1	17.1
50	19.3	18.1	17.8	18.3	12.6
60	15.7	17.4	11	14.6	10
70	10	13	10.3	10.6	7.7
80	8.9	9.8	7.5	8.9	5.67
90	8.1	7.7	5.6	7.3	4.3
100	7.0	6.2	4.7	4.5	3.6

Table 6: pH of Indomethacin Loaded Nanosuspension

Formulation	pH
FN-1	6.4
FN-2	6.2
FN-3	6.5
FN-4	6.3
FN-5	5.8

Table 7: Stability study data for FN3

S. No.	Days	% R.D.C. $4^{O}C$	% R.D.C. 27±2ºC	% R.D.C. $40 \pm 2^{O}C$
1	0	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
2	15	99.97 ± 0.038	99.95 ± 0.038	99.94 ± 0.038
3	30	99.84 ± 0.012	99.81 ± 0.010	99.73 ± 0.025
4	45	99.63 ± 0.011	99.54 ± 0.031	98.44 ± 0.026
5	60	99.24 ± 0.023	98.93 ± 0.021	98.80 ± 0.031
6	90	98.54± 0.041	98.47± 0.021	98.33± 0.013

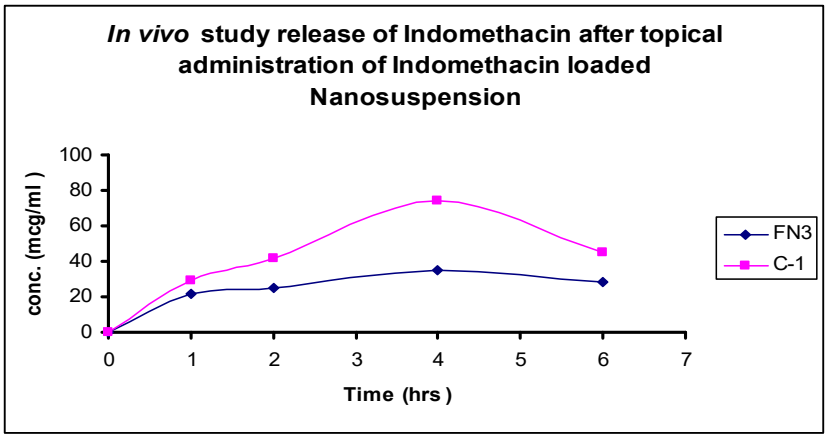
R.D.C. =Remaining Drug Content in 7.4 pH buffer

Table 8: Release kinetic models of different formulation

Formulations	Zero order		Higuchi's		Peppa's	
	Slope (K_0)	Correlation (r^2)	Slope (K_H)	Correlation (r^2)	Slope (n)	Correlation (r^2)
FN_1	5.4034	0.9877	23.9148	0.9953	0.5113	0.9849
FN_2	6.3096	0.9961	24.3149	0.9824	0.5204	0.9927
FN_3	6.4195	0.9919	24.5834	0.9916	0.5053	0.9983
FN_4	5.6238	0.9854	24..6364	0.9912	0.5019	0.9578
FN_5	5.4966	0.9978	23.9104	0.9819	0.5061	0.9831

Table 9: *In vivo* Release of Indomethacin after topical administration of Indomethacin loaded Nanosuspension

Time in hrs	Controlled sample		Formulation FN3	
	AUC	Conc. µg/ml	AUC	Conc. µg/ml
1	138994±1.88	21.30±1.41	148994±1.79	29.54±1.79
2	175558±1.72	17.87±1.44	271579±1.61	41.71±1.74
4	222457±1.77	13.96±1.39	459267±1.68	73.80±1.71
6	183512±1.70	19.97±1.47	301579±1.68	45.19±1.71



CONCLUSION

In the present study an attempt was made to develop the ophthalmic Nanosuspension of Indomethacin with improved bioavailability, avoidance of repeated administration and dose reduction. From the experimental finding, it is concluded that:

Eudragit RS 100 is a good film forming biodegradable polymer and is a promising agent for ocular delivery.

The pH of all formulations was found to be satisfactory thus there would be no irritation to the patient upon administration of the formulation.

The particle size analysis revealed that the nanoparticles were in nanometer range and all the formulations showed ideal surface morphology. Particle size of formulation FN-3 was smallest and discrete.

The *in vitro* release studies showed biphasic release pattern for all formulation, with an initial burst effect, which may be attributed to the drug loaded on the surface of the particles.

The optimum drug to polymer ratio was found to be in FN-3 depending on the particle size, entrapment efficiency, and in vitro release profile. There was no significant increase drug release with increase in drug to polymer ratio.

Formulation FN-3 showed zero order release and followed Higuchi matrix and showed release through diffusion, it also showed that the diffusion is through Non Fickian mechanism.

On the basis of drug content, particle size, morphology, in vitrorelease and satisfactory release kinetics, formulation FN-3 was selected as an optimum formulation for in vivo and stability studies.

In vivo release profile indicated that polymeric system of Indomethacin has achieved the objectives of increased contact time, prolonged release, and decreased frequency of administration, avoidance of eye-irritation and redness of the rabbit eye.

The DSC study showed that complete disappearance of the melting endotherm of Indomethacin, which could indicate the complete amorphization of the drug as well as loss of drug crystallinity.

Zeta potential study proved that the formulation FN-3 have excellent stability. The positive value + 45mv indicate that the Eudragit RS 100 Nanosuspension was stabilized by electrostatic repulsion forces.

By these facts, study can be concluded by saying that Nanosuspension prepared from Eudragit RS 100 using different polymer concentration is a promising approach to enhance the bioavailability of Indomethacin

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

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