Formulation and Characterization of Glucosamine Sulphate Loaded Carbopol Based Hydrogels for the Management of Osteoarthritis

Sundareswara Kumar Chellaswamy*, Satheesh Babu Natraj
Department of Pharmaceutics, Lincoln University College, Malaysia

**ABSTRACT**

Osteoarthritis is emerging as the most ordinary form of arthritis, affecting 22-39% of the Indian population. A wide range of medications and therapies are available for the treatment of osteoarthritis. With a desire to develop a therapeutically effective dosage form, the present study was carried out to formulate glucosamine sulfate loaded carbopol based hydrogel. Hydrogels H1 to H6 were formulated without permeation enhancers while formulations H7 to H12 were developed with a different class of permeation enhancers such as PEG400, oleic acid, Tween 40, DMSO and PG. Based on viscosity, it was detected that formulation H4 containing polymer 1% was ideal for incorporating drug. Considering H4 as a placebo, H6 was used for further evaluation. Drug content was found to be 99.2±0.64, with in vitro drug release of 15±0.86, 22±1.59, 28±0.72, 35±0.68, 40±0.31, 47±0.83, 58±1.59, and 70±0.9% at a duration of 1, 2, 3, 4, 5, 6, 7, 8 hours respectively. Skin irritation tests carried out on Wistar rats revealed that skin was intact with no inflammation or erythema detected, compared to untreated site. By diffusion disc method, it was evident that the levels of microbial load were relatively low, and no harmful microorganisms were identified. There were no significant changes in physicochemical properties on stability studies. Due to a simple method of preparation and effective drug delivery, glucosamine sulfate loaded hydrogels could be contemplated as a prominent formulation in the beneficiary treatment of osteoarthritis.

INTRODUCTION

Osteoarthritis, the most common form of arthritis, is a degenerative joint disease characterized by cellular stress and deterioration of the extracellular matrix. These, in turn, activate inflammatory pathways. The anatomic modifications include degradation of cartilage and bone remodelling accompanied by inflammation of joints and formation of osteophytes. And thus, affect the normal routine activities. Osteoarthritis was believed to be caused only due to mechanical degradation of cartilage. But it was later discovered to be a complex condition affecting the joint. According to the Global Burden of Disease study conducted in 2010, osteoarthritis was the 11th highest contributor to global disability (for Disease Control and Prevention, 2018; Sharma, 2016; Glyn-Jones et al., 2015). It is also the most cited reason for locomotor impairment in elders (Thamvasupong and Viravaidya-Pasuwat, 2016). Various strategies are being employed for the treatment of osteoarthritis, which includes lifestyle modification, surgical pro-
cedures and drug treatment. It is usually treated with nonsteroidal anti-inflammatory drugs. Chondroitin and glucosamine are being widely studied as they exhibit anti-inflammatory and anticatabolic properties. They tend to provide symptomatic relief and delay anatomic degradation (Glyn-Jones et al., 2012). Glucosamine, an amino saccharide, decreases the production of prostaglandin E2 which is responsible for inflammatory response. Shreds of evidence have demonstrated that administration of glucosamine sulfate orally tends to reduce the symptoms of osteoarthritis (Reginster et al., 2012). Hydrogels are 3-D, cross-linked networks of water-soluble polymers that are highly preferred for topical delivery. These biocompatible formulations are hydrophilic, which holds potential in controlled drug release (Chellaswamy and Natrajan, 2020). Once the drug is incorporated into the hydrogel, it can be injected in a liquid state. On achieving the body temperature, it converts into a gel to release the drug content at the site of administration (Thamvasupong and Viravaidya-Pasuwat, 2016). Glucosamine sulphate being a potential candidate in the treatment of osteoarthritis, the present study was designed to formulate glucosamine sulphate into hydrogels, which are considered as promising drug carriers. The hydrogel was constructed using carbopol (Ultrez 20) due to the ability to increase elasticity and bio adhesion. The optimized formulation was further added with permeation enhancers, and the various evaluation results were compared.

