Formulation and Evaluation of Glucosamine Sulphate Transdermal

Alaa Mohammad Abdel Azim [1*], Manal El-Ashmoony [2], Abdel Moneim Swealem [3] and Ragia Ali shoukry [2]

1. Department of toxic and narcotic drug forensic medicine, Cairo, Egypt
2. Department of pharmaceutics, faculty of pharmacy, Cairo university, Cairo, Egypt
3. Department of Pharmaceutics, National Organization for Drug Control and Research, Cairo, Egypt.

Corresponding author Email : alaa_mohammad_one@yahoo.com

ABSTRACT: The purpose was to develop transdermal delivery systems (TDS) of Glucosamine Sulphate using Pluronic F-127 (PF-127) and mucoadhesive polymer sodium carboxymethylcellulose (Na CMC) in order to optimize its release profile, and the clinical performance. Glucosamine (G) is known to be formulated in an oral dosage form, but it suffers from hepatic metabolism which greatly affects its bioavailability, in addition to its side effects on the gastrointestinal tract (GIT). Gel formulae were subjected to rheological studies, *in-vitro* release, and effect of 1% dimetylsulphoxide (DMSO) on rat skin permeation. Higuchi diffusion model was the best fitted model for the release results. PF127 and Na CMC gels showed high permeation results, and 25% PF-127 gel showed no skin irritation or histological change of skin layers. The results obtained suggest the feasibility of designing a successful TDS providing constant (G) input and overcoming the disadvantages of oral administration.

Keywords: transdermal, Glucosamine**.**

INTRODUCTION

Osteoarthritis (OA), previously called degenerative joint disease, is the most common form of arthritis and is often associated with disability, functional impairment, and impaired quality of life (Poolsup N., 2005; Creamer P., 1997; Carr AJ., 1999). A new perspective in OA management is to delay disease progression by modifying joint structure. Much attention has been focused on biological compounds, particularly glucosamine. Some evidence suggests it has both analgesic and disease-modifying effects without affecting cyclooxygenase, and so stabilize cell membrane and increase the intracellular ground substance (Kanwischer M., 2005; Fox B.A., 2006).

Glucosamine (G), an amino monosaccharide, is a natural component of glycoprotein found in connective tissue and gastrointestinal mucosal membranes (Habashi A.A., 2002a; Hawker G., 1997). Each person naturally produces a certain amount of glucosamine within his or her body, but the amount might not be sufficient for healthy joint maintenance, especially as age increases (Sullivan C., 2005).

The compound GS can be derived from chitin. Chitin is the second most abundant polymer on earth and is available from, for example, crab, lobster, shrimp or oyster shells. It can also be produced by synthetic means (Freeze H.H., 2002).

Directly or indirectly, glucosamine plays a role in the formation of articular surfaces, tendons, ligaments, synovial fluids, skin, bone, nails, heart valves, blood vessels and mucous secretion within the digestive, respiratory and urinary systems (De los Reyes G.C. et al., 2000).

G has been evaluated as a therapeutic agent for OA in Germany since 1969 (Freeze H.H., 2002). It has been used to treat OA for more than 20 years.

Approximately 70% of the oral GS is absorbed through the intestine and excreted through the renal system. The majority of clinical trials on oral glucosamine have used a standard dosage of glucosamine, 500mg taken three times daily, with or without rescue pain medication as required by the patient (Hungerford D.S, Jones L.C., 2003).

The benefit of GS in patients with osteoarthritis is likely the result of a number of effects including its anti-inflammatory activity (Largo R., 2003; Chan P.S., 2006], the stimulation of the synthesis of proteoglycans (Bassleer C. et al. 1998), and the decrease in catabolic activity of chondrocytes inhibiting the synthesis of proteolytic enzymes and other substances that contribute to damage cartilage matrix and cause death of articular chondrocytes (Dodge G.R., 2003; Chan P.S., 2005; Uitterlinden E.J. , 2006; S.C. Chu, 2006]. Recently, glucosamine has been shown to inhibit the expression of inducible nitric oxide synthase (iNOS), thereby suppressing the excess production of NO (Meininger C.J., 2000).

