

PHARMACEUTICAL PREFORMULATION- OSTEOARTHRITIS THERAPY VIA TWO
DRUG DELIVERY APPLICATIONS: THE MUTUAL PRODRUG AND THE
TRANSDERMAL DELIVERY OF A WELL-KNOWN NUTRACEUTICAL, GLUCOSAMINE

by

SOLOMON T. GARNER, JR

(Under the Direction of Anthony C. Capomacchia, Ph.D.)

ABSTRACT

As a preface to this research, it was important to look at the challenges facing industry during new product development. There was an attempted to familiarize this research with current directives and maintain product driven research, via pharmaceutical development by re-engineering an effective product/s. Per this directive, the research herein was directed toward improving the biopharmaceutical properties of an effective osteoarthritis therapy, glucosamine. Initially our studies were focused on transdermally delivering glucosamine to improve its bioavailability, leading to how a delivery method can influence a chemical entity's biopharmaceutical properties. These studies failed early on and later succeeded. Another prospective of drug delivery arose from the synthesis of mutual prodrugs based on a NSAID (non-steroidal anti-inflammatory drug) and a glucosamine molecule covalently bound via an ester linkage(s) to modify each active chemical entity's biopharmaceutical properties.

The first project was geared towards the transdermal/percutaneous delivery of glucosamine, a nutraceutical used to treat osteoarthritis (OA). Published, unpublished and our studies have shown that the orally administered glucosamine salts, its parent molecule or highly recognized metabolite chondroitin cannot be transdermally/percutaneously delivered via traditional means.

These studies were completed with via the transdermal delivery of a glucosamine metabolite, N-acetyl-D-glucosamine (NAG). These studies investigated the permeability of NAG in various permeation enhancer suspensions and simple pluronic-organo gel formulations utilizing shed snakeskin as a model membrane *via* Franz-type cell diffusion studies. Findings show NAG to be an excellent candidate for the transdermal transport of glucosamine.

The synthesis of NSAID-glucosamine derivative mutual prodrugs has been an attempt to improve the biopharmaceutical properties of the parent compounds such as permeability, solubility and stability. These chemical entities were produced as models towards the delivery of glucosamine and NSAIDs as concomitant agents that halt the progress of OA and produce known anti-inflammatory effects respectively. The synthesized compounds were characterized and evaluated '*in silico*' to predict the biopharmaceutical properties. Initial predictive findings show these products to be mutual prodrug candidates to concomitantly deliver synergistic OA therapies.

INDEX WORDS: Pharmaceutical Preformulation, Drug Delivery, Osteoarthritis, Mutual Prodrug, Nutraceutical, Transdermal Delivery/Transport, Biopharmaceutical, NSAID (nonsteroidal anti-inflammatory drug), Glycosaminoglycan, Glucosamine, N-acetyl-D-glucosamine (NAG), Permeation Enhancers, Pluronic-organo Gels, Percutaneous Absorption, Franz-type cell, Diffusion Studies, Pharmacological, Pharmacokinetic, Physicochemical, Differential Scanning Calorimetry (DSC)

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SOLOMON T. GARNER, JR

B.S., Jackson State University, 1999

M.S., The University of Georgia, 2001

A Dissertation Submitted to the Graduate Faculty of The University of Georgia in Partial
Fulfillment of the Requirements for the Degree

DOCTOR OF PHILOSOPHY

ATHENS, GEORGIA

2005

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SOLOMON T. GARNER, JR

Major Professor: Anthony C. Capomacchia

Committee: Parastoo Azadi
Michael G. Bartlett
J. Warren Beach
James C. Price

Electronic Version Approved:

Maureen Grasso
Dean of the Graduate School
The University of Georgia
May 2005

DEDICATION

Dedicated to my grandparents Peyton Gaddis Sr. and loving memory of the late- Bertha Dixon-Gaddis, Sylvester and Doretha Clincy-Garner, Sr. Finally, it is impossible to have embarked upon any of my endeavors without my immediate and extended family's love and support, as well as my friends' encouragement. This dissertation is dedicated to them. To all of you, thank you.

There is, it seems to us,
At best, only a limited value
In the knowledge derived from experience.
The knowledge imposes a pattern, and falsifies,
For the pattern is new in every moment
And every moment is a new and shocking
Valuation of all we have been. - *S. Eliot, Four Quartets, East Coker*

... at this sound, the crowd gathered, all bewildered because each one heard his own language being spoken. They were amazed, and in their astonishment exclaimed, 'Why, they are all Galileans, are they not, these men who are speaking? How is it then that we hear them, each of us in his own native language? Parthians, Medes, Elamites; inhabitants of Mesopotamia, of Judaea and Cappadocia, of Pontus and Asia, of Phrygia and Pamphilia, of Egypt and the districts of Libya around Cyrene; visitors from Rome, both Jews and proselytes, Cretans and Arabs, we hear them telling in our own tongues the great things God has done.' And they were all amazed and perplexed, saying to one another, 'What can this mean?' Others said contemptuously, 'They have been drinking!'- *The Acts of the Apostles, ch. 2*

Jesus answered, '... My task is to bear witness to the truth. For this I was born; for this I came into the world, and all who are not deaf to truth listen to my voice.' Pilate said, 'What is truth?'- *The Gospel according to John, ch. 18*

We shall not cease from exploration
And the end of all our exploring
Will be to arrive where we started
And know the place for the first time- *T. S. Eliot, Four Quartets, Little Gidding*

For now we see through a glass, darkly; but then face to face: now I know in part; but then shall I know even as also I am known.- *1 Corinthians 13.12*

ACKNOWLEDGEMENTS

I would like to express me deepest gratitude to my major professor Dr. Anthony C. Capomacchia, Ph.D. Thanks to Dr Capomacchia's tireless effort to recruit me, I was able to earn a M.S. in Pharmacy (Medicinal Chemistry) under Chung K. Chu, Ph.D. (2001) and finally complete doctoral studies under his tutelage. As he is winding down his career, mine is beginning to commence. It is my wishes that Dr. Capomacchia is able maintain and increase his ability to matriculate African-American students at the Ph.D. level. His guidance, support and mentorship allowed me to garner his four keys to success- teaching, research, service and outreach. Our adventures in the Pharmaceutical Development Laboratory have hopefully have laid a foundation for the success of product driven scientific experimentation, discovery and implementation in the delivery of drug products to improve and maintain the health of individuals as well animals. I am also very thankful for financial support I received from the Department of Pharmaceutical and Biomedical Sciences as well as the Graduate School for its generous dissertation completion award.

I am also very grateful to my advisory committee members, Drs. Parastoo Azadi, Michael G. Bartlett, J. Warren Beach, Won Jun and James C. Price for their help, advice and valuable suggestions throughout my work. Their knowledge and expertise were indispensable for the successful accomplishment of my studies. I am very thankful to Dr. Azadi's and Trina Abney for their tremendous help with the use of high pH anion exchange chromatography (HPAEC-PAD). Without their assistance, I would not have completed all of my studies.

My educational matriculation has been an exceptional journey- one that I had never dreamed of. I am very grateful to place where my foundation was laid, my Alma Mater- Jackson State University (JSU) and the life-long lessons learned there. Here is where I earned my B.S in Chemistry (1999) under the most awesome faculty and staff anyone can imagine- Drs. Ken Lee, Booker Spurlock, Eric Noe, Margaret Wodetzski, Jeffery D. Zubkowski, Bennye Henderson, Yiming Liu, Ms. Sandra Hall and Ms. Maxine Simpson. At JSU research career began under Hongtao Yu, Ph.D. He wanted compensate me financially but was unable initially. I think much to his amazement; I was not worried about being paid to do research. I just had a desire to learn via the hands on approach, in the laboratory environment. A few months after beginning a research project, he acquired a slot for me in the National Institute of General Medical Sciences, Minority Biomedical Research Support (MBRS) program at JSU. To Dr. Yu, I am deeply indebted for his encouragement, advice, mentoring, and research support throughout my undergraduate and graduate studies. I also truly appreciate his patience and tolerance, such as when the lab flooded during one of my overnight experiments.

Words alone can express the thanks I owe to my immediate and extended family. My family has been the ultimate influence on my life. From the imparted wisdom of my late great-uncle Wesley to the friendship of my cousins such as LaTongya, Starlette and others whom been by my side physically and in spirit always. To my immediate family I owe much gratitude. I would be nothing without the support of my parents Lavern and Solomon Sr.; siblings- Tamika, Logan, and Alicia (Yogi); aunts and uncle- Regina, Tracey, Sylvester Jr., Jean, Greg, Malinda, Hattie, Della Lee, the late Wesley, Ethel, Lucy, Rosie, Leola, Elnora, Leroy, Henry, Peyton, Jr., Robert, Berthard, Elex, the late Arthur Lee, Cornelius and Clarence as well my numerous cousins. I must also express thanks to my wife Malaika D. Amedee-Garner, her family and my extended family- the Fletcher and Amedee

clans. I also extend many expressions of thanks to the Watson family... and to my Baker's Grove M.B. Church family... Special thanks to my cousin Victoria and her husband Yusuf whom opened their doors, supported and mentored me throughout my years at the University of Georgia. As a couple, they have provided me an excellent example or that old paraphrased saying "...live and show yourselves to be a model after which others may follow in a positive light that uplifts mankind..." Finally, for the people whom made that have impacted my life and/or graduate student experience and/or to I theirs, I am grateful to have met each of you- Willie J. Watson, Jr., Kevin Dudley, Tamara Watson, Roosevelt "Andre" Davis, James "Jimmy" Brooks, Cory Watts, Terry Stovall, "The Chemaholics", Elvis Jones, Guyla Johnson, Kiesha D. Wright, Otis Horton, Robert A. Rhymes, Chico Knight, Tomekia Logan, Lakiesha Bland, Terri Lynn Robinson, Zakiya S. Wilson, Toria Crawford, Tiffany Turner, Carlos Butler, Roderick Mosley, Veronica Stewart, Chris, Dedra Sims, Ramona Heard, Tina Steele, Sumona V. Smith, Christopher Harvey and others who I have not mentioned. Special thanks should be given to my colleagues from the Department of Pharmaceutical and Biomedical Sciences as well as the persons I've had the awesome opportunity serve with in the leadership of student run organizations such as the National Black Graduate Student Association, Inc., the Graduate and Professional Scholars (GAPS) and Graduate Student Association (GSA). These person are Jay Houston, Kimberly Hill, Babatunde Olubajob, Monica Grandison, Chakita K. Williams, Sandra Granade, Jarrod Collier, Mervin Williams, Tamara Bertrand, Adrienne Dixon, Carey Hines, Summer Lewis, Carey Hines, Oscar Solis, Jarrett Ngemi-Landor, Johnetta Farrar, Neal and Emily Ware, Casey Bethel, Kevin and Shamita Williams, Bridg'ette Johnson, Katrina Landau, George Felis, Chandra Brown, Frank E. Marley, III, Dwayne Wright, Renita Ward, Tiffany Adams, Eric M. Bridges, J. Steven Cooperwood, Curtis Byrd, Giuseppe Gumina, Sureyya Olgen, Yun Ho Jin, Yongseok Choi, Terrell Neal, Lou Edward Matthews, Adrienne Dixon, Akesha Horton, Tamara Bertrand, Oscar Solis, Ted Bunch, Kmt Shockley and to others I may have excluded..

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CHAPTER I

PHARMACEUTICAL PREFORMULATION OVERVIEW AND CURRENT FDA FOCUS

Introduction

As a preface to this research it was important to look at the challenges facing industry during new product development. Thus, we attempted to familiarize our challenge with current issues related to preformulation. Whereas typically, preformulation investigations are designed to identify physicochemical, physico-mechanical and biopharmaceutical properties of drug substances, excipients and packaging materials which may influence: 1) formulation design, 2) method of manufacture of drug substance and drug product; 3) pharmacokinetic/biopharmaceutic properties of the resulting product, and 4) packaging of the product. [1, 2] Formal and experimental preformulation process stages are the following. [1, 2]:

- **Solubility** (aqueous, pK_a , log P, log D)
- **Molecular optimisations** (salt, hydrate, solvate, new analogues, etc.)
- **Crystal Engineering** (polymorph, habit, size, surface characteristics)
- **Crystal structure determination**
- **Biopharmaceutical classification (BCS)** (solubility, dissolution, absorption)
- **Complete Physical and Chemical Characterisation**
- **Drug stability evaluation** (physical, chemical, solution phase, solid phase)
- **Compatibility analyses** (drug substance, excipient, packaging materials)
- **Technical characterisation** (flowability, compactability, etc)

Contemporary preliminary preformulation studies in industry typically begin with the calculation/prediction 'in silico' of many of the important physicochemical characteristics of the new chemical entity (NCE). The studies characteristically include studies of the NCE's solubility [2], polymorphism [3] and morphology of the particles [4, 5]. Literature searches of similar or related compounds provide understandings of possible degradation processes, adverse conditions relevant to the drug, bioavailability, pharmacokinetics and formulation of similar or related compounds. [1-4] This allows for careful consideration of the number of experiments to be performed and the stages at which to perform them in developing a preformulation master plan.

Preformulation starts when 1) a newly synthesised drug shows pharmacological evidence that requires further evaluation in humans, 2) formulation and dosage form changes are required and 3) dosage changes of a delivery system (DS) are required. The end goal is to produce innovative, stable, safe, cost-effective dosage forms capable of delivering the substance for its intended use. This is achieved by producing sufficient information, especially physicochemical data, on a proposed entity, excipients and packaging materials. Preformulation studies give 1) directions for development of formulation in choice of drug form, excipients, composition, physical structure; 2) helps in adjustment of pharmacokinetic and biopharmaceutical properties; 3) support for process development of drug substance (e.g. yield, filtration) and industrialization of product (e.g. compactability, flowability), and 4) produce necessary and useful data for development of analytical methods. The strategic points are that we gain an understanding associated with the behaviour and physicochemical properties of the drug substances, excipients and packaging materials. This may assist in preventing generic competition by patenting molecule modifications, crystal modifications, formulations and physical structures. It would also shorten the time following drug development processes and improve know-how of strategic NCEs towards the preformulation studies support of a product's life cycle management. [1-6]

Current Focus

The FDA produced draft guidance [7] in 2002 for industry in entitled “Guidance for Industry: *Process Analytical Technology (PAT)- A Framework for Innovative Pharmaceutical Development, Manufacturing and Quality Assurance*”. The FDA’s mission was to develop rational, science-based requirements for drug substances and excipients, which are appropriate to a specific quality and performance objective. The guidance explains a science-based, risk-based framework to support innovation and efficiency in pharmaceutical development, manufacturing, and quality assurance. This framework was founded on process understanding, with the goal of facilitating innovation and risk-based regulatory decisions by industry and the FDA to develop more flexible protocols. The expectation is that industry will make strides towards implementing more robust technology, thus reducing waste reduction from batch to batch discrepancies and reducing the need for recalls or plant shutdowns. Working with existing regulations, this guidance described a regulatory approach that would enable the agency and the pharmaceutical industry to address technical and regulatory issues and questions anticipated during the implementation of PAT.

Discussions were held amongst the FDA, United States Pharmacopoeia (USP) (7) and the American Association of Pharmaceutical Sciences (AAPS) Preformulation Focus Group. These dialogues led to distinct directives in these areas:

1) Bulk Substance Characterization: Investigate critical physicochemical factors that assure identity, purity and stability of drug substances at the preformulation stage, both for new chemical entities, and for drug substances with proposed synthetic, purification, stereochemical or other changes, as well as changes in analytical methodology used to assess such factors. Also, investigate properties of bulk drug substances, which demonstrate "sameness", formulatability, and eventual product performance and quality (e.g., particle size, polymorph, hydrate/solvate, crystal habit, impurity profile).

- 2) Excipients and Vehicles: Characterize excipient and vehicle functionality and possible pharmacological properties of excipients and vehicles, which may impact product quality and performance.
- 3) Chiral Chemistry: Investigation of the mechanisms of chiral separations and their application to the control of chiral drug substances and drug products.
- 4) Outcomes and Impact: Completion of program objectives will strengthen the scientific foundation of the “Guidance for Industry BACPAC I: Intermediates in Drug Substance Synthesis Bulk Actives Postapproval Changes: Chemistry, Manufacturing, and Controls Documentation” (BACPAC) (9) by providing regulatory relief and conserve resources in the drug development and evaluation process, improve public safety standards, enhance, product quality and facilitate the implementation of new technologies as well as facilitate policy development and regulatory decision making.

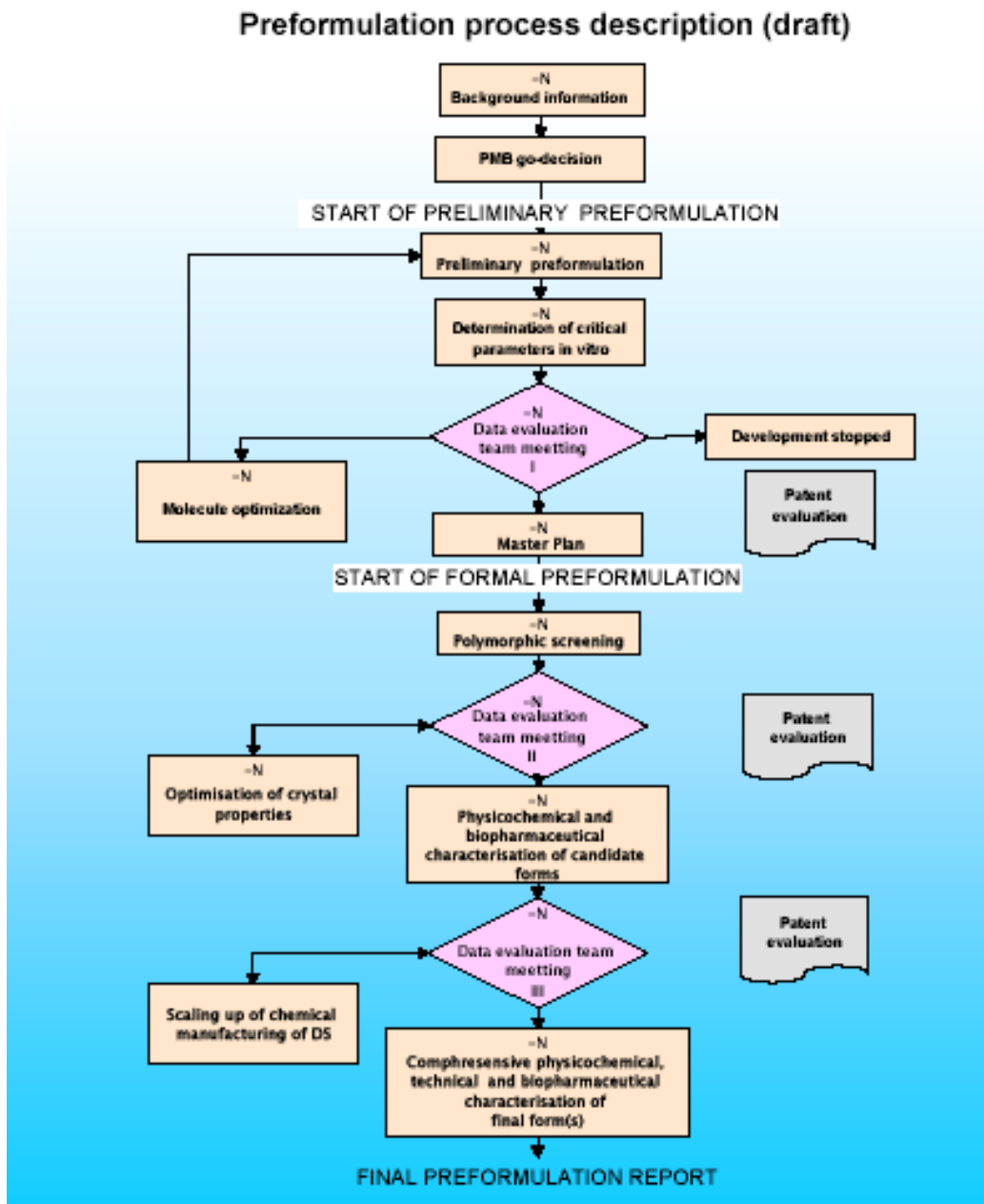
In of September 2004, the FDA published the finalized “Guidance for Industry: *Process Analytical Technology* (PAT)- A Framework for Innovative Pharmaceutical Development, Manufacturing and Quality Assurance” (10). This is a non-binding guidance that is a science-based, risk-based framework to support innovation and efficiency in pharmaceutical development, manufacturing, and quality assurance. Orion Pharma is an example of how industry is beginning to implement this guidance. Working with existing regulations, they have derived a system shown in **Chart I.1** below to address technical and regulatory issues before the implementation of the PAT.

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Chart I.1: An example of Orion Pharma’s draft structure towards implementing Guidance for Industry: PAT — A Framework for Innovative Pharmaceutical Development, Manufacturing, and Quality Assurance.



CHAPTER II

PERSPECTIVES TOWARDS POTENTIAL OSTEOARTHRITIS THERAPEUTICS

Introduction

Osteoarthritis (OA) is a debilitating, incurable joint disease caused by the deterioration of joint cartilage. . It is one of the most symptomatic medical problems for middle aged and older people. [1-3] Symptoms experienced by OA victims include localized pain, inflammation, decreased mobility, and joint deformity. In addition, OA patients must change and carefully manage their lifestyles to prevent further joint deterioration. The Centers for Disease Control reports (CDC) arthritis diseases are the #1 **[Figure II.1]** disability in the United States today, affecting more persons than cancer, diabetes, or heart disease. It affects more than 20 million people and is accountable for more than 7 million doctor visits in the United States. [1, 2] OA results from the breakdown and eventual loss of cartilage. [1, 2] The Arthritis Foundation, headquartered in Atlanta, Georgia, reported that over 21 million Americans are affected by OA in 2002 that spent over \$6 billion on products and services to treat their OA. [2] **[Figure II.2]**

Osteoarthritis therapy, beyond the non-prescription drugs available e.g. acetaminophen, NSAIDS such as ibuprofen etc. and prescription medications like Celebrex® (generic name celecoxib) in the United States; has recently turned to oral nutraceutical glycosaminoglycan products such as glucosamine, chondroitin, and n-acetylglucosamine (NAG). The use of glucosamine became popular after being featured in the book, The Arthritis Cure by Jason Theodasakis, MD. [4] Currently, glucosamine and its metabolites are not classified as drugs but as nutraceutical/dietary supplements under United States Food and Drug Administration's Dietary Supplement Health and

Education Act of 1994 (DSHEA). [5] Oral dosage formulations of N-acetyl-D-glucosamine (NAG) and its parent compound glucosamine in salt form (sulfate, hydrochloride, etc.) are commercially available nutraceuticals, and are commonly administered in conjunction with chondroitin sulfate, also a readily available nutraceutical. Glucosamine and chondroitin have been reported effective in the oral treatment of osteoarthritis but have not undergone the rigorous studies needed for FDA approval as pharmaceuticals. The National Institutes of Health (Bethesda, MD, USA) has an ongoing multi-center study- GAIT (Glucosamine/Chondroitin Arthritis Intervention Trial) that is currently evaluating the efficacy of orally, administered glucosamine and chondroitin oral supplements. The GAIT study results are to be announced during the fall of 2005.

