Research Article

Development and evaluation of moxifloxacin hydrochloride loaded microspheres for controlled release ophthalmic delivery

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ABSTRACT

Poor bioavailability and therapeutic response exhibited by conventional ophthalmic solutions due to precorneal elimination of the drug may be overcome by the use of ocular gels enriched with microspheres. Microspheres involving drug loaded particles may enhance dosage form acceptability by providing sustained release in ocular milieu. The objective of the present study was to prepare and characterize microspheres formulation of Moxifloxacin Hydrochloride which is a synthetic broad spectrum flouroquinolone antibacterial agent used for treatment of conjunctivitis caused by susceptible strains of aerobic gram positive, negative and other organisms. Moxifloxacin Hydrochloride loaded microspheres were prepared by emulsion solvent evaporation technique using acrylate methacrylate copolymer Eudragit RS 100. The 3^2 factorial design along with response surface methodology was used to optimize microsphere formulation. The optimized microspheres were characterized for parameters like appearance, particle shape, particle size, percentage yield, drug loading, differential scanning colorimetry, Scanning electron microscopy (SEM) and *in-vitro* drug release. The drug release kinetics from developed microspheres was determined by calculating the correlation coefficients of percentage drug release versus time plot, percentage drug release versus square root time plot and log percentage drug retained versus time plots. The microspheres of Moxifloxacin Hydrochloride were successfully prepared for controlled ophthalmic drug delivery. These microspheres were able to sustain the *in-vitro* release of the drug. These optimized microspheres showing controlled drug release can be further incorporated into bioadhesive polymer to prepare ophthalmic gel. Controlled release with this formulation may reduce dose frequency and side effects as well as improve the patient compliance.

Keywords: Controlled release, microspheres, moxifloxacin hydrochloride, ophthalmic

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INTRODUCTION

Moxifloxacin Hydrochloride is a synthetic broad spectrum flouroquinolone antibacterial agent available in the form of conventional eye drops for treatment of conjunctivitis caused by susceptible strains of aerobic gram positive, negative and other organisms. In ocular drug delivery the physiological barriers to diffusion and productive absorption of topically applied drug exists in precorneal and corneal spaces. The precorneal constraints responsible for poor ocular bioavailability of conventional ophthalmic dosage forms are solution drainage, lacrimation, tear dilution, tear turn over, and conjuctival absorption. This results in poor absorption of drugs with very small fractions of instilled dose penetrating the cornea and reaching the intraocular tissues. Therefore the objective of the present research work was to develop and evaluate Moxifloxacin Hydrochloride loaded Eudragit RS100 microspheres for the controlled ophthalmic delivery of drug which may reduce dose frequency and side effects of conventional ocular dosage forms. Microspheres are one of the particulate delivery systems used to achieve sustained or controlled drug

delivery, to improve bioavailability and stability and to target drug to specific sites. Microspheres also offer advantages such as limiting fluctuation within a therapeutic range, reduction in side effects, decreased dose frequency and hence improved patient compliance [1]. One of the popular methods for the encapsulation of drugs within water-insoluble polymers is the emulsion solvent evaporation method [2]. The emulsion solvent evaporation technique has been successfully used in the preparation of microspheres made from several biocompatible polymers such as poly (d,l-lactide-co-glycolide) [3-6], poly(" caprolactone) [7-9], Chitosan [10] and Eudragit [2,11-13]. This technique offers several advantages and is preferable to other preparation methods such as spray drying, sonication and homogenization because it requires only mild conditions such as ambient temperature and constant stirring. Thus, a stable emulsion can be formed without compromising the activity of the drugs [2]. Eudragits are another class of biocompatible copolymers synthesized from acrylic and methacrylic acid esters [13]. Eudragit RS, is insoluble in aqueous media but permeable, and as such have been shown to be suitable for sustained-release microencapsulated dosage forms. Eudragit RS have been investigated as a suitable polymer for ophthalmic delivery of drug to increase the ocular drug bioavailability. Acyclovir loaded Eudragit microparticles [14], ciprofloxacin-loaded Eudragit RS100 nanoparticles [15], gentamicin-Eudragit microspheres [16], Ibuprofen Carried by an Eudragit RS100 Nanoparticle Suspension [17], Cloricromene loaded Eudragit nanosuspension [18], Piroxicam loaded Eudragit nanoparticles [19]. Thus Eudrragit RS100 polymer was selected to prepare Moxifloxacine Hydrochloride loaded microspheres for ophthalmic delivery.