Long term use of (G) may reduce radiographic progression of OA of the knee, suggesting that it may be a chondroprotective, disease modifying agent in OA of the knee (Reginster J.Y., 2001).

Although rapidly absorbed from the gastrointestinal tract, pharmacokinetic data show that when administered orally, G could be subjected to uptake and degradation by the liver and uptake into non-joint tissues so that the dose reaching the articular cartilage is a fraction of a percentage of the oral dose (Setnikar I. et al., 1993; Habashi A.A., 2002).

In the present study, a transdermal gels for GS were prepared using 1% dimethyl sulphoxide (DMSO), as a penetration enhancer, incorporated in PF-127 gel and Na CMC gel. in addition to *In-vitro* permeation across rat skin, evaluation of skin irritation and histological toxicity were studied.

MATERIALS AND METHODS

Materials

GS was purchased from MP Biomedicals, Inc. (France). Ninhydrine was purchased from RFCL, Limited, (India). Na CMC, Potassium dihydrogen ortho phosphate, disodium hydrogen phosphate, Sodium hydroxide, orthophosphoric acid were purchased from El-Nasr Pharmaceutical Chemicals Co.(Adwic, Egypt). P F-127 was purchased from Sigma-Aldrich, (Germany). DMSO was purchased from Sd fine-chem. Limited, Mumbai, (India). Male albino adult rats. (The post conduction approval for this experimental study was attached, approval from Research Ethical Committee, Faculty of Pharmacy, Cairo University, EGYPT).

Gel preparation

Na CMC at different concentrations was sprinkled into a calculated amount of distilled water stirred using magnetic stirrer. GS 5% was dissolved in distilled water and added to the previous mixture. The poloxamer gel was prepared using the cold preparation method (Schmolka I.R., 1972). DMSO was added to each formula. Different gel formulations are illustrated in table (1).

Rheological Measurements and Data Analysis:

A sample of the freshly prepared gel was placed in the cup of Brookfield Viscometer; using spindle 52 and temperature of 25°C. Measurements were made over the whole range of speed setting ranging from 0.1 to 250 rpm with 30 seconds between each two successive measurements.

In-vitro Release of Glucosamine Sulphate from different Gel Formulations:

This study was carried out using the paddle method. Three grams of the gel containing 5% GS were spreaded over the surface of watch glass of 8 cm diameter and covered with equally sized wire screen mesh. The gel loaded watch glass and the covering screen were held together by equally spaced 3 inert plastic binder clips and placed at the bottom of dissolution vessel (Jones D.S., 2000; El-Nabarawi M.A., 2003).

The *in-vitro* release was carried out using Sotax apparatus, release medium was 400 ml of phosphate buffer at pH 5.5, adjusted at temperature of 32 ± 0.5 °C, paddle speed was 100 rpm.

At predetermined time intervals, aliquots of the release medium were withdrawn, filtered through a Millipore filter of 0.45 μm and analyzed for GS content. Equal volumes of the buffer solution were added to the release medium to maintain the total dissolution medium constant.

Kinetic Analysis of the Drug Release:

Kinetic analysis of GS release data from the prepared gel formulae was determined by finding the best fit of the release data to distinct models (Zero order, First order and Simplified Higuchi Diffusion Model).

Release efficiency (RE), which describes the whole release profile (rate and extent of release), was calculated for each gel formula, values of RE were calculated for the maximum release time (RE 210 based on 210 minutes).

The obtained RE data of different gel formulae were analyzed using one-way analysis of variance (ANOVA), in order to detect the difference in release profile of the studied formulae, at $p \leq 0.05$ using SPSS® software.