Osteoarthritis

Osteoarthritis is a slowly progressive degenerative disease caused when cartilage deteriorates or is damaged. It is characterized by cartilage breakdown that causes bones to rub against each other, producing pain, swelling, and loss of movement. [1, 2] Part of the problem is that the concentration of hyaluronic acid declines within the joints. [1, 7] Characteristics of osteoarthritis cartilage also include an increase in the extracellular matrix, including alteration of the proteoglycans.[8] Bits of bone or cartilage can also break and float inside the joint space causing even more pain and inflammation. [1] The joint cartilage deteriorates and the formation of bone spurs (osteophytes) form.[9, 10] Joints are designed to protect bone ends from wearing away and to absorb shock from movement like walking or other repetitive movements. **[Figure II.3]** Joints are composed of cartilage, joint capsule, synovium, synovial fluid, muscles, ligaments, and tendons. Cartilage is composed of a complex extracellular matrix of collagen and elastic fibers within a hydrated gel of glycosaminoglycans (GAG) and proteoglycans. This specialized network is stabilized by means of intermolecular cross-links. These structures harness the swelling pressure exerted by the high

concentration of negatively charged aggregates. The network accounts for more than 98% of the articular cartilage volume and cellular components constitute the remaining 2%. Proteoglycans are large macromolecules consisting of multiple chains of GAGs and oligosaccharides attached to a central protein core. Proteoglycans provide a framework for collagen and also bind water and cations, forming a viscous, elastic layer that lubricates and protects cartilage. The GAGs that are most common in human connective tissue include keratin sulfate, dermatan sulfate, heparin sulfate, chondroitin sulfate, and hyaluronic acid. They consist mostly of amino sugars, which are repeating disaccharide units composed of hexuronic acid (D-galactosamine). [8] The joint capsule is a tough membrane sac that contains a thin membrane of synovium. Synovial fluid lubricates the joint and keeps the cartilage smooth and healthy. Together muscles and connective tissues keep bones stable and allow the joint to bend and move. Ligaments connect one bone to another and tendons connect muscle to bones. [1]

Pharmacological Management

The traditional pharmacological management of OA has been primarily via NSAIDS and other ethical pharmaceuticals that treat the associated pain and not OA's root cause. [11] Marketed ethical pharmaceuticals have major drawback in their use as long-term therapeutics. For example the FDA issued "black-box" warnings and product label changes for the drug Bextra® (valdecoxib). Pharmacia and Pfizer, in full cooperation with the FDA, sent letters on November 13, 2002, to thousands of physicians in an effort to alert them to the new warnings. Classified also as a COX-2 inhibitor, Bextra® was approved by the FDA on November 16, 2001, and was marketed in March, 2002, with an indication for relief of the signs and symptoms of osteoarthritis and adult rheumatoid arthritis, as well as treatment of primary dysmenorrhea. From post marketing experience, the FDA reportedly received about 20 reports of serious reactions which include the skin diseases: Stevens-

Johnson syndrome, toxic epidermal necrolysis, exfoliative dermatitis, erythema multiforme as well as hypersensitivity reaction, anaphylactic reactions and angioedema. The adverse reactions are considered rare, but some patients did require hospitalization. Since the reactions can be life threatening, it has been recommended that people who start Bextra® and develop a rash should discontinue the drug immediately. Since the reported adverse reactions occurred in patients with and without a history of allergic-type reactions to sulfonamides, the FDA has stated that Bextra® should not be used by patients with a known allergy to sulfa or sulfa-containing drugs.

More recently as of September 30, 2004 the FDA recognized Merck & Company's voluntary withdrawal of its product Vioxx® (chemical name rofecoxib), a non-steroidal anti-inflammatory drug (NSAID). There were great claims made upon the development of COX-2 selective NSAIDS such as Vioxx®, owing to a reported reduction the rate of gastrointestinal (GI) ulcers and bleeding. On the other hand adverse drug reactions (ADRs) such as increased risk of colon polyps, cardiovascular events, heart attacks and stroke were seen in comparison of Vioxx® to naproxen in Merck's VIGOR (Vioxx Gastrointestinal Outcomes Research) study. Generally medical treatment is based on symptomatic treatment, whereas OA's etiology is not fully understood towards producing more effective therapies. [12] As of 2005's first quarter, a FDA expert panel endorsed the use of Vioxx® but recommend the utilization of more patient counseling and a significant reduction in overt marketing.

The most common therapies used to treat osteoarthritis include acetaminophen, corticosteroids, and NSAIDS as well as topical pain relieving creams, rubs, and sprays. Acetaminophen (Tylenol) is a non-anti-inflammatory pain reliever that relieves mild pain. [1, 2] Adverse effects associated with acetaminophen include rashes and hepatotoxicity. [3] Patients with ulcers, asthma, kidney, or liver disease may not be able to safely take this type of medication. [11] Corticosteroids are powerful anti-inflammatory hormones that are typically injected into affected

joints to relieve pain. This is a short-term measure of treatment, not recommended for more than two or three times per year. [1] Complications of corticosteroids injections include infection, cutaneous atrophy, and acceleration of joint degeneration. [3] NSAIDS work to fight inflammation and temporarily relieve pain [1], but cause gastrointestinal events and adverse drug reactions with long-term use. [13] It has also been found that NSAIDS can slow the rate of proteoglycan and glycosaminoglycan synthesis, potentially exacerbating the loss of cartilage and aggravating the disease for which they are meant to treat. [14]

In studies that compared glucosamine and chondroitin sulfate to NSAIDS, patients who received glucosamine and chondroitin sulfate exhibited long-term reductions in pain and the overall responses it gave after treatment lasted longer. The patients treated with the NSAID showed a prompt reduction of clinical symptoms; however, symptoms reappeared quickly after the discontinuation of treatment. [15] Hyaluronic acid, an intra-articularly injected therapy, provides relief temporarily and works to restore joint levels of hyaluronic acid. [1, 7] Hyaluronic acid produced by the synovium is the major organic constituent of synovial fluid and is primarily responsible for its high viscosity and its protective lubricating and shock-absorbing properties. Although synovial fluid volume tends to be increased in osteoarthritis, its viscosity is diminished, owing primarily to a reduction in both the concentration and molecular weight of hyaluronic acid. [16] Glucosamine offers hope to OA sufferers because it has been shown in a number of scientific studies to alleviate both the pain and inflammation.

Glucosamine and/or Chondroitin: Building Blocks?

Glucosamine salts and chondroitin sulfates are agents marketed as nutritional supplements in the United States, that are considered to have beneficial effects on cartilage. [20] These agents are widely utilized for the OA treatment of the knee, hip, and hands; but the degree of their

effectiveness is unclear. It is hypothesized that these compounds produce glycosaminoglycan (GAGs) that inhibits degradative enzymes, up regulate cartilage metabolism and proteoglycan matrix production. [6]. There are many factors that contribute to osteoarthritis. Age is definitely a risk factor. Obesity is also a risk factor that might lead to osteoarthritis of the knees. People with joint injuries caused by sports, work related activities or accidents might be at an increased risk of developing osteoarthritis. Genetics, as well, play a role in the risk factor of developing osteoarthritis, mainly in the hands. However, some persons are born with defective cartilage or with slight joint defects. As a person ages, these defects may cause early cartilage breakdown in the joint. [2]. The treatment plans for osteoarthritis comprise exercising, rest and joint care, pain relief, weight control, medications, surgery, and nontraditional methods. The objective of these treatment plans are to alleviate pain, maintain or enhance joint mobility, increase strength of the joints, and reduce the disabling affects of the disease. [18]

Europeans have used Glucosamine for more than thirty years osteoarthritis dating back to the 1970s, as a therapeutic agent for. [13, 16] Hence, glucosamine–sulfate is approved as a prescription drug in some European countries. [17] In vitro experiments have shown that the administration of glucosamine sulfate to human chondrocytes in tissue culture leads to its incorporation into GAG composition as well as to the activation of core-protein synthesis, thus promoting proteoglycan production. [8]

Glucosamine, which is formed in the body as glucosamine 6-phosphate as shown in **Scheme II.1**, is the most fundamental building block required for the biosynthesis of compounds such as glycolipids, lipoproteins, GAG, hyaluronates and proteoglycans. Glucosamine is a normal constituent in cartilage matrix and synovial fluid. [19] The formation of galactosamine, N-acetylglucosamine, and chondroitin sulfate all require glucosamine 6-phosphate. Keratan sulfate and hyaluronates play a central role in cartilaginous matrix assembly and are essential for maintaining the

structural and functional integrity of articular cartilage. Hyaluronic acid, the backbone of proteoglycans, requires glucosamine 6-phosphate for its synthesis. [15] Glucosamine has no known toxicity at high dose levels. An example of glucosamine's pharmacokinetic profile via a chart depicting its oral bioavailability is shown in **Figure II.4**. Whereas excretion is primarily via urine and feces, with 87% orally administered glucosamine being absorbed. [23] The general central hypothesis is that glucosamine bypasses the rate-limiting metabolic block in the conversion of glucose to glucosamine and stimulates the biosynthesis of the GAGs and the hyaluronic acid backbone used in the formation of the proteoglycans found in the structural matrix. [14, 15]

Chondroitin is primarily manufactured from shark and bovine cartilage and regulated by federal drug agencies in Europe but not in the United States. [17] For over two decades chondroitin sulfate injection, known as Arteparon, has been used in Europe as veterinary medicine for the treatment of degenerative joint disease. Studies, which include humans, showed that the use of Arteparon effectively relieved osteoarthritis of hip joints. [15] In vitro studies have shown that chondroitin sulfate inhibits enzymes that break down cartilage. [19] As a long chain polymer of a repeating disaccharide unit of galactosamine sulfate and glucuronic acid, chondroitin is the predominant glycosaminoglycan (GAG) found in articular cartilage. [15,19] Different from glucosamine, chondroitin sulfate is thought to stimulate GAG and proteoglycan synthesis by extracellular as well as intracellular mechanisms. Chondroitin sulfate as the major constituent of cartilage, provides structure, holds water and nutrients and allows molecules to travel throughout cartilage. This is an important property because there is no blood supply to cartilage. [20] Chondroitin sulfate, by virtue of its long chain length, competitively inhibits degradative enzymes of proteoglycans in cartilage and synovial fluid. In degenerative joint disease, there is a loss of chondroitin sulfate as the cartilage erodes. [16] Chondroitin sulfate has been shown to be effective in reducing the symptoms of degenerative joint disease in many clinical studies and in many

randomized, double-blind, controlled clinical trials. [20-23] The metabolic fate of orally administered chondroitin sulfate is still unknown, but it is hypothesized that chondroitin molecules break down in the gut into biochemical components that enter the bloodstream and joints. [23, 24]

Glucosamine and Chondroitin: Action

Although glucosamine and chondroitin have not been approved by the FDA in the United States, the combination of glucosamine and chondroitin sulfate has been used in veterinary medicine for about four years in the United States to treat degenerative joint disease with favorable results and no side effects. [20] Most data provided concerning glucosamine and chondroitin is from studies performed in Europe and Asia. These studies have examined the effects of glucosamine and chondroitin sulfate on mild-to-moderate osteoarthritis. Several human trials show promising reduction in pain with the use of glucosamine and chondroitin. [20] One recently published study examined a combination of glucosamine hydrochloride, chondroitin sulfate, and manganese ascorbate in 93 patients with knee osteoarthritis. The effect size in this study on function, a value of 0.98 (WOMAC subscale) was considerably high. [17] Hanson et. al. [20c] investigated the effect of administration of a glucosamine chondroitin sulfate compound in horses with degenerative joint disease. Within two weeks from the start of administration, the lameness grade, flexion test, and stride length were significantly improved. Most available clinical data is difficult to interpret due to serious deficiencies in the study design. Many of the studies were short, included relatively few patients, or had methodological flaws. [20, 21] Because of this more studies are needed to examine the mechanism of action of glucosamine and chondroitin sulfate in combination and the long-term beneficial effects that these compounds have on joint structure and function. [21, 23] It is hypothesized that glucosamine and chondroitin sulfate work together in a synergistic, rather than an additive effect. Together they work to form GAGs, which inhibit degradative enzymes initiating up

regulation of cartilage metabolism and matrix production. [20] In addition chondroitin sulfate possesses extracellular and anti-inflammatory properties not found in glucosamine. [8, 21] Correspondingly glucosamine is a fundamental component of skin, tissue and cartilage. Its biochemical pathway also shown in **Scheme II.1** begins with glucose and is completed with the chain elongated glycosamino products of sialic acid, keratin sulfate, hyaluronates, dermatan sulfate and chondroitin sulfate. These products along with proteins to form proteoglycans, which are the formal components of skin, tissue and cartilage and well as synovial fluids. [25, 26]

Glucosamine Focus

Though glucosamine and its analogues therapeutic benefits are poorly understood [27], exogenous glucosamine may serve in an anti-inflammatory capacity by reducing joint swelling and pain levels comparable to that observed via NSAID usage.[28-30] Researchers have found that in degenerating cartilage, pro-inflammatory cytokines such as IL-1 β and TNF α are associated with an increased degradation of cartilage matrix. [31, 34] These events are also correlated with the reduction in cartilage matrix gene expression and synthesis in vitro. [35, 36] Some conclude that exogenous glucosamine counteracts the degenerative effects that IL-1 β has on proteoglycan synthesis.[31, 35]. Whereas exogenous glucosamine reduces nitric oxide production induced by IL-1 β and TNF α [36] and suppresses the synthesis of cyclooxygenase-2 by human chondrocytes in response to IL-1 β . [37]

In conclusion, exogenous glucosamine is considered to promote glycosaminoglycan synthesis towards the production of proteoglycans by avoiding the rate-limiting steps of its conversion from glucose to glucosamine and ultimately to N-acetyl-D-glucosamine (NAG) by glutamine (fructose-6-phosphate amidotransferase). [38] Since glucose and glucosamine are the other substrates of glucokinase [39], the phosphorylated glucosamine product, glucosamine-6-phosphate inhibits glucokinase and alters both glucose and subsequent glucosamine metabolism. [40] Miwa et

al. reported that glucokinase has a low affinity for NAG. [41] Thus, NAG kinase mediates the phosphorylation of NAG to produce NAG-6-phosphate that does not affect glucokinase activity. [42] Therefore, concluding that NAG-6-phosphate does not affect glucokinase activity thus allowing glucose and glucosamine to proceed unrestricted through metabolism.. The biosynthesis of glycosaminoglycans from this perspective would be better promoted with the use of NAG or some other rate-limiting glucosamine analogue rather than by glucosamine. [36]

Therapeutic Horizons

There are delivery perspectives towards therapeutic horizons derived as this dissertations major content which are: I) *Model Mutual Prodrugs- Synthesis and Basic Physicochemical Characterization Towards Preformulation Development* and II) *N-Acetyl Glucosamine Preformulation Study Towards its Development as Transdermally Delivered and/or Percutaneous Absorbed Agent (Drug)*.

The topic, *Model Mutual Prodrugs- Synthesis and Basic Physicochemical Characterization Towards Preformulation Development*, is centered at the interdisciplinary interface of pharmaceutical preformulation. In perspective, the synthesis of a glucosamine molecule has been covalently bound to an NSAID presenting a mutual prodrug. This based on the old idea of concomitant delivery of two or more drugs to offset the non-therapeutic side effects of either drug. The objective was attained via the synthesis of two compounds as models aimed towards the treatment of osteoarthritis. The synthesis and basic physicochemical characteristic of these new chemical entities have been described from physical and 'in silico' methods.

The topic, *N-Acetyl Glucosamine Preformulation Study Towards its Development as Transdermally Delivered and/or Percutaneous Absorbed Agent (Drug)*, was arrived at from the challenge of attaining a simple solution to transdermally deliver osteoarthritis therapies via the means of using glucosamine and/or chondroitin. The most difficult challenge was to utilize these compounds in a transdermal

delivery and/or percutaneous delivery system. Tasks were completed to arrive at the conclusion of identifying NAG as the glucosamine product that our studies utilized. Correspondingly, a basis towards further studies has been established. Our studies may lead to a product(s) with commercialization potential in light of its timing in relationship to the recent NSAID catastrophes as a potential alternative osteoarthritis therapeutic and the GAIT study to which the NIH has recently ended a bioavailability study enrollment period.

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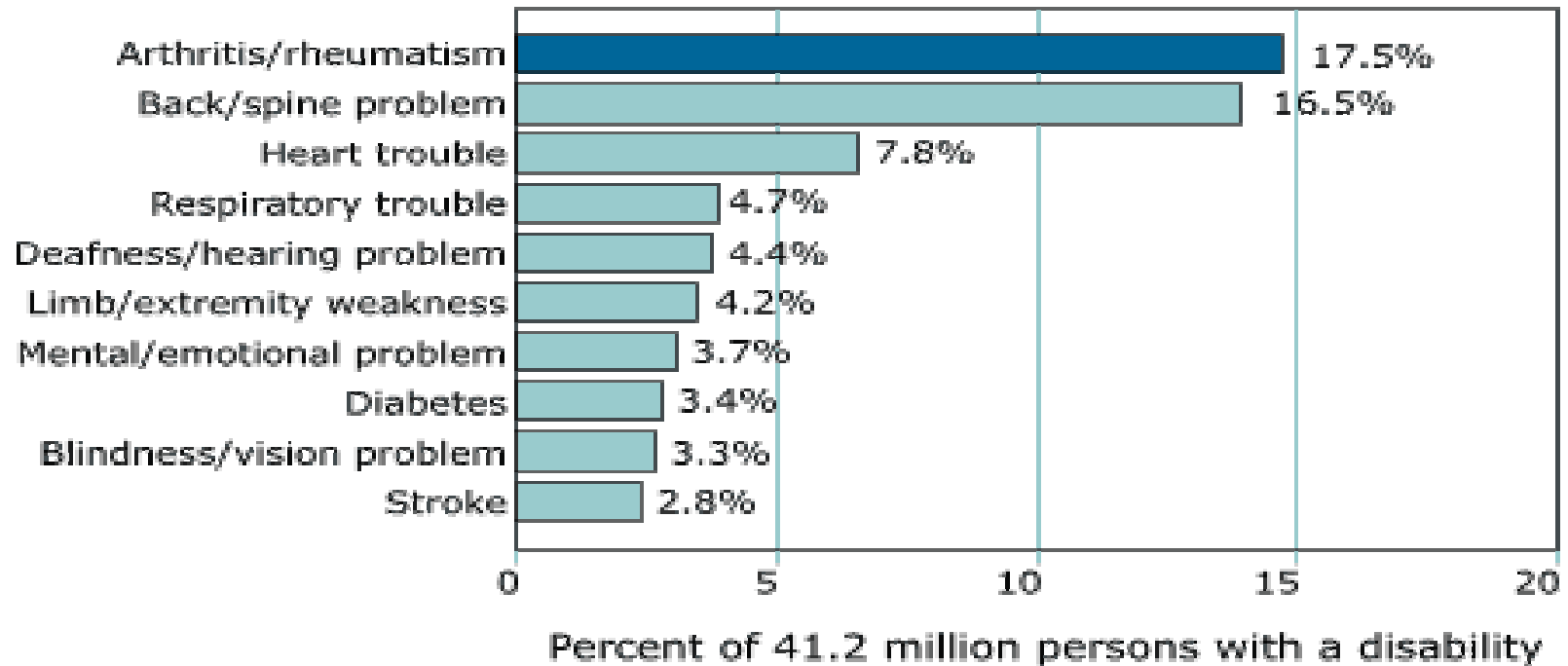


Figure II.1: The graph above is courtesy of *Source: Centers for Disease Control and Prevention (www.cdc.gov/nccdphp/arthritis/index.htm)*

Catastrophic Effects

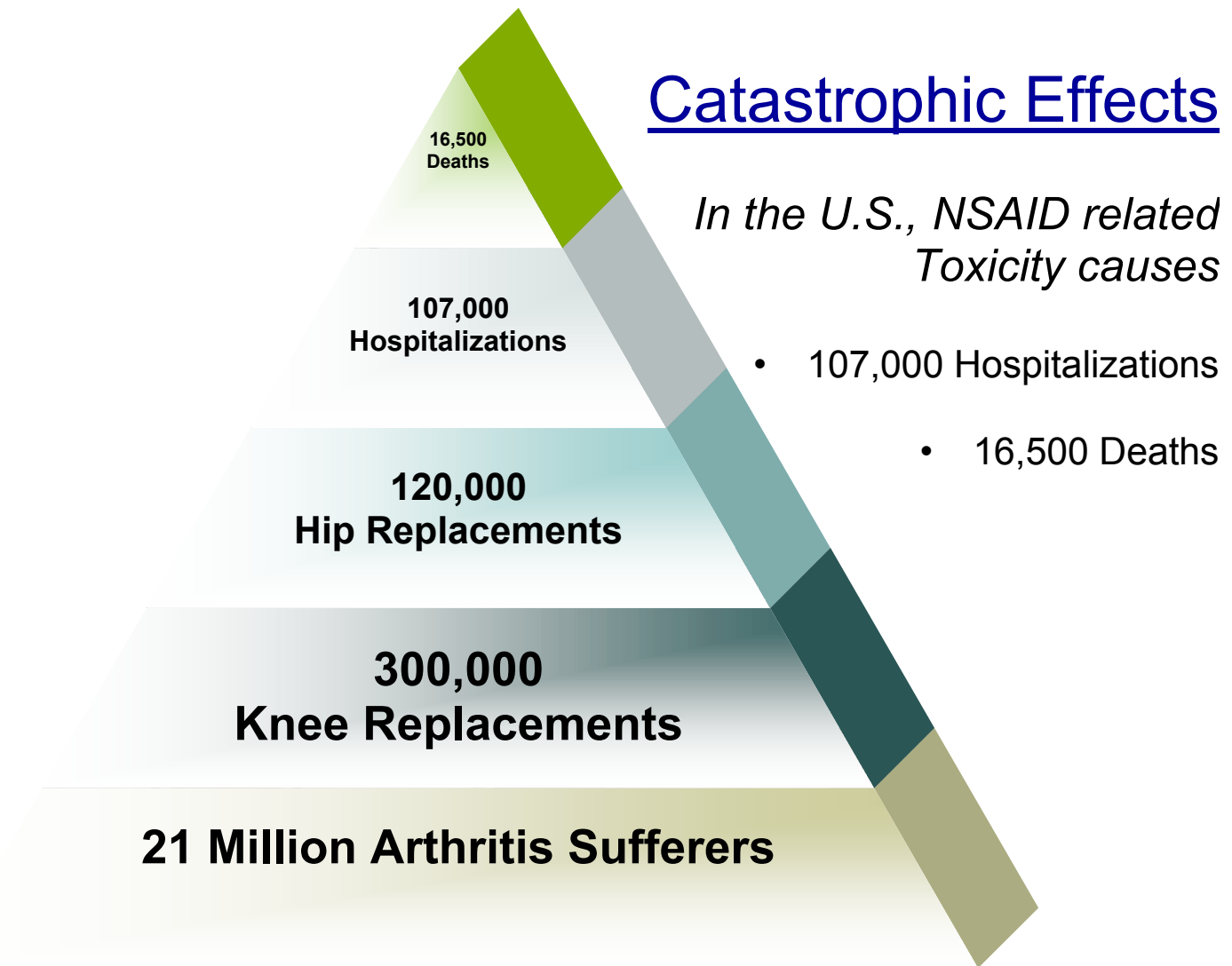


Figure II.2: The chart above gives general breakdown of arthritis sufferers. *Data source: Wolfe, M., N Engl J Med; 340:1888-1899, Jun 17, 1999*