MATERIALS:

Moxifloxacin Hydrochloride (MXF) was gifted by Ciron drugs Pharmaceuticals. The polymer Eudragit RS 100, a gift sample from the Degussa India Pvt. Ltd. All the other reagents used were of analytical grade.

METHOD:

MXF loaded Eudragit microspheres were prepared by emulsification solvent evaporation method [3]. Eudragit was dissolved in acetone by sonication for 10mins. MXF was dissolved in methanol. Ratio of acetone:methanol used was 1:1 as internal phase. MXF solution was then slowly added to the polymer solution. This solution containing drug and polymer was then added to 250 ml beaker containing a mixture of 160ml light liquid paraffin and 0.2 percentages of span 80. The stirring of resultant emulsion was allowed to continue at speed of 2500rpm until complete evaporation of organic solvents. The resultant microspheres were collected by vacuum filtration, washed with n-hexane and dried microspheres were sieved carefully, weighed, labeled and stored in air tight container which were finally stored in desiccator and the yield of microspheres preparation was calculated using the following formula: [2]

Percent yield = (the amount of microspheres obtained (g)/ the theoretical amount (g) of non volatile material) X 100

The prepared MXF loaded microsheres were subjected for optimization process. Optimization of Moxifloxacin Hydrochloride loaded Eudragit microspheres were done in two stages. Initially optimization of drug to polymer ratio was done. The effect of the ratio of drug: polymer ratio on mean particle size, percentage yield and percentage drug loading is shown in [**Table 1**]. Further optimization of Moxifloxacin Hydrochloride loaded Eudragit microspheres were done by using 3^2 factorial design. The 3^2 factorial design along with response surface methodology was used to optimize the effect of input variables viz. internal:external phase ratio, stirring speed and surfactant concentration on the various physicochemical parameters of microspheres like mean particle size, percentage drug content and percentage entrapment efficiency.

Particle size analysis and morphological studies:

The mean particle size of the MXF loaded microspheres were determined by optical microscopy. At least 200 microspheres were analyzed for each preparation and the

mean diameter was calculated. The surface morphology and appearance of the microspheres were examined by a scanning electron microscopy Philips XL 30SEM setup at 10kv with a till of 45°. The specimens were mounted on a metal stub (with double-sided adhesive tape) and coated under vacuum with gold in nitrogen atmosphere prior to observation. Using the suitable magnification the micrographs for the sample were recorded.

Drug entrapment efficiency:

20 milligrams of dried microspheres were dissolved in 10ml methanol by sonication for 10min. The samples were assayed for MXF content by an Jasco UV-spectrophotometer at 294 nm after suitable dilution.

%EE= (Percent of real loaded drug/Percent of theoretical loaded drug) X 100.

Drug polymer interaction:

Determined by Differential Scanning Calorimetry (DSC) by using a Mettler – Model DSC 821. DSC analysis of MXF, Eudragit RS polymer and MXF loaded microspheres was done. The samples were weighed in an aluminum cuvette and sealed with an aluminum lid. An empty standard aluminum cuvette was used as reference. The containers were placed in the DSC apparatus and heated at a constant rate of 10°C/min, over a temperature range of 30°C to 300°C. The thermograms were plotted by the instrument directly and peak temperature, initiation temperature and recovery temperature were determined.