In-vitro permeation studies of GS through full thickness rat skin

A vertical diffusion cell with double open sided tube was used (Devi, K., 1999). The donor vehicle was 3 gm medicated gel placed on the dorsal side of the excised skin of newly born albino rats. The available skin diffusion area was 6.16 cm^2. The receptor medium was 100 ml of phosphate buffer pH 7.4 (Sung K.C. et al, 2000; Fang J. et al, 2004; Huang J., 2005; Tetsuya H. et al., 2008) agitated by magnetic stirrer at 300 rpm (by the aid of 2 cm Teflon coated magnetic bar). Experiments were carried out at 37°C using water bath. Samples were withdrawn at predetermined time intervals up to 24 h and compensated immediately with fresh receptor medium. Samples were assayed spectrophotometrically at 570 nm, depending on Yunqi Wu, et al (2005) (Wu Y. et al., 2005) method, using a Shimadzu UV Spectrophotometer (UV-1601). The data were analyzed statistically by the one-way ANOVA. This statistical analysis was carried out using SPSS®software.

In-vitro **Skin Permeation Study After Addition of Permeation Enhancer:**

The permeation enhancer DMSO was added to the selected gel formulae to produce a concentration of 1% of the total gel weight. These formulae were subjected to skin permeation study as mentioned before.

Skin Irritation and Histological evaluation of the skin Test

The skin irritation test was carried out on male albino adult rats weighing 250 ±10 g. The animals were kept under standard laboratory conditions, with temperature of 25 ± 1 °C and relative humidity of 45-60 %, with free access to water and food. The back of the animals was clipped free of fur with an electric clipper 24 hours before application of the sample. For the experiment, GS gel was applied to one shaved site; another site was used as control.

Both the treated and controlled sites were covered by gauze and the back of the rabbit was wrapped with a non-occlusive bandage. The application of the gel was daily, and the animals were observed for the reactions, defined as erythema and edema, and evaluated to the scoring system for skin reactions (Table 2). "Total score" was calculated by summing up each participant's higher score between the 5 assessments (3, 7, 14, 21 and 30 days). A skin irritation index was calculated using the formula "skin irritation index = (total score) / (number of participants) × 100" based on the scores yielded by the assessment. A skin irritation index was classified according to the criteria for irritation caused by medicines for external application (table 2). In Shin Iri et al this classification, a safe, acceptable, and mildly irritating product was defined as a skin irritation index of 5.0 or lower, 15.0 or lower, and above 15.0, respectively (Iri S. et al. , 2006; Hayakawa R., Ninagawa Y., 2000).

Four animals were sacrificed at after 3rd, 7th, 14th, 21st and 30th day and the excised skin was withdrawn. Tissues were fixed in 10% neutral buffered formalin. Histological evaluation was performed by microscopical examination after staining with haematoxylin and eosin (Alemdaroglu C. et al, 2006).

RESULTS AND DISCUSSION

Rheological Measurements and Data Analysis:

Visual inspection revealed clear translucent gels. The exhibited non-Newtonian thixotropic, shear thinning, pseudo-plastic flow behavior, where there is a decrease in viscosity by increasing the shear rate (figures 1, 2).

In-vitro **Release of Glucosamine Sulphate from different Gel Formulations:**

From the obtained data, it was observed that there was an inverse proportional relationship between the polymer concentration and the percentage of GS released (figures 3 and 4). This may be attributed to the increase in the viscosity of the formulations upon increased entangled nature of the polymeric network which is associated with the increase in polymer concentration. This is in accordance with Jones et al (Jones D.S. et al., 1997; El laithy H.M., El-Shaboury K.M.F., 2002).

The difference in the release of GS from the studied polymers may be due to chemical factors such as molecular weight, nature of the polymer and affinity of the vehicle to bind to the drug (Campos-Aldrete M., Villafuerte-Robles L., 1997).

It is generally recognized that if the active substance is held firmly by the vehicle, the release of the active substance is slow. As the release of the active substance generally occurred through the spaces or channels within the hydrogen network (Shojaei H., 1998), it was likely that at higher polymer concentrations or increasing the polymer molecular weight, GS was more trapped in the polymer network (Tas C. et al, 2006). In addition, the

increase in polymer concentration could increase the density of chain structure, thereby limiting the movement area of GS (Tas C., 2003).

In-vitro permeation studies of GS through full thickness rat skin

The cumulative amount (Q) of GS penetrated into the receptor compartment per unit area ($\mu g/cm^2$) was plotted as a function of time (hours). The permeation profiles are represented in figures (5 and 6).