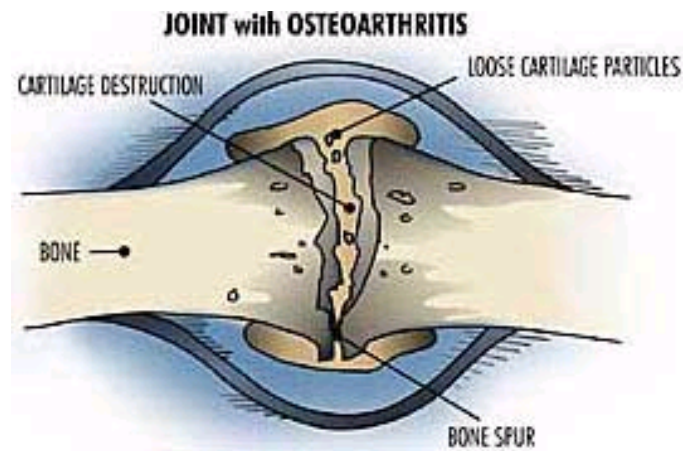
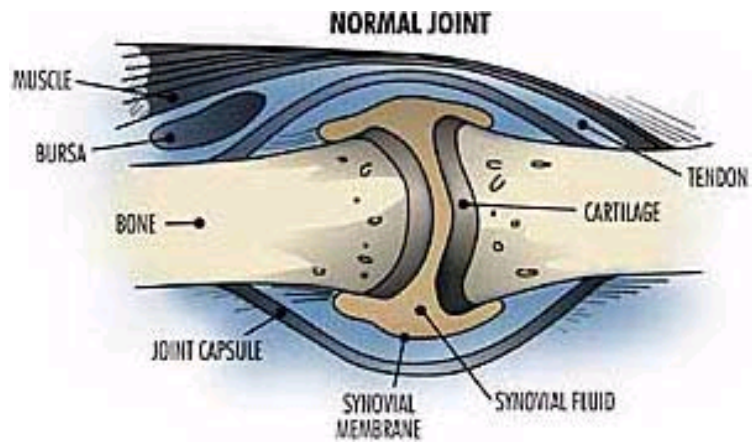
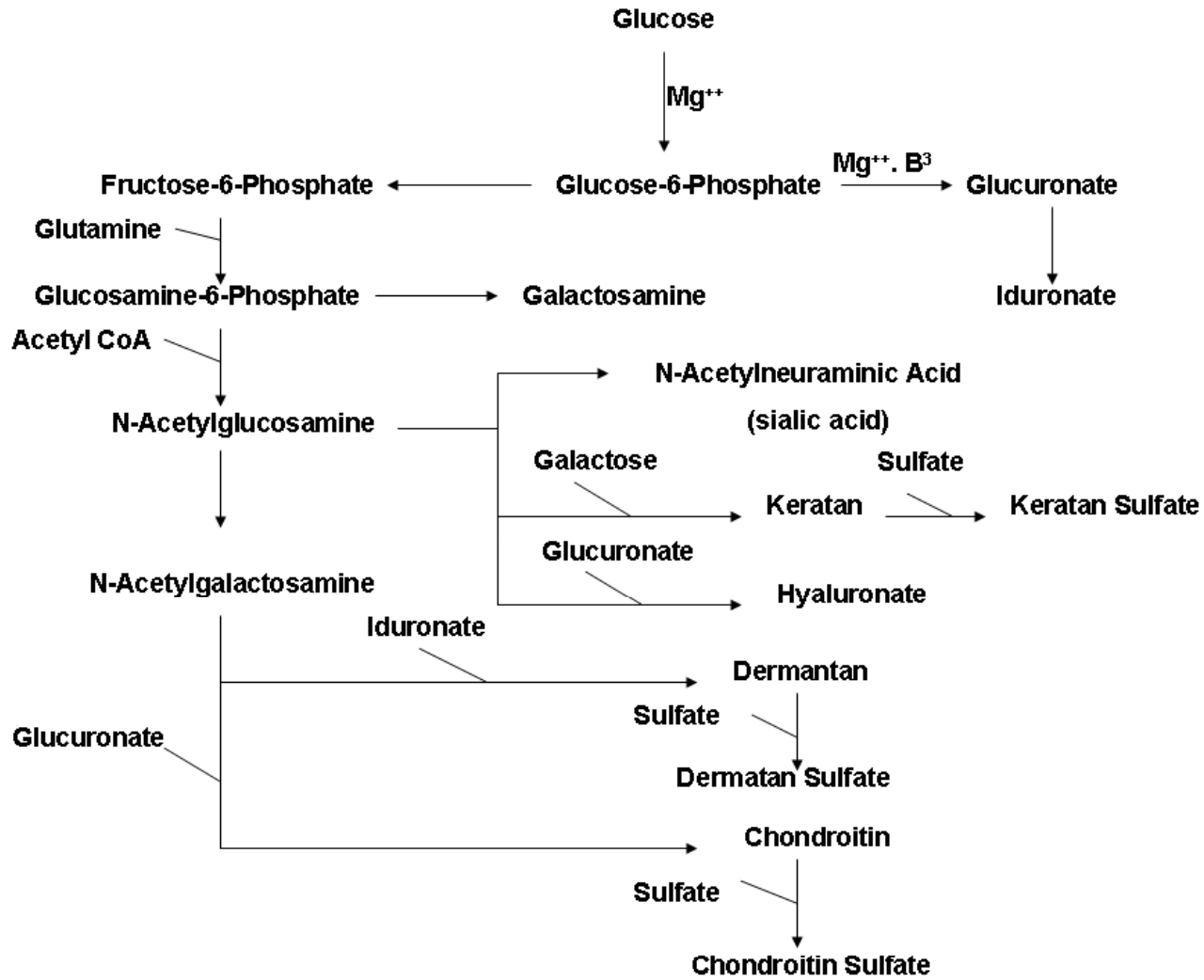


Figure II.2. Shown are the schematics and x-rays of healthy and osteoarthritic joints.

Scheme II.1. Glucosamine's biochemical pathway beginning with Glucose



OA Sufferer ingests Glucosamine supplement orally

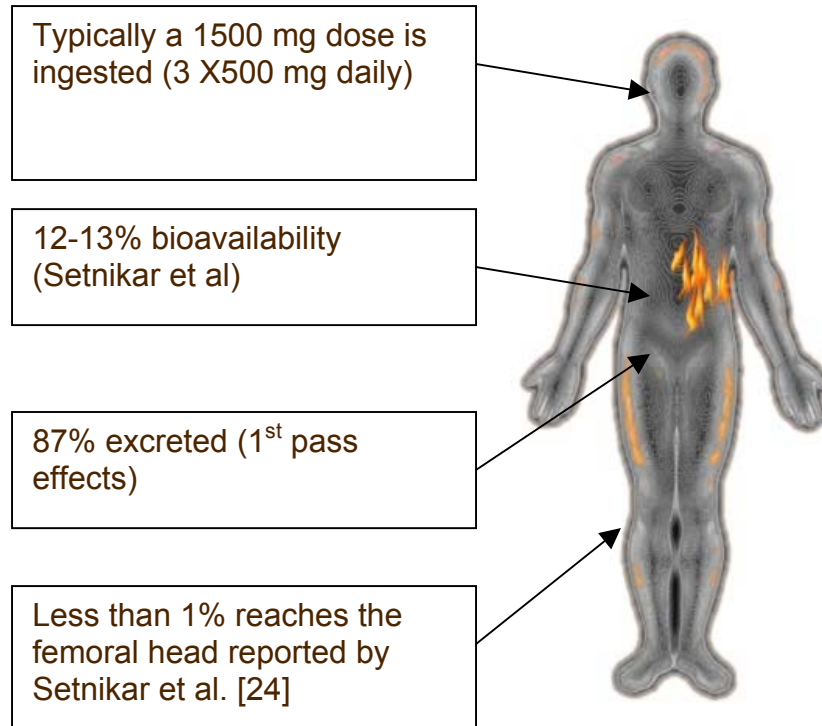


Figure II.4. The diagram above shows the general pharmacokinetic fate of orally ingested glucosamine.

CHAPTER III
MODEL MUTUAL PRODRUGS- SYNTHESIS AND BASIC PHYSICOCHEMICAL
CHARACTERIZATION TOWARDS PREFORMULATION DEVELOPMENT¹

¹Garner, Jr. S. T. and Capomacchia, A. C. To be submitted to the Journal of Pharmaceutical

Research

Introduction

The original objectives of prodrug synthesis and development were to improve drug stability and to target drug delivery for drugs administered orally and IV. Stability is the key to drug activity; and for water and enzyme labile drugs stability is achieved by protecting the drug from chemical hydrolysis and enzyme degradation subsequent to drug administration. Targeted delivery for prodrugs is based on enhancing drug solubility and permeability and is especially important in lipid membranes in order to penetrate the very hydrophobic blood brain barrier. The most common form of prodrug utilizes an ester linkage formed synthetically through reaction of a carboxylic acid with an alcohol or phenol to modify the parent drug's *in vivo* metabolic fate. Moreover, the ester prodrug may possess other advantages like reduced side effects. For example gastric distress should be reduced if the prodrug were formulated with a nonsteroidal anti-inflammatory drug (NSAID) as compared to the NSAID alone.

Historically, a prodrug is composed of one active drug compound and a second non-active compound. In contrast, a typical mutual prodrug (MP) is composed of two active drug compounds linked by an ester linkage. [1] Ueda and Tani reintroduced the MP concept in 1990 more clearly than previous authors as a means to produce a more efficacious product. Their work is based on the same principles as for prodrug synthesis; that is, chemically bonding two drugs, usually *via* an ester linkage that is easily degraded by mammalian esterases, so that after administration each drug is released to produce its respective pharmacological action. At first, it was named a chimera prodrug, since it is composed of two parts; however mutual prodrug is a better description since both entities are active. [1] The linking of the two drugs apparently imparts a protective effect so that side effects or unwanted degradation may be prevented. This particularly important if an NSAID is one of the drugs. Also, the MPs will also exhibit different aqueous/lipid solubility profiles, which may aid in formulation and/or delivery, just as for ester prodrugs. Ueda and Tani have combined a non-

steroidal anti-inflammatory drug (NSAID) with an anti-ulcer drug, a histamine H2 antagonist, *via* an ester linkage, for oral administration. Their goal was reached in the development of a drug candidate designed to deliver an NSAID without producing stomach ulcers linked to an anti-ulcer agent as a rational attempt to offset any gastric distress caused by the NSAID. [1]

Our current research uses what appears to be a modified MP model; however, our inspiration for the work was developed independently and in ignorance of Ueda and Tani's work. It was only after an exhaustive literature search that their synthesis of an orally delivered drug agent was discovered. The initial goal was to develop a means for transdermal/percutaneous delivery of glucosamine to osteoarthritic joints with the knowledge that an oral formulation would also be a viable delivery method. Synthesis of glucosamine-NSAID drug compounds as used in historically synthesized prodrugs was employed; Ueda and Tani's work provided us with a name for our newly synthesized drug agents and justification for the validity of our approach. The two novel compounds, which are mutual prodrugs of glucosamine and an NSAID; are potentially active osteoarthritis therapeutic agents since they release both glucosamine and an NSAID following esterase cleavage of the ester bond. One novel compound covalently links ibuprofen to the amide nitrogen of glucosamine using a short chain acetyl linker; and the other compound covalently links ketoprofen to the amide nitrogen on glucosamine (compounds 6 and 9, respectively in Schemes I and II).

Background

Mutual prodrugs (MP) are best described as the conjugation of two drugs having different pharmacological activities. The concept arises from the practice of clinically co-administering two drugs in order to enhance pharmacological activity or prevent clinical side effects. [2] MPs are synthesized towards a pharmacological objective of improving each drug's efficacy, optimizing

delivery, and lowering toxicities. MPs are linked to the prodrug concept initially developed by Albert. [3] MPs follow the prodrug accordingly whereby it is converted into the active drug within the body through enzymatic and non-enzymatic reactions; and may behave as carrier-linked prodrugs; bio-precursor prodrugs, or chemical activation prodrugs. [3, 4] At the site of action, the side effects of the original drug would be masked allowing the drug to work more effectively. [3] MPs follow the same processes/methodologies as single active agent prodrugs in regard to pharmaceutical and pharmacological activity such as absorption, disposition, metabolism, and excretion. Positive characteristics associated with prodrug usage include: stable covalent ester linkage, less intrinsic activity compared to the parent drug, lower toxicity, and better release kinetics at the binding site to ensure effective drug levels; thus achieving the objective of two active drugs reaching their respective active sites to provide the desired pharmacological effects while minimizing adverse metabolic and/or toxicological events.

For many years patients have been administered mutual prodrugs as therapeutic agents before the term prodrug had been placed into the research domain, as expressed by Singh and Sharma. [5]. Advances have arisen from the production of sulphasalazine related to the modern advances in antibiotic prodrugs. Essentially, this has led to mutual prodrugs of β -lactam antibiotics and their potentiating agents to produce, for example ampicillin-mecillinam MP and ampicillin-sulbactam MP to form sultamicillin, and Dual Action Cephalosporins as well as other agents not typically referred to as MPs. [5] Mutual prodrug research is sparsely available unlike its precursor, the prodrug. One example is Estramustine® (Pharmacia, La Roche) developed in the early 1970's as an anti-neoplastic agent that shows mutual prodrug characteristics shown in **Figure 1**. [6] This has gained some attention as a prodrug research niche with topics such as the synthesis of 5-fluorouracil/cytarabine mutual prodrugs aimed towards reducing the resistance mechanisms at work in the delivery of single nucleoside drugs. [7] Researchers such as Bhosale and co-workers have

made attempts to produce mutual prodrugs of ibuprofen/paracetamol and ibuprofen/salicylamide. This work was aimed towards prodrugs of NSAIDs to reduce the associated side effects. [8] Their approach was unique from the inherent perspective of producing mutual prodrugs vis-à-vis physicochemical modifications towards simplistic NSAID delivery [8], much like sulphasalazine, currently used more so as a ulcerative colitis therapeutic consisting of sulphapyridine and 5-aminosacrylic acid covalently bound via an azo bond. [9] Examples of these mutual prodrugs are shown in **Figure 2**.

NSAID-Glucosamide MP Development

Our concept to produce NSAID-Glucosamide MPs was derived to deliver an NSAID and glucosamine concomitantly that would target osteoarthritis by either oral or transdermal administration. The goal in the former case was to produce model MPs to avoid the oral delivery effects such as adverse drug reactions (ADRs)/adverse gastrointestinal (GI) effects experienced with NSAIDs [10] and metabolism/excretion events observed with glucosamine [11]. There have been many reviews and articles concerning the ADRs/GI affects associated with NSAIDs mainly due to their long-term use. This is especially evident in osteoarthritis sufferers living with this chronic disease. [12] In pursuit of safer therapies, many patients have begun to utilize dietary supplements such as glucosamine due to its benefits. [13] Along these lines the concept of a transdermal dosage form is very attractive since almost all potential side effects are eliminated. In essence, our concept evolved as the marriage of the dietary supplement (DS), glucosamine, and an active pharmaceutical ingredient (API), NSAID, as defined by the U.S. Food and Drug Administration.

After extensive SciFinder Scholar®, Beilstein® and patent searches, much to our surprise a MP example existed in terms of synthetic outcome in the form of glucametacine. Glucametacine (*glucosamine linked indomethacin via an imido ester bond*) is the glucosamide moiety and alternative to

indomethacin to its parent drug [Figure 3]. [14] Glucametacine was developed in the 1970's and is not approved for use in the U.S., but is marketed and prescribed currently in some European, South American and Asian countries as well as Mexico under the Teorema® and other brand names. It is used as an effective orally administered anti-inflammatory agent in patients with chronic rheumatic conditions or as an analgesic, whereas few and usually mild side effects were experienced when compared to its parent moiety. [14] Glucametacine's pharmacokinetic data has been found to be unavailable. It is not promoted or indicated as a mutual prodrug to deliver both glucosamine and indomethacin via its patents or studies. We were unable to obtain glucametacine to complete our studies. So, rather than synthesizing it to determine physicochemical parameters as well as pharmacokinetic properties, the objective became to synthesize a directly and a chained –linked NSAID-glucosamide MP models.

Experimental

Materials and Methods

All reagents and solvents utilized were purchased from Fisher Scientific. TLC and preparative TLC chromatographs were performed on Analtech Co. Uniplates™. Melting points were determined on a Fisher-Johns apparatus and are uncorrected. Nuclear magnetic resonance spectra were recorded on Varian Inova 500 MHz spectrometer for ¹H NMR and ¹³C NMR with tetramethylsilane as an internal standard. Chemical shifts (δ) are reported in parts per million (ppm) and signals are reported as s (singlet), d (doublet), t (triplet), m (multiplet), or br (broad singlet). A Beckman DU-650 and a Thermo Electron Corp. Aquamate were used to record the UV spectra™. Staff at the University of Georgia's Chemical and Biological Sciences Mass Spectrometry Facility completed ESI (electrospray ionization) mass spectra. Column chromatographs were performed using silica gel >440 mesh. Differential Scanning Calorimetry (DSC) was performed on a Perkin-

Elmer DSC 7 with TAC 7/DX utilizing PYRIS Thermal Analysis System (Rev. E/March 2002) software.

Synthesis

Synthesis of the MP models were successfully completed to produce compound products **6** & **9** in **Schemes I** and **II**. Methods were explored that would preserve the carbohydrate's β -conformation, protect the hydroxy (OH) groups to allow the amine (NH₂) to undergo selective addition to produce primary intermediates **4** and **7**. Compounds **1- 3** were synthesized from procedures adapted from 1) Bergman, M., and Zervas, L., Chem. Ber. 1932, 975 2) Silva, D. J., et al. J. Org. Chem. 1999, 64, 5926-5929. (Supplemental Material) and Charviere, G. et al. J. Med. Chem. 2003, 46, 427-220 as starting materials towards compound **6**.

*Preparation of 1,3,4,6-tetra-O-acetyl-2-deoxy-2-{\{[2- (4-isobutylphenyl) propanoyl] oxy\} (phenyl) acetyl} amino}- β -D-glucopyranose (6), **Scheme I***

2-deoxy-2-amino-1, 3, 4, 6-tetra-O-acetyl- β -D-glucopyranosyl (4) Compound **3** (100 g, 0.26 mol) was titrated with triethylamine to yield a white precipitate. The precipitate was filtered and washed with CH₂Cl₂ (2x150 ml). The filtrate was dried under vacuum for 24 hrs. The organic layer was washed with brine (2x100) and dried with MgSO₄. The solvent was removed via reduced pressure rotary evaporation and product dried for 24 hrs under vacuum. Compound **4** was obtained as a white solid (87.9 g, 97% yield). ¹H NMR (d-acetone) 9.21 s, 1H), 6.15 (d, 1H), 5.38 (t, 1H), 5.08 (t, 1H), 4.31 (dd, 1H), 4.11-4.03 (m, 2H), 3.56 (t, 1H), 3.03-2.05 (dd, 6H), 2.25 (d, 3H), 2.10-2.09 (m, 3H). ¹³C NMR (d-acetone) 205.7, 170.1, 169.87, 169.39, 169.0, 95.20, 74.85, 72.28, 68.61, 61.90, 55.46, 19.98, 19.85, 19.82, 19.77. ESI for C₁₄H₂₁NO₉: FW 347 found m/z 348 [M + H]⁺ Mp 134° C

2-deoxy-2-(2-chloro-2-phenyl)acetylamino-1, 3, 4, 6-tetra-O-acetyl-β-D-glucopyranosyl (5) α-Chlorophenylacetyl chloride (20g, 0.105 mol) was added drop-wise to a stirring solution of compound 4 (29.88g, 0.105 mol), triethylamine (12.4 ml, 0.90 ml) in 50 ml CH₂Cl₂ at -10° to r.t. for 24 hours. The reaction mixture was washed with HCl (1.5 N, 2 x 7 ml), H₂O (1 x 100 ml) and brine (1 x 100 ml). The organic phase was dried with Mg₂SO₄ and solvent removed via reduced pressure rotary evaporation. The resultant syrup was crystallized with ice-cold acetonitrile and dried under vacuum for 24 hrs. Compound **5** (27.4g, 93.5%) was obtained as a white solid. ¹H NMR (d-acetone) 7.80 (s, 1H), 7.37(s, 2H), 7.25 (s, 2H), 5.79 (s, 1H), 5.33 (d, 2H), 4.90 (s, 1H), 4.09 (d, 2H), 3.95 (s, 1H), 3.84 (s, 1H), 3.17 (s, 1H) 1.87-1.64 (m, 12H). ¹³C NMR (d-acetone) 205.55, 16.88, 169.69, 169.18, 168.51, 167.69, 128.85, 128.61 (2C), 127.81 (2C) 91.03, 68.54, 61.70, 60.60 53.13, 19.73, 19.70 (2C), 19.62. ESI for C₂₂H₂₇NO₁₀: FW [M + H]⁺ 499 found *m/z* 500 [M + H⁺]. Mp >200(238)° C

1,3,4,6-tetra-O-acetyl-2-deoxy-2-[[[2-(4-isobutylphenyl)propanoyl]oxy](phenyl)acetyl]amino}-β-D-glucopyranose (6) Compound **5** (653 mg, 1.45 mmol) and α-methyl-4-[isobutyl]phenylacetic acid-Na salt in anhydrous CH₂Cl₂ (10 ml) was stirred at room temperature for 16 hours. The solution was washed with brine (2 x 10 ml) and concentrated via rotary evaporation to give **6** (763 mg, 93.5%) as a white powder. ¹H NMR (d-acetone) 7.73 (s, 1H), 7.41-7.13 (m, 9H), 5.81 (d, 2H), 5.38 (m, 1H), 5.04 (m, 1H), 4.26 (s, 2H), 4.10 (s, 1H), 3.96 (s, 2H), 2.48 (s, 2H), 2.01 (s, 6H), 1.84 (s, 6H), 1.65 (s, 1H), 1.53 (s, 3H), 0.90 (s, 6H) . ¹³C NMR (d-acetone) 205.69, 173.37, 169.61, 168.73, 168.70, 140.38, 138.43, 138.03, 129.25, 128.36, 127.31, 126.84, 91.96, 75.91, 72.54, 71.66, 68.31, 52.07, 44.61, 21.72. 19.61, 17.96: UV 203λ nm. ESI for C₃₅H₄₃NO₁₂: FW 669 found *m/z* 522 [M + H]⁺ w/loss of (C₁₁H₁₅).