***In-vitro* drug release:**

Drug release kinetics from the prepared MXF loaded microspheres was determined by using specially designed Franz diffusion cell. Diffusion media used was Phosphate buffer pH 7.4. Microspheres equivalent to dose were mounted on top of 0.45µm cellulose nitrate membrane and wetted with 3-4 drops of diffusion media. Water at 37 ^{0}C was circulated through the water jacket surrounding the receptor cell and Teflon coated magnetic stir bar kept at bottom of the receptor cell created a homogeneous receptor volume. 2ml sample was withdrawn at regular time intervals and replaced with the equal volume of prewarmed medium. The withdrawn samples were analyzed for MXF content at 294nm using an Jasco ultraviolet spectrophotometer.

Release Kinetic:

Data obtained from in vitro release studies were fitted to various kinetic equations to find out the mechanism of MXF release from microspheres. The kinetics models used were zero order, first order, and Higuchi models. The rate constants were also calculated for the respective models.

RESULT AND DISCUSSION

MXF loaded Eudragit microspheres were produced by emulsification solvent evaporation technique which is a good technique and seemed to be promising for the preparation of MXF microspheres because it is easy, reproducible, rapid method and has an advantage of avoiding solvent toxicity. From effect of drug to polymer ratio on mean particle size, percentage yield and percentage drug loading, it was observed that as the drug: polymer ratio was increased from 1:3 to 1:8, percentage yield and percentage drug loading were increased with the decrease in mean particle size [**Table 1**]. Amongst the series of batches from Eu1-Eu6, batch Eu6 having drug:polymer ratio of 1:8 showed maximum drug loading 50.5%, percentage yield 85.4% and mean particle size of 10.2 µ [**Table 1**]. Hence batch Eu6 with drug: polymer ratio of 1:8 was selected and used for further optimization processes.

In case of further optimization with 3^2 factorial design, the effect of three input variables viz. stirring speed, surfactant concentration and ratio of internal to external phase on mean particle size, percentage drug content and percentage entrapment efficiency of microspheres was determined. It was observed that when the stirring speed was increased from 2000 to3000rpm, surfactant concentration was increased from 0.1 to 0.3% and the ratio of internal to external phase was increased from 1:8 to 1:24, all the three response variables viz. the mean particle size, drug content and entrapment efficiency of microspheres were decreased [**Table 2,3,4**].

Table 1: Effect of drug:polymer ratio on and mean particle size, percentage yield and percentage drug loading

Batch No.	Drug: Polymer Ratio	Mean particle size ± S.D(μ)	Percentage (%) Yield	Drug loading (%)
Eu1	1:3	14.5±2.2	60.5	30.2
Eu2	1:4	13.8±3.1	64.3	35.6
Eu3	1:5	12.5±1.5	69.3	38.5
Eu4	1:6	11.1±2.2	75.5	42.4
Eu5	1:7	10.8±2.4	78.9	45.2
Eu6	1:8	10.2±2.0	85.4	50.5

Table 2: Effect of stirring speed and internal:external phase ratio on mean particle size, percentage drug content and percentage entrapment efficiency for batches E1-E9

Batch no:	Stirring speed (rpm)	Internal: external phase ratio (ml)	Mean particle size ± S.D (μ)	Drug content (%)	Drug entrapment (%)
E1	2000	1:8	15.5±0.84	84.1±0.81	48.2±0.89
E2	2000	1:16	14.1±0.63	82.3±0.84	47.5±0.78
E3	2000	1:24	13.4±0.78	77.2±0.92	46.1±0.83
E4	2500	1:8	12.5±0.92	82.5±0.93	47.6±0.89
E5	2500	1:16	10.4±0.79	81.4±0.88	46.5±0.79
E6	2500	1:24	10.2±0.87	75.2±0.85	43.2±0.82
E7	3000	1:8	10.1 ±0.88	64.3±0.78	33.8±0.80
E8	3000	1:16	09.5±0.76	62.1±0.86	32.1±0.82
E9	3000	1:24	09.2±0.79	60.2±0.90	30.3±0.84

