Formulation and characterization of dexmedetomidine HCL liposomes in gel for intraarticular administration

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Abstract---Rheumatoid arthritis (RA) is a musculoskeletal disorders that distresses joints and cartilage and may lead to bone degeneration. Intraarticular administration of the drug directly in joints causes relief but is limited by the half-life of the administered drug. The objective of the present investigation therefore was to prepare Dexmedetomidine HCl containing liposomes which were then loaded in xanthan gum gel for intraarticular administration to prolong the duration of drug release. Liposome formulations were prepared by using various ratio of 1,2-Dierucoyl-snglycero-3-phosphatidylycholine, 1,2-Dipalmitoyl-snglycero-3-phospho-rac-glycerol and cholesterol using thin film hydration method in a Rota evaporator. The liposomes were evaluated for size distribution, surface charge potential, entrapment efficiency for establishing the levels of formulation components and process parameters. Scanning electron micrographs of the liposomes indicated the spherical topography of the prepared liposomes. The liposomes were then loaded in gel formulated using xanthan gum as the gelling agents. Viscosity and gel strength of the formulation was evaluated by rheometer. In Vitro Dissolution in simulated synovial fluid media indicated that the liposomes in gel could prolong the drug release for a period of 7 days. The formulation was studied for stability over a period of 6 months for pH, viscosity, drug release at 4 hours and 4 days at two different conditions i.e. 2 to
8°C and 25°C±2°C and 60% RH±5%RH. No significant difference was found out in these parameters when the formulation was stored at 2 to 8°C.

**Keywords**—Liposome, Dexmedetomidine HCl, Xanthan gum Gel, In vitro release, intraarticular injection, rheumatoid arthritis.

**Introduction**

A joint is well known as “articulation”, where two or more bones and cartilage comes together and allows movement. Synovial joints are the chief functional joints of the body and are freely movable (diarthroses). Joint bones are covered by articulating cartilage and enclosed with joint cavity that contains synovial fluid. Main function of the articular is the suppress friction upon joint movement and absorb shock. Synovial fluid is produced by synovial membrane which lubricates the joints and provide nutrition [1].

Arthritis is an autoimmune degenerative disease which involves pain, swelling, tenderness and stiffness of joints and subsequently interruption in normal joint functioning and mobility. Rheumatoid arthritis (RA) and osteoarthritis (OA) are most common types [2]. Rheumatoid arthritis is a type of autoimmune disease in which the immune system attacks its own tissue and joints, starting with the lining of synovium joints. By blood test, C-reactive protein (CRP), Erythrocyte sedimentation rate (ESR) and Rheumatoid factor (RF) test RA can be diagnosed. While in osteoarthritis cartilage (natural cushioning between joints) wears away and subsequently disruption of joint bones, which can be detected by physical examination, X-ray and magnetic resonance imaging (MRI) [3] [4] [5]. The chemicals which are released in joint cavity like Interleukin (IL)-1, IL-6, IL-8 and tumor necrosis factor alpha (TNF-α) are responsible to induce inflammation [6]. Pro-osteoclastogenic effects resulted in bone erosion is occurs due to involvement of inflammatory cytokines, IL-1β, IL-6, IL-17 and TNF- α. In rheumatoid arthritis mononuclear cell infiltration may also occurs in innermost layer because of B-cells, T-cell, macrophages, mast cells, fibroblast cells, fibroblasts and dendritic cells [7].

Primary treatment targets to minimize pain, reduce joint damage, and improve functioning of joints. Depends on severity and stage, commonly used medications are analgesics, non-steroidal anti-inflammatory drugs (NSAIDs), disease-modifying antirheumatic drugs (DMARDs), corticosteroids, visco-supplementation and biologics. Recent data shows, globally more than 350 million people have arthritis. In the US, every 4 adults one has arthritis and it is estimated that by 2040 approximately 78 million adults will have arthritis [8].