Preparation of 2-deoxy-2-[2-(3-benzoylphenyl)propanoic acid]amino-β-D-glucopyranosyl (9), Scheme II

2-deoxy-2-amino-1, 3, 4, 6-tetra-O-triethylsilyl-β-D-glucopyranosyl (7) Glucosamine hydrochloride (7.32 g, 33.95 mmol) and DMAP (cat.) was stirred in 100 ml anhydrous pyridine for three hours at room temperature. Chlorotriethylsilane (20.47 g, 136.27 mmol) was added drop wise while the solution stirred on an ice bath at -5° C to room temperature for 16 hrs and at 40-45° C for 2 hrs. The pyridine was removed and the resulting oil was washed with 1:1 water and ethyl acetate and subsequently with 1:1 ethyl acetate and brine. The final organic layer was dried with Na₂SO₄ and solvent removed via rotary evaporation to give a colorless oil, which was dried overnight under vacuum, affording **8** (21.82g; 97%) as a white foam. ¹H NMR (CD₃OD): 5.39 (d, 1H), 4.83-4.80 (m, 2H), 3.89-3.47 (m, 3H), 3.09-3.01 (dd, 1H), 1.04-0.53 (m, 60H). ESI for C₃₀H₆₉NO₅Si₄: FW 635 found *m/z* 636 [M + H]⁺ and 522 [M + H]⁺ w/loss of (C₆H₁₅Si).

2-deoxy-2-[2-(3-benzoylphenyl)propanoic acid]amino-β-D-glucopyranosyl (9) Compound **7** (28 g, 43.5 mmol) and DMAP (cat) was stirred in 10 ml acetonitrile on an ice bath. In a separate vessel, (2*S*)-2-(3-benzoylphenyl)propanoic acid (11 g, 43.55 mmol) in 10 ml acetonitrile was stirred on an ice bath with BuNMe₂ (8.75 ml, 120 mmol), Me₂NSO₂Cl (9.67 ml, 90 mmol) until the solution became clear. Both solutions were mixed and stirred at 0° C to r. t. over 12 hours. The acetonitrile was removed via rotary evaporation. The resulting syrup was washed with 2 x 150 water/ethyl acetate (1:1) and the organic layer 2 x 100 ml brine. The organic layer was removed via rotary evaporation and the resulting syrup dried *in vacuo* for 12 hours to give 2-deoxy-2-[2-(3-benzoylphenyl)propanoic acid]amino-1, 3, 4, 6-tetra-O-triethylsilyl -β-D-glucopyranosyl (**8**) (43 g), quantitative: ESI for C₄₆H₈₁NO₇Si₄: FW 871 found *m/z* 857 [M + H]⁺ w/loss of CH₃ [M + H]⁺, as a yellow liquid with the consistency of syrup. **9** was directly de-protected with t-butyl ammonium fluoride/MeOH to give white precipitate that was filtered, washed with methanol and recrystallized from hot ethanol to

give compound 9 (17.3 g, 85%) as an opaque solid. ¹H NMR (DMSO-d₆) 7.63-7.24 (m, 9H), 6.17 (s, 1H), 4.65 (s, 1H), 4.56 (s, 1H), 4.44 (s, 1H), 4.21 (s, 1H), 3.61 (s, 1H), 3.45-2.10 (m, 4H), 2.83 (d, 2H), 2.25 (d, 3H), 1.1 (s, 3H). ¹³C NMR (DMSO- d₆) 197.13, 176.39, 141.67, 137.64, 137.34, 132.54, 131.73, 129.69 (2C), 128.79, 128.47, 128.41 (2C) , 128.19, 95.20, 74.85, 72.28, 68.61, 61.90, 55.46, 45.09, 18.12 . ES1 for C₂₂H₂₅NO₇: FW 415 found *m/z* 416 [M + H]⁺. Mp 164° C

Results and Discussion

Compounds 6 and 9 are projected to be mutual prodrugs to treat osteoarthritis via the delivery of both a NSAID and glucosamine moiety. Compound 6 is ibuprofen covalently bound via a linker to the amide of glucosamine; and compound 9 is a ketoprofen molecule directly linked to glucosamine, each a model MP. Physiological enzymatic and hydrolysis reactions are expected to affect each MP's ester and imido-ester linkage respectively. Thus making them ideal MPs used to target osteoarthritis' associated pain and perhaps root cause by either oral or transdermal administration.

Though glucosamine is not recognized as a pharmaceutical in the United States, studies have shown that orally administered glucosamine promotes glycosaminoglycan synthesis and the production of proteoglycans that compose the lubricating fluids and support joint tissues i.e. cartilage, thus treating osteoarthritis' root cause. [15, 16] Glucose and glucosamine are substrates of glucokinase. [16] Phosphorylated glucosamine, glucosamine-6-phosphate inhibits glucokinase and alters both glucose and subsequent glucosamine metabolism. [17] Miwa et al. reported that glucokinase has a low affinity for NAG. [18] Thus, NAG kinase mediates the phosphorylation of NAG to produce NAG-6-phosphate that does not affect glucokinase activity. [19] Concluding that NAG-6-phosphate does not affect glucokinase activity thus allowing glucose and glucosamine to proceed through metabolism unrestricted. The biosynthesis of glycosaminoglycans from this perspective would be better promoted with the use of NAG or some other rate-limiting glucosamine

analogue rather than by parent glucosamine. [20] Anastassiades, et al. reports that glucosamine and analogues thereof such as NAG as well as glucosamine with varying N-linkage-chains have shown degrees of human chondrocyte cell culture growth via matrix gene expression *in vitro*. [21] From the pharmaceuticals perspective per review and taking consideration of Anastassiades patents and patent applications [22]; our assumed hypothesis states that by protecting the glucosamine's amide, the half-life of the glucosamine molecule is increased. Chain linkage effects have been shown in numerous literature studies, such as coupling a polymer to a molecule via an ester bond, to increase its half-life as method to modify a chemical entity's dissolution properties and/or biopharmaceutical properties. [23] The bioavailability of glucosamine is a problem since it is approximately 12-13%, though studies seem to indicate potency in mild to moderate cases of OA. [24, 25]

Our prediction is that these two synthesized MPs will undergo *in vivo* hydrolysis to give the parent compounds. Then, each parent compound should provide its therapeutically recognized effect. Studies have not been completed to determine whether the new MPs dissolution profile is faster than their hydrolysis or enzymatic degradation rate constants. Although this is expected, the rates are predicted to vary since the linkages depend on the chemical nature of the covalent bonds, structure of the compounds and the surrounding conditions *in vivo/in vitro*. Compound 6, the tripartite entity may undergo hydrolysis more easily than the bipartite compound 9. The ibuprofen molecule of compound 6 has the potential to undergo hydrolysis due to the linking group. The linking group's benzyl moiety could possibly then undergo an intramolecular reaction and/or enzymatic reaction that release the glucosamine molecule. Compound 9, the bipartite compound is expected to hydrolyze to the parent compounds ketoprofen and glucosamine.

These predictions however have been made based on the 'in silico' calculated pka values of compounds 6 and 9, **Figures 4 & 5** respectively. Predictive calculations may provide an estimation of the biopharmaceutical properties. The pka calculations reported are derived from algorithms

derived from known pKa's or various chemical groups. Here we mainly focus on the pKa of the amide and/or ester linkage/s. The negative pKa of the amide in compound 6 suggests a large Ka value, which implies that the equilibrium constant lies to the right for the dissociation of the imido-ester bond via a hydrolysis type cleavage lending itself more to enzymatic cleavage, which can also be the case with the imido-ester of compound 6. On the other hand, the amide also has a high pKa value and depending on its ionization, the imide-ester bond may be hydrolyzed. Furthermore, the pKa values near -2.5-3.0 are usually due to the protonation of the negatively charged oxygen. Our predictions show this to be possible on the imido ester's carbonyl-oxygen. More studies are needed to determine the true pathway of whether or not these MPs have substrate specificity, hydrophilicity and/or other influences that affect the release rates of the parent drugs. Development based on their physicochemical properties and understandings of their mechanisms of release are the primary determining factors towards further MP development. The '*in silico*' log P predictions of the compound 6 and 9 are 6.45 ± 0.82 and 3.81 ± 0.48 respectively. Currently, using Lipinski's Rule of 5 [25], compounds 6 and 9 rate a number of 4 and 2 respectively. Whereas, these numbers are solubility ranking based on a collection of chemical compounds estimated from data mining, e.g. using the "rule of five" to determine "drug likeness". The "rule of 5" states that: poor absorption or permeation is more likely when:

- A. There are more than 5 H-bond donors (expressed as the sum of OHs and NHs);
- B. The MW is over 500;
- C. The Log P is over 5 (or MLog P is over 4.15);
- D. There are more than 10 H-bond acceptors (expressed as the sum of Ns and Os).

Poor absorption or permeability is possible since neither compound satisfies more than two criteria. Though more drug likeness is indicated for compound 9 than compound 6 in regard to their aqueous solubility and intestinal permeability based on the “rule of 5”. Since these MPs contain carbohydrate moieties, they potentially could fall into classes that are substrates for biological transporters, which are exceptions to the rule.

This study was primarily commenced as a pharmaceuticals study to synthesize and evaluate the physicochemical properties of two proposed MP candidates. Though the dissolution characteristics have not been discussed, we found the DSC data to be intriguing. In order to evaluate these MPs dissolution characteristics, a consistent crystal form must be established of which we failed to produce. We encountered commonly observed carbohydrate phase transitions such as glassy state dynamics, which follow trends of the Tool-Narayanaswamy-Moynihan model. [27] This model is an attempt to explain the glassy state transitions observed with carbohydrates and other chemical entities such as sucrose, trehalose, (poly) vinylpyrrolidone and sucrose. Its application is widely used in the pharmaceutical industry to investigate the physicochemical stability and the quantitative relationship between the width of the glass transition and fragility/activation energy for structural relaxation. [28]

In **Figure 6** the DSC thermograph of compound 6, a powder, shows three transitions indicative of polymorphs that look to be thermally stable, with phase transitions at 226.93°C, 233.09°C and 237.52°C respectively. All thermographs were consistent with heating at various rates. After opening all of the DSC pans, we found that the compound had sublimed and decomposed. The DSC thermographs of compound 9 were difficult to obtain. Compound 9’s uncorrected melting point was determined to be 164°C. A representative DSC thermograph of compound 9 shows exothermic activity and many phase transition points indicative of sublimation,

which was proven upon visual inspection [Figures 7 & 8]. The uncorrected melting point is actually the decomposition point in DSC thermograph. Visually, compound 6 was obtained as syrupy liquid, which was recrystallized under high vacuum pressure and/or solvent recrystallization as opaque crystals with elliptical surfaces. To obtain a clearer, picture of compound 6, we tried x-ray crystallography, which failed due to the opaqueness and complexity of the crystal structure/s. No distinct polymorph and glassy transition states were observed. Pikal et al.'s hypothesis, "... because physical and chemical degradation processes require atomic and molecular mobility, just as structural relaxation requires similar mobility, instability processes are correlated or 'coupled' to structural relation..." [28] This theory of glassy state transitions is applicable to compound 6 as observed via the DSC thermographs, but further studies may verify its validity.

Conclusion

The objectives of this study were complete with the synthesis of two MP models as targets that improve the pharmaceutical properties of each parent compound directed towards improving and/or modifying each drug's permeability, solubility and stability in relationship to the parent glucosamine and NSAIDs. These modifications alter the physiochemical properties that affect delivery methods, route of administration and formulation

Glucosamine is currently not regulated as a drug in the U.S. as an active pharmaceutical ingredient (API). The current trend with glucosamine is to find better dosage forms due to its poor bioavailability as an orally administered nutraceutical. The main problems with the use of glucosamine and its sister analogues in pharmaceutical applications such as SYNVISIC (hyaluronic acid derivative), a FDA approved therapy (medical device) for the local treatment of pain associated with osteoarthritis of the knee, is the establishment of protocols other than pain studies to prove

efficacy to the FDA. For example, though SYNVISC is only approved for use in the knee, often times it is administered “off-label” to other joints. NSAIDS, on the other hand, are well documented for pharmacological, toxicological and biopharmaceutical properties.

The two MP models have been synthesized to follow the same processes/methodologies as prodrugs in attempts to overcome pharmaceutical and pharmacological problems such as incomplete absorption, too rapid absorption and excretion observed with NSAIDS and glucosamine. The end objective is to have an NSAID and glucosamine reach a site providing its pharmacological effect while minimizing the adverse drug events/effects. Though *in vitro* or *in vivo* studies have not been presented, these studies will determine the outcome of these new synthesized MPs and/or analogues.

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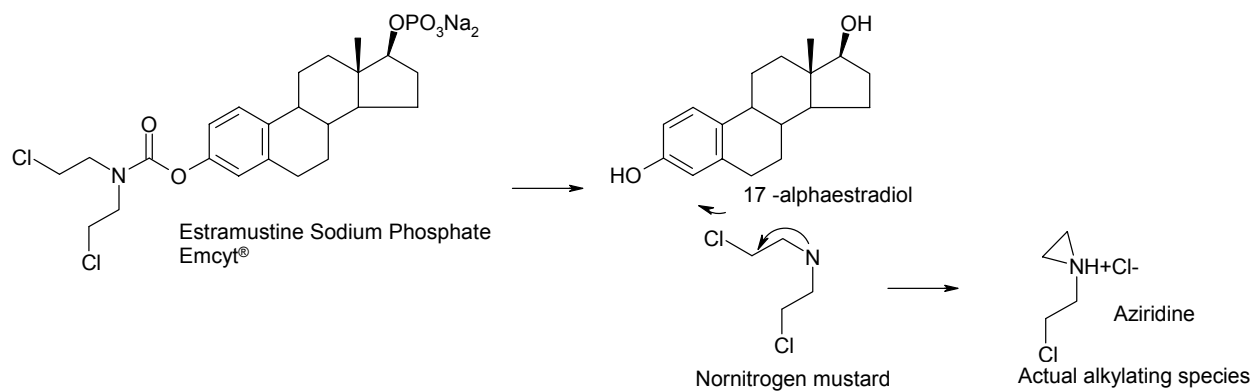
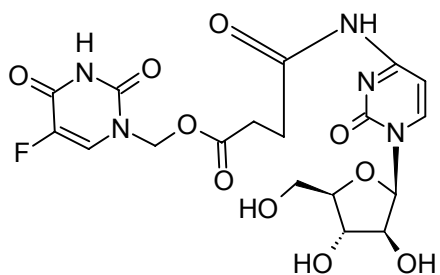
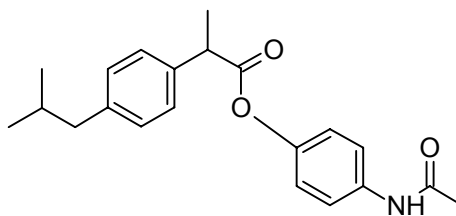


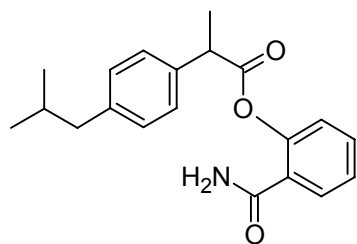
Figure 1. Estramustine is used for metastatic carcinoma of the prostate. Whereas the pro-moieties are also drugs. The prodrug is selectively taken up into estrogen receptor positive cells and then the urethane linkage is hydrolyzed to give 17- α -estradiol that slows prostate cell growth and nornitrogen mustard as a weak alkylating agent.



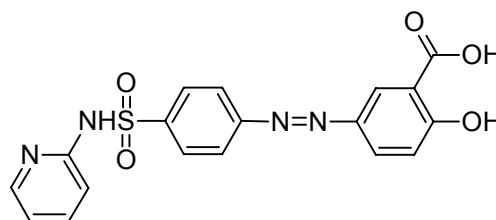
5-Fluorouracil/Cytarabine



Ibuprofen/Paracetamol



Ibuprofen/Salicylamide



Sulphasalazine

Figure 2. Examples of projected mutual prodrug models and the prodrug sulphasalazine

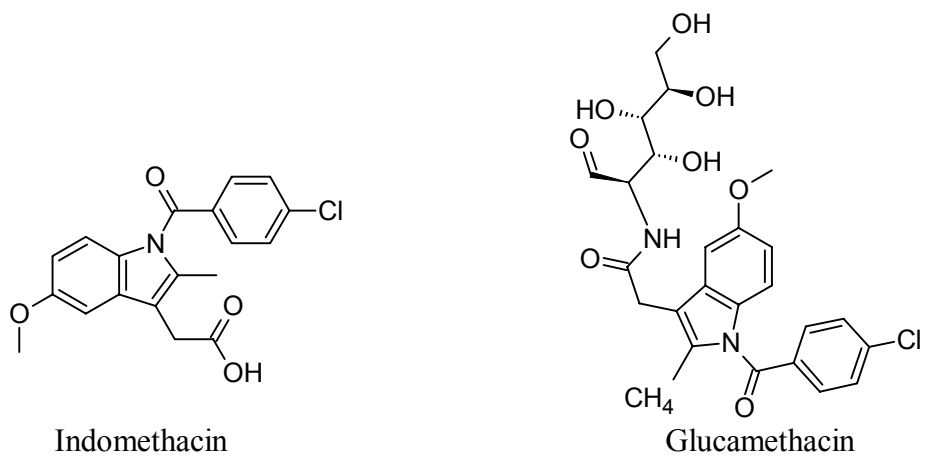
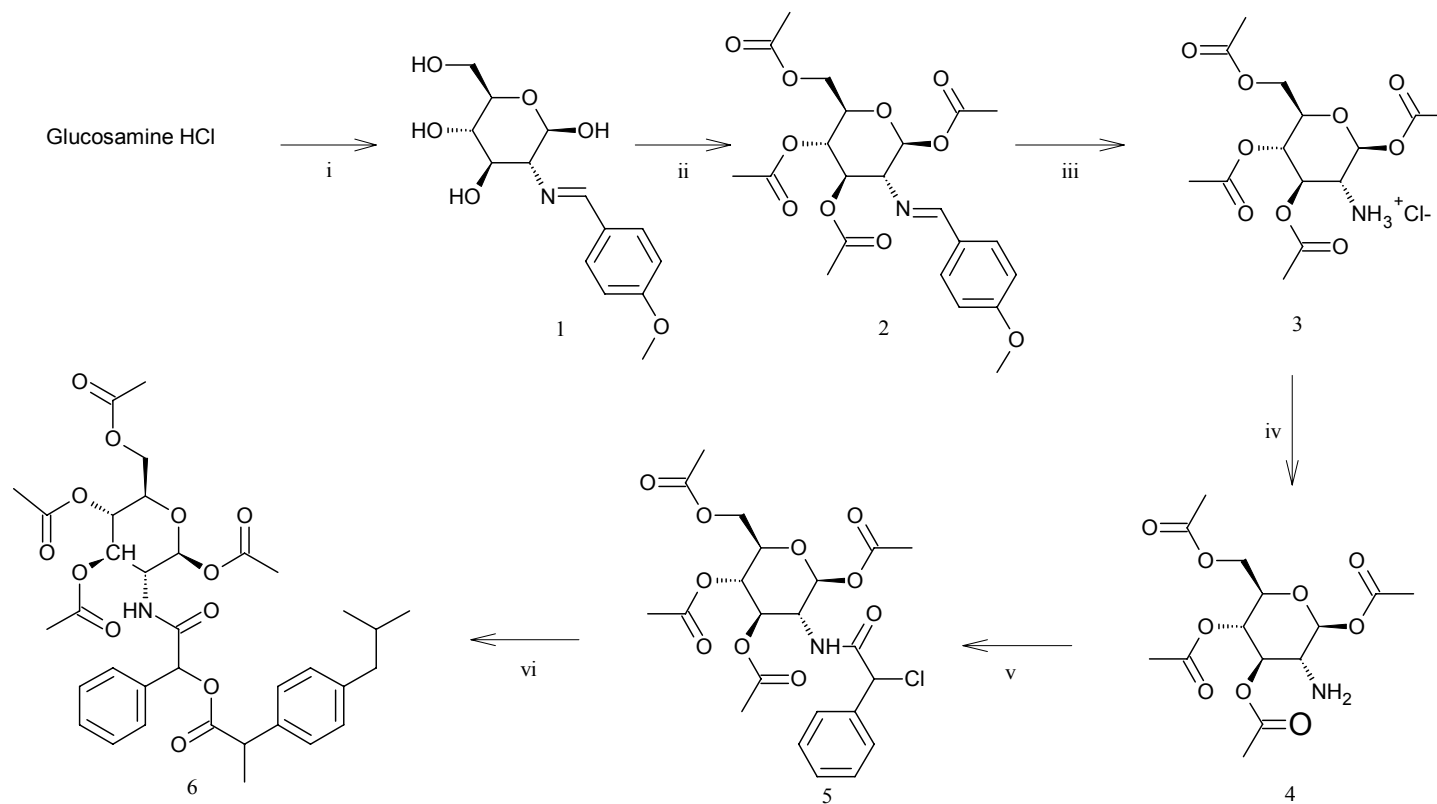


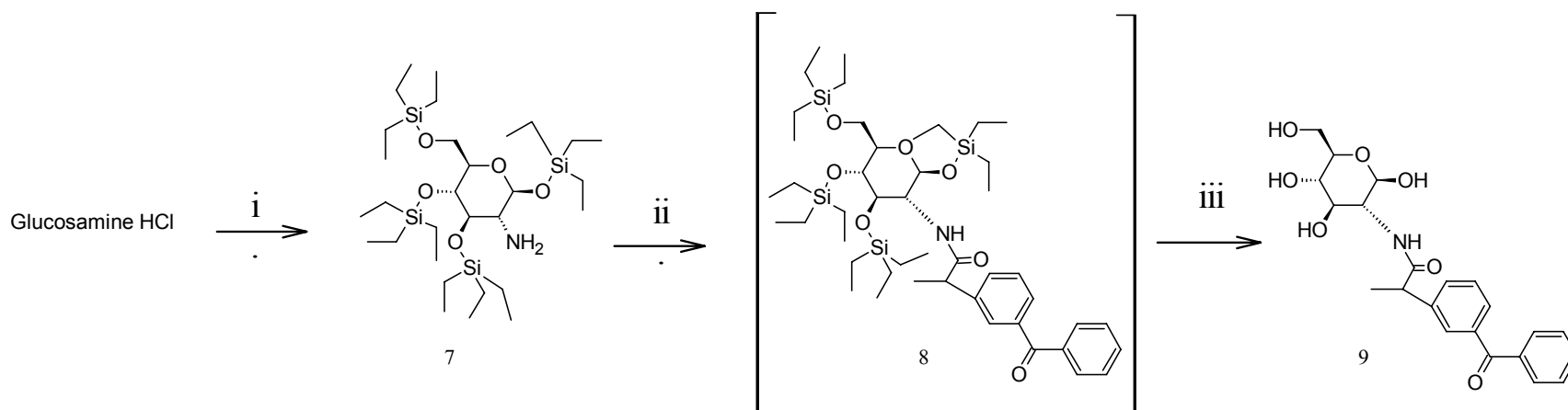
Figure 3. These are the structures of indomethacin and its glucosamide prodrug, glucamethacin.