Table 3: Effect of stirring speed and surfactant concentration on mean particle size, percentage drug content and percentage entrapment efficiency for batches E10-E18

Batch no:	Stirring speed (rpm)	Surfactant Concentration (%)	Mean particle size ± S.D (μ)	Drug content (%)	Drug entrapment (%)
E10	2000	0.1	15.3±0.89	83.6±0.82	47.8±0.91
E11	2000	0.2	14.2±0.79	82.4±0.86	46.2±0.90
E12	2000	0.3	13.8±0.99	75.3±0.89	45.2±0.79
E13	2500	0.1	13.2±0.82	81.2±0.79	46.9±0.81
E14	2500	0.2	12.2±0.78	79.1±0.90	43.5±0.79
E15	2500	0.3	11.9±0.87	74.1±0.94	42.0±0.76
E16	3000	0.1	11.2±0.90	64.6±0.87	34.2±0.84
E17	3000	0.2	10.8±0.92	62.2±0.79	33.5±0.86
E18	3000	0.3	10.6±0.88	61.1±0.89	31.2±0.79

The MXF loaded Eudragit microspheres were found to be free flowing and pale yellow colored. All the microspheres showed a normal distribution of sizes with the mean particle size of 10.1 μ. Scanning electron microscopy images [**Figure 1**] showed that microspheres were spherical and having smooth surface.

By using 3^2 Factorial design E1-E27 batches of MXF loaded Eudragit microspheres were prepared and evaluated for mean particle size, percentage drug loading and percentage drug entrapment. From all the responses of factorial design study it was observed that batch E5 was better in terms of particle size 10.4 μ, drug content 81.4% and entrapment efficiency 46.5% [**Table 2**]. As microspheres are intended for

ophthalmic drug delivery and eye being a very sensitive organ the large particles could cause irritation. Thus the particle size of microsphere is a critical parameter. As a result aesthetic appeal of the final formulation was improved.

Table 4: Effect of surfactant concentration and internal: external phase ratio on mean particle size and percentage drug content and entrapment efficiency for batches E19-E27

Batch no:	Stirring speed (rpm)	Surfactant Concentration (%)	Mean particle size ± S.D (µ)	Drug content (%)	Drug entrapment (%)
E10	2000	0.1	15.3±0.89	83.6±0.82	47.8±0.91
E11	2000	0.2	14.2±0.79	82.4±0.86	46.2±0.90
E12	2000	0.3	13.8±0.99	75.3±0.89	45.2±0.79
E13	2500	0.1	13.2±0.82	81.2±0.79	46.9±0.81
E14	2500	0.2	12.2±0.78	79.1±0.90	43.5±0.79
E15	2500	0.3	11.9±0.87	74.1±0.94	42.0±0.76
E16	3000	0.1	11.2±0.90	64.6±0.87	34.2±0.84
E17	3000	0.2	10.8±0.92	62.2±0.79	33.5±0.86
E18	3000	0.3	10.6±0.88	61.1±0.89	31.2±0.79

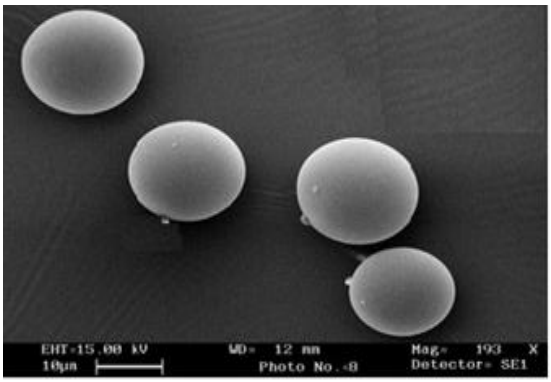

Figure 1: Scanning Electron Microscopy of MXF Loaded Eudragit microspheres

Differential scanning colorimetry: Physico-chemical characterization of microspheres was performed by DSC analysis. DSC thermogram of pure drug MXF and polymer Eudragit showed a characteristic endothermic peak at the temperature corresponding to their melting points of 254 and 196°C [**Figure 2 and 3**]. DSC thermogram of drug loaded microspheres showed a single characteristic endothermic peak at 213 °C [**Figure 4**] which was found to be present in the range of melting points of drug and polymer indicating the possible merging of peaks.