An inverse proportional relationship was observed between the polymer concentration and Q values of GS permeated from Na CMC gels; that was expected from the results obtained from *in-vitro* release data. The permeation data of PF-127 gels were unexpected; it was noticed that there was an increase in Q values of GS as the polymer concentration increased (i.e direct proportional relationship).

These results may be explained depending on the non-ionic surfactant nature of PF-127 (Attwood and Florence, 1983) which increases both the solubility of drug, and their ability to bind to the stratum corneum proteins and disruption of the intercellular lipid matrix (Breuer M.M., 1979, Imokawa G., 1989), selective loss of intercellular lipids, and an increase in the hydration levels of the tissues as a result of more exposure of water binding sites (Rhein L.D., 1986).

In-vitro Skin Permeation Study After Addition of Permeation Enhancer:

Several enhancement techniques have been developed to overcome the impervious nature of the stratum corneum. A popular technique is the use of the permeation enhancers, which alter reversibly the permeability barrier of the stratum corneum. DMSO has been extensively used as permeation enhancer in the permeation of hydrophilic and lipophilic drugs (Dey S. er al, 2009; Chandar A., Sharma P.K., 2009; Pathan I.B., Setty C.M., 2008; Ammar H.O. et al, 2007).

The transport of GS through skin was increased significantly in presence of 1% DMSO this was found to be in agreement with Abdul Faruk et al (Abdul Faruk et al, 2009) (Abdul Faruk et al, 2009). The permeation profiles are represented in figures (7, 8).

The percentage of permeation efficiency data was statistically analyzed using independent sample t-test at $p \leq 0.05$. It was found that PE_{24} of GS increased significantly after addition of 1% DMSO to gel formulae. The permeation efficiency (PE_{24}), which describes the whole permeation profile (rate and extent of permeation), was calculated (table 3) and represented in figure (9). Depending on $PE_{24,}$ F6, was found to has the highest value and was chosen for skin irritation and histological evaluation of the skin test.

Skin Irritation and Histological evaluation of the skin Test

Assessment of irritation of pharmaceutical and cosmetic products with natural compounds is a significant step in the evaluation of their biocompatibility. Researchers and regulatory agencies recognize the important role of *in vitro* and animal tests play in the biological evaluation of transdermal products (Kamkaen N., 2007).

F6 was found to produce almost no skin reactions, neither erythema nor edema, in the tested rats.

Histological examination of the excised skin under binocular light microscope showed no apparent change in the skin layers that treated with F6 when compared to control one during the whole tested period (figure 10).

In conclusion, Na CMC and PF-127 were suitable bases for preparation of GS gels. On the basis of the highest drug permeation, formulation F6 was selected, which contained PF-127 (25% w/w) in cmbination with (1% w/w) DMSO, for skin irritation and histological evaluation of the skin test. *In-vitro* skin irritation and histological evaluation of the skin test revealed no meaningful skin irritation or histological toxicity manifestations. Based on the previous results it is possible to conclude that GS could be formulated safely in a stable TDS using 25% PF-127. Further studies will be warranted to assess the safety of F6 in the real clinical setting and permeation studies across human skin.

Table (1): Quantitative Composition of Glucosamine Sulphate gels (%wt/wt)

Ingredients (g %)	Gel Formulae					
	F1	F2	F3	F4	F5	F6
NaCMC	5	5.5	6	-	-	-
PF-127	-	-	-	15	20	25
DMSO	1	1	1	1	1	1
dist. H₂O to	100	100	100	100	100	100

Table 2: Japanese criteria for patch test

Condition	Assessment	Score
No reaction	-	0
Mild erythema	±	0.5
Erythema	+	1
Erythema and edema	++	2
Erythema and edema with papular rash or vesicles	+++	3
Bulla	++++	4

