In Vitro Characterization of Solid Nano Particles containing Methotrexate and Doxycycline

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Abstract

Rheumatoid arthritis is a chronic inflammatory illness that affects many elderly people's quality of life. Combinational DMARDs are a new therapeutic approach in managing disease conditions, based on the concept that the two medications work in two distinct routes to diminish (or) delay disease development.This study aimed to develop and improve the pharmacokinetic and safety margin of SLN as an MTX carrier in vitro. As a result, an attempt was made to construct SLN for the delivery of MTX and DOX and to evaluate size, stability, and drug release characteristics.Methods: supplied the methotrexate (Chennai, India). Micro Labs Pvt. Ltd provided the Doxycycline (Hosur, India). Tokyo Chemicals Industries provided the tristearin. Himedia Laboratories Pvt. Ltd provided stearic acid, Pluronic F68, Dialysis Membrane, and Membrane Filter (Mumbai, India).

SD fine chem. Lyophilization eliminates excess water from SLN and improves its storage stability. A calibration curve for the medicines MTX/DOX was created using standard solutions of MTX and DOX, each with final concentrations of 10, 20, 30, 40, and 50 g/mL. An experiment using a simple and sensitive spectrophotometric approach was used to determine the number of pharmaceuticals present in the SLN formulation.Both MTX and DOX were discovered to have higuchi first-order kinetics and Higuchi zero-order kinetics, respectively. The release was initially relatively low (up to 3 hours) since it takes time for the medications to diffuse out of the lipid matrix, suggesting a strong embodiment of pharmaceuticals in the lipid matrix and surrounding poloxamer coating & thickness (dependent on concentration). The release has become concentration-dependent as the MTX loaded in the SLN has decreased.Preliminary data show that the designed SLNs can administer dual medicines MTX and DOX without incompatibility. However, in vivo studies relevant to RA are required to validate their efficacy in vivo

Key Words: Methotrexate, Doxycycline, Hydrophilic Drugs, Amino groups

Introduction:

Rheumatoid arthritis is a chronic inflammatory illness that affects many elderly people's quality of life. Combinational DMARDs are a new therapeutic approach in managing disease conditions, based on the concept that the two medications work in two distinct routes to diminish (or) delay

disease development. It comes in handy when you don't know your illness status in the early stages of rheumatoid arthritis. The combination of MTX (methotrexate) and DOX (Doxycycline), proposed in the literature, was chosen for the current study. These tiny molecules of matrix metalloid proteinase inhibitors (MMPIs) (e.g., DOX) have been shown to replace biological DMARDs in combination therapy partially. DOX is a tetracycline that inhibits matrix metalloproteinases (MMPs) and is immunomodulatory in addition to its antibacterial activity. MTX, which is used as a parent medication in combination with DMARD therapy, is an antineoplastic antimetabolite with immunosuppressive effects. The standard combination drug delivery of DMARDs is ineffective in decreasing the combined treatment's negative effects. The use of Nanocarriers for the administration of dual medications, on the other hand, has been demonstrated to be beneficial for treating the chronic inflammatory symptoms of RA by improving the efficacy of both drugs by sustaining drug activity for a more extended period with a probable reduction in adverse effects. Increased drug stability, increased drug payload, and integration of lipophilic and hydrophilic medicines are some of the advantages of solid lipid nanoparticles (SLNs). The efficacy of SLNs for single or combined delivery of a variety of medicinal drugs via various administration routes has been demonstrated**.**The main requirement in the long-term therapy of RA has sustained efficacy without increasing drug toxicity. As a result, the use of SLN for dual drug delivery of MTX and DOX should help lower the toxicity of both medications while also increasing their therapeutic efficacy. Furthermore, DOX is unstable in water and irritates the mucosal membrane.

Aim and Objective:

This study aimed to develop and improve the pharmacokinetic and safety margin of SLN as an MTX carrier in vitro. As a result, an attempt was made to construct SLN for the delivery of MTX and DOX and to evaluate size, stability, and drug release characteristics.

Material and Methods:

Madras Methods: supplied the methotrexate (Chennai, India). Micro Labs Pvt. Ltd provided the Doxycycline (Hosur, India). Tokyo Chemicals Industries provided the tristearin. Himedia Laboratories Pvt. Ltd provided stearic acid, Pluronic F68, Dialysis Membrane, and Membrane Filter (Mumbai, India). SD fine chem. Limited provided the hydrochloric acid, sodium hydroxide, and methanol (Mumbai, India). The solubility of pharmaceuticals (MTX and DOX) in different solid lipids was tested by dissolving a known amount of medication (5mg MTX and 50mg DOX) in 100mg of lipid melts, specifically tristearin and stearic acid, at a temperature above the lipid melting point.