Methodology

Formulation Of Glucosamine Loaded Hydrogel

The gels (0.2, 0.5 and 1% w/w, respectively) were prepared by the following procedure (Škalko et al., 1998). Carbopol (Ultrez 20) is found to have outstanding dispersion ability and forms gels rapidly. Carbopol resin (w/v) was dispersed in distilled water. The dispersion was stirred using mechanical stirrer at 500 rpm until achieving uniform dispersion and then neutralized by using triethanolamine (drop-wise addition) to form gel consistency. Once the polymer concentration was optimized, drug glucosamine sulphate 1 % w/v was incorporated. Hydrogel formulation was developed with a different class of permeation enhancers such as PEG400, oleic acid, Tween 40, DMSO and PG. Benzalkonium chloride was used as a preservative, which is added to all the batches. The prepared hydrogel formulations were stored at room temperature for 24 hours to stabilize.

Characterization Of Hydrogel

Physical appearance

Initial characterization involves analyzing physical appearance. The formulations were tested for their homogeneity by visual appearance after the hydrogels have been set in a suitable container. Also, a small quantity of each formulation was pressed between the thumb and the index finger, then the consistency of the hydrogel was noticed homogeneity (Vasudevan and Rajan, 2012).

pH analysis

The pH of hydrogel formulations was determined using pH meter. pH meter was calibrated before each use with standard buffer solutions. A quantity of 1 g of hydrogel was dissolved in 100 mL freshly prepared distilled water and stored for 2 hours. The electrode was inserted into the sample solution 10 min before recording the reading at 25°C temperature. Each analysis was carried out in triplicate (Zakaria et al., 2016).

Viscosity

The viscosity of the hydrogel formulations was determined using Brookfield viscometer with spindle no. 7 at 100 rpm at the temperature of 25°C (Monica and Gautami, 2014).

Spreadability

Spreadability (g.cm/sec) is expressed in terms of time consumed in seconds by two slides to slip off from the hydrogel placed between them, under a specific load. The standardized load tied on the upper plate was 20g, and the length of the glass slide was 7.5 cm. Spreadability was calculated by using the following formula Spreadability = (Weight × Length) / Time

Drug content analysis

A specific quantity of each drug-loaded hydrogel (Code: H6-H11) was weighed and transferred into a volumetric flask containing 100ml of methanol. The hydrogel was stirred for 1 hour on a magnetic stirrer, at 250 rpm, to get complete solubility of the drug. The solution was filtered to remove the undissolved particles and analyzed the drug content using UV spectrosocopical method (Aly, 2012).

In vitro drug release analysis

A sample of 1g hydrogel was accurately weighed and placed on a semipermeable standard cellophane membrane (previously immersed in phosphate buffer, pH 7.4, for 24 hours). The loaded membrane was stretched over the lower open end of a glass tube of 3 cm diameter and sealed with a rubber band. The glass cylinder was then immersed in a 250 ml beaker containing 200 ml of the phosphate buffer solution (pH 7.4) in such a manner that the membrane was located just below the surface.
of the sink solution. The whole dialysis assembly was placed in a thermostatically controlled shaker water-bath adjusted at $37\pm1^\circ\text{C}$ with constant stirring at 50 rpm. At predetermined time intervals aliquots of 2ml, was collected and immediately replaced by an equal volume of the fresh phosphate buffer solution at the same temperature to keep the volume of the sink solution constantly during the experimentation. Samples were then assayed UV spectrophotometrically at 240 nm (Aly, 2012; Muthukumar et al., 2019).

**Accelerated stability studies**

Stability studies were carried out on optimized hydrogel (Code: H8) according to International Conference on Harmonization (ICH) guidelines. The hydrogel tightly packed in an aluminium tube was subjected to accelerated stability testing for three months duration as per ICH norms at a temperature ($40\pm2^\circ\text{C}$) and relative humidity 75 $\pm$ 5%. Samples were taken at every one month for three months and investigated for the change in physical appearance, pH, viscosity and drug content.

**Skin Irritation test**

Wistar rats (200-250 g) of either sex were used for skin irritation test for hydrogel formulation. The animals were maintained on standard animal feed and had free access to water. The animals were kept under standard conditions, and hair was gen-
RESULTS AND DISCUSSION

Formulation Of Glucosamine Loaded Hydrogel

Around 11 formulations were prepared and coded as H1 to H11. Formulations H1 to H6 were prepared using different concentrations of carbomer and without permeation enhancers. After determining the optimum concentration of carbomer, glucosamine sulphate was incorporated with the addition of permeation enhancers (Table 1).

