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# To design and development of nanoparticles of meloxicam using chitosan

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Meloxicam is non steroidal anti-inflammatory drug having BCS class II category. Meloxicam inhibits cyclooxygenase (COX-2), the enzyme responsible for converting arachidonic acid into prostaglandin (PG-$H_2$) the first step in the synthesis of prostaglandins, which are mediators of inflammation. In the present study, Polymeric nanoparticles were prepared using Emulsion Cross-linking technique to overcome poor aqueous solubility and low oral bioavailability. The W/O emulsion containing drug and chitosan in aqueous phase was cross-linked with aldehyde groups of gluteraldehyde with amine group of chitosan. The physicochemical properties like zeta sizes, zeta potential, polydispersity index of formulated nanoparticles were evaluated. The preparation of MLX nanoparticles was initiated by optimizing the drug: polymer on the basis of size, polydispersity index and encapsulation efficiency. The final optimized formulation was found to be MC8, MLX: Chitosan (10:400) with mean size of 84±1.7 nm and having polydispersity index of 0.086. The shape and surface morphology of the nanoparticles were evaluated by the use of scanning electron microscopy (SEM). The SEM photomicrograph revealed that the carrier system was more spherical in shape and uniformly distributed without any aggregation or adhesion of nanoparticles. The crystalline state evaluation was done by DSC of Meloxicam (plain drug), optimized formulations MC8.

According to the research the nanoparticles of Meloxicam with smaller particle size can be effectively produced by using Emulsion cross linking technique. The nanoparticles produced by this technique resulted in marked increase in solubility and dissolution rate of the drug and the particle size obtained (84 nm) was suitable even for i.v. administration. This technique was shown to be simple and adequate for drug particle size reduction and did not seem to alter the crystalline state of the drug.

*Keyword:* Meloxicam, COX-2, prostaglandin, nanoparticles etc.

## Introduction

Meloxicam is non-steroidal anti-inflammatory drug having BCS class II category. Meloxicam inhibits cyclooxygenase (COX-2), the enzyme responsible for converting arachidonic acid into prostaglandin (PG-$H_2$) the first step in the synthesis of prostaglandins, which are mediators of inflammation. Meloxicam has been shown, especially at its low therapeutic dose, selectively to inhibit COX-2 over COX-1. It is well absorbed from the gastrointestinal tract, which is reflected by a high absolute bioavailability of 89% following oral administration. Following single dose administration, mean maximum plasma

concentrations are achieved within 5-6 hours for the tablets. The half life of the drug is 15-20hrs and the recommended dose is 7.5 to 15 mg daily by oral route. The empirical formula of the drug is $C_{14}H_{13}N_3O_4S_2$. The IUPAC name of the drug is 4-hydroxy-2-methyl-N-(5-methyl, 2-thiazolyl)-2H-1, 2-benzothiazine-3-carboxamide-1, 1-dioxide. The chemical structure of Meloxicam is given below.

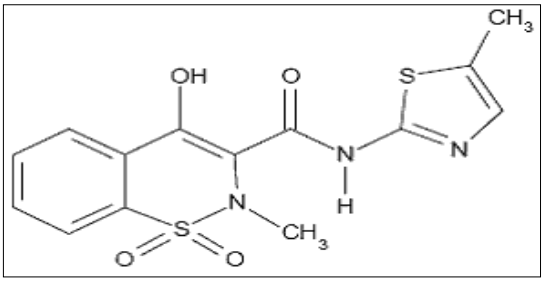



**Fig 1:** Chemical structure of Meloxicam

It is a pale yellow crystalline powder which is bitter in taste and odourless. The molecular weight of the drug is 351.401 having melting point 243-247 °C. It is soluble in dimethyl fomamide, slightly soluble in acetone and practically insoluble in water. The dissociation constant of the drug is 3.02 and the partition coefficient is 4.08. It undergoes extensive hepatic biotransformation having plasma protein binding is 99%. It is used in the treatment of Osteoarthritis and Rheumatoid Arthritis.