Intra-articular (IA) injection offers many advantages as it bypasses first pass and cleansing effect, provides 100% bioavailability. Rapid onset of action and higher concentration of drug is possible because of IA route provides direct access to the joint cavity. It is most effective therapy compared with physical therapy, oral analgesics and other non-operative treatments [9]. For patients who want to postponement or avoid surgical treatment and who do not bear pharmacological
oral therapy due to higher systemic exposure related side effects and when drugs are no longer effective by this route, IA therapy is recommended. Additionally, compared to surgical treatment, IA treatment is more safe as it causes fewer adverse reactions and less invasive. Though many Intra-articular (IA) therapies are approved by well-recognized regulatory bodies like Food and Drug Administration (FDA) and European Medicines Agency (EMA), it remains limited and controversial for several drug molecules [10].

Currently available IA therapies and drug delivery vehicles are not as beneficial and potential due to rapid clearance of the drug from site of injection. IA drug delivery platforms should offer extended bioavailability of therapeutic drug, joint retention, sustained delivery, minimum safety concerns, and should be readily translatable from lab to market. In spite of current developments, no single IA therapy meets all these properties. This is the reason of short coming of therapy [11].

Dexmedetomidine HCl (Dex) is a highly selective, specific and potent agonist of α2-adrenoceptors. Dexmedetomidine HCl has been stated to have a different of pharmacological effects. It offers analgesic effects as well as anti-inflammatory. In human rheumatoid arthritis, data shows Dex inhibits expression of IL-1β, IL-6 and TNF-α [12]. In rat model it is also reported that when Dex (1 and 3 μg/kg) is given by IA route, TNF-α levels decreased significantly and histological data shows no further damage to the synovial membrane [13]. Many randomized controlled trials (RCTs) described that Dex was able to significantly decrease pain score and increase duration of analgesic effect in knee arthroscopy [14]. Liposomal bodies are synthetic vesicles made up of one or more phospholipids which surround an internal aqueous core. Liposome provides longer retention time of drug in synovial cavity to provide site specific and targeted drug delivery [15].

Xanthan gum (XG) is a high molecular weight anionic polysaccharide produced from microbial fermentation. It is more stable and less degrade in vivo compared to hyaluronic acid. Earlier experiments proven that IA injection of XG could relieve the synovitis, shield the joint cartilage, and decrease the OA progression in an OA model [16] [17].

In the present investigation liposomes containing Dex were prepared and loaded in to Xanthan gum gel for intraarticular administration. The prepared gel was investigated using In Vitro parameters like drug release for a proposed duration of 7 days. The prolonged release of drug was attributed to the composition of liposomes and viscosity of the prepared gel. The gel was found to be stable over a period of 6 months when stored at 2-8°C.

Materials and Methods

Materials

Liposomes are made from 1,2-Dierucoyl-sn-glycero-3-phosphatidylcholine (DEPC), 1,2-Dipalmitoyl-sn-glycero-3-phospho-rac-glycerol (DPPG) (Lipoid GmbH, USA) and cholesterol (Dishman, India). The drug was the Dexmedetomidine HCl which purchased from the Strem Chemicals (USA). Propylene glycol, boric acid, chromatographic grade acetonitrile were purchased from Merck reagents (USA).
Xanthan gum procured from SR Chemie (India). Purified water was prepared in the laboratory. All other reagents used in liposome gel were of analytical grade.

**Method**

**Preparation of Liposome**

Liposome were prepared by dry film hydration/thin film evaporation technique. First of all, lipids were dissolved in organic solvent (dichloromethane) and mixed to get clear solution. Then solution was transferred into round bottom flask to remove organic solvent under vacuum by using rotary evaporator to form thin lipid film. To ensure complete removal of solvent traces, the dry lipid film was kept under vacuum overnight. Then lipid film was hydrated by using 1% Dexmedetomidine HCl solution using vortex mixing to ensure recovery of all lipid from the flask. The pH was adjusted to 7.0 by using 0.5% Lysine solution. Subsequently dispersion was homogenized by using high shear homogenizer (Polytron, Kinemetica AG) to form the liposome. Homogenization speed adjusted to 10,000 RPM and batch size was 100 mL. Then liposomal dispersion was further extruded through polycarbonate filters (Whatman Neuclepore, pore size of 200 nm) by using an extruder (Lipex Extruder, Evonik, Canada). Then nitrogen gas purged through the liposomal solution. Liposomes were prepared and evaluated for entrapment efficiency by using different concentration of lipids. The lipid film was formed at different process conditions and entrapment efficiency was checked.

