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# Characterization and optimization of GMO-based gels with long term release for intraarticular administration

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ABSTRACT

Osteoarthritis is characterized by slow degenerative processes in the articular cartilage within synovial joints. It could be interesting to develop a sustained-release formulation that could be effective on both pain/inflammation and restoration of mechanical integrity of the joint. Recently, an injectable system based on glycerol monooleate (GMO), containing clonidine as a model hydrophilic analgesic/anti-inflammatory drug and hyaluronic acid as a viscoelastic scaffold, showed promising potential as a biodegradable and biocompatible preparation to sustain the drug activity. However, drug release from the system is relatively fast (complete within 1 week) and the underlying drug release mechanisms not fully understood. The aims of this study were: (i) to significantly improve this type of local controlled drug delivery system by further sustaining clonidine release, and (ii) to elucidate the underlying mass transport mechanisms. The addition of FDA-approved inactive ingredients such as sodium oleate or purified soybean oil was found to be highly effective. The release rate could be substantially reduced (e.g., 50% release after 10 days), due to the increased hydrophobicity of the systems, resulting in slower and reduced water uptake and reduced drug mobility. Interestingly, Fick's second law of diffusion could be used to quantitatively describe drug release.



## 1. Introduction

Osteoarthritis (OA), the most common form of arthritis, is characterized by slow degenerative processes in the articular cartilage within synovial joints, which could be associated with hypertrophy of bones (subchondral bone and osteophytes sclerosis) and thickening of the capsule. This degenerative disease involves an irreversible and progressive loss of articular cartilage, changes in synovial membrane, and an increased volume of synovial fluid with modification of its composition and reduction of its viscoelastic/lubrication properties (Balazs, 1974; Gerwin et al., 2006; Sarzi-Puttini et al., 2005). Clinically, the main symptoms are characterized by joint pain, tenderness, limitation of movement, crepitus, occasional effusion, and variable degrees of local inflammation (Symmons et al., 2000; Buckwalter and Martin, 2006).

Currently available pharmacological therapies target palliation of pain and include analgesic drugs (e.g., non-steroidal anti-inflammatory drugs (NSAIDS) and opioids) (Sarzi-Puttini et al., 2005; Gerwin et al., 2006; Uthman et al., 2003). Different compounds, such as glucocorticoids and hyaluronic acid, have usually been used intraarticularly (Gerwin et al., 2006; Uthman et al., 2003). Nevertheless, glucocorticoids are thought to have many side effects on the cartilage (Bellamy et al., 2006a) and hyaluronan products need several injections to be effective (Scale et al., 1994).

Nevertheless, $\alpha_2$-adrenergic agonists such as clonidine (CLO) could represent a new suitable alternative to the administration of corticosteroids. Indeed, the intraarticular (IA) use of $\alpha_2$-adrenergic receptor agonists are thought to produce analgesia mainly through an inhibition of the transmission of nociceptive stimulation (Sullivan et al., 1987; Gentili et al., 1996; Lavelle et al., 2007; Larsen et al., 2008). Moreover, Bastianelly et al. (2009) have recently shown that the combination of CLO and hyaluronan decreased the secretion of cytokines such as IL-1β and inflammatory cell proliferation, providing an anti-inflammatory effect. Indeed, cytokines produced by the synovium and chondrocytes,

*Abbreviations:* GMO, glyceryl monooleate; CLO, clonidine; HA, hyaluronic acid; ET, ethanol; PG, propylene glycol; IA, intraarticular; OA, osteoarthritis; Mw, molecular weight.

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especially interleukine 1 (IL-1) and tumor necrosis factor alpha (TNF-α), seem to play a significant role in the degradation of the cartilage (Sarzi-Puttini et al., 2005). Unfortunately, it was demonstrated that small therapeutic molecules, such as CLO, are rapidly cleared from the synovial space after IA injection (Larsen et al., 2008).

Therefore, it seems that there is a need to improve the design of formulations. More particularly, it could be useful to develop a sustained-release formulation that could be effective both on pain/inflammation and on restoration of the mechanical integrity of the joint.

Lipids have recently received considerable attention as an alternative to polymeric carrier or coating materials in the development of drug delivery systems. Glyceryl monooleate (GMO), also called monolein, allows the formation of an in situ gel that is able to sustain the delivery of various water-soluble and water-insoluble drugs, based on its capacity to form viscous systems in the presence of water (Engstrom, 1990; Shah et al., 2001). Interestingly, GMO is biocompatible and biodegradable. Moreover, it is characterized by bioadhesive properties and can be used to enhance the therapeutic efficacy of drugs by increasing their residence time at the site of administration (Nielsen et al., 1998). Generally, injectable formulations based on GMO that were described in the literature are not able to sustain the release of a drug for more than a couple of days (Shah et al., 2001). Nevertheless, a new injectable formulation containing CLO as a model hydrophilic drug and hyaluronic acid (HA) as a viscoelastic scaffold was recently described as being able to sustain the release of this active drug for about one week (Réeff et al., 2012). Moreover, it was demonstrated that this formulation provided a similar release profile to that obtained from a cubic reference formulation ($f_2$ = 61.3), containing 75% (w/w) GMO and 25% (w/w) water, which is reported by many authors as presenting very high viscosity and as a consequence poor syringeability properties but the best sustained-release capacity (Shah et al., 2001; Ouedraogo et al., 2008). Nevertheless, it was suggested that the selected formulation could be optimized to improve the sustained release of CLO. For instance, it was reported by Shah et al. that the addition of ingredients characterized by a low HLB value could potentiate the formation of crystalline phase structures (Shah and Paradkar, 2007). Another approach could be the addition of sodium oleate, which can form insoluble complexes with many drugs (Chang and Bodmeier, 1997; Ouedraogo et al., 2008). For a stability point of view, α-tocopherol acetate could also be added to protect lipid compounds from oxidation.