## Materials and Methods

Meloxicam was obtained as gift sample from M/S Intas Pharmaceuticals, Ahmedabad, whereas Chitosan was generously provided by M/S Leo chem., Bangalore, Karnataka. Other chemicals used in this study were of suitable analytical grade. Demethyl formamide, petroleum ether, hydrochloric acid and potassium dihydrogen phosphate were purchased from M/s Rankem, Ltd, New Delhi. Tween 80, liquid paraffin, sodium bicarbonate, potassium chloride, potassium hydrogen phthalate, talc, sodium saccharine and formaldehyde solution were purchased from M/s Nice Chemicals Pvt. Ltd, Cochin, and Kerala.

## Experimental Methods

### Preparation of Polymeric Nanoparticles of Meloxicam

Meloxicam loaded Chitosan nanoparticles were prepared by emulsification-cross-linking method, which utilizes the reactive functional amine group present in chitosan to cross-link with aldehyde groups of the glutaraldehyde (cross-linking agent). The different compositions of nanoparticles are given below.

**Table 1:** Formulation Used for the Preparation of Nanoparticles

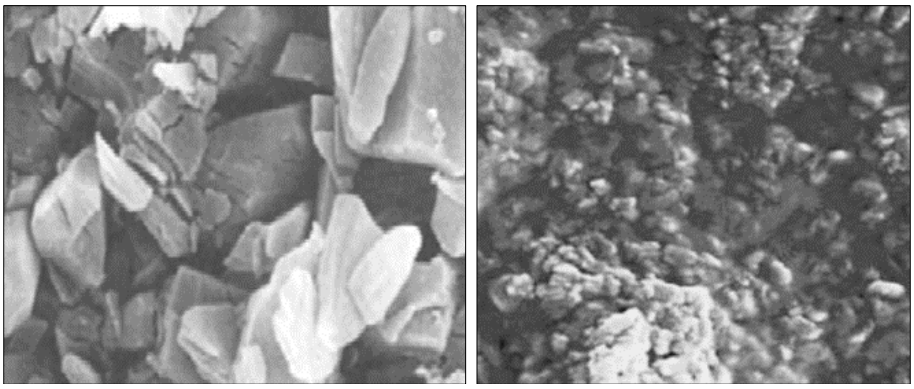
| S. No. | Formulation Code | Meloxicam (mg) | Chitosan (mg) | Liq. Paraffin (mL) | DMF (mL) | Span 80 (mL) | Glutaraldehyde (mL) |
|---|---|---|---|---|---|---|---|
| 1 | MC1 | 5 | 100 | 80 | 1 | 2 | 1 |
| 2 | MC2 | 5 | 200 | 80 | 1 | 2 | 1 |
| 3 | MC3 | 5 | 300 | 80 | 1 | 2 | 1 |
| 4 | MC4 | 5 | 400 | 80 | 1 | 2 | 1 |
| 5 | MC5 | 10 | 100 | 80 | 1 | 2 | 1 |
| 6 | MC6 | 10 | 200 | 80 | 1 | 2 | 1 |
| 7 | MC7 | 10 | 300 | 80 | 1 | 2 | 1 |
| 8 | MC8 | 10 | 400 | 80 | 1 | 2 | 1 |
| 9 | MC9 | 15 | 100 | 80 | 1 | 2 | 1 |
| 10 | MC10 | 15 | 200 | 80 | 1 | 2 | 1 |
| 11 | MC11 | 15 | 300 | 80 | 1 | 2 | 1 |
| 12 | MC12 | 15 | 400 | 80 | 1 | 2 | 1 |

### Characterization of Nanoparticles

The particle size and size distribution, polydispersity index, zeta potential and entrapment efficiency are shown given below in the table.