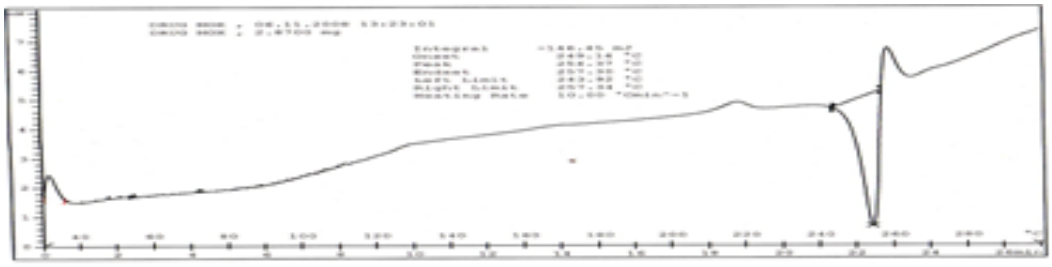

Figure 2: DSC thermogram of MXF

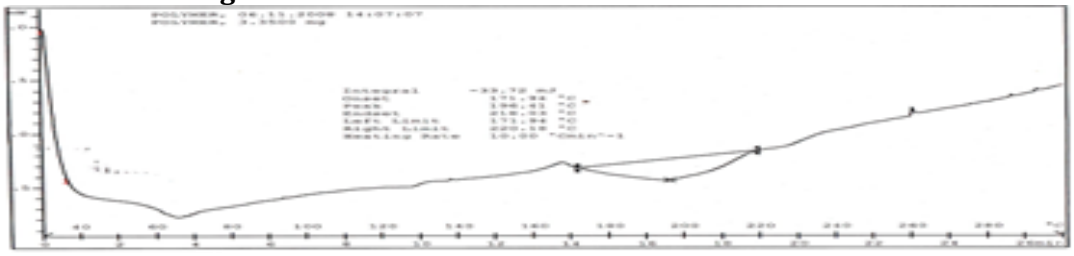

Figure 3: DSC thermogram of Eudragit

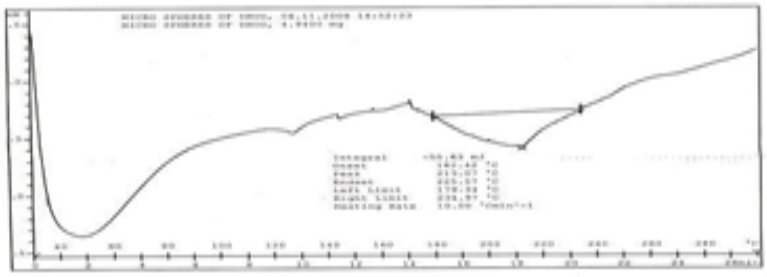

Figure 4: DSC thermogram of MXF loaded microspheres

***In-vitro* release study:**

In-vitro release of MXF from Eudragit microspheres was examined in phosphate buffer pH 7.4 at 37°c using Franz diffusion cell. The percentage of drug released from various batches with varying drug: polymer ratios at different time points were plotted in [**Figure 5**].