Each time the lipid melt was raised by 100mg, the medicines were entirely dissolved. The production of a clean, transparent solution was deemed the solubility study's final aim. The high shear homogenization process was used to make SLNs. Methotrexate and Doxycycline were added to the lipid melts of stearic acid and tristearin in this approach by keeping the temperature above the melting point of these lipids (about 80°C). This hot lipid phase was then dispersed in a 50ml hot surfactant solution (Poloxamer 1.5 per cent w/w) for 3 minutes at 15000rpm at 80°C using a high-speed stirrer (Ultra Turrax, IKA homogenizer). After that, the pre-emulsion was diluted up to 200ml with cold de-ionized water and swirled for 2 minutes at 10,000 rpm. After cooling in an ice bath, the resulting nanoemulsion (O/W) was lyophilized to obtain solid lipid nanoparticles. Lyophilization eliminates excess water from SLN and improves its storage

stability. A calibration curve for the medicines MTX/DOX was created using standard solutions of MTX and DOX, each with final concentrations of 10, 20, 30, 40, and 50 g/mL. An experiment using a simple and sensitive spectrophotometric approach was used to determine the number of pharmaceuticals present in the SLN formulation. Powder X-ray diffraction study (Analytical XPERT PRO powder diffractometer (Cu κ radiation) operating at a voltage of 40V.XRD) was used to determine the degree of crystallinity of entrapped pharmaceuticals MTX/DOX. The geometric scattering of radiation from crystal planes within a solid can be used to detect the presence or absence of the latter, allowing the degree of crystallinity to be measured.

Results and Discussion:

The functional group peaks of MTX and DOX have been present in their physical mixture's FITR in this instance. The peaks of MTX were found at wavenumbers 3335.2.1644cm-1, 1606cm-1, 1551cm-1 (hydroxyl and amino groups)

Concerning the FITR of SLN containing MTX & DOX, the functional group's peaks of lipid tristearin such as 2914cm-1 (methylene C-H Stretching), 1701cdg.m-1 (simple ester group), 1701cm-1 & 722cm-1 elicited the functional group's peaks of lipid tristearin such as 2914cm-1 (methylene C-H Stretching), 1701cm-1are suggestive of a long linear aliphatic chain. The physicochemical features of SLNs were shown to be retained after 30 days of refrigeration. As a result, it is advised that MTX-DOX-SLNs be stored. At a temperature that is cooler than room temperature SLNs' physical characteristics. Monitoring was used to establish the amount of time spent in storage.Particle size changes as a function of time. The subatomic particle. Table 3 shows the size analysis at various time points.

Table 1: Particle Size of formulations F3 upon storage at refrigerator

Formulation	Day 0	Day 15	Day 30
F3	153.2	158.6	161.2

Table 4 shows the findings of the in vitro release tests. Both MTX and DOX were discovered to have Higuchi first-order kinetics and Higuchi zero-order kinetics. Initially (up to 3 hours), the discharge was slight.

Table 2: Release Kinetics Methotrexate and Doxycycline from SLN (F3)

Kinetics Model	R^2 (MTX)	R^2 (DOX)
Zero order	0.9142	0.9745
Higuchi	0.9523	0.9325
First Order	0.9986	0.9475
Kors MeyerPeppa's	0.9331	0.8745
Hixson Crowell	0.9265	0.5462

The medications take time to diffuse out of the lipid, indicatinga robust drug encapsulation in the lipid matrix &the poloxamer coating's surrounding area and thickness (which isbased on concentration)

The table shows the findings of the in vitro release investigations. Both MTX and DOX were discovered to have higuchi first-order kinetics and Higuchi zero-order kinetics, respectively. The release was initially relatively low (up to 3 hours) since it takes time for the medications to diffuse out of the lipid matrix, suggesting a solid embodiment of pharmaceuticals in the lipid matrix and surrounding polaxamer coating & thickness (dependent on concentration). The release has become concentration-dependent as the MTX loaded in the SLN has decreased. The loaded amount of DOX was large, and it was released at a steady pace, regardless of DOX concentration. It might be said that the release kinetics data are appropriate for their pharmacokinetics behaviour in vivo. This SLN formulation was found to be suitable for the prolonged effect of medications MTX and DOX in all modes of administration, which may aid in reducing drug toxicities in vivo.

Conclusion:

The hot homogenization approach was effective in producing SLNs with a nanosized of 157.2nm and successfully combining a lipophilic and hydrophilic medication with a triglyceride (Tristearin) and a surfactant (Polaxamer). Since the procedure was accompanied by heat, SLNs have not been sterilized. For MTX-DOX-SLN, high-speed stirring resulted in the optimal zeta potential and size. In SLN, PXRD research revealed that MTX and DOX are stable crystallines. Positively charged MTX-DOX-SLNs have elicited longer-lasting and independent MTX and DOX release from the SLNs. As a result, preliminary data show that the designed SLNs can administer dual medicines MTX and DOX without incompatibility. However, in vivo studies relevant to RA are required to validate their efficacy in vivo.

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