**Preparation of Xanthan gum gel**

To prepare 5% Xanthan gum phase, required quantity of xanthan gum was dissolved in boric acid and propylene glycol containing purified water at 37°C and allow it for swelling for two hours under gentle stirring. Xanthan gum phase was further filtered with 0.6 µ filter to remove any undissolved particle and then sterilized by steam sterilization method at 110°C for 40 min.

**Preparation of Liposomal gel**

Extruded dispersion was poured into sterilized xanthan gum gel phase. Then mixed gently to get homogenous liposomal gel. Then it was further diluted with water to prepare 0.5 mg/mL Dexmedetomidine HCl liposomal gel containing 1% xanthan gum gel phase. Product was stored at 2-8°C for further characterization.

**Evaluation Methods for Liposome**

**The Particle size distribution**

The liposomal gel was determined by Particle size distribution (DLS), using a Zetasizer (NanoZS, Malvern, UK) at a temperature of 25 ± 0.1 °C. At an angle of 173°, the intensity of the light scattered by the particles was detected. Liposomal gels were diluted with purified water and sonicated before the measurement. From this analysis, the z-average value (intensity based distribution) and PDI were obtained [18].
**Surface charge Potential**

The zeta potential (surface charge) of the liposomal gel was determined after measurement of the electrophoretic mobility (20 s, 25°C) by (Zeta Seizer, Malvern Instruments, UK). Sample preparation was prepared by diluting with purified water and vortex mixing of liposome gel. Sample was taken in specific cuvette and ensured no any air bubble was present before analysis at room temperature.

**HPLC analysis of Dexmedetomidine HCl**

The HPLC system comprised of auto sampler, binary pump, and Waters dual absorbance detector (Waters, USA). The mobile phase consisting of acetonitrile, water (30:70) was pumped through the Zobra X SB C-8 (150mm × 4.6mm × 5µm) at a flow rate of 1.5 ml/min and the eluent was monitored at 220 nm.

**Determination of %Drug Entrapment Efficiency**

Dex liposome (5 ml) formulation was transferred to the internal chamber of Amicon® centrifuge tubes fitted with an ultrafilter (molecular weight cut-off 30 kDa, Sigma Aldrich) and centrifuged at 9000 rpm for 45 min. The aqueous phase collected at the bottom of ultrafilter membrane was subjected to high-performance liquid chromatography (HPLC) analysis to determine the Dex content. The entrapment efficiency was calculated by the following equation:

\[
\text{%Drug Entrapment Efficiency} = \frac{W_{\text{total}} - W_{\text{free}}}{W_{\text{total}}} \times 100
\]

Where, “\(W_{\text{total}}\)” = mass of the total quantity of drug present in the formulation and “\(W_{\text{free}}\)” = mass of the free drug identified in the filtrate of lower compartment of Amicon® post-centrifugation of the aqueous liposomal dispersion. In order to verify the total amount of drug present in the system, Dex liposome formulation was diluted suitably with methanol to destroy the liposome structure, releasing the drug into the solution and then final clear solutions were analyzed for drug content by HPLC.

**Evolution Methods for liposomal Gel pH**

pH of bulk solution was measured at room temperature (25°C±2°C) by using Thermo Scientific™ Orion Star™ A121 Portable pH Meter.

**Rheology**

Liposomal rheology was evaluated by using Rheometer (Model: MCR302, Make: Anton PAAR). For sample preparation, 1 ml sample was poured on the surface of stage and CP-50-1 spindle was used. Analysis was carried out at 25°C.