Scheme I: Spacer Linked Glycosaminoglycan –NSAID Mutual Prodrugs

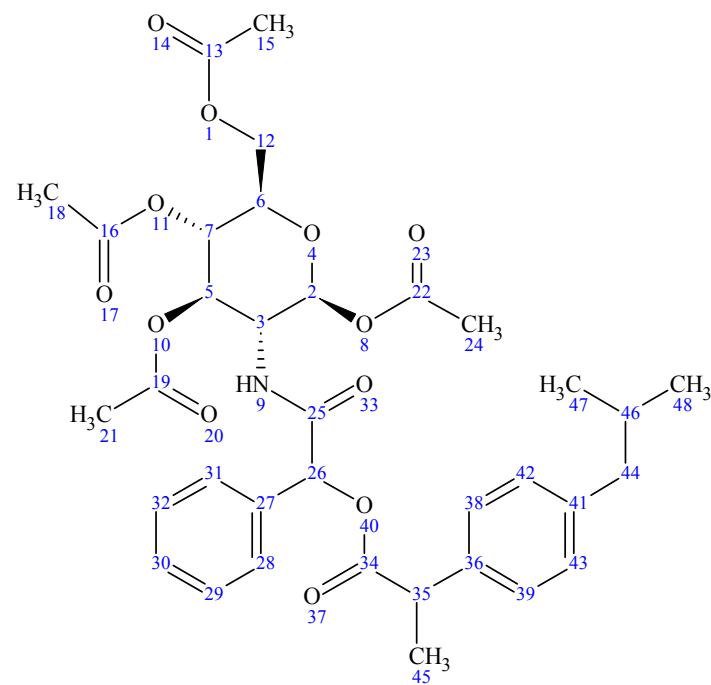


i) 1 N NaOH, p-methoxybenzaldehyde/ 0° C - r.t. 1 hrs **ii)** pyridine (anhydrous), acetic anhydride/ r.t. 24 hrs **iii)** acetone (reflux) and 5 N HCl **iv)** triethylamine, water **v)** chlorophenylacetyl chloride (linker), triethylamine, **vi)** NSAID, CH_2Cl_2

Scheme II: Directly Linked Glycosaminoglycan-NSAID Mutual Prodrugs



i) Trichloroethylsilane, pyridine (anhydrous) **ii)** NSAID, $\text{Me}_2\text{NSO}_2\text{Cl}$, MeCN, BuNMe_2 , DMAP **iii)** TBAF, Methanol



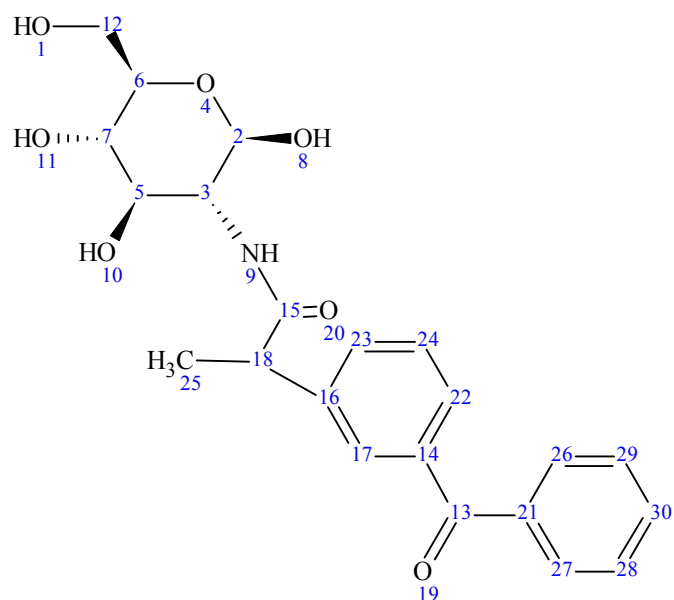
Ionic form: HL

$$\text{pK}_{a1}(\text{HL}/\text{H}+\text{L}; 9) = 10.79 \pm 0.70$$

$$\text{pK}_{a2}(\text{H}_2\text{L}/\text{H}+\text{HL}; 9) = -3.67 \pm 0.70$$

$$\text{Calculated log } P: 6.45 \pm 0.82$$

Figure 4. The *in silico* pka and log P calculations of compound **6** were obtained as a result of ACD/pka v8.02 using the ACD/I-Lab Service.



Ionic form : H5L

$$\begin{aligned} \text{pKa}_1(\text{HL}/\text{H}+\text{L}; 9) &= 15.53 \pm 0.70 \\ \text{pKa}_2(\text{H2L}/\text{H}+\text{HL}; 11) &= 14.69 \pm 0.70 \\ \text{pKa}_3(\text{H3L}/\text{H}+\text{H2L}; 11) &= 14.33 \pm 0.70 \\ \text{pKa}_4(\text{H4L}/\text{H}+\text{H3L}; 10) &= 13.53 \pm 0.70 \\ \text{pKa}_5(\text{H5L}/\text{H}+\text{H4L}; 8) &= 12.03 \pm 0.70 \\ \text{pKa}_6(\text{H6L}/\text{H}+\text{H5L}; 9) &= -2.40 \pm 0.70 \end{aligned}$$

$$\text{Calculated log } P: 3.81 \pm 0.48$$

Figure 5. The *in silico* pka and log P calculations of compound **9** were obtained as a result of ACD/pka v8.02 using the ACD/I-Lab Service.

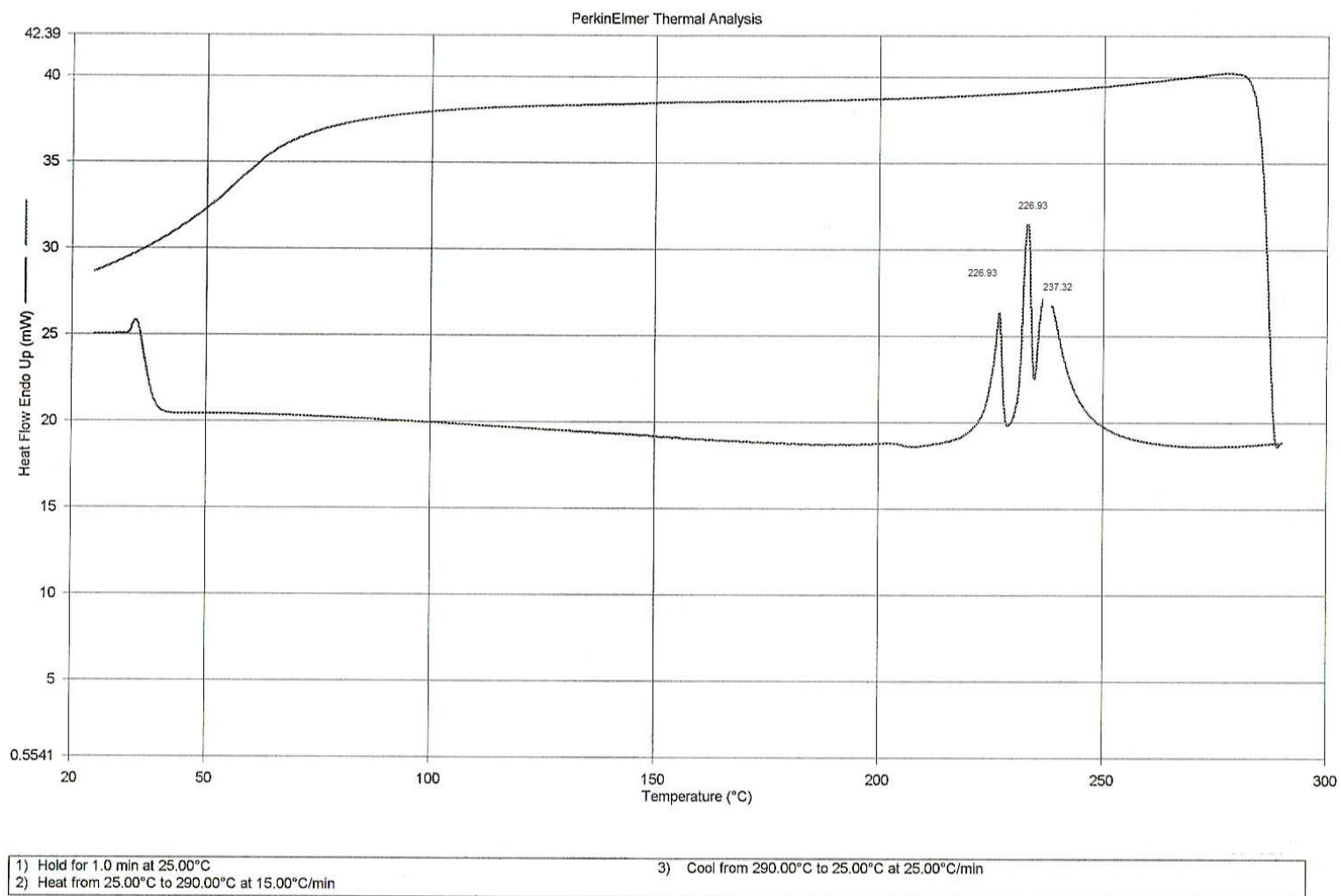


Figure 6. DSC thermograph of compound 6

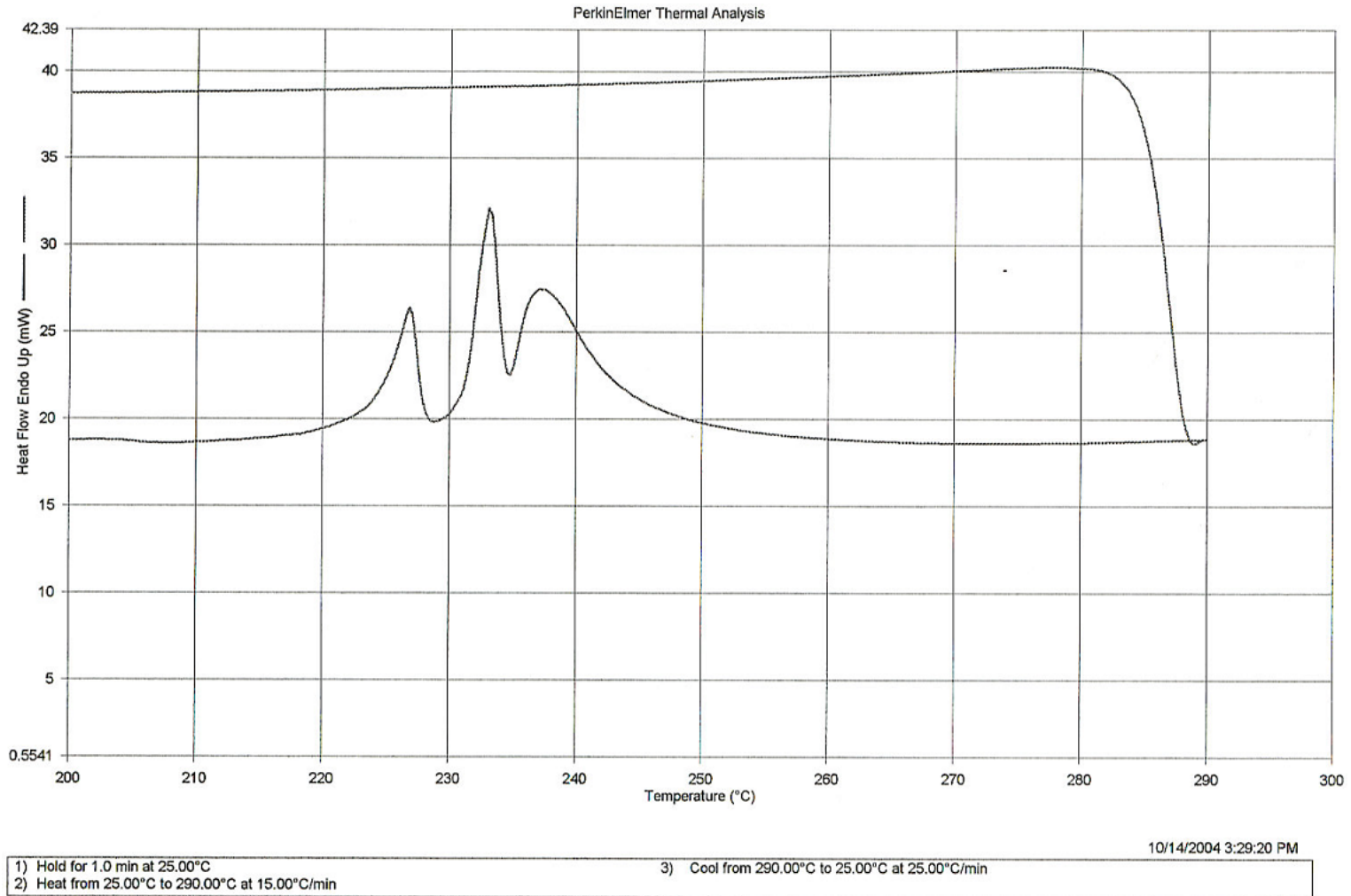


Figure 7. Expanded DSC thermograph of compound 6

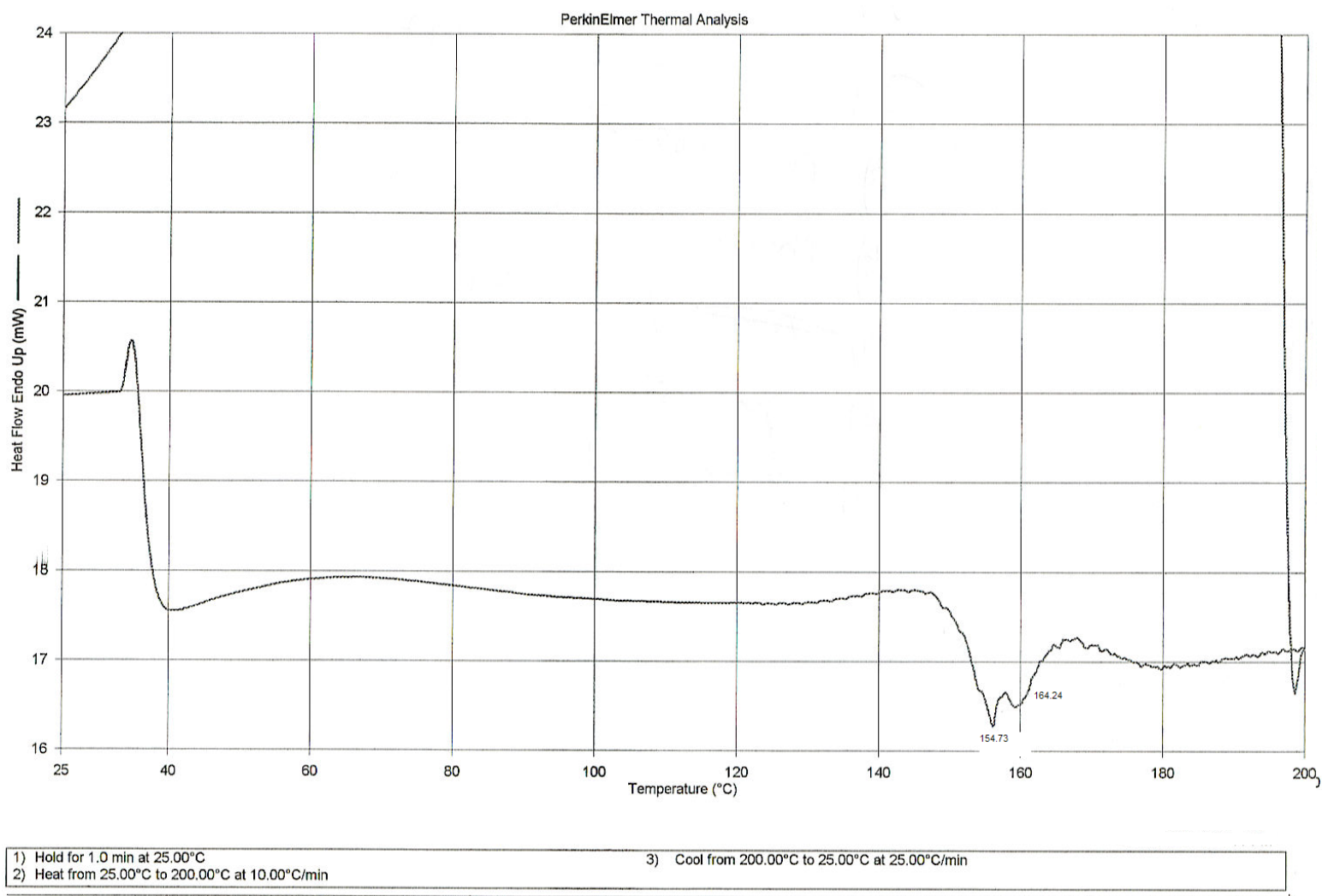


Figure 8. DSC thermograph of compound 9 exhibiting definite exothermic characteristics

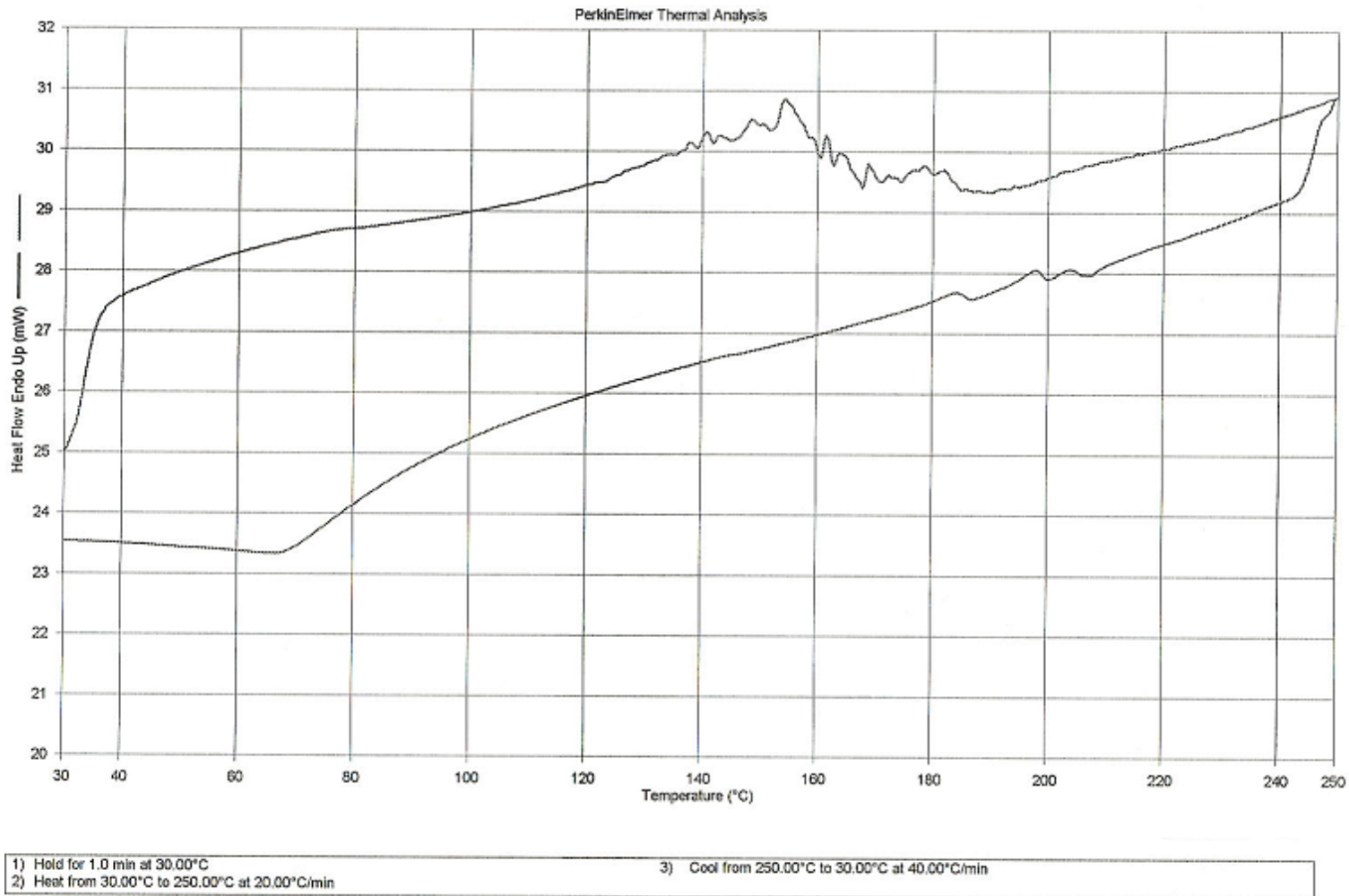


Figure 9. DSC thermograph of compound 9 exhibiting the sublimation, phase change and degradation phenomena

CHAPTER IV
PRELIMINARY STUDIES ON TRANSDERMAL PERMEABILITY OF N-ACETYL-D-
GLUCOSAMINE (NAG): A GLUCOSAMINE METABOLITE¹

¹Garner Jr., S. T, Abney, T., Azadi, P. and., Capomacchia, A. C. To be Submitted to the Journal of
Pharmaceutical Research

Abstract

The purpose of this study was to investigate the feasibility of the transdermal delivery of N-acetyl-D-glucosamine (NAG), a metabolite of glucosamine. Glucosamine salts are nutraceuticals used in the treatment of osteoarthritis. Published and unpublished studies show that glucosamine salts cannot be transdermally delivered or percutaneously absorbed through traditional means. Insignificant information is available regarding NAG's transdermal transport or percutaneous absorption. This study investigates the permeability of NAG in various enhancer suspensions utilizing shed snakeskin as a model membrane *via* Franz-type cell diffusion studies. Negligible permeability was observed for NAG suspensions of known membrane permeation enhancers ethanol, oleic acid, isopropyl myristate, and isopropyl palmitate; as well as from saturated solutions of NAG in water or phosphate buffers. Permeability measurements were obtained from saturated solutions of NAG in DMSO and phosphate buffer solutions containing ethanol at 2%, 5%, 10%, 25%, and 50%. Permeability coefficients of the phosphate buffer/ethanol solutions at 5%, 10% and 25% were found to be close in value and four times greater than that of the saturated DMSO solution. Observation of such a large flux through shed snakeskin provides that further studies of this compound be completed in novel formulations designed for local percutaneous delivery of NAG to osteoarthritic joints.