**Table 2:** Physical Characteristics of Drug Loaded Nanoparticles

| Sr. No. | Formulation code | Particle size (nm) (Mean±SD) n=3 | Polydispersity index (Mean±SD) n=3 | Zeta potential (mV) (Mean±SD) n=3 | Entrapment efficiency (%) (Mean±SD) n=3 |
|---|---|---|---|---|---|
| 1 | MC1 | 97±0.6 | 0.14±0.003 | 48.8±0.19 | 68.76±1.8 |
| 2 | MC2 | 118±0.3 | 0.074±0.003 | 42.8±0.17 | 82±2.4 |
| 3 | MC3 | 284±1.7 | 0.066±0.004 | 41.7±0.06 | 95.6±2.7 |
| 4 | MC4 | 342±0.2 | 0.066±0.005 | 42.3±0.16 | 97.4±1.4 |
| 5 | MC5 | 209±1.4 | 0.17±0.016 | 46.2±0.17 | 78.1±1.6 |
| 6 | MC6 | 346±1.9 | 0.11±0.008 | 48.3±0.12 | 81.4±2.8 |
| 7 | MC7 | 98±1.5 | 0.16±0.017 | 46.8±0.19 | 84.7±1.3 |
| 8 | MC8 | 84±1.7 | 0.086±0.013 | 39.8±0.27 | 86.8±1.5 |
| 9 | MC9 | 158±1.4 | 0.088±0.024 | 49.7±0.08 | 74.3±1.9 |
| 10 | MC10 | 268±1.6 | 0.086±0.017 | 54.3±0.14 | 79±1.9 |
| 11 | MC11 | 448±0.8 | 0.1±0.004 | 52.9±0.29 | 82.6±0.5 |
| 12 | MC12 | 634±1.5 | 0.18±0.007 | 49.5±0.12 | 77.11±1.7 |



SEM of Pure drug (Meloxicam) SEM of Meloxicam Nanoparticles

**Fig 2:** Morphological Studies

## Encapsulation Efficiency of Nanoparticles

The encapsulation efficiency was found to be directly proportional to the preparative parameters tested. Among these the efficiencies of batches MC2 to MC4 with increasing chitosan concentration and MC6 to MC8 with increasing chitosan: Glutaraldehyde ratio were more significant ($p< 0.05$). It ranged from 82±2.4 to 97.4±1.4 and 81.4±2.8 to 86.8±1.5 respectively. The mechanism of meloxicam association to chitosan nanoparticles was mediated by an ionic interaction between both macromolecules. The electrostatic interactions between the meloxicam and the amino groups of CS played a role in association of meloxicam to the chitosan nanoparticles. High chitosan concentration (15mg/mL) showed increased encapsulation efficiency, while low concentration of chitosan showed smaller nanoparticles with lower entrapment efficiency.

## *In-vitro* Drug Release

**Table 3:** *In vitro* drug release study of Meloxicam Loaded Nanoparticles (MC1-MC6) (mean ± S.D.; n=3)

| Time (min.) | Control Pure Meloxicam | MC1 | MC2 | MC3 | MC4 | MC5 | MC6 |
|---|---|---|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 5 | 3.59±0.22 | 34.97±0.15 | 36.22±0.40 | 36.21±0.69 | 38.34±0.56 | 35.84±0.28 | 45.21±0.82 |
| 10 | 7.04±0.33 | 39.41±0.38 | 42.15±0.81 | 41.87±0.21 | 44.45±0.57 | 41.40±0.40 | 53.87±0.41 |
| 15 | 8.62±0.52 | 44.72±0.36 | 48.47±0.40 | 48.10±0.29 | 50.41±0.51 | 48.47±0.54 | 61.74±0.89 |
| 20 | 12.77±0.76 | 49.56±0.26 | 53.48±0.54 | 55.50±0.53 | 58.03±0.45 | 54.76±0.36 | 68.71±0.44 |
| 25 | 13.87±0.54 | 54.74±0.74 | 59.14±0.49 | 59.77±0.80 | 63.49±0.29 | 60.13±0.51 | 73.23±0.52 |
| 30 | 15.22±0.54 | 60.37±0.59 | 66.21±0.39 | 66.78±0.93 | 69.52±0.44 | 66.24±0.47 | 75.60±0.46 |
| 60 | 21.35±0.15 | 65.91±0.44 | 70.95±0.12 | 72.93±0.39 | 74.96±0.88 | 72.10±0.59 | 81.59±0.46 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 90 | 26.72±0.34 | 71.77±0.74 | 75.97±0.17 | 79.18±0.67 | 80.39±0.45 | 78.19±0.43 | 86.42±0.41 |
| 120 | 30.37±0.45 | 77.58±0.34 | 79.01±0.80 | 86.13±0.55 | 84.88±0.77 | 85.78±0.53 | 92.58±0.70 |