**Residual solvent Analysis by Gas Chromatography**

Residual solvent concentration was detected by using gas chromatography. The Liposomal dispersion (2 mL) was taken in a GC head space 20 mL glass vial. GC
column (DB-624; Dimensions Length: 60 m, diameter: 0.53 mm, temperature: -20°C to 260°C, Agilent Technologies, Japan) was used. By using a micro-syringe heated to 65 °C, the gas phase was evaporated from the vial and quantified by GC [19].

**Scanning Electron Microscopy (SEM)**

SEM images were captured by using scanning electron micrograph (magnification: 10,000; accelerating voltage: 20.0 kV, Jeol). The liposomes were diluted and freeze at-10°C and then placed on the grid. After the samples was placed and vacuum was applied, the images were recorded.

**In-vitro drug release studies by Bottle Rotating Apparatus**

In vitro drug release studies were conducted by using dialysis semi permeable membrane bags (Molecular weight cut off: 30 KD, Hercules, USA) using a Bottle Rotating dissolution apparatus (Tianjing, China) for 72 h with the help of 500 ml of phosphate buffer saline (PBS) (pH 7.4) as the dissolution medium. Cellulose membranes were soaked overnight in the dissolution medium. To the pre-swollen cellulose membrane bags, 1 ml of the liposome formulation was placed and both the ends of bags were tied to prevent any leakage. Later, dialysis bags were gently inserted at the bottom of the dissolution vessel and the paddles were rotated at 100 rpm for 72 h at 37.0 ± 0.1 °C. At regular time intervals 1 ml of the sample was collected and replaced with an equal volume of the dissolution medium. The amount of Dex released in to the medium was determined with the help of HPLC analysis. The analysis was carried out in triplicate.

**In-vitro drug release studies in simulated synovial fluid media by Franz diffusion cell**

The use of simulated synovial fluids (SSFs) is a promising in-vitro drug release method to predict the release mechanisms and possible in vivo behavior of drug formulations. In the literature, the wide variety of SSFs described to replicate synovial environments. The composition is provided in Table 1. Three different formulations are tested and compared for drug release in simulated synovial fluid media by using Franz diffusion dissolution apparatus [20] [21].

These formulations are as per below:
- Formulation A: API is mixed in Xanthan gum gel phase as per required dose
- Formulation B: Liposome formulation (without gel phase)
- Formulation C: Liposome encapsulated gel formulation (liposome embedded in gel phase)

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Reagent</th>
<th>Amount (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Hyaluronic acid</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>Bovine serum albumin</td>
<td>10</td>
</tr>
</tbody>
</table>

Table 1
Composition of simulated synovial fluid
Storage stability studies

The stability studies were conducted at different temperature conditions on optimized Dex loaded liposomal gel. A 1 mL product of Dex liposomal gel dispersion with drug concentration of 0.5 mg/mL was taken into glass PFS, stoppered with fluorotec plunger stopper and stored at 2-8°C and 25°C/60%RH for 6 months. The stability assessment was evaluated on the basis of pH, particle size, viscosity, and % drug release in the dispersion, at different predetermined time points [22].

Result and Discussion

Optimization of formulation parameters for Liposomal Gel

Selection of Lipid to drug ratio

Different concentration of Phosphatidyl choline (DEPC) and cholesterol were taken to evaluate % drug entrapment efficiency. Liposome film was formed at 37±2 °C, 200 mmHg vacuum and 150 RPM. Then film was hydrated with API containing aq. Phase and processed for size reduction by high pressure homogenization. Unentrapped drug was removed by centrifugation. Outcome results are mentioned in Table 2.

<table>
<thead>
<tr>
<th>Batch No.</th>
<th>DEPC (mg/mL)</th>
<th>Cholesterol (mg/mL)</th>
<th>Entrapment efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DL1</td>
<td>9</td>
<td>4</td>
<td>22.6%</td>
</tr>
<tr>
<td>DL2</td>
<td>10</td>
<td>5</td>
<td>39.3%</td>
</tr>
<tr>
<td>DL3</td>
<td>15</td>
<td>5</td>
<td>66.1%</td>
</tr>
<tr>
<td>DL4</td>
<td>10</td>
<td>10</td>
<td>51.5%</td>
</tr>
<tr>
<td>DL5</td>
<td>15</td>
<td>10</td>
<td>61.9%</td>
</tr>
</tbody>
</table>

As increasing in the concentration of DEPC, drug entrapment efficiency also increased. But in case of cholesterol drug entrapment efficiency increased at certain level and then further increase in concentration, interferes with the drug loading in the liposome structure. Formulation DL3 concentration was found
maximum entrapment efficiency and was finalized for further process optimization.