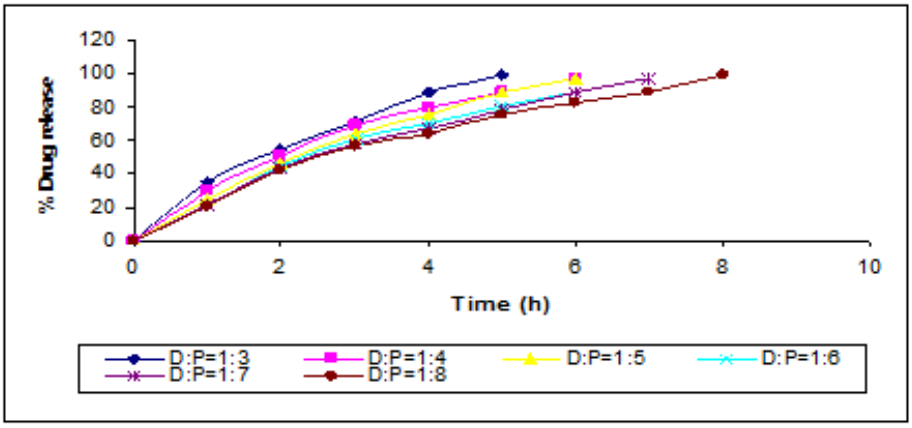

Figure 5: Comparative *in-vitro* drug release of MXF loaded microspheres with the varying ratios of Drug to polymer

Microspheres prepared with drug to polymer ratio of 1:3 showed a burst release with $t_{10\%}$ of 18.5min whereas microspheres with drug to polymer ratio of 1:8 showed much lesser burst effect with $t_{10\%}$ of 42min. Also microspheres having drug:polymer ratio of 1:3 showed $t_{90\%}$ of 4.17 h while microspheres with drug:polymer ratio of 1:8 showed more sustained drug release with $t_{90\%}$ of 7.15 h and drug release being prolonged by releasing 96% of MXF at the end of 8 h. With the increase drug:polymer ratio from 1:3 to 1:8, the drug release was found to be sustained and prolonged [**Figure 5**]. The percentage of drug released from various batches (E1-E27) with varying stirring speed, ratio of internal to external phase and surfactant concentration did not show much variable effect on *in-vitro* drug release [**Figure 6,7,8,9,10,11**].

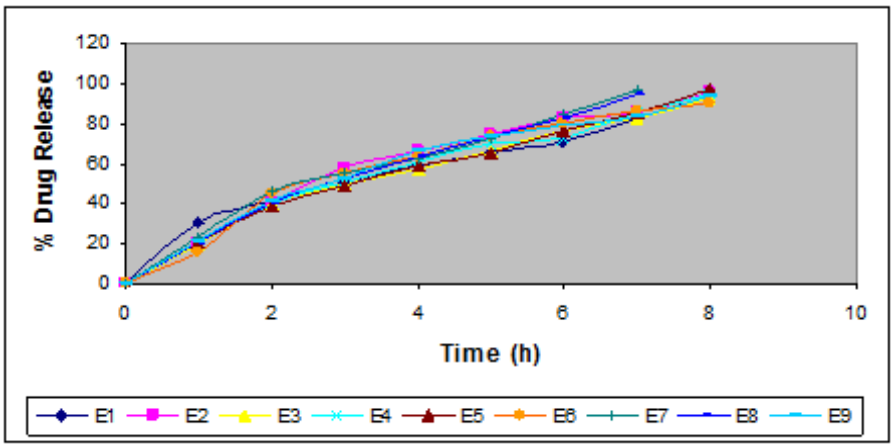

Figure 6: Comparative in-vitro drug release profile of drug loaded microspheres batches E1-E9