Table 3. Permeation efficiency of GS from gel bases

Formulae	In Absence of DMSO	In Presence of DMSO
F1	19.083 ± 0.020	22.238 ± 0.005
F2	14.082 ± 0.052	19.603 ± 0.064
F3	9.111 ± 0.052	13.435 ± 0.099
F4	16.960 ± 0.033	18.362 ± 0.120
F5	18.456 ± 0.012	21.000 ± 0.027
F6	21.480 ± 0.015	22.704 ± 0.005
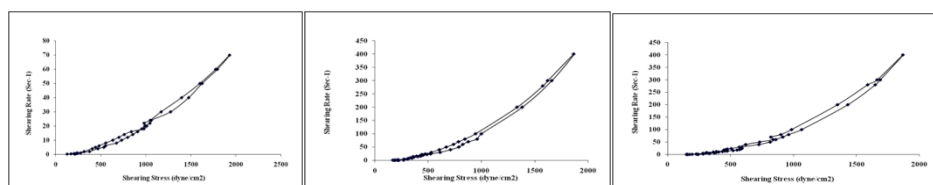
Figure 1. Rheogram of formula (a) F1, (b) F2 and (c) F3.
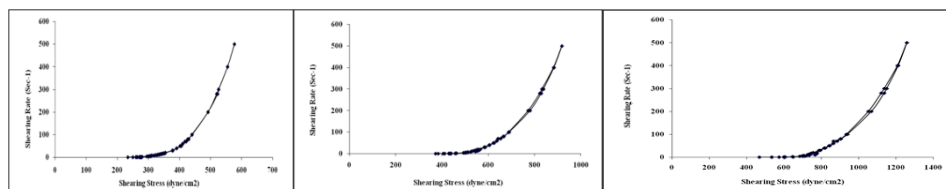
Figure (2): Rheogram of formula (a) F4, (b) F5, (c) F6
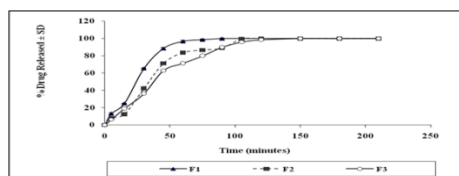
Figure 3. Release Profile of GS from NaMC bases in Phosphate Buffer (pH 5.5), where F1 represents 5% Na CMC, F2 represents 5.5% Na CMC and F3 represents 6% Na CMC
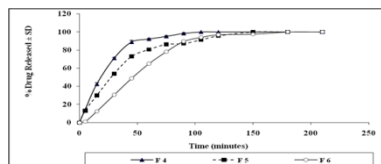
Figure 4. Release Profile of GS from PF-127 bases in Phosphate Buffer (pH 5.5), where F4 represents 15% PF-127, F5 represents 20% PF-127 and F6 represents 25% PF-127
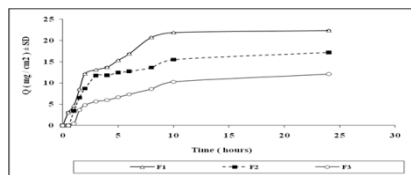
Figure 5. Permeation profile of GS from F1, F2 and F3, where F1 represents 5% Na CMC, F2 represents 5.5% Na CMC and F3 represents 6% Na CMC
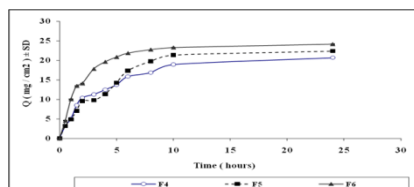

Figure 6. Permeation profile of GS from F4, F 5 and F 6, where F4 represents 15% PF-127, F5 represents 20% PF-127 and F6 represents 25% PF-127
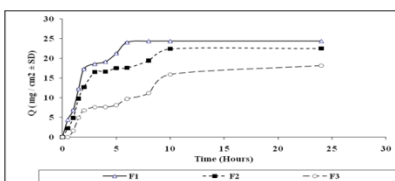

Figure 7. Permeation profile of GS from F1, F2 and F3 containing 1% DMSO, where F1 represents 5% Na CMC, F2 represents 5.5% Na CMC and F3 represents 6% Na CMC
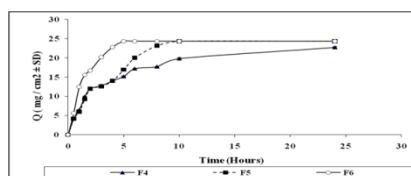