The aim of this study was to substantially improve the sustained-release properties of a GMO based injectable formulation, previously described by Réeff et al., and containing CLO as a model drug for the treatment of OA (Réeff et al., 2012), and to better understand the underlying mass transport mechanisms. The present work evaluates the influence of different inactive ingredients such as sodium hyaluronate, propylene glycol, sodium oleate or purified soybean oil and their concentrations on the release profile of the CLO. Physico-chemical properties such as rheological properties, syringeability, release profiles, and the water uptake of each formulation were also evaluated.

## 2. Materials and methods

Glyceryl monooleate (Rylo MG 20 characterized by 99% purity) was used as an in situ gelling-agent and was supplied by Danisco® (Grinsted, Denmark). Sodium hyaluronate (Mw 1.9 MDa) was used as a reference viscoelastic polysaccharide and was supplied by European Technologies Inc.® (Dolni Dobrouc, Czech Republic). Clonidine HCl was used as a small, hydrophilic model drug and supplied by Chemicals International Group® (Holte, Denmark). Ethanol absolute was used as a solvent to improve the syringeability of the formulation and was supplied by VWR® (Leuven, Belgium). Propylene glycol was used as a co-solvent and was supplied by Certa® (Braine l'Alleud, Belgium). Sodium oleate and purified soybean oil were kindly donated by Lipoid® (Cham, Switzerland). These kinds of excipients could be able to improve the sustained-release properties of the formulation by complexing the drug or potentiating the formation of crystalline phase structures. α-Tocopherol acetate was used as an antioxidant to protect lipid compounds and was supplied by Certa® (Braine l'Alleud, Belgium). Sterile, apyrogenic, and endotoxin-free water was distributed from a Purelab apparatus (Analis®, Namur, Belgium) and was found to be essential to dissolve sodium hyaluronate.

### 2.1. Preparation of the gel

All operations required in the preparation of the formulations were carried out under aseptic conditions in a class IIB biosafety cabinet (ADS Laminaire®, Le Pre-Saint-Gervais, France). Moreover, laboratory glasswares used were previously sterilized by steam sterilization using an autoclave (Systec® D-65, Wettenberg, Germany).

GMO was gently melted at a maximal temperature of 45 ± 2 °C. Afterwards, co-solvents, such as ethanol (ET) and propylene glycol (PG), were added to dissolve the GMO. Alternatively, organic oil (e.g., soybean oil) or sodium oleate and an antioxidant (e.g., α-tocopherol) could also be incorporated. HA-parenteral grade was then dispersed using a T25 Ultra-Turrax® (IKA® Werke GmbH & Co. KG. Janke & Kunkel, Staufen, Germany) with a rotational speed fixed at 24,000 rpm for 3 min. Simultaneously, an aqueous solution of CLO was prepared by dissolving the drug in purified, apyrogenic water. This aqueous solution was finally added to the solution of GMO at 45 ± 2 °C under magnetic stirring. The gels were stored at 6 °C for 48 h before performing any experiment.

### 2.2. Quantification of clonidine in the gel formulations and in residues of gel at the end of the release studies

Two validated High Performance Liquid Chromatography (HPLC) methods adapted from USP 25 monograph of CLO, which is developed to verify the uniformity of dosage units, were previously described (Réeff et al., 2012). Briefly, the HPLC system (Series 1200, Agilent® Technologies, Diegem, Belgium) was equipped with a quaternary pump, a degasser, an ALS auto sampler equipped with a TCC thermostated column oven set at 25 °C, and a diode-array detector (DAD) set at 210 nm. The separation system was an Agilent® Zorbax Eclipse Plus C8 4.6 mm × 150 mm (3.5 μm) column. The isocratic mobile phase was composed of a 50:50 (v/v) ratio of water/methanol, adjusted to pH 3.0 with 1 ml phosphoric acid 85% (w/w) (Riedel-de Haën®, Seelze, Germany) and 1 N sodium hydroxide solution (Merck®, Overijse, Belgium). 1.1 g of sodium octanesulfonate (Sigma–Aldrich®, Bornem, Belgium) was added as an ion pair. The flow rate was set at 1 ml/min and the injection volume was set at 5 μl or 50 μl depending on the study performed.

### 2.3. Extraction of clonidine from the gel formulations and in residues of gel at the end of release studies

Two liquid–liquid extraction steps of CLO from the gel formulations and residues of gels at the end of release studies were previously described to remove potential impurities such as GMO and HA before doing HPLC analysis (Réeff et al., 2012). Briefly, 2 g of gel was weighed and placed in 25.0 ml of dichloromethane with 2.0 ml or 0.5 ml of a tizanidine HCl solution (200 μg/ml) used as the internal standard. When the entire amount of gel was dissolved, CLO was extracted twice in 10 ml of citrate buffer pH 3.0 and the