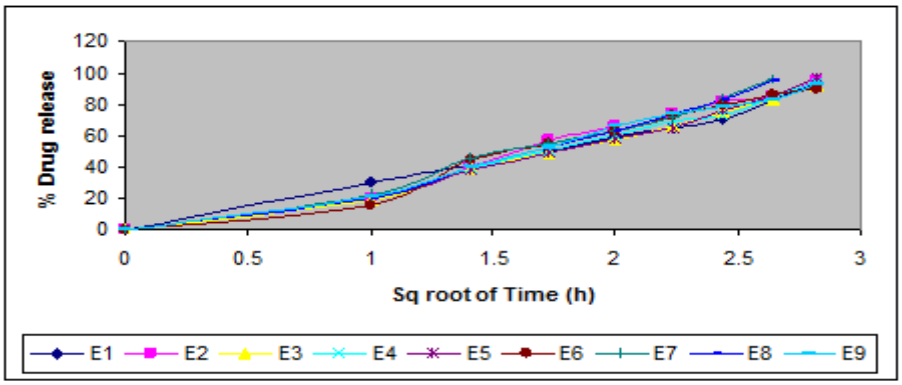

Figure 7: Comparative Higuchi plot of drug loaded microspheres batches E1- E9

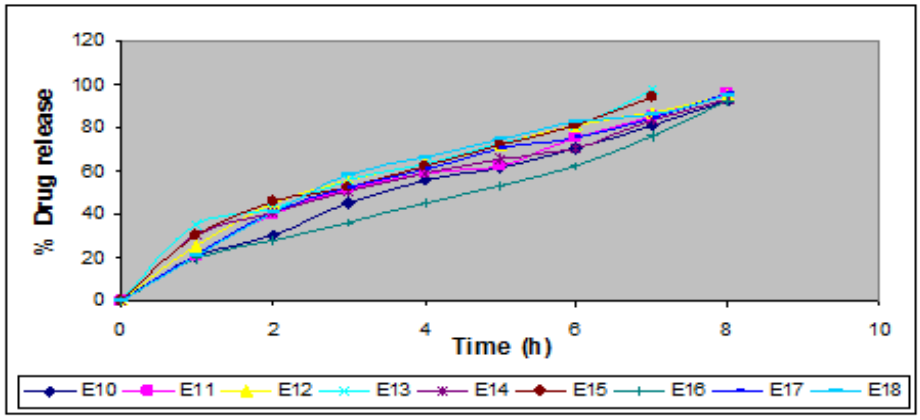

Figure 8: Comparative in-vitro drug release profile of drug loaded microspheres batches E10-E18