Figure 8. Permeation profile of GS from F4, F 5 and F 6 containing 1% DMSO, where F4 represents 15% PF-127, F5 represents 20% PF-127 and F6 represents 25% PF-127
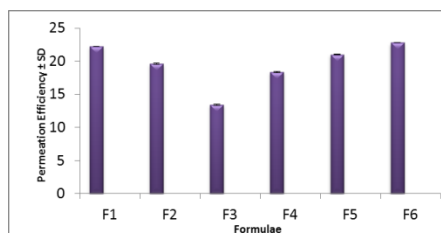

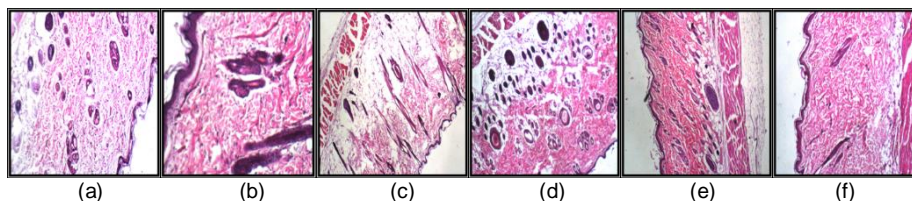Figure 9. In-vitro permeation efficiency of gel formulae after addition of 1 % DMSO

(a)	(b)	(c)	(d)	(e)	(f)

Figure 10. Light microscopic photographs of rat skin; (a) control, (b) following 3 days of daily application of GS gel, (c) following 7 days of daily application of GS gel, (d) following 14 days of daily application of GS gel, (e) following 21 days of daily application of GS gel and (f) following 30 days of daily application of GS gel

REFERENCES

Abdul Faruk, Singh G., Ishar M.P.S. 2009. Effect of drug concentration and permeation enhancer on iontophoretic transport of salbutamol sulphate in vitro, Int. J. Pharm. Sci. Nanotechnology, 1, 4, 341-348.

Alemdaroglu C., Degim Z., Celebi N., Zor F., Ozturk S., Erdogan D. 2006. An investigation on burn wound healing in rats with chitosan gel formulation containing epidermal growth factor, Burns. 32, 319-327.

Ammar H.O., Ghorab M., El-Nahhas S.A., Kamel R. 2007. Evaluation of chemical penetration enhancer for transdermal delivery of aspirin, Asian J. Pharmaceut. Sci. 2, 3, 96-105.

Bassleer C., Rovati L., Franchimont P. 1998. Stimulation of proteoglycan production by glucosamine sulfate in chondrocytes isolated from human osteoarthritic articular cartilage in vitro, Osteo. Cart. 6, 427-434.

Breuer M.M. 1979. The interaction between surfactants and keratinous tissues, J. Soc. Cosm. Chem., 30, 41-64.

Campos-Aldrete M., Villafuerte-Robles L. 1997. Influence of the viscosity grade and the particle size of HPMC on metronidazole release from matrix tablets, Eur. J. Pharm. Biopharm., 43, 2, 173-178.

Carr AJ. 1999. beyond disability: measuring the social and personal consequences of osteoarthritis, Oste. Cart. 7, 230-238.

Chan P.S. 2005. Effect of glucosamine and chondroitin sulfate on regulation of gene expression of proteolytic enzymes and their inhibitors in interleukin-1-challenged bovine articular cartilage explants, Am. J. Vet. Res., 66, 11, 1870-1876.

Chan P.S. 2006 Short-term gene expression changes in cartilage explants stimulated with interleukin beta plus glucosamine and chondroitin sulfate, J. Rheumatol. 33, 7, 1329-1340.

Chandar A., Sharma P.K. 2009. Transdermal delivery of ketorelac, The Pharm. Societ. Jap., 129, 3, 373-379.

Chu S.C. 2006. Glucosamine sulfate suppresses the expressions of urokinase plasminogen activator and inhibitor and gelatinases during the early stage of osteoarthritis, Clin. Chim. Acta. 372, 1-2, 167-172.

