Hyaluronic Acid – Creation of Slow-release Formulations for Osteoarthritis Treatments

Osteoarthritis (OA) is the world’s most common degenerative joint disorder, affecting more than 60% of the population over the age of 65. During this condition, bone surfaces come into contact under ordinary loads, leading to severe pain and disability, and symptoms including subchondral bone sclerosis, progressive articular cartilage loss and synovial fluid viscosity decrease. Economically, the cost of arthritis as a whole is extortionate, with the total economic burden estimated to be 2.5% of the gross national product of Western nations. OA accounts for a large portion of this - in the United States alone, the costs have been estimated to be more than 60 billion dollars per year.

Although the symptoms of OA can be managed, no disease-modifying drugs have yet been approved for its treatment. However, for moderate to advanced OA, local intra-articular (IA) treatments are currently available. One of these is the injection of hyaluronic acid (HA) preparations, which restore the viscoelastic properties of the synovial fluid and increase the lubricant properties of the joint. In this way, pain is reduced and joint functionality significantly improved, dramatically improving a patient’s quality of life. In addition, exogenously administered HA can reduce the production of pro-inflammatory cytokines, meaning it continues to have an effect beyond its actual residence time in the joint.

Current Complications in the Use of HA

Despite its increasing popularity, sourcing high-quality HA has not proven to be an easy task. Many HAs that are traditionally used in OA applications are produced from rooster comb extraction or various strains of Streptococcus bacteria. Streptococci are innately pathogenic to human beings, thanks to their secretion of toxins and resulting haemolytic properties. In an attempt to solve this issue, HA from these sources is often purified using harsh organic solvents, but even this technique can pose further health issues. The potential threats are of particular concern for regulatory bodies, such as the Food and Drug Administration (FDA) in the US and European Medicines Agency (EMA), causing them to put pressure on companies to look for alternative sources of HA.

In response to these challenges, new technologies have been developed, one of which is Hyasis®. This highly-pure HA is manufactured with high-quality, animal-free ingredients through an innovative Q7 cGMP biomanufacturing process, and provides manufacturers with a ready source of HA with exceptional quality and safety levels.

Introducing Bacillus-derived HA

Hyasis® has been developed using a groundbreaking Bacillus subtilis fermentation process. As a non-pathogenic host, Bacillus subtilis’ products fit the Generally Recognized as Safe (GRAS) criteria outlined by the FDA, and the resulting Hyasis® is characterised by low amounts of nucleic acids, proteins, bacterial endotoxins, exotoxins and microbial contamination. Not only does this reduce the incidence of hypersensitivity reactions when the material is injected, but it also produces a number of disease-modifying properties, including analgesic, anti-inflammatory and chondroprotective qualities, which make it well suited to use in OA therapies.

Hyaluronic acid (HA)

Hyaluronic acid (HA) is a naturally-occurring polysaccharide capable of providing structure to bodily tissues such as skin and cartilage. The understanding of its properties and functions has grown from the 1980s onwards, and today HA’s value as a biological material is well recognised, along with its many uses within medical and pharmaceutical applications. These applications are mostly due to its extensive moisturising properties, ability to extend drug retention time and promotion of tissue healing.

In addition, HA is a natural choice when considering possible therapy components. Several significant structural and rheological properties make it an attractive carrier for drug delivery applications. HA can act as a depot matrix, where it slowly releases the active substance of a therapy locally and, as a result, prolongs the therapeutic period of the active pharmaceutical ingredient (API) in the formulation. By adding HA into a formulation with steroid drugs, therapeutic effects can be achieved, potentially leading to more effective treatment of symptoms.
To demonstrate the efficacy of Hyasis® in viscosity measurements combining HA with APIs – and results of these investigations. Here we will discuss the methodology combination products for OA treatment. A recent study was conducted to evaluate the effectiveness of HA with three model APIs, which could serve as model combination products for OA treatment. We will now discuss the methodology and results of these investigations.

Combining HA with APIs – Methodology

Viscosity Measurements

To demonstrate the efficacy of Hyasis® in the formulation of OA therapies, 1, 2 and 3% HA solutions were initially prepared using a molecular weight of 0.85 MDa. Three model APIs were used across release studies: diclofenac, dexamethasone phosphate and triamcinolone hexacetonide. The diclofenac used was the commercial injectable Voltaren® (Novartis International AG) with a diclofenac concentration of 25 mg/mL, while both the dexamethasone phosphate and triamcinolone hexacetonide were pure API. Dexamethasone phosphate was dissolved in phosphate buffered saline (PBS) pH 7.4 yielding a 4 mg/mL solution. A 10 mg/mL triamcinolone hexacetonide suspension was made with 10% propylene glycol, 10% ethanol, 0.1% sodium lauryl sulphate (SLS) and 1% ethanol was added to the medium for triamcinolone hexacetonide all equilibrated at 37°C. The flow rate was set to 4 mL/min and a high stirring speed was used. The released API was measured online with UV absorbance. Three replicates were performed for each formulation and a drug release above 95% was considered complete.

Results and Discussion

Viscosity Measurements

When treating OA, the viscous and elastic behaviour of HA-based formulations is crucial, as effective lubrication is the key to relieving pain and alleviating symptoms. However, flow curves show that HA solutions behave as non-Newtonian fluids with shear thinning properties, which can create obstacles in the easy injection and administration of therapies. The effects of these properties on the ease of syringe injection were determined by measuring the force needed to inject different concentrations of HA solutions in a texture analyser.