Characterization Of Hydrogel

Physical appearance

Each formulation is expected to have a uniform appearance and elegant. Formulations H1 to H6 were formulated without incorporation of the drug. One evaluation, they appeared clear and transparent. Formulations H7 to H11 were observed to be white and opaque (Figure 1). This was due to the presence of glucosamine. All the formulations exhibited good homogeneity without any clumps. This denotes complete dispersion of polymer and added drug.

pH analysis

As hydrogel is intended for topical application, pH plays a vital role. The pH of all formulations ranged within 5.1-6.1. It was found that the observed pH range is compatible with skin and will not produce any redness or irritation.

Viscosity

Viscosity reflects the consistency of the formulation. The viscosity of formulation depicts the penetration of the drug into skin and ease administration—concentration and type of polymer influence the viscosity of the product. Increase in viscosity was noticed with an increase in the concentration of polymer. When the polymer was used at 0.25% concentration, viscosity was found to be 3712 cps. On 0.5% and 0.75%, viscosity was 7204 cps and 9827cps respectively. And at 1 % and 1.25 %, it increased to 12529 cps and 13454 cps. It was detected that formulation H4 containing polymer 1% was ideal for incorporating drug. And formulation H5 seemed to be very thick, and it was not feasible in terms of administration.

Spreadability

Spreadability is considered as a critical parameter for topical formulations. It is predicted that the lesser the time consumed for separation of the two slides, the better its spreadability. H7 formulation showed higher spreadability with 35±1.14 g.cm/Sec (Table 2).

Drug Content
Table 1: Formulation of glucosamine loaded hydrogel

| Table 1: Formulation of glucosamine loaded hydrogel |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Formulation Code | Formulation without Permeation Enhancers | Formulation with Permeation Enhancers |
|                 | H1   | H2   | H3   | H4   | H5   | H6   | H7   | H8   | H9   | H10  | H11  |
| Carbomer Ultrace 20 | 0.25 | 0.5  | 0.75 | 1.0  | 1.25 | 1.0  | 1.0  | 1.0  | 1.0  | 1.0  | 1.0  |
| Glucosamine sulphate (W/V) | -    | -    | -    | -    | 1    | 1    | 1    | 1    | 1    | 1    | 1    |
| PEG-400 | -    | -    | -    | -    | -    | 2    | -    | -    | -    | -    | -    |
| Oleic acid | -    | -    | -    | -    | -    | -    | 2    | -    | -    | -    | -    |
| Tween 40 | -    | -    | -    | -    | -    | -    | -    | 2    | -    | -    | -    |
| DMSO | -    | -    | -    | -    | -    | -    | -    | -    | 2    | -    | -    |
| Propylene Glycol | q.s  | q.s  | q.s  | q.s  | q.s  | q.s  | q.s  | q.s  | q.s  | q.s  | q.s  |
| TEA | q.s  | q.s  | q.s  | q.s  | q.s  | q.s  | q.s  | q.s  | q.s  | q.s  | q.s  |
| Distilled water (q.s. to) | 100 ml | 100 ml | 100 ml | 100 ml | 100 ml | 100 ml | 100 ml | 100 ml | 100 ml | 100 ml | 100 ml |
| Benzalkonium chloride (%W/V) | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |

Table 2: Physical characterization of formulations

| Table 2: Physical characterization of formulations |
|-----------------------------------------------|
| Code | Physical Appearance | Homogeneity | pH       | Viscosity (cps) | Spreadability (g.cm/Sec) |
| H1   | Clear, Transparent  | Homogenous   | 6.8±0.37 | 3712±5.41      | 3±0.41                     |
| H2   | Clear, Transparent  | Homogenous   | 6.3±0.15 | 7204±3.51      | 15±0.28                    |
| H3   | Clear, Transparent  | Homogenous   | 6.4±0.41 | 9827±2.1       | 21±1.2                     |
| H4   | Clear, Transparent  | Homogenous   | 6.1±0.72 | 12529±5.17     | 28±0.69                    |
| H5   | Clear, Transparent  | Homogenous   | 5.8±0.55 | 13454±4.55     | 31±0.57                    |
| H6   | White, opaque       | Homogenous   | 5.9±0.3  | 14345±2.58     | 29±0.32                    |
| H7   | White, opaque       | Homogenous   | 5.1±0.48 | 13307±4.54     | 35±1.14                    |
| H8   | White, opaque       | Homogenous   | 5.7±0.75 | 14610±2.9      | 31±1.83                    |
| H9   | White, opaque       | Homogenous   | 5.5±0.52 | 15112±2.57     | 29±2.65                    |
| H10  | White, opaque       | Homogenous   | 6.1±0.27 | 13539±4.36     | 32±0.53                    |
| H11  | White, opaque       | Homogenous   | 5.9±0.93 | 15419±5.68     | 34±0.91                    |

Values are expressed as mean ±SD, n=3

Based on the viscosity analysis, we considered a placebo formulation (H4) and hydrogel (H6) developed with the same variables and added glucosamine (1%W/V). Formulations H7-11 used different permeation enhancer with other variables constant. The drug content was in the limit. It ranged from 97 to 99% (Table 3). The formulations also exhibited content uniformity.