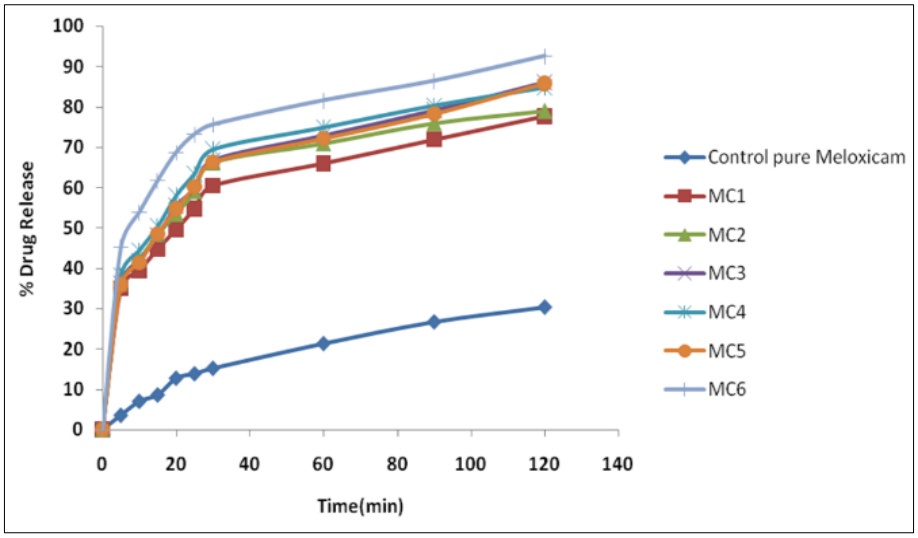



**Fig 3:** Percent release of Meloxicam from different batches MC1-MC6

**Table 4:** *In vitro* drug release study of Meloxicam Loaded Nanoparticles (MC7-MC12) (mean ± S.D.; n=3)

| Time (min.) | Control Pure Meloxicam | MC7 | MC8 | MC9 | MC10 | MC11 | MC12 |
|---|---|---|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 5 | 3.59±0.22 | 40.94±0.56 | 39.05±0.79 | 37.92±0.52 | 41.84±0.57 | 38.74±0.63 | 35.73±0.94 |
| 10 | 7.04±0.33 | 50.00±0.87 | 46.13±0.74 | 43.99±0.48 | 48.61±0.45 | 44.17±0.19 | 40.54±0.54 |
| 15 | 8.62±0.52 | 53.84±0.36 | 51.19±0.80 | 50.06±0.60 | 55.64±0.76 | 51.29±0.85 | 47.30±0.71 |
| 20 | 12.77±0.76 | 60.13±0.35 | 59.64±0.23 | 56.92±0.13 | 59.68±0.56 | 56.97±0.84 | 54.50±0.45 |
| 25 | 13.87±0.54 | 64.36±0.90 | 63.07±0.16 | 63.55±0.65 | 64.96±0.44 | 61.80±0.19 | 58.25±0.58 |
| 30 | 15.22±0.54 | 70.16±0.71 | 67.64±0.50 | 70.07±0.57 | 70.86±0.31 | 69.18±0.61 | 63.46±0.03 |
| 60 | 21.35±0.15 | 76.97±0.20 | 73.62±0.32 | 76.94±0.34 | 76.89±0.94 | 74.58±0.92 | 78.29±0.49 |
| 90 | 26.72±0.34 | 82.82±0.30 | 79.11±0.36 | 81.06±0.42 | 81.96±0.88 | 80.45±0.78 | 82.34±0.95 |
| 120 | 30.37±0.45 | 88.80±0.29 | 83.90±0.75 | 87.94±0.20 | 87.58±0.24 | 85.42±0.62 | 87.38±0.34 |