Key Words: N-acetyl-D-glucosamine (NAG); transdermal permeation; osteoarthritis; penetration enhancers; dietary supplement

Background

The use of glucosamine became popular after being featured in the book, The Arthritis Cure by Jason Theodasakis, MD, (1). Currently, glucosamine and its metabolites are not classified as drugs

but as nutraceutical/dietary supplements under United States Food and Drug Administration's Dietary Supplement Health and Education Act of 1994 (DSHEA). Oral dosage formulations of N-acetyl-D-glucosamine (NAG), and its parent compound glucosamine in salt form (sulfate, hydrochloride etc.) are commercially available nutraceuticals, and are commonly administered in conjunction with chondroitin sulfate, also a readily available nutraceutical. Glucosamine and chondroitin have been reported effective in the oral treatment of osteoarthritis but have not undergone the rigorous studies needed for FDA approval as pharmaceuticals. (1, 2) The National Institutes of Health (Bethesda, MD, USA) has an ongoing multi-center study, Glucosamine/Chondroitin Arthritis Intervention Trial (GAIT), which is currently evaluating the efficacy of orally, administered glucosamine and chondroitin oral supplements, (3).

Glucosamine and chondroitin salts are charged, highly polar, water/aqueous soluble, and poor candidates for transdermal absorption. Currently, there are topical products containing these ingredients as salts, marketed as nutraceuticals for the treatment of osteoarthritis, which contain ingredients whose effects may be mistaken in the short term as being therapeutic. NAG, an acetylated glucosamine metabolite, is less polar and neutral. It appears to be a more likely candidate for transdermal delivery and percutaneous absorption.

Glucosamine and its orally delivered salt forms are metabolized to NAG via the hexosamine pathway. Glucosamine or galactosamine, plus a uronic acid, is incorporated as a disaccharide unit into all macromolecules requiring amino sugars such as keratan sulfates, dermatan sulfates, chondroitin 4- and 6-sulfates, hyaluronates, and heparin and heparan sulfates, to produce glycosaminoglycans (GAGs). GAGs are highly negatively charged molecules, with an extended conformation, and demonstrate high viscosity and low compressibility ideal as a lubricating fluid for

anatomical joints. The majority of GAGs in the body are linked to core proteins, to form proteoglycans or mucopolysaccharides, which are basic components of skin, tissue and cartilage. (4,5)

Introduction

The objective of this research is to evaluate transdermal permeability of NAG, in order to assess the feasibility of pursuing a percutaneous formulation for local therapy to osteoarthritic joints. In connection, a provisional patent has been filed with the US Patent and Trademark Office, (6). Permeability was evaluated by employing NAG suspensions of various known membrane transport enhancing reagents, ethanol, oleic acid, isopropyl myristate, isopropyl palmitate; NAG solutions of water and phosphate buffer; and NAG saturated DMSO solution.

Oral administration of glucosamine, its salts, and NAG are affected by the liver's first-pass metabolism, (7). However, a more recent report indicates that these agents may be metabolized mostly in the gut rather than solely by the liver, (8). Few pharmacokinetic literature reports exist on the disposition of these agents in articular cartilage. Setnikar *et al.* (9, 10) has reported on the pharmacokinetic properties of glucosamine in dogs and man. It is estimated that approximately 87% of the original glucosamine oral dose is absorbed and excreted; <13% is widely distributed in the body; and <1% reaches osteoarthritic joints. Chondroitin is known to degrade into its basic disaccharide components within the gut prior to further metabolism, (11). Although only a small fraction of glucosamine reaches the articular cartilage target site, it is reported to exhibit a high potency; and together glucosamine and chondroitin therapy demonstrate therapeutic efficacies over time, (2). NAG was selected because it is an active metabolite and prodrug of glucosamine; and owing to its commercial availability, stability and relatively low cost. It possesses the following physical and chemical characteristics making it a reasonable candidate for transdermal delivery and

percutaneous absorption: a) high potency, b) reasonably lipid soluble, c) low molecular weight, d) unique biochemical pathway with active transport from blood into articular cartilage. (5)

Materials and Methods

Chemicals

99.9+% purity NAG was purchased from MP Biomedical (Aurora, Ohio). All enhancer reagents purchased for this study were at 99.9+% purity. All other reagents were of analytical grade and used without further purification.

Analysis

NAG analysis was carried out using high-performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD); Dionex, Sunnyvale, CA USA); on a Dionex DX-500 HPLC system consisting of a GP40 gradient pump, ED40 Electrochemical detector, AS3500 autosampler and PeakNet Chromatography Workstation, (12-15). The HPAE-PAD was equipped with CarboPac™ PA20 (3 x 150 mm), analytical anion-exchange column for the rapid, high-resolution separation of monosaccharides and disaccharides, using pulsed amperometric detection, (12-15). A CarboPac PA20 analytical guard column (3 x 30 mm) and a carbonate trap column (25 x 15 mm). Mobile phase (A) was degassed and deionized water. The mobile phase (B) consisted of 0.02 N NaOH prepared with deionized water and filtered with 0.45 µm filters in a solvent filtration apparatus (Waters-Millipore, Milford, MA, USA), which was finally degassed under vacuum. The mobile phase system was run at a gradient concentration of 16 mM NaOH at a flow rate of 0.5 ml/min. A standard calibration curve of NAG (Figure 1) was obtained with linear regression and had a value of $R^2=0.9936$. Each sample set was run with external standards. The sample concentration values were obtained *via* the Peak Net software. These values were compared with to

those obtained by calculations of the peak area and peak height observe as functions of the standard curve's linear regression equation. The instrument sensitivity was approximately 10^{-4} units.

Solubility measurements

An excess amount of NAG (pKa 6.73) was placed in separate vials containing 10 ml water, 10 ml n-hexane and 10 ml phosphate buffer (pH 6, 6.73, and 7.4; 1 M) and stirred at 37°C for 24 hours. The solutions were centrifuged for 5 min at 9000 rev/min and the supernatant filtered with cellulose acetate membrane filters (0.45 μ m pore size). The NAG concentration in each filtrate was determined by HPAE-PAD after the appropriate dilution. NAG highly soluble in the water and aqueous solutions.

Determination of partition coefficients

The oil/water partition coefficient for NAG was determined using n-hexane/phosphate buffer (pH 5.5, 6, 6.73, and 7.4, 0.1 M) and n-hexane/water, (16). In each case 5 ml of n-hexane was mixed with aqueous solutions containing NAG and shaken at 37°C for 24 hours. The mixture was afterwards centrifuged and the organic and aqueous phases separated. The NAG concentration in the filtrates was determined by HPAE-PAD after the appropriate dilution. .

In-vitro Membrane Permeation

Shed snakeskins were used as a model membrane for all permeation studies using the NAG suspensions in known membrane permeation enhancers; ethanol, oleic acid, isopropyl myristate, and isopropyl palmitate; saturated solutions of NAG in water and in phosphate buffer; as well as in a saturated DMSO solution and phosphate buffer (pH 5.5) containing ethanol at 2%, 5%, 10%, 25%, and 50% solutions. The skins were hydrated in 0.1% aqueous sodium azide solution at room

temperature for 48 hrs. Franz-cell diffusion experiments were carried out. In general the receptor cell was filled with 7.4 pH 0.1 M phosphate buffer and the donor cell filled with a solution or suspension. For the phosphate buffer (pH 5.5) containing ethanol at 2%, 5%, 10%, 25%, and 50% solutions in the donor phase, the receptor phase consisted of phosphate buffer (pH 5.5, 0.1 M). The receptor solution was maintained at 37°C and stirred with a magnetic stirrer. The snakeskins were mounted between the receptor and donor cells. The surface exposed to diffusion was 2.54 cm² (diameter 1.8 cm) and the receptor cell volume was 6 cm³. The top of the donor cell was covered with plastic film. The system was allowed to equilibrate at 37°C for two hours before each experiment. To the donor cells, 5 ml of the NAG-enhancer suspension or solution was added. Samples were taken at intervals over a 24-hour period, 200- μ l samples of receptor solutions were taken and replaced with fresh buffer; experiments were conducted in triplicate. The amounts of NAG that permeated through the snakeskin were determined by HPAE-PAD.

Data Treatment

Steady state flux (J_{ss}) for NAG (mg/cm²/h) was calculated from its increasing amount in the receptor medium, (17). NAG's permeability coefficient (K_p) in cm/h was calculated from known physiochemical parameters, (18). NAG's experimental permeability coefficients (K_p) in cm/hr were determined by dividing the steady-state flux by the cumulative donor concentration. Lag time (t_{lag}) was determined graphically from the cumulative amount of drug released per unit area (mg/cm²) versus time plots. A square root of time ($t_{1/2}$) versus cumulative amount of drug released per unit area (mg/cm²) was obtained to monitor NAG in vitro release rate (mg/cm²), (19).

Results and Discussion

Initial permeability investigations were carried out using shed snakeskin as a model membrane to

human skin; a widely recognized and sufficient model for preliminary studies due to its similarity in composition to the human stratum corneum, (20). Negligible NAG transport was observed from suspensions of membrane permeability enhancers; ethanol, oleic acid, isopropyl myristate and isopropyl palmitate. No permeation was observed from the aqueous solutions of NAG in water or phosphate buffer solutions (pH 5.5, 6.0, 6.73, and 7.4; 0.1 M). As a qualitative correlation to a selection of NAG's partition coefficients shown in **Table 1**, permeation of NAG from the aforementioned membrane penetration enhancer suspensions or aqueous solutions was expected. NAG was highly soluble in the aqueous solutions, but highly insoluble in the organic solvent including ethanol. This data is not reported but its solubility in DMSO has been recorded as 0.45 g/ml.

DMSO was chosen for evaluation as a benchmark permeation enhancer due to its physical properties and well-documented enhancement properties, (21). Enhancers are reported to disrupt intercellular lipids of the stratum corneum, by increasing a drug's partitioning into the stratum corneum with a concomitant increase in drug permeation through the intercellular junctions via percutaneous absorption. (22-24). From the plot containing cumulative NAG concentration per unit area (mg/cm^2) versus square root of time ($t_{1/2}$) NAG's *in vitro* release rate was shown to be $73.48 \text{ mg}/\text{cm}^2$ (Figure 1) with high linearity in transport thus exhibiting no lag time, (Table 2). The assumption taken is that NAG's high polarity and its low permeation coefficient (Table 1) contributes to its inability to be transported efficiently by means of a single permeation enhancer or in aqueous solution. Our study shows that DMSO allows NAG to be transported immediately and continuously as seen in **Figure 1** with a linear concentration increase over time.

This study also incorporated NAG into an ethanol/buffer solution at various concentrations. Ethanol as an enhancer is known to promote the transdermal penetration and percutaneous absorption of many drugs, (25). The oil/water partition coefficient increases with the decrease in pH of the buffer solution [Table 1]. NAG's transdermal transport was not observed from phosphate buffer or ethanol where it is highly soluble and insignificantly soluble respectively. Its permeation was observed in sink conditions from the phosphate buffer (pH 5.5) containing ethanol at 2%, 5%, 10%, 25%, and 50% (Figure 1). The cumulative concentration of the solutions containing 5%, 10%, and 25% ethanol are very similar after 24 hrs. Whereas the 2% and 50% ethanol in buffer solutions delivery significantly less NAG. [Figure 2] Beyond 50% ethanol concentration in buffer, the NAG precipitated. The flux values for 10% and 25% ethanol concentration are similar, whereas the 5% is half that of both. Graphically, each has a slightly different linear permeation profile up to the approximate experimental mid-point. At the conclusion of the 24-hour endpoint each solution had delivered similar amounts of NAG.

In conclusion, the results overall suggest that thermodynamic and solubility effects affect the permeation of NAG in correlation to use of DMSO at 100% (26) and the varying concentrations of ethanol in buffer solutions. NAG's *in vitro* flux and release rates from ethanol in buffer solutions overall exceeded those permeation values obtained from DMSO. This shows that 5-25% concentration of ethanol as an enhancer in delivery vehicles to be an excellent starting point towards the formulation of a transdermally delivered/percutaneously absorbed NAG product. The current study also shows DMSO to be a skin penetration enhancer for NAG. DMSO is generally used in veterinary drug delivery (27). The use of DMSO in NAG formulations may be useful for localized osteoarthritis treatment in animals, since DMSO is not an FDA approved excipient for human use in topical/transdermally delivered pharmaceutical products. Furthermore, unpublished preliminary

results in our laboratory show that NAG may have adequate permeation from other drug delivery vehicles. Further *in vitro* studies will determine whether or not other NAG formulations will effectively demonstrate transdermal towards percutaneous absorption.

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Table 1. Experimentally determined partition coefficients for N-acetyl-D-glucosamine (NAG) [pKa 6.73] and permeability coefficient (k_p)

n-hexane/pH	n-hexane/pH	n-hexane/pH	n-hexane/pH	Octanol/water ¹⁵	k_p (cm/hr)
5.5 buffer	6.0 buffer	6.73 buffer	7.4 buffer		
0.252	0.194	0.092	0.091	0.017	0.731

Table 2. Physiochemical data obtained for the permeation of N-acetylglucosamine (NAG) through shed snake's skin *via* a saturated dimethyl sulfoxide (DMSO) solution in the donor phase and pH 7.4 phosphate buffer in receptor phase.

Parameter	
J_{ss} (mg/cm ² /h)	73.48
t_{lag} (h)	
In Vitro release rate (mg/cm ²)	186.64
Solubility (g/ml)	0.45
K_p (cm/hr)	0.20
R^2	0.9736

Table 3. Physicochemical data obtained for the permeation of N-acetylglucosamine (NAG) through shed snake’s skin *via* phosphate buffer (pH 5.5) containing ethanol at 2%, 5%, 10%, 25%, and 50% solutions in the donor phase and pH 5.5 phosphate buffer in the receptor phase.

Parameter (% Ethanol)	2	5	10	25	50
J_{ss} (mg/cm ² /h)	112.61 ±	119.53 ±	211.61 ±	205.93 ±	77.96 ±
In Vitro release rate (mg/cm ²)	286.03	303.61	537.49	523.06	198.02
t_{lag} (h)	-	-	-	-	-
K_p (cm/hr)	0.057	0.021	0.037	0.033	0.068
R^2	0.9778	0.8751	0.7966	0.9924	.9836

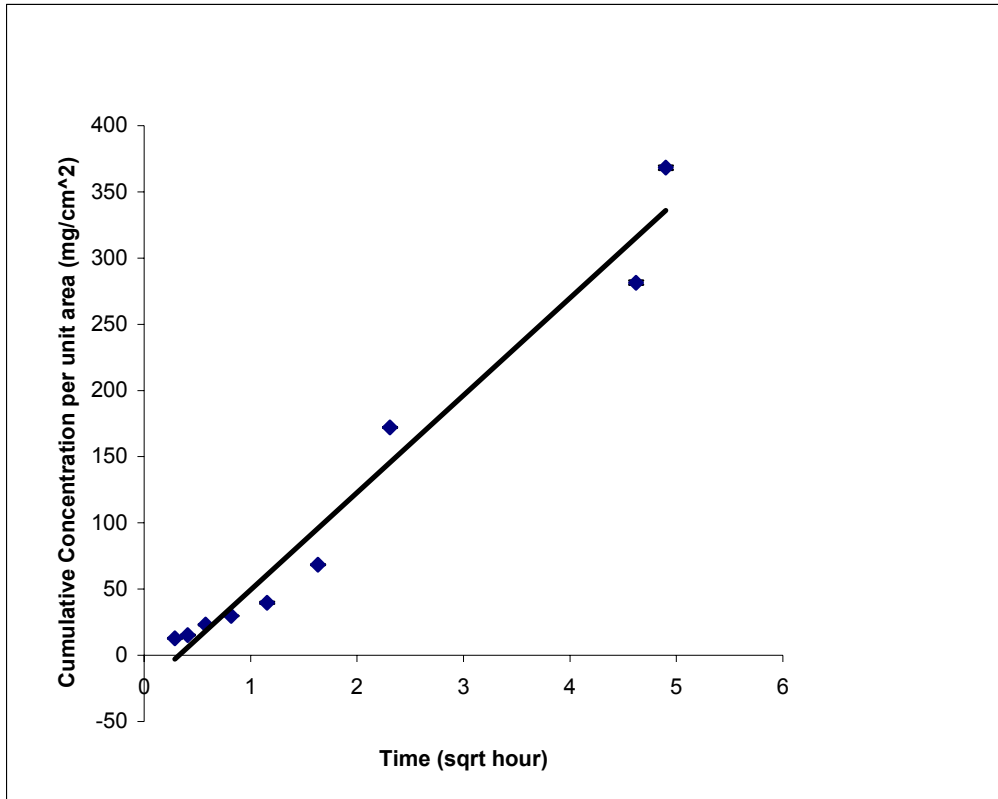


Figure 1. Effect of DMSO on the cumulative permeation of N-acetylglucosamine (NAG) at 37.5°C through shed snake skin (cumulative concentration versus the square root of time). Each point represents the mean \pm s.d., n=3.

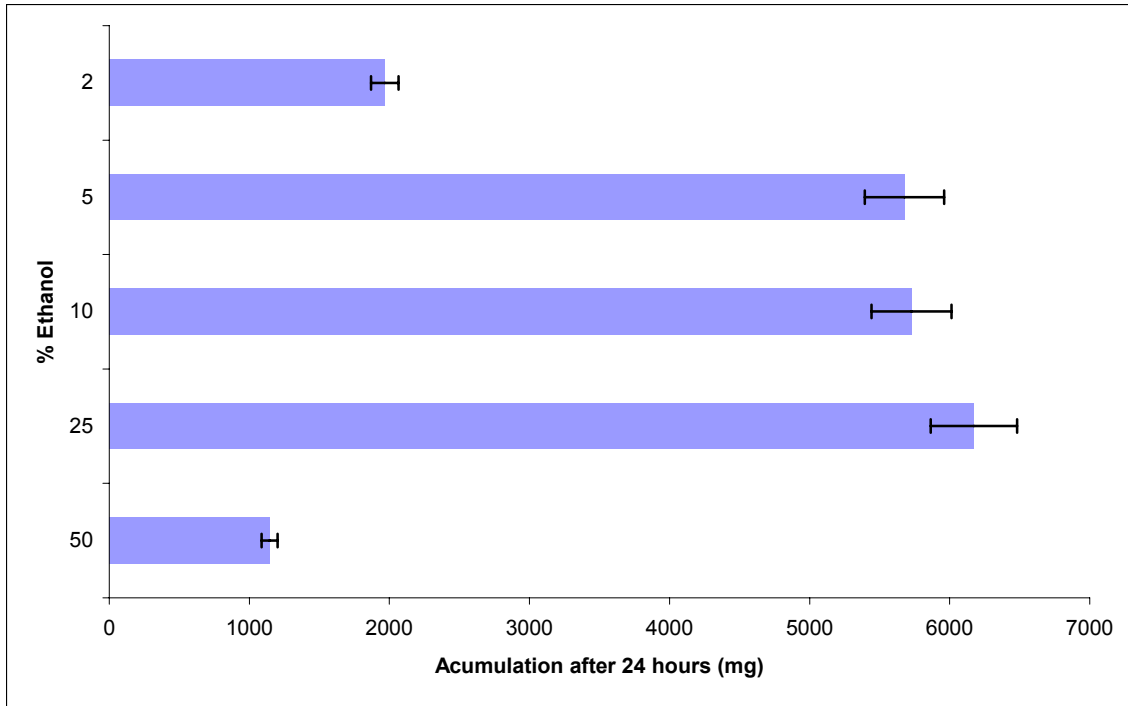


Figure 2. Accumulation of N-acetylglucosamine (NAG) through shed snake skin in the Franz type receptor cell from phosphate buffer (pH 5.5) containing ethanol at 2%, 5%, 10%, 25%, and 50%. Each point represents the mean \pm s.d., n=3.