**In vitro drug release**

For the treatment of osteoarthritis, we aimed to develop glucosamine hydrogel with better penetration through various skin membranes and the simultaneously prolong therapeutic effectiveness. Since glucosamine sulphate is highly soluble in an aqueous medium, there was a necessity of addition to permeation enhancer. Various permeation enhancers like PEG 400, Oleic acid, Tween 40, DMSO and PG were chosen for the formulation of glucosamine hydrogel. The results compared with a reference coded as R. It was observed that 15-20% of drug released at a first one-hour time. At 6th hour, 50% drug was released. And at the end of
Table 3: Drug content of formulations

| Formulation Code | Drug Content |
|------------------|--------------|
| H1               | -            |
| H2               | -            |
| H3               | -            |
| H4               | -            |
| H5               | -            |
| H6               | 99.2±0.64    |
| H7               | 98.1±0.45    |
| H8               | 97.5±0.72    |
| H9               | 98.9±1.33    |
| H10              | 99.2±1.15    |
| H11              | 97.6±0.39    |

Table 4: In vitro drug release studies using diffusion membrane method

| Code | Cumulative (%) drug release | 1Hr | 2Hr | 3Hr | 4Hr | 5Hr | 6Hr | 7Hr | 8Hr |
|------|----------------------------|-----|-----|-----|-----|-----|-----|-----|-----|
| H6   |                           | 15±0.86 | 22±1.59 | 28±0.72 | 35±0.68 | 40±0.31 | 47±0.83 | 58±1.59 | 70±0.9 |
| H7   |                           | 17±2.35 | 24±1.32 | 30±0.93 | 35±1.53 | 43±0.75 | 57±2.35 | 69±0.73 | 73±1.46 |
| H8   |                           | 18±1.75 | 22±1.94 | 31±2.74 | 42±1.9 | 58±0.18 | 65±0.66 | 71±0.18 | 75±2.05 |
| H9   |                           | 21±1.93 | 25±3.63 | 32±2.1 | 39±0.54 | 52±1.87 | 58±0.21 | 63±0.51 | 68±1.39 |
| H10  |                           | 19±2.94 | 23±1.16 | 29±2.02 | 36±0.97 | 43±2.29 | 54±1.55 | 61±1.22 | 65±2.25 |
| H11  |                           | 19±0.47 | 25±1.22 | 28±1.58 | 32±0.11 | 37±0.08 | 48±1.28 | 59±1.83 | 72±1.83 |
| R    |                           | 10.23 | 17.23 | 22.02 | 27.30 | 35.78 | 43.79 | 51.76 | 58.62 |

Table 5: Stability testing for Glucosamine loaded hydrogel (Code: H8)

| Test                        | Initial | 1st month | 2nd month | 3rd month |
|-----------------------------|---------|-----------|-----------|-----------|
| Physical appearance         | White, opaque | White, opaque | White, opaque | White, opaque |
| pH                          | 5.7±0.75 | 5.6±1.7 | 5.6±4.31 | 5.4±0.22 |
| Viscosity (cps)             | 14610±2.9 | 13459±3.1 | 13292±7.4 | 13124±1.83 |
| Drug content (%)            | 97.5±0.72 | 97.3±1.49 | 97.1±4.61 | 96.9±3.15 |

8th hour, approximately 75% drug was released (Table 4, Figure 2). As a result, the performance of permeation enhancers was comparable. Formulation H8 containing oleic acid permeation enhancer was selected, as oleic acid is acknowledged for topical delivery and well tolerated by skin compared to DMSO and Tween 40. Therefore, H8 was considered for stability studies.

Skin irritation studies

Animals were treated with H6 formulation. The treated skin was intact; no inflammation and erythema compared to untreated site. There was no significant inflammation at the time of application, and after 4 hours (Figures 3, 4 and 5). All the animals were tolerated with applied hydrogel, and there were no signs of irritations/ redness noticed during the whole period of study.