**Optimization of Solvent Evaporation by using Rota evaporator**

Liposomes were formulated by dry film hydration technique as described earlier. Here Solvent removal process was carried out by Rota evaporator under vacuum to form thin lipid film. Rota evaporator process was further evaluated for solvent removal. On the basis of % entrapment efficiency process parameters were optimized as mentioned in Table 3.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Time (Hr)</th>
<th>Temperature (°C)</th>
<th>RPM</th>
<th>Vacuum (mmHg)</th>
<th>% Entrapment efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>DL-6</td>
<td>4 hr</td>
<td>37±2 °C</td>
<td>200</td>
<td>300</td>
<td>67.9%</td>
</tr>
<tr>
<td>DL-7</td>
<td>6 hr</td>
<td>37±2 °C</td>
<td>250</td>
<td>300</td>
<td>71.2%</td>
</tr>
<tr>
<td>DL-8</td>
<td>6 hr</td>
<td>37±2 °C</td>
<td>250</td>
<td>200</td>
<td>76.3%</td>
</tr>
<tr>
<td>DL-9</td>
<td>15 hr</td>
<td>37±2 °C</td>
<td>250</td>
<td>200</td>
<td>77.2%</td>
</tr>
<tr>
<td>DL-10</td>
<td>6 hr</td>
<td>37±2 °C</td>
<td>250</td>
<td>200</td>
<td>83.5%</td>
</tr>
<tr>
<td>DL-11</td>
<td>6 hr</td>
<td>37±2 °C</td>
<td>250</td>
<td>100</td>
<td>92.3%</td>
</tr>
</tbody>
</table>

Process parameters of DL-11 formulations supports the maximum drug loading in nano liposome. The rate of Solvent removal under vacuum conditions has significant impact on drug entrapment efficiency of liposome.

**Particle size distribution**

In the present work, dry film hydration method was used for the preparation of liposome because of its high entrapment efficiency, reproducibility and simplicity. The average particle size of the liposomal dispersion was estimated to be 453.5
nm and polydispersity index of 0.104 indicating uniform particle size distribution. As per Fig. 2, it shows that single peak of particle size distribution indicates uniform and PDI value shows size distribution is narrow. There was no further significant change in the particle size of liposomes were observed when they are dispersed in xanthan gum gel phase.

![Size Distribution by Intensity](image)

Fig. 2 Particle size Distribution by DLS (NanoZS, Malvern) of formulation DL-14. A) Dex liposome B) Dex liposomal gel

**Zeta Potential**

As per fig3, the ζ-potentials of the liposome preparation observed -28 mV. There was no any significant difference observed in the zeta potential value of liposomes and liposomal gel formulations. It was obtained in the range of -26 mV and -34 mV. Generally, particles are stable above 30 mV or below -30 mV in their dispersion media. Single sharp peak shows uniform charge distribution on liposomes. Negative zeta potential value indicates liposomes carries negative surface charge due to presence of DPPG lipid. Due to presence of surface charge, liposomes are remaining suspended form in the dispersive media during storage.

![Zeta Potential Distribution](image)

Fig. 3 Zeta potential of Dex Liposome (DL-14)
**Liposomal Gel Characterization**

**pH**

pH of the different liposomal gel formulation obtained were in the range of 5.5 to 7.5.

**Rheology**

Rheology was evaluated by Rheometer on formulation DL-14. It was observed that Gel strength value 4.55 Pa. In other batches it was observed in the range of 4.2 to 5.5 Pa. Additionally, rheology with reference to storage modulus G' vs shear stress evaluated on stability batch DL-14. It was observed that storage modulus value is lesser at 25°C/60% RH compared to results observed at 2-8°C (Fig. 4).