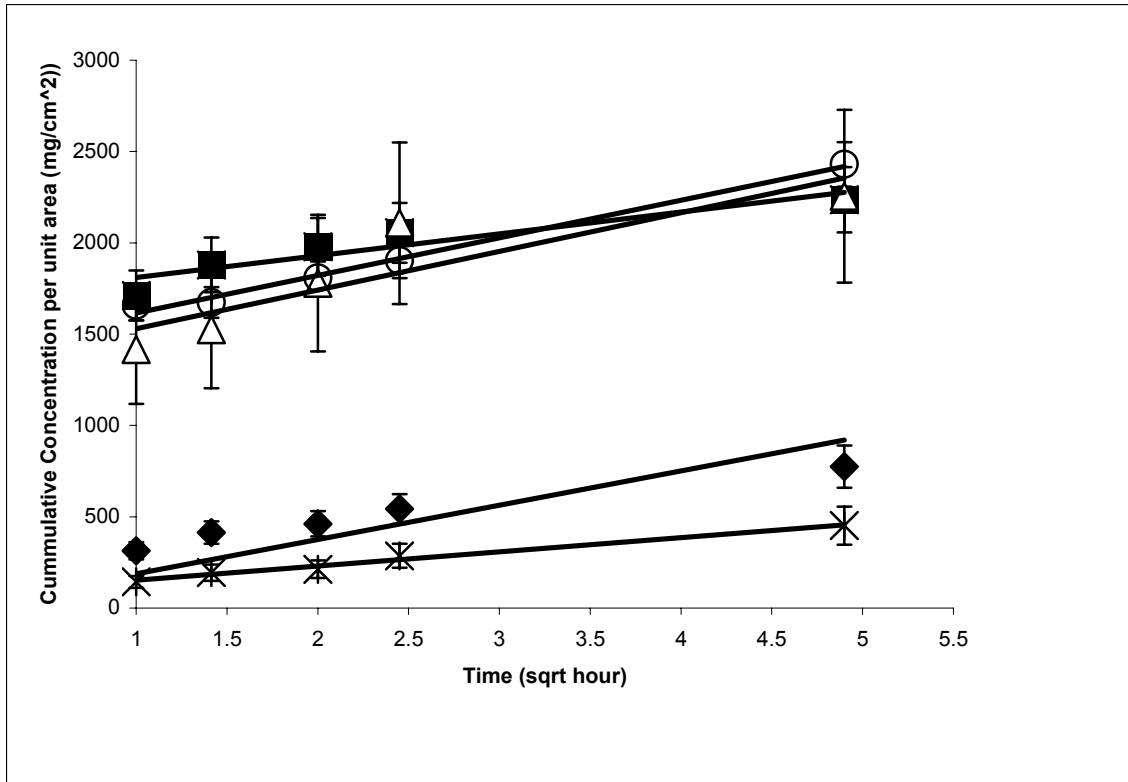


Figure 3. Effect of ethanol concentration cumulative permeation of N-acetylglucosamine (NAG) at 37.5°C through shed snake skin (cumulative concentration versus the square root of time). Each point represents the mean \pm s.d., n=3. 2% ethanol: ◆, 5% ethanol: ■, 10% ethanol: △, 25% ethanol: ○, 50% ethanol: ×

CHAPTER V

PERMEATION STUDIES OF NAG FROM PLURONIC-ORGANO-GELS

Introduction

The objective of this study was to further evaluate the permeation of NAG in pluronic-organo-gel formulations across shed snake's skin. These pluronic gel formulations each contained lecithin-isopropyl palmitate and/or lecithin-vitamin E component as the enhancer and organic phase. Isopropyl palmitate is a well-studied enhancer. [1] Literature reports concerning the permeation enhancement effects of vitamin E [2] and soy lecithin [3] are sparse other than patents and patent applications describing various dosage forms.

This study arose as an experiment while conducting transdermal feasibility studies of metabolites and prodrugs of glucosamine. [4] For these studies, NAG was chosen as the compound of choice, even though it has an octanol-water partition coefficient of 0.017 (pH 7.4). [5] Mahjour and co-workers [6] studied the effect of lecithins on *in vitro* skin permeability of several drugs with various NAG's octanol-water partition coefficients. They found that soy lecithins improved the permeability of all the drugs including procaterol and oxymorphone which both have a comparatively low octanol-water partition coefficient of -0.37 (pH 7.7) and 0.0 (pH 7.4) respectively. The most impressive finding was that the soy lecithins enhancement effect was the highest for procaterol, which has the lowest octanol-water partition coefficient of the drugs studied. [6] As for vitamin E, it is postulated to act as a permeation enhancer by intercalating within the lipid bilayer of the stratum corneum to alter the membrane's permeability. [7] Whereas, vitamin E has a two-fold effect within biological membranes as an antioxidant and membrane stabilizer. [7] Structurally,

vitamin E's chromanol ring's hydroxyl group is believed to situate in the polar head environment of the phospholipid membrane, while the phytol chain intercalates with the lipid acyl chains. [7] Trivedi and co-workers observed vitamin E's permeation enhancement. [7] Their conclusion was that overall improvement in the permeability of the stratum corneum is moderate. This is a result from the limited insertion of vitamin E within the ceramide-rich bilayer structure. Thus their final concluding statement was that overall vitamin E enhancement effect "...will not be tremendous but discernible nonetheless." [7]

Experimental

Materials

NAG was purchased from MP Biomedicals, Inc (Aurora, OH). Poloxamer 407 (Pluronic® F127; Polyethylene-Polypropylene Glycol), soy lecithin and isopropyl palmitate were purchased from Spectrum Chemical Mfg. Corp. (Gardena, CA). Vitamin E (mixed tocopherol complex) was purchased from Solgar Vitamin and Herb (Leonia, NJ). Water used for the preparation was double distilled and deionized by a Millipore purification system (Continental Water Systems Corp., El Paso, TX).

Vehicle Preparation

Twenty percent (20%) poloxamer 407 (Pluronic® F127; Polyethylene-Polypropylene Glycol) gels were made by standard methods e.g. dissolving the poloxamer into cold water under refrigeration for 24 hours to produce the pluronic gel phase. NAG was incorporated into the pluronic gel phase. The final formulations were prepared via the emulsification of the select organic phase with the pluronic gel phase using luer-lock connected syringes.

In-vitro Membrane Permeation Studies

Shed snakeskins were used as the model membrane for all permeation studies using the NAG semi-solid gel formulations. The skins were hydrated in 0.1% aqueous sodium azide solution at room temperature for 48 hrs. Franz-cell diffusions experiments were carried out. In general the receptor cell was filled with a 7.4 pH 0.1 M phosphate buffer. The receptor solution was maintained at 37°C and stirred with a magnetic stirrer. The snakeskins were mounted between the receptor and donor cells. The surface exposed to diffusion was 2.54 cm² (diameter 1.8 cm) and the receptor cell volume was 6 cm³. The donor cell top was covered with plastic film. The system was allowed to equilibrate at 37°C for two hours before each experiment. The donor cells were filled with 5 ml of the semisolid NAG pluronic-organo-gel formulation. Samples (N=3) were taken at intervals over a 48-hour period, 200- μ l samples of receptor solutions were taken and replaced with fresh buffer; experiments were conducted in triplicate. The amounts of NAG that permeated through the snakeskin were determined by HPAE-PAD.

HPAE-PAD (HPLC) Analysis

NAG analysis was carried out at the University of Georgia, Complex Carbohydrate Research Center. High-performance anion exchange chromatography with pulsed amperometric detection (HPAE-PAD); Dionex, Sunnyvale, CA, USA); on a Dionex DX-500 HPLC system consisting of a P40 gradient pump, ED40 Electrochemical detector, AS3500 autosampler and PeakNet Chromatography Workstation was utilized. The HPAE-PAD was equipped with CarboPac™ PA20 (3 x 150 mm), analytical anion-exchange column for the rapid, high-resolution separation of monosaccharides and disaccharides, using pulsed amperometric detection and a CarboPac PA20 analytical guard column (3 x 30 mm) and a carbonate trap column (25 x 15 mm). Mobile phase (A) was degassed and deionized water. The mobile phase (B) consisted of 0.02 N

NaOH prepared with deionized water and filtered with 0.45 μm filters in a solvent filtration apparatus (Waters-Millipore, Milford, MA, USA) that was degassed under vacuum. The mobile phase system was run at a gradient concentration of 16 mM NaOH at a flow rate of 0.5 ml/min. A standard calibration curve of NAG (Figure 1) was obtained with linear regression ($R^2=0.9936$). Each sample set was run with external standards. The sample concentration values were obtained *via* the Peak Net software. These values were compared with to those obtained by calculations of the peak area and peak height observe as functions of the standard curve's linear regression equation.

Data Analysis

Steady state flux (J_{ss}) for NAG ($\text{mg}/\text{cm}^2/\text{h}$) or ($\mu\text{g}/\text{cm}^2/\text{h}$) was calculated from its increasing amount in the receptor medium. [8] NAG's permeability coefficient (k_p) in cm/h was calculated from known physiochemical parameters. [9] NAG's experimental permeability coefficients (K_p) in cm/hr were determined by dividing the steady-state flux by the cumulative donor concentration. Lag time (t_{lag}) was determined graphically from the cumulative amount of drug released per unit area (mg/cm^2) or ($\mu\text{g}/\text{cm}^2$) versus time (h) plots. A square root of time ($t_{1/2}$) *versus* cumulative amount of drug released per unit area (mg/cm^2) or ($\mu\text{g}/\text{cm}^2$) was obtained to monitor NAG in vitro release rate (mg/cm^2) or ($\mu\text{g}/\text{cm}^2$). [10]

Discussion and Results

These *in vitro* studies have demonstrated that N-acetyl-D-glucosamine (NAG), a metabolite of glucosamine, exhibits excellent membrane transport towards transdermal transport and/or percutaneous absorption. The studies have also demonstrated that NAG can be transported effectively *in vitro*.

Initially NAG transport was observed from lecithin-isopropyl palmitate, vitamin E, and

lecithin-vitamin E (1:1) suspensions (100 mg/ml). Negligible (below the limit of detection) transport was observed from all of these suspensions, excluding the permeation of NAG from the lecithin-vitamin mixed E (1:1) suspension, which is shown in **Figure V.1**. Relatively, linear transport was observed from the lecithin-vitamin mixed E (1:1) suspension. NAG's release rate= $13.71 \mu\text{g}/\text{cm}^2$, steady state flux $J_{ss}=4.23 \mu\text{g}/\text{cm}^2/\text{h}$ and its permeability coefficient $k_p=5.012 \times 10^{-3} \text{ (cm/h)}$ were recorded. [**Table V.1**]

For the pluronic organo-gels, each vehicle was composed of a pluronic gel and organic phase, as shown in **Table V.2** along with its respective physicochemical data obtained for the permeation of N-acetylglucosamine (NAG). Each NAG vehicle exhibited lag (t_{lag}) time as reported in **Table V.2**. The cumulative concentration versus time plot to obtain t_{lag} is not shown. As exhibited in **Figure V.2**, the cumulative concentration per unit area versus the square root of time plot shows a comparison of all the vehicles. Overall graphically, the pluronic gel formulations containing organic phase mix of lecithin and isopropyl palmitate out-performed the pluronic gel containing lecithin and vitamin E organic phase vehicles. Formulation III A, which contained the pluronic gel to lecithin-vitamin E mix at a 93:7 exhibited the best release rate, steady state flux and permeability coefficient values, [**Table V.2**]. The data shows that as the organic enhancer phase is decreased, NAG permeation increases for both formulations III A and III B. However, the release rates of formulations II B and III B are comparable. Comparatively, NAG's steady state flux and permeation coefficient is significantly higher from vehicle III A than from III B. A simplified graphical comparison of the physicochemical data obtained is shown in **Figure V.3**. Overall, a more in depth statistical analysis should provide an analysis to determine a starting towards optimal formulation development.

In experiments not reported here, NAG was not transported across shed snake's skin from aqueous vehicles, such as water or buffers. The conclusion from our observations is that

permeation enhancers are needed to effectively transdermally transport NAG. Although this study reports higher permeability for vehicles containing lecithin-isopropyl palmitate as the organic phase; there is reason to further explore this systems mechanism in enhancing NAG's transport.

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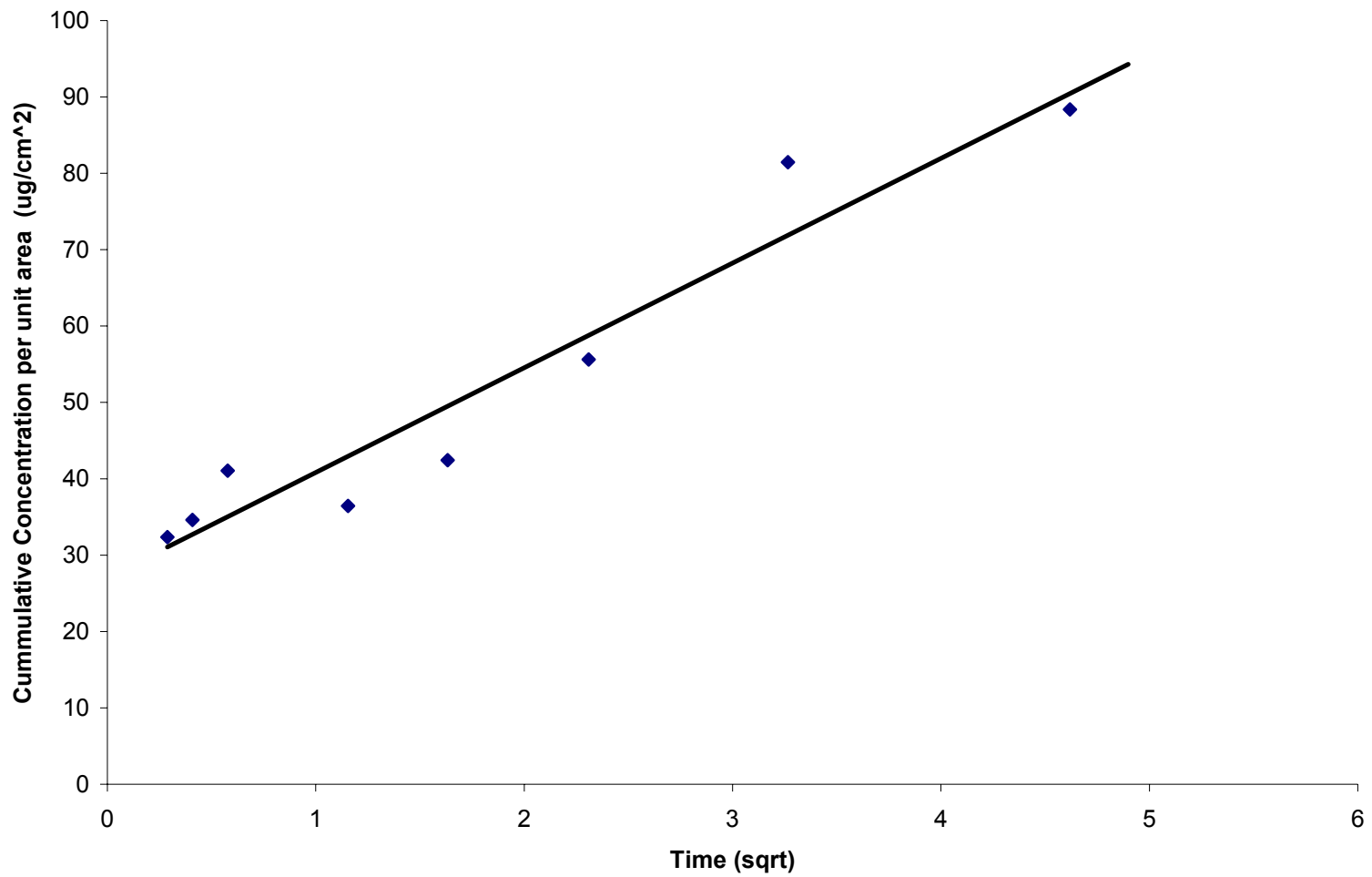


Figure V.1. Effect of soy lecithin-vitamin E on NAG permeation across shed snakeskin. Cumulative concentration ($n=3$) per unit area \pm standard deviation as a function of the square root of time.

Table V.1. Physicochemical data obtained for the permeation of N-acetylglucosamine (NAG) from a lecithin-vitamin E suspension vehicle through shed snakeskin.

Vehicle	J_{ss} ($\mu\text{g}/\text{cm}^2/\text{h}$)	Release rate ($\mu\text{g}/\text{cm}^2$)	k_p (cm/h)	t_{lag} (h^2)
Lecithin-Vitamin E (1:1)	4.23 $\mu\text{g}/\text{cm}^2/\text{h}$	13.71 $\mu\text{g}/\text{cm}^2$	5.012×10^{-3}	0

Table V.2. Physicochemical data obtained for the permeation of NAG in pluronic-organic phase vehicles through shed snakeskin.

Vehicle	Graphical Label	Organic Phase	J_{ss} (mg/cm ² /h)	Release rate (mg/cm ²)	k_p (cm/h)	t_{lag} (h ²)
Pluronic Gel: Organic Phase I A (1:1)	I A	Lecithin: Vitamin E (1:1 w/w)	1.01	6.88	3.2×10^{-3}	6.45
Pluronic Gel: Organic Phase II A (4:1)	II A	Lecithin: Vitamin E (1:1 w/w)	1.06	8.94	3.1×10^{-3}	3.98
Pluronic Gel: Organic Phase III A (93:7)	III A	Lecithin: Vitamin E (1:1 w/w)	10.3	74.9	3.2×10^{-1}	0.85
Pluronic Gel: Organic Phase I B (1:1)	I B	Lecithin: Isopropyl palmitate (1:1w/w)	2.16	14.6	3.1×10^{-4}	5.33
Pluronic Gel: Organic Phase II B (4:1)	II B	Lecithin: Isopropyl palmitate (1:1 w/w)	6.98	38.5	2.5×10^{-4}	6.98
Pluronic Gel: Organic Phase III B (93:7)	III B	Lecithin: Isopropyl palmitate (1:1 w/w)	4.46	43.1	6.8×10^{-4}	2.49

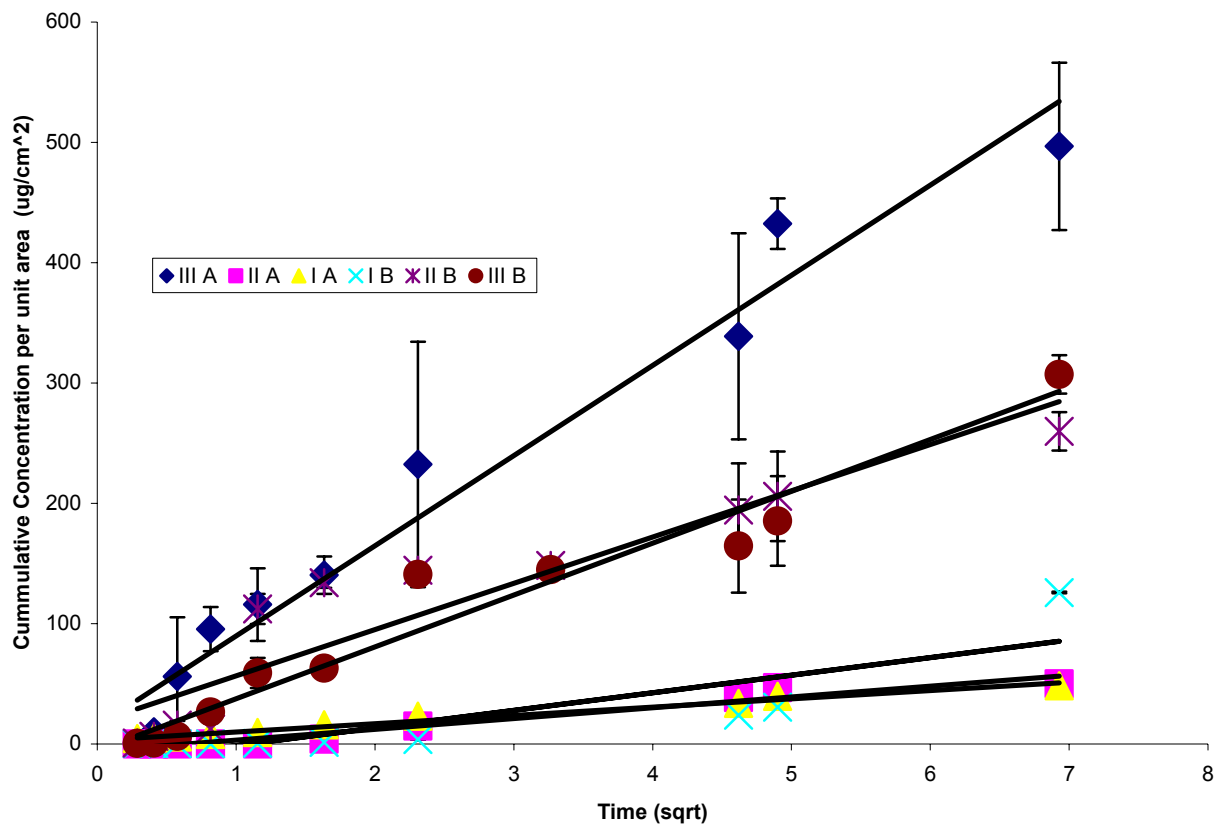


Figure V.2. Physicochemical data obtained for the permeation of NAG in pluronic gel-organic phase vehicles through shed snakeskin. Cumulative concentration (n=3) per unit area \pm standard deviation as a function of the square root of time. **I A** \blacktriangle : Pluronic Gel: Organic Phase I A [Lecithin: Vitamin E (1:1 w/w)] (1:1); **II A** \blacksquare : Pluronic Gel: Organic Phase II A [Lecithin: Vitamin E (1:1 w/w)] (4:1); **III A** \blacklozenge : Pluronic Gel: Organic Phase III B [Lecithin: Vitamin E (0.93:0.07 w/w)] (0.93:0.07); **I B** \times : Pluronic Gel: Organic Phase I B [Lecithin: Isopropyl palmitate E (1:1 w/w)] (1:1); **II B** \times : Pluronic Gel: Organic Phase II B [Lecithin: Isopropyl palmitate E (1:1 w/w)] , **III B** \bullet : Pluronic Gel: Organic Phase III B [Lecithin: Isopropyl palmitate E (1:1 w/w)] (0.93:0.07)]