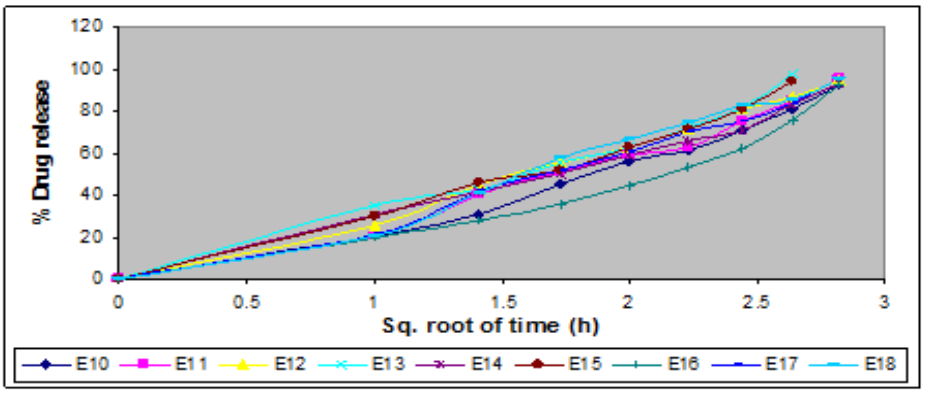

Figure 9: Comparative Higuchi plot of drug loaded microspheres batches E10-E18

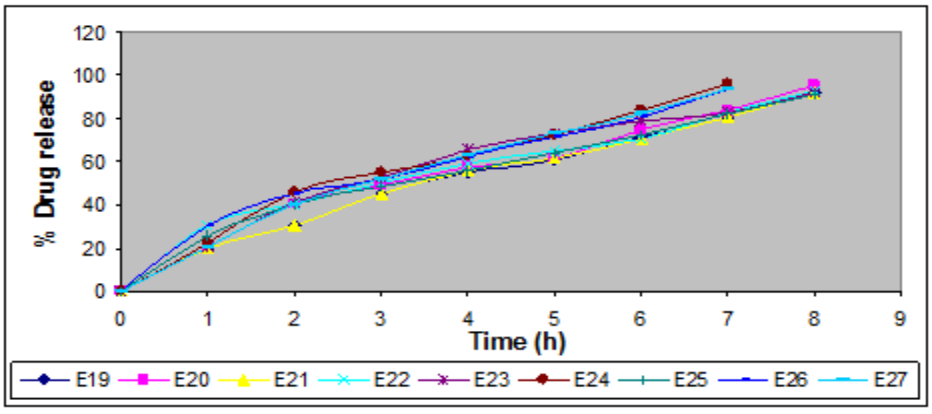

Figure 10: Comparative *in-vitro* drug release profile of drug loaded microspheres batches E19-E27

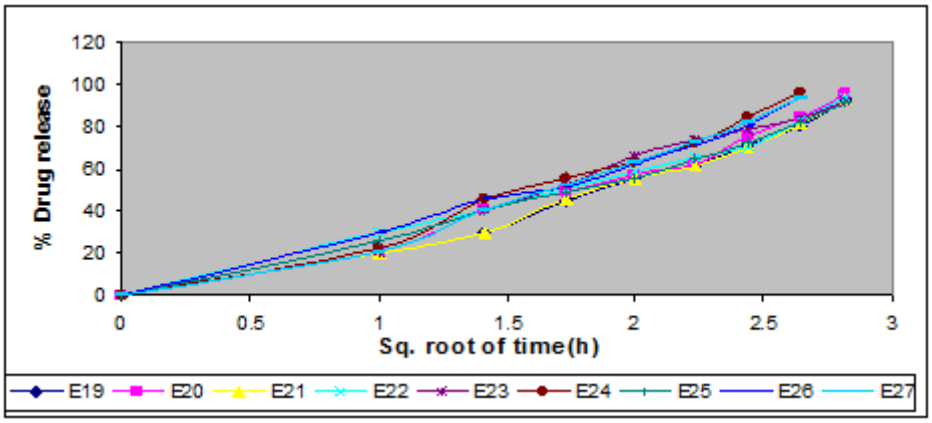

Figure 11: Comparative Higuchi plot of drug loaded microspheres batches E19-E27

Release Kinetics

The release mechanism of MXF from formulation was determined by comparing their respective correlation co-efficient. It was observed that all formulations showed higher coefficient of correlation and better linearity with percentage drug release versus square root time plot [**Figure 7,9,11**], indicating that they followed Higuchi model of matrix diffusion controlled drug release.

CONCLUSION

The MXF loaded Eudragit microspheres were prepared successfully using the emulsion solvent evaporation technique. The microspheres were pale yellow in color, spherical; smooth surfaced, free flowing with rigid morphology. Microspheres showed high percentage yield in the range of 75.5-80.2%. The drug loaded microspheres showed 30.3-48.2% of entrapment and *in-vitro* drug release up to 8 h. The differential scanning calorimetry thermographs showed stable character of drug in the drug-loaded microspheres and revealed the absence of drug-polymer interactions. The best-fit release kinetics was achieved with Higuchi plot and followed Higuchi model of matrix diffusion controlled drug release. By analyzing all the evaluation parameters of E1-E27 batches it was observed that batch E5 was better in terms of particle size 10.4 µ , drug content of 81.4%, entrapment efficiency of 46.5% and *in-vitro* drug release up to 8 h. As microspheres are intended for ocular drug delivery and eye being a very sensitive organ the large particles could cause irritation. Thus the particle size of microsphere is a critical parameter. E5 batch showed the smallest particle size of 10.4 µ and also showed the controlled drug release of 8h. These optimized microspheres showing controlled drug release can be further incorporated into bioadhesive polymer to prepare ophthalmic gel. Controlled release with this formulation may reduce dose frequency and side effects as well as improve the patient compliance. As a result aesthetic appeal of the final formulation was improved.

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