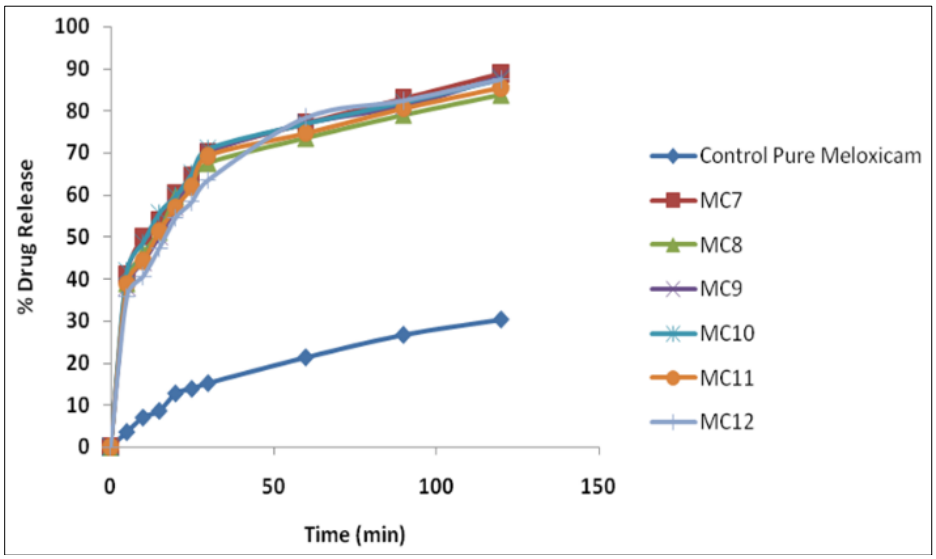



**Fig 4:** Percent release of Meloxicam from different batches MC7-MC12

**Summary**
In the present study, Polymeric nanoparticles were prepared using Emulsion Cross-linking technique to overcome poor aqueous solubility and low oral bioavailability. The W/O emulsion containing drug and chitosan in aqueous phase was cross-linked with aldehyde groups of gluteraldehyde with amine group of chitosan. The physicochemical properties like zeta sizes, zeta potential, polydispersity index of formulated nanoparticles were evaluated. The preparation of MLX nanoparticles was initiated by optimizing the drug: polymer on the basis of size, polydispersity index and encapsulation efficiency. The final optimized formulation was found to be MC8, MLX: Chitosan (10:400) with mean size of 84±1.7 nm and having polydispersity index of 0.086. The shape and surface morphology of the nanoparticles were evaluated by the use of scanning electron microscopy (SEM). The SEM photomicrograph revealed that the carrier system was more spherical in shape and uniformly distributed without any aggregation or adhesion of nanoparticles. The crystalline state evaluation was done by DSC of Meloxicam (plain drug), optimized formulations MC8.

**Conclusion**
According to the research the nanoparticles of Meloxicam with smaller particle size can be effectively produced by using Emulsion cross linking technique. The nanoparticles produced by this technique resulted in marked increase in solubility and dissolution rate of the drug and the particle size obtained (84 nm) was suitable even for i.v. administration. This technique was shown to be simple and adequate for drug particle size reduction and did not seem to alter the crystalline state of the drug. Nanoparticles were prolonged blood circulation time of drug and observed different pharmacokinetic parameters as compared to Meloxicam solution. The bioavailability has been found to be increased. These results suggested that Meloxicam nanoparticles would be good candidate with improved bioavailability. In future work, the development of stealth nanoparticles laced with functionalized surface coatings capable of eliciting passive or active targeting as per the requirement can be regarded as the future step in the nanoparticles research.

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