![RheoCompass](image)

Fig. 4. Rheology of Dex Liposomal gel (DL-14). A) gel strength, B) Rheological behavior on stability
Residual Solvent analysis by Gas Chromatography (GC)

As per ICH guidelines [Q3C(R8)], DCM is categorized as class-2 residual solvent and recommendation for permitted daily exposures (PDE) should be ≤ 6.0 mg/day (600 ppm). As per GC chromatograph (Fig. 5), Dex-liposome gel, DCM concentration was observed <400 ppm.

Scanning Electron Microscopy (SEM)

The scanning electron micrograph of Dex liposome was shown in Fig.6. Obtained SEM images shows that the particles are spherical in shape and have a uniform size distribution. The size of liposomes detected by SEM analysis were comparable with size distribution obtained from DLS method [23].

Stability Study of Liposome Gel

A stability study was carried out on liposomal gel formulations (DL-14). Liposomes were filled in glass PFS and stored under horizontal conditions at 2-8°C and (room temperature) 25°C/60%RH over a period of six months.
From stability, it was observed that pH value, Viscosity, Assay of API and Particle size (Z-average) decreased significantly at 25°C/60%RH condition. While there was no any change major change was observed in pH value, Viscosity, Assay of API and Particle size (Z-average) at 2-8°C stability storage condition. Initial burst release and drug release at day-4 was observed higher at 25°C/60%RH condition compared with 2-8°C stability storage condition during in-vitro study (Fig 7).

The magnitude of drug retention within the vesicles, on storage under defined conditions, ultimately governs the shelf life of the developed formulation. Acceleration in drug leakage at higher temperatures, as observed in storage-stability studies, suggested keeping the liposomal product in the refrigeration conditions, to minimize the drug leakage from liposomal-systems. Loss of drug from the vesicles stored at elevated temperatures may be attributed to the effect of temperature on the gel to liquid transition of lipid bilayers together with possible chemical degradation of the phospholipids, leading to defects in the membrane arrangement.

![Graph showing pH stability conditions with time points and temperature](image-url)
B

Stability Conditions (Time points and Temperature)

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Assay of API (%)

C

Stability Conditions (Time points and Temperature)

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#### Particle Size (Z-average - nm)

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#### In-Vitro Drug Release (4 Hrs)

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Fig. 6. Stability studies of Formulation DL-14. A) pH, B) Viscosity, C) Assay of API, D) Particle size (Z-average), E) In-vitro Drug Release (4 Hrs.) and F) In-vitro Drug Release (Day-4)

**In-Vitro Dissolution in simulated synovial fluid media**

Drug release in simulated synovial media was evaluated up to 7 days. It was observed that initial burst was higher in Dex Gel and DEX Liposome compared to Dex liposomal gel formulation. Maximum drug released from Dex Gel and DEX Liposome (>80%) in 4 days. While in case of Dex liposomal gel formulation, >80%
drug released in 5 days. It can be concluded from these data that; xanthan gum gel phase provides additional sustained release effect from liposome phase.

**Conclusion**

Liposomal gel as delivery system can play an important role in improving the need for developed delivery of Dexmedetomidine HCl for targeted and local action. Optimization loading of Dex is not only dependent on the physico-chemical nature of these compounds, but also on factors such as the lipid ratio and the manufacturing method. Thin film hydration method liposomes facilitate a significant loading of Dex into the nano liposome compared to Ethanol injection method. Liposomal gel drug cargos were found to be stable at 2-8°C temperature from stability data. In-vitro release data supports and proves the uniform and sustained drug release of liposomal gel carriers for targeting Intra articular route. The Dex encapsulated in to Xanthan gum gel found to be promising formulation capable of effective sustained delivery, which require further preclinical and clinical investigations. Future investigation should focus on developing relevant mathematical models to estimate drug release performance and mechanisms of release related to a wide-ranging of nano-sized formulations.

**Conflict of Interest**

The authors declare no conflict of interest, financial or regarding the publication of this paper.

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**Ethical Approval**

Not applicable

**References**