The microbial load of the hydrogel by disc diffusion method

It was observed that levels of microbial load were relatively low, and no harmful microorganisms were identified. The total microbial count was found to lie within the specified limit (Figures 6, 7 and 8). Hence the formulation passed the microbial quality test.

Stability analysis

Stability analysis showed no significant changes in physicochemical properties of optimized formulation even after its exposure to accelerated conditions of temperature (40°C and 75 ±5%RH). The optimized formulation was found to be stable.
after subjecting to accelerated stability conditions (Table 5).

CONCLUSIONS

A versatile, biodegradable hydrogel has been successfully prepared using Carbomer Ultrez 20 loaded with Glucosamine sulphate for topical delivery for the treatment of osteoarthritis. Selection of Suitable polymers and their concentration is a prerequisite for formulating an effective transdermal drug delivery system. Various concentrations of polymer and different permeation enhancers have been tested successfully to optimize the formulation. The optimized glucosamine loaded hydrogels exhibited agreeable formulation characteristics such as physical appearance, pH, drug uniformity, rheology, microbial load, skin irritation, stability, and in vivo drug release. Further preclinical and clinical studies can be performed to support the use of this hydrogel for the patients suffering from osteoarthritis.

Funding Support

The authors declare that they have no funding support for this study.

Conflict Of Interest

The authors declare that they have no conflict of interest for this study.

REFERENCES

Aly, U. F. 2012. Preparation and evaluation of novel topical gel preparations for wound healing in diabetics. Int J Pharm Pharm Sci, 4(4):76–77.

C, S., S. M., G. A., R. V., Manimekalaim, M., Simson, S. G. 2020. Evaluation of Methanolic Extract of Hypericum Mysorense Ointment for its Wound Healing Activity. Global Journal of Medical Research, pages 41–45.

Chellaswamy, S. S. K., Natrajan, B. 2020. An Overview Of Microparticles Loaded Hydrogel System For Transdermal Delivery. Journal of Global Trends in Pharmaceutical Sciences, 11(1):7505–7513.

for Disease Control, C., Prevention 2018.

Glyn-Jones, S., Palmer, A. J. R., Agricola, R., Price, A. J., Vincent, T. L., Weinans, H., Carr, A. J. 2015. Osteoarthritis. The Lancet, 386(9991):376–387.

Monica, A. S., Gautami, J. 2014. Design and evaluation of topical hydrogel formulation of diclofenac sodium for improved therapy. International Journal of Pharmaceutical Sciences and Research, 5(5):1973–80.

Muthukumar, S., Sankar, C., kumaran, G. A., Shalini,

S., Vinesha, R., Varghese, S. 2019. Formulation and Comparative Evaluation of Etoricoxib Loaded Osmotic Drug Delivery Systems. Research Journal of Pharmacy and Technology, 12(11):5223–5223.

Register, J.-Y., Neuprez, A., Lecart, M.-P., Sarlet, N., Bruyere, O. 2012. Role of glucosamine in the treatment for osteoarthritis. Rheumatology International, 32(10):2959–2967.

Sharma, L. 2016. Osteoarthritis year in review 2015: clinical. Osteoarthritis and Cartilage, 24(1):36–48.

Thamvasupong, P., Viravaidy-Pasuwat, K. 2016. Controlled Release of Glucosamine from Pluronic-Based Hydrogels for the Treatment of Osteoarthritis. International Journal of Chemical and Molecular Engineering, 10(7):841–845.

Vasudevan, D., Rajan, R. 2012. Effect of permeation enhancers on the penetration mechanism of transfusional gel of ketoconazole. Journal of Advanced Pharmaceutical Technology & Research, 3(2):112–112.

Škalko, N., Bouwstra, J., Spies, F., Stuart, M., Frederik, P. M., Gregoriadis, G. 1998. Morphological observations on liposomes bearing covalently bound protein: Studies with freeze-fracture and cryo electron microscopy and small angle X-ray scattering techniques. Biochimica et Biophysica Acta (BBA) - Biomembranes, 1370(1):151–160.

Zakaria, A. S., Afifi, S. A., Elkhodairy, K. A. 2016. Newly developed topical cefotaxime sodium hydrogels: antibacterial activity and in vivo evaluation. BioMed research international.