Dissolution

The release of API from the HA formulations was assessed by dissolution method using a closed loop system configuration and a USP apparatus 4 equipped with 22.6 mm cells at 37°C. Glass beads were added to fill the sample cells and 1 mL of each formulation was added with a syringe. PBS buffer pH 7.0 was used as medium for diclofenac and dexamethasone phosphate, while 1% SLS and 1% ethanol was added to the medium for triamcinolone hexacetonide all equilibrated at 37°C. The flow rate was set to 4 mL/min and a high stirring speed was used. The released API was measured online with UV absorbance. Three replicates were performed for each formulation and a drug release above 95% was considered complete.

Following these preparations, a texture analyser was used to test the injection forces needed to inject the varying concentrations of HA solution. Syringes containing the solutions were placed in the texture analyser and exposed to constant injection speeds of 4 and 6 mL/min, equivalent to an injection of 1 mL over 10- to 15-second periods. The average force needed to inject the material at 4 and 6 mL/min using 22G 1” needle sizes was then calculated across the three solution measurements.

Dissolution

In addition to viscosity, dissolution testing is a critical parameter that must be carefully monitored when developing new formulations. Initially developed for solid oral-dosage forms, this technique is becoming increasingly used in the analysis of other formulations, including pioneering controlled release formulations, where in vitro drug release is crucial. Among the different in vitro methods available for dissolution testing, the flow through system (USP apparatus 4) offers the best characteristics for parenteral formulations. This system is recognised as a contemporary method for drug release from injectable controlled release formulations, as shown in the schematic diagram (Figure 2).
Diclofenac

Diclofenac is a non-steroidal anti-inflammatory drug (NSAID) that can be given orally, intravenously or ocularly to reduce inflammation and pain. It is often applied in the treatment of conditions such as arthritis and acute injury. In this instance, the sodium salt of diclofenac was used. Figure 3 shows the accumulative in vitro release profiles of diclofenac, determined using the USP apparatus 4 method.

For the diclofenac formulation, a complete release was obtained after 10 minutes, reflecting a reasonable water solubility and fast distribution in the dissolution system. This is the typical profile of fast-release formulations. By adding HA to the formulation, the release time was extended to three, six and nine hours in an HA concentration-dependent manner. This can be put down to the increased viscosity of the formulations containing HA, which in turn results in a slower diffusion of the API. No initial burst release of diclofenac was observed for all three HA concentrations. Overall, the diclofenac formulation containing 3% HA showed a steady release profile with a 50 times slower release compared to a formulation without HA.

Dexamethasone Phosphate

Dexamethasone phosphate is a corticosteroid that is also used as an anti-inflammatory. The collective in vitro release profiles of dexamethasone phosphate, determined using the USP apparatus 4 method, are given in Figure 4. The API dissolved in PBS once again showed a fast distribution in the dissolution system, with a complete release obtained after 10 minutes. The effect of HA in the formulation was similar to that achieved when it was combined with diclofenac, in that a complete release of dexamethasone phosphate was obtained after 9-10 hours when formulated with 3% HA. No burst release was observed, although the release profile of dexamethasone phosphate showed greater curvature than seen with diclofenac.

Triamcinolone Hexacetonide

The final therapeutic to be tested with HA was triamcinolone hexacetonide. This second corticosteroid is used in the treatment of arthritis and designed for a slow release, thanks to its low aqueous solubility – a trait which was observed when triamcinolone hexacetonide was formulated as a suspension without HA. Here the in vitro release profile was significantly different from diclofenac and dexamethasone phosphate (Figure 5). After two hours of an initial, relatively fast release period, 80% API was released. 95% had been released through a six-hour slower release phase and 100% after 12 hours. By adding HA to the formulation, the release rate in the initial phase decreased while the rate in the second phase remained constant. Subsequently, the initiation of the second phase was shifted from two hours without HA to 10 hours with 3% HA in the formulation and a 95% drug release was not achieved during the 12 hours.

Conclusion

It has been recognised that the increasing number of effective therapies for OA will prompt a rise in advanced and multifunctional solutions, many of which may utilise a combination of HA and APIs. In this investigation, three suitable APIs were formulated with Hyasis®.
as potential models for such future combination products.

All three tested APIs showed a slower release time when formulated with HA, and this release was shown to be in a concentration-dependent manner. The effect of HA on diclofenac and dexamethasone phosphate release was similar, leading to 50 times slower release. For triamcinolone hexacetionate, the effect was smaller but resulted in the longest total release time of the three APIs. It is therefore shown that combining HA and an API could lead to a more effective treatment which remains in the patient’s system longer. In the case of OA treatment, the viscoelastic properties of HA and the anti-inflammatory effect of the API can be combined in a controlled release formulation to provide a potentially effective therapy.

In an industry setting, Bacillus-derived HA offers new opportunities by providing a pure and biocompatible source which confers numerous advantages to manufacturers, practitioners and patients, creating a safer, purer alternative to the differently sourced HA of the past. The technology offers pioneering ease of administration, effectiveness and an exceptional safety profile, making it highly suitable for joint care applications.

For further information on Novozymes’ Hyasis, please visit http://www.biopharma.novozymes.com

References

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