CreamerP. 1997. Intra-articular corticosteroid injections in osteoarthritis: do they work and if so, how?, Ann. Rheum. Dis. 56, 634-636.

De los Reyes G.C., Koda R.T., Lien E.J. 2000. Glucosamine and chondroitin sulfates in the treatment of osteoarthritis, Prog. Drug Res., 55, 81–103.

Devi K., K.L.K. 1999. Paranjothy, Pharmacokinetic profile of a new matrix type transdermal delivery system: diclophenac diethyl ammonium patch, Drug Dev. Ind. Pharm. 25, 695-700.

Dey S., Mazumder B., Patel J.R. 2009. Enhanced percutaneous permeability of acyclovir by DMSO from topical gel formulation, Int. J. Pharm. Sci. Drug Res. 1, 1, 13-18.

Dodge G.R. 2003. Glucosamine sulfate modulates the levels of aggrecan and matrix metalloproteinase-3 synthesized by cultured human osteoarthritis articular chondrocytes, Osteo. Cart. 11, 6, 424-432.

El laithy H.M., El-Shaboury K.M.F. 2002. The Development of Cutina Lipogels and Gel Microemulsion for Topical Administration of Fluconazole, AAPS PharmSciTech., 3, 4, article 35. (http://www.aapspharmscitech.org)

El-Nabarawi M.A., Nesseem D.I., Sleem A.A. 2003. Delivery and analgesic activity of tramadol from semi-solid (topical) and solid (rectal) dosage forms, Bull. Fac. Pharm. Cairo Univ.41, 2, 25-31.

Fang J., Hung C., Fang Y., Chan T. 2004. Transdermal iontophoresis of 5-fluorouracil combined with electroporation and laser treatment, Int. J. Pharm. 270, 241-249.

Fox B.A., Schmitz E.D., Wallace R. 2006 . Glucosamine and Chondroitin for Osteoarthritis, Amer. Fam. Phys. Health Module 73, 7, 1245-1246.

Freeze H.H. 2002. Sweet solution: sugars to the rescue, hypothesis on how glucosamine may work in treating OA, J. of Cell Biol., 158, 4, 615-616.

Habashi A.A. 2002 a. HPLC determination of glucosamine in rat plasma, J. Pharm. Pharmaceut. Sci. 5, 2, 176-180.

Habashi A.A. 2002. Single dose, pharmacokinetics and bioavailability of glucosamine in the rat, J. Pharm. Pharmaceut. Sci., 5, 2, 181-184.

Hawker G. 1997. Update on the epidemiology of the rheumatic disease, Curr. Opin. Rheumatol., 9, 90-94.

Hayakawa R., Ninagawa Y. 2000. Skin safety evaluation of nadifloxacin (OPC-7251) ointment, Skin Res., 42, 509–513.

Huang J., Sung K.C., Hu OY, Wang J., Lin Y., Fang J. 2005. The effects of electrically assisted methods on transdermal delivery of nalbuphine benzoate and sebacoyl dinalbuphine ester from solutions and hydrogels, Int. J. Pharm. 297, 162-171.

Hungerford D.S, Jones L.C. 2003 Glucosamine and chondroitin sulfate are effective in the management of osteoarthritis, J. Arthropl. 18, 3, suppl. 1:5-9.

Imokawa G., Akasaki S., Minemastu Y., Kawai M. 1989. Importance of intercellular lipids in water-retention properties of the stratum corneum: induction and recovery study of surfactant dry skin. Arch. Dermat. Res., 281, 45-51.

Iri S., Tsukikawa1 H., Nabeshima T., Usami M., Karino T., Komatsu S., Akasaki M. 2006. Skin Tests of a Novel Nicotine Patch, PHK-301p, in Healthy Male Volunteers: Phase I, Placebo-Controlled Study, J. Pharmacol. Sci. 102, 72 – 76.

Jones D.S., Woolfson A.D., Brown A.F. 1997. Textural, viscoelastic and mucoadhesive properties of pharmaceutical gels composed of cellulose polymers, Int. J. Pharm., 151, 223-233.

Jones D.S., Woolfson A.D., Brown A.F., Coulter W.A., Melland C., Irwin C.R. 2000 Design, characterization and preliminary clinical evaluation of a novel mucoadhesive topical formulation containing tetracycline for the treatment o periodontal disease, J. Cont.Release. 67, 357-362.

Kamkaen N., Phuntuwate W., Samee W., Boonrod A., Treesak C. 2007 The Investigation of the Rabbit and Human Skin Irritation of Herbal Anti-wrinkle Cream, Thai. Pharmaceut. Health Sci. J., 2, 1, 20-25.

Kanwischer M., Kim S., Bian S. 2005. Evaluation of the physicochemical stability and skin permeation of glucosamine sulphate. Drug Dev. Ind. Pharm. 1, 91-97.

Largo R. 2003. Glucosamine inhibits IL-1beta-induced NFkappaB activation in human osteoarthritic chondrocytes, Osteo. Cart. 11, 4, 290-298.

Meininger C.J., Kelly K.A., Li H., Haynes T.E. 2000 Wu G., Glucosamine inhibits inducible nitric oxide synthesis, Biochem. Biophys. Res. Commun. 279, 234–239.

Pathan I.B., Setty C.M. 2008. Chemical permeation enhancers for transdermal delivery system, Top. J. Pharmaceut. Res. 8, 2, 173-179.

Poolsup N., Suthisisang C., Channark P., Kittikulsuth W., 2005. Glucosamine Long-Term Treatment and the Progression of Knee Osteoarthritis: Systematic Review of Randomized Controlled Trials, The Ann. Pharm. 39, 1080-1087.

Reginster J.Y., Deroisy R., Rovati L.C. 2001. Long-term effects of glucosamine sulphate on osteoarthritis progression: a randomised, placebo-controlled clinical trial, Lancet, 357, 251-256.

Rhein L.D., Robbins C.R., Fernee K., Cantore R. 1986. Surfactant structure effects on swelling of isolated human stratum corneum, J. Societ. Cos. Chem. 37, 125-139.

Schmolka I.R. 1972. Artificial skin. I. Preparation and properties of pluronic F-127 gels for treatment of burns, J. Biomed. Mater. Res.6, 571–582.

Setnikar I., Pertusi R., Canali S., Zanolo G., 1993. Pharmacokinetics of glucosamine in man, Arzneimittel-Forschung, 43, 1109-1113.

Shojaei H. 1998 Buccal mucosa as a route for systemic drug delivery: a review, J. Pharm. Pharm. Sci., 1, 15-30.

Sullivan C., Sherma J. 2005. Development and validation of an HPTLC-Densitometry method for assay of glucosamine of different forms in dietry supplement tablets and capsules, ACTA Chromatographica. 15, 119-130.

Sung K.C., Fang J. 2000. Hu OY, Delivery of nalbuphine and its prodrugs across skin by passive diffusion and iontophoresis, J. Contr. Rel. 67, 1-8.

Tas C., Ozkan C.K., Savaser A., Baykara T. 2003. In vitro release studies of chlorpheniramine maleate from gels prepared by different cellulose derivatives. IL. Farmaco., 58, 605-611.

Tas C., Ozkan C.K., Savaser A., Ozkan Y., Tasdemir U., Altunay H. 2006 Nasal absorption of metoclopramide from different Carbopol 981 based formulations: In vitro, ex vivo and in vivo evaluation, Eur. J. Pharm. Biopharm., 64, 246-254.

Tetsuya H., Asako K., Jaori M., Go Y., Teruki H., Masayuki A., Hiroaki T., kenji S. 2008. Analysis of skin disposition and metabolism of ethyl nicotinate after topical application using dual ager gel disc-inserted rats, Biol. Pharm. Bull., 31, 1, 85-89.

Uitterlinden E.J. 2006. Glucosamine decreases expression of anabolic and catabolic genes in human osteoarthritic cartilage explants, Osteo. Cart. 14, 3, 250-257.

Wu Y., Hussain M., Fssihi T. 2005. Development of a simple analytical methodology for determination of glucosamine release from modified release matrix tablets, J. Pharm. Biom. Anal., 38, 263-269.