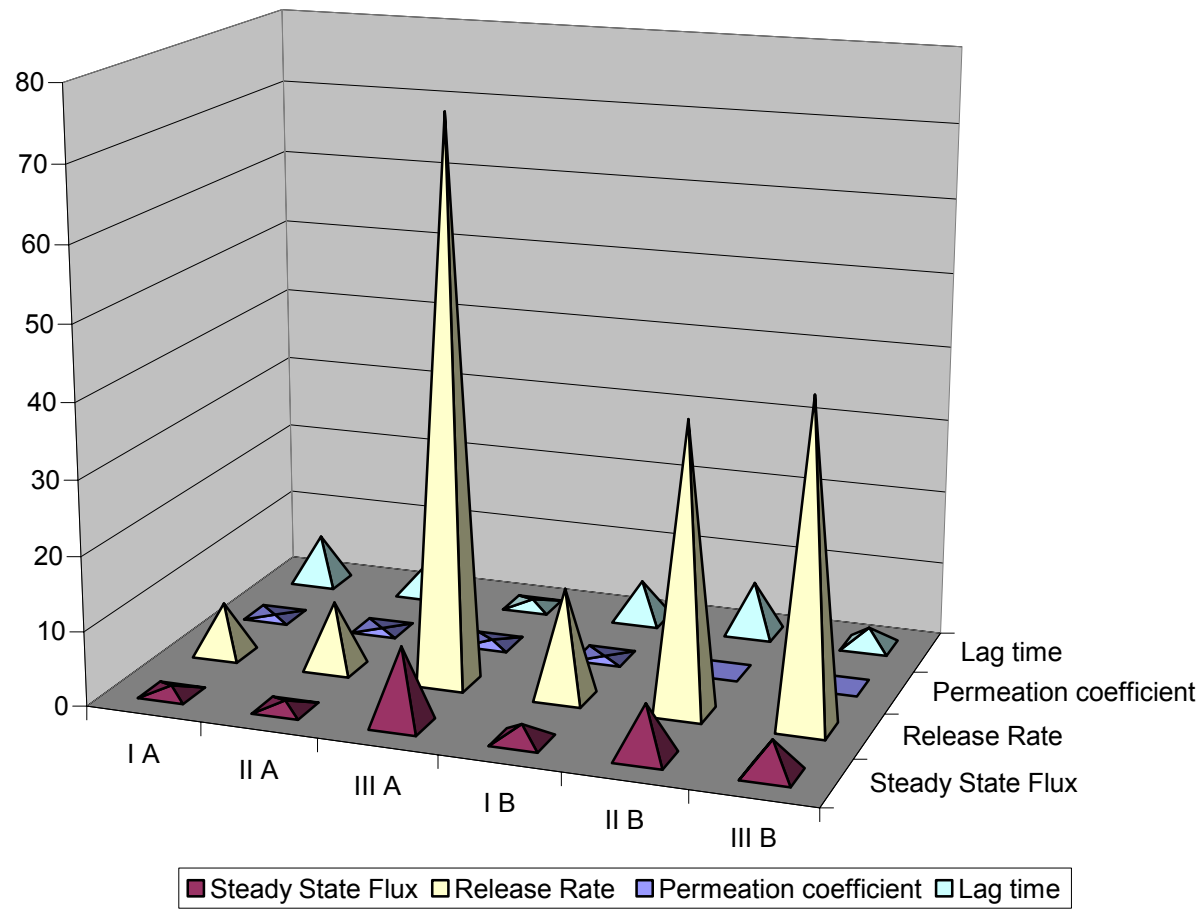


Figure V.3. Graphical comparison of the physicochemical data obtained for the permeation of NAG in pluronic gel-organic phase emulsions through shed snakeskin

CHAPTER VI

SUMMARY AND CONCLUSIONS

Part of this research is about osteoarthritis (OA) therapy beyond the over-the-counter (OTC) and prescription drugs available, e.g. acetaminophen and ibuprofen and prescription medications like Celebrex® (generic name celecoxib). O A sufferers in United States have recently turned to oral nutraceutical glycosaminoglycan products such as glucosamine, chondroitin, and n-acetylglucosamine (NAG). The use of glucosamine became popular after being featured in the book, The Arthritis Cure by Jason Theodasakis, MD. Currently, glucosamine and its metabolites are not classified as drugs but as nutraceutical/dietary supplements under United States Food and Drug Administration's Dietary Supplement Health and Education Act of 1994 (DSHEA). Oral dosage formulations of N-acetyl-D-glucosamine (NAG) and its parent compound glucosamine in salt form (sulfate, hydrochloride etc.) are commercially available nutraceuticals, and are commonly administered in conjunction with chondroitin sulfate, also a readily available nutraceutical. Glucosamine and chondroitin have been reported effective in the oral treatment of osteoarthritis but have not undergone the rigorous studies needed for FDA approval as pharmaceuticals. The National Institutes of Health (Bethesda, MD, USA) has an ongoing multi-center study- GAIT (Glucosamine/Chondroitin Arthritis Intervention Trial) that is currently evaluating the efficacy of orally, administered glucosamine and chondroitin oral supplements. This study will be released later this year. Whereas the enrollment period for a NIH sponsored bioavailability study of glucosamine and chondroitin recently ended.

This research evolved from a challenge-based attempt to initiate development of topical applications of the nutraceuticals, chondroitin and glucosamine. Each project was derived from initial failures encountered towards developing a solid pharmaceuticals research project centered on the initial challenge, which was geared towards product development. In meeting the challenge, two new chemical entities were synthesized and NAG was selected towards preformulation studies. This research for potential osteoarthritis therapy has led to the initial filing of a provisional patent and patent applications with the US Patent and Trademark Office

The synthesis and initial physicochemical evaluation of model mutual prodrugs as potential osteoarthritis therapeutics show some theoretical promise. Similar applications are of the pharmaceutical industry's interest. One objective was met with the synthesis of NSAID-glucosamine mutual prodrugs. These models may or may not improve the pharmaceutical properties of NSAIDs and glucosamine such as their permeability, solubility and stability in relationship to each drug's pharmacological and pharmacokinetic properties. The modifications have altered the physicochemical properties that will affect delivery methods by optimizing the drug's delivery, pharmacodynamic and pharmacokinetic properties. A drawback to the use of a concomitant (mutual prodrug) application of a product delivering an NSAID and glucosamine is that it currently would immediately raise questions by FDA, since glucosamine is not considered a drug and its efficacy has not been proven. Hence, further *in vitro* and especially *in vivo* studies must be undertaken. Therefore, if these products or similar products show clinical significance a new drug application (NDA) would have to be filed due to the claims needed to protect the glucosamine entity. Predictive science inherently says that, like the "statin-drugs", that were developed from nutraceuticals, glucosamine based products will be developed into "blockbuster" drugs with proven efficacies under FDA protocol.

In conclusion, the permeation studies almost never occurred due to: 1) there not being an initial identification of a glucosamine deliverable 2) the lack of in-house analytical equipment capable of analyzing carbohydrates rapidly and efficiently. Whereas once we identified N-acetyl-glucosamine (NAG) as our transdermal candidate, pre-column derivations initially failed due to NAG's N-acyl group and the numerous hydroxy groups that could not be selectively protected effectively.

The collaboration with Dr. Parastoo Azadi group at the University of Georgia's Complex Carbohydrate Research Center was established. Hence progress was made towards the objective of evaluating the transdermal permeability of NAG, in order to assess the feasibility of pursuing a percutaneous absorbed product. The transdermal and percutaneous absorption of NAG will have to be determined in an animal model such as the rabbit, which is model for the study of osteoarthritis. Snakeskin studies, from some researchers perspective, are mainly a tool to identify whether or not a compound will partition well within the skin's dermis and epidermis as indicator of plausible an *in vivo* transdermal transport and percutaneous absorption. Ethanol as a marker for enhancement and the pluronic- organic phase vehicle studies has identified NAG as transdermally transportable chemical entity. These preformulation studies have laid a foundation towards product development for a non-invasive locally delivered osteoarthritis therapy.

APPENDIX A
PROVISIONAL PATENT APPLICATION FILING¹

¹Capomacchia, A. C., Garner, S. T. JR., Beach, W. J. NSAID/Glucosamine Mutual Prodrug and Uses Thereof. US Provisional Patent Application No. 60/560,128. 2004. [Patent Application Filing Priority Date 04/07/2004]

NSAID/GLUCOSAMINE MUTUAL PRODRUG AND USES THEREOF

A United States Provisional Patent Application

NSAID/GLUCOSAMINE MUTUAL PRODRUG AND USES THEREOF

Glucosamine, an amino monosaccharides naturally occurring in the connective and cartilage tissues, contributes to maintaining the strength, flexibility, and elasticity of these tissues. Glucosamine is a precursor to a molecule called a glycosaminoglycan, which is used in the formation and repair of cartilage. In recent years, glucosamine has been used widely to treat osteoarthritis in humans and animal models. *In vivo*, glucosamine is typically converted to N-acetyl-glucosamine.

Non-steroidal anti-inflammatory drugs (NSAIDS) are effective in reducing inflammation but are not highly soluble and, in addition, may have undesirable side effects. Efforts have been made to improve the pharmaceutical properties of NSAIDs, such as permeability, solubility and stability, by creating NSAID prodrugs. Prodrugs are typically evaluated in relation to the drug's pharmacological and pharmacokinetic properties. These modifications may alter the physicochemical properties of the drug, which may in turn effect the administration options that optimize drug delivery.

“Mutual prodrug” is a relatively new term in medicinal chemistry, pharmaceuticals and drug delivery. Ueda and Tani (1990) chemically linked selected known pharmaceutically active compounds to produce a chimeric drug now known as a mutual prodrug. Specifically, they linked an anti-ulcer drug-histamine H₂ antagonist via an ester linkage to yield a new pharmaceutical agent producing a desired pharmaceutical profile. Glucametacine, the glucosamide pro moiety and alternative to its parent drug indomethacin, is another example of a mutual prodrug (capable of delivering both glucosamine- a glycosaminoglycan, and indomethacin) although it is neither promoted nor marketed

as such. Glucamethacin is marketed in Europe as an effective anti-inflammatory agent in patients with chronic rheumatic conditions, and is usually associated with fewer side effects than the parent moiety, indomethacin.

The present invention provides for the topical delivery of glucosamine and glucosamine derivative, preferably, N-acetyl glucosamine; 2-Amino-1, 3, 4, 6-acetyl- β -D-glucopyranosyl, 2-Acetamido-2-deoxy- β -D-glucopyranose 1, 3, 4, 6-tetraacetate; 2-Acetamido-2-deoxy- β -D-glucopyranose: or their acetylated analogues. Optionally, topical application of a glucosamine or glucosamine derivative can be accompanied by co-administration of anti-inflammatory compound such as NSAID. When administered together, the NSAID and the glucosamine derivative may, but need not, be covalently linked to form a mutual prodrug as described below.

The invention further provides a pharmaceutical composition that includes a mutual prodrug containing two pharmaceutically active substances. We have developed and performed preliminary preformulation studies of other glucosamine-NSAID analogues as a delivery system for glucosamine and NSAIDs to produce anti-inflammatory effect as well as cartilage growth and/or maintenance in osteoarthritis sufferers. Mutual prodrugs that include modified NSAIDs coupled with a glycosaminoglycan or ester of glycosaminoglycans or glycosaminoglycan esters, covalently linked to an NSAID, including derivatives thereof. The NSAID can be, for example, ibuprofen or ketoprofen. The two structural components of the mutual prodrug are preferably joined by an ester linkage, which can be cleaved after delivery to a patient by endogenous esterases. Cleavage of the mutual prodrug after administration to the patient releases a plurality of pharmaceutically active components.

The novel pharmaceutical composition of the invention can be administered to a patient using any convenient route, without limitation. In a preferred embodiment, the mutual prodrug is administered to a patient topically via local application to the skin or other external or internal

membrane. In some formulations, transdermal delivery is achieved, allowing the mutual prodrug or its metabolic products to be delivered to the bloodstream and circulated systemically. In another preferred embodiment, the mutual prodrug is administered orally. Other forms of administration, such as subcutaneous, intramuscularly and intravenous injections are also included.

The complete disclosures of all patents, patent applications including provisional patent applications, and publication, and electronically available material (e.g., GenBank amino acid and nucleotide sequence submissions) cited herein are incorporated by reference. The following detailed description and examples have been provided for clarity of understanding only. No unnecessary limitations are to be understood therefrom. The invention is not limited to the exact details shown and described; many varied will be apparent to one skilled in the art.

Novel Use Prodrugs for the Transdermal Delivery of Glucosamine (esters of glycosaminoglycans) Novel Mutual Prodrugs That Concomitantly Deliver Both Glucosamine (or N-Acetyl Glucosamine) and an NSAID (non-steroidal anti-inflammatory drug) like Ibuprofen or Aspirin; and Novel Use Prodrugs to Transdermally Deliver Glucosamine and N-Acetyl Glucosamine

The invention is in two parts: First, 2-Amino-1, 3, 4, 6-acetyl-beta-D-glucopyranosyl [1] and 2-Acetamido-2-deoxy-beta-D-glucopyranose 1, 3, 4, 6-tetraacetate [2] or their acetylated analogues may be conjugated, via an imide ester or ester linkage, to carbonyl containing chemical entities such as those found in non-steroidal anti-inflammatory drugs (NSAIDs, i.e. ibuprofen, aspirin) to concomitantly deliver two pharmaceutically active entities, Glucosamine (or N-acetyl Glucosamine) and an NSAID. Drug delivery of the two agents occurs after enzymatic cleavage of the ester linkage; via transdermal and/or oral formulations, not excluding buccal or subcutaneous formulations. Second, compounds [1] and [2] are themselves glucosamine prodrugs. The active drug glucosamine is linked to carrier acetyl group(s), and glucosamine (or N-Acetylglucosamine) are also released by enzymatic action as stated. Compound [1] is known but not commercially available; Compound [2] is known and commercially available. Transdermal delivery of the mutual prodrugs as described and synthesized, and Compounds [1] and [2], may be used for treatment of osteoarthritis, and is viewed as the primary commercial use. However, oral and other drug delivery routes, as mentioned, may also be commercially viable.

EXAMPLES

Novel Use Prodrugs for the *present invention is illustrated by the following examples which describe inter alia (1) mutual prodrugs and (2) topical and/or transdermal application of glycosaminoglycans and esters thereof. It is to be understood that the particular examples, materials, amounts and procedures are to be interpreted broadly in accordance with the scope and spirit of the invention as set forth herein.*

Transdermal Delivery of Glucosamine (esters of glycosaminoglycans) Novel Mutual Prodrugs That Concomitantly Deliver Both Glucosamine (or N-Acetyl Glucosamine) and an NSAID (non-steroidal anti-inflammatory drug) like Ibuprofen or Aspirin; and Novel Use Prodrugs to Transdermally Deliver Glucosamine and N-Acetyl Glucosamine

I. Abstract: Mutual Prodrugs

The present invention provides modified NSAIDS coupled with a glycosaminoglycan or ester of glycosaminoglycan. According to the invention mutual prodrugs are provided. The mutual prodrugs may work under a number of mechanisms to provide their parent or active metabolites as a 1) Carrier-linked Prodrugs- the active drug molecule is linked to the carrier moiety and usually the active drug is released by enzymatic means. [All the necessary functional groups on the drug molecule are present in the prodrug.]; 2) Bio-precursor Prodrugs- the prodrug does not contain the active functionality, but after metabolic conversion, the active drug is generated; or 3) Chemical Activated Prodrugs- the prodrug is converted into the active species by chemical mean. Following conversion the agents mutually provide the therapeutic benefits of NSAIDS and

glycosaminoglycans. Therefore pain is managed via COX-1/COX-2 inhibition mechanisms of NSAIDS and the maintenance and/or repair of tissues *via* regulation of cellular events and/or physiological processes, such as cell-cell and cell-matrix interactions, and cell proliferation/differentiation is managed by glycosaminoglycans or esters of glycosaminoglycans.

Background

Ueda and Tani were the first to develop the mutual prodrug concept in 1990. The concept is based on chemically bonding two drugs, usually *via* an ester linkage that is easily degraded by mammalian esterases, so that after administration each drug is released to produce its respective pharmacological action. At first it was named a chimera prodrug, since it is composed of two parts; however mutual prodrug is a better description since both entities are active. The linking of the two drugs may impart a protective effect so that unwanted degradation, usually by stomach acids may be prevented. Also, the mutual prodrug will usually exhibit different aqueous/lipid solubility profiles, which may aid in formulation and/or delivery. Ueda's and Tani's mutual prodrug combined a non-steroidal anti-inflammatory drug (NSAID) with an anti-ulcer drug (histamine H₂ antagonist *via* an ester linkage). The goal was to design a way to deliver an NSAID without producing stomach ulcers, therefore the NSAID was linked to an antiulcer agent as a rational attempt to offset any gastric distress caused by the NSAID. Using this concept as a template, our group has developed potential osteoarthritis' therapeutics via the mutual prodrug approach.

Description

The objective with the synthesis of NSAID prodrugs has been to improve the pharmaceutical properties of drugs such as permeability, solubility and stability in relationship to a drug's pharmacological and pharmacokinetic properties. These modifications alter the physiochemical

properties which may affect delivery methods i.e. route of administration in optimizing drug delivery, pharmacodynamic and pharmacokinetic properties. For example, glucametacine is the glucosamide promoiety and alternative to its parent drug indomethacin marketed in Europe as an effective anti-inflammatory agent in patients with chronic rheumatic conditions. It has few and usually mild sides effects in comparison to its parent moiety. Currently it is not promoted, nor indicated as a mutual prodrug to deliver both glucosamine -a glycosaminoglycan, and indomethacin.

The synthesis of glycosaminoglycan/s and ester/s of glycosaminoglycan/s covalently bonded to NSAIDs has commenced. Thus the concept of these chemically entities being mutual prodrugs exist. The chemical entities exist as concomitant delivery systems for glucosamine or ester/s of glycosaminoglycan/s and NSAIDS. Changes and /or improvements in the pharmaceutical properties of the parent moieties such as permeability, solubility and stability in relationship to the pharmacological and pharmacokinetic properties exist. Furthermore the concomitant delivery of these prodrugs can provide for example anti-inflammatory affects and connective tissues repair in the treatment of osteoarthritis.

Synthesis of Mutual Prodrugs

Example I

Compounds 1- 3 were synthesized from procedures adapted from 1) Bergman, M., and Zervas, L., Chem. Ber. 1932, 975 2) Silva, D. J., et. al J. Org. Chem. 1999, 64, 5926-5929. (Supplemental Material) and Charviere, G. et. al J. Med. Chem. 2003, 46, 427-220. Final product examples are compounds 6, 7 and 10.

2-deoxy-2-amino-1, 3, 4, 6-tetra-O-acetyl- β -D-glucopyranosyl (4) 3 (100 g, 0.26 mol) was titrated with triethylamine to yield a white precipitate. The precipitate was filtered and washed

CH₂Cl₂ (2x150 ml). The filtrate was dried under vacuum for 24 hrs. The organic layer was with brine (2x100) dried with MgSO₄. The solvent was removed via reduced pressure rotary evaporation and product dried for 24 hrs under vacuum. Compound **4** was afford as a white solid (87.9 g, 97% yield). ¹H NMR (d-acetone) 9.21 (s, 1H), 6.15 (d, 1H), 5.38 (t, 1H), 5.08 (t, 1H), 4.31 (dd, 1H), 4.11-4.03 (m, 2H), 3.56 (t, 1H), 3.03-2.05 (dd, 6H), 2.25 (d, 3H), 2.10-2.09 (m, 3H). ¹³C NMR (d-acetone) 205.7, 170.1, 169.87, 169.39, 169.0, 95.20, 74.85, 72.28, 68.61, 61.90, 55.46, 19.98, 19.85, 19.82, 19.77. ES1 for C₁₄H₂₁NO₉: FW 347 found *m/z* 348 [M + H⁺]. Mp 134° C

2-deoxy-2-(2-chloro-2-phenyl)acetylamino-1, 3, 4, 6-tetra-O-acetyl-β-D-glucopyranosyl (5) α-Chlorophenylacetyl chloride (20g, 0.105 mol) was added drop-wise to stirring solution of **4** (29.88g, 0.105 mol), triethylamine (12.4 ml, 0.90 ml) in 50 ml CH₂Cl₂ at -10° to r.t. for 24 hours. The reaction mixture was washed with HCl (1.5 N, 2 x 7 ml), H₂O (1 x 100 ml) and brine (1 x 100 ml). The organic phase was dried with Mg₂SO₄ and solvent removed via reduced pressure rotary evaporation. The resultant was crystallized with ice cold acetonitrile and dried under vacuum for 24 hrs. **5** (27.4g, 93.5%) was obtained as a white solid. ¹H NMR (d-acetone) 7.80 (s, 1H), 7.37(s, 2H), 7.25 (s, 2H), 5.79 (s, 1H), 5.33 (d, 2H), 4.90 (s, 1H), 4.09 (d, 2H), 3.95 (s, 1H), 3.84 (s, 1H), 3.17 (s, 1H) 1.87-1.64 (m, 12H). ¹³C NMR (d-acetone) 205.55, 16.88, 169.69, 169.18, 168.51, 167.69, 128.85, 128.61 (2C), 127.81 (2C) 91.03, 68.54, 61.70, 60.60 53.13, 19.73, 19.70 (2C), 19.62. ES1 for C₁₄H₂₁NO₉: FW [M + H⁺] 499 found *m/z* 500 440 [M + H⁺]. Mp >200(238)° C

2-deoxy-2-[(4-Isobutyl-phenyl)-2-propionic acid-2-phenyl]acetylamino-1, 3, 4, 6-tetra-O-acetyl-β-D-glucopyranosyl (6) **5** (653 mg, 1.45 mmol) and α-methyl-4-[isobutyl]phenylacetic acid-Na salt in anhydrous 10 ml CH₂Cl₂ was stirred at room temperature for 16 hours. The solution was washed with brine (2 x 10ml) and reduced via rotary evaporation to give **6** (763 mg, 93.5%). ¹H NMR (d-acetone) 7.73 (s, 1H), 7.41-7.13 (m, 9H), 5.81 (d, 2H), 5.38 (m, 1H), 5.04 (m, 1H), 4.26 (s,

2H), 4.10 (s,1H), 3.96 (s, 2H), 2.48 (s, 2H), 2.01 (s, 6H), 1.84 (s, 6H), 1.65 (s, 1H), 1.53 (s, 3H), 0.90 (s, 6H) . ¹³C NMR (d-acetone) 205.69, 173.37, 169.61, 168.73, 168.70, 140.38, 138.43, 138.03, 129.25, 128.36, 127.31, 126.84, 91.96, 75.91, 72.54, 71.66, 68.31, 52.07, 44.61, 21.72. 19.61, 17.96 ES1 for C₃₅H₄₃NO₁₂ FW 669 found *m/z* 522 [M + H⁺] w/loss of (C₁₁H₁₅).

2-deoxy-2-[(4-Isobutyl-phenyl)-2-propionic acid-2-phenyl]acetyl-amino-1, 3, 4, 6-tetra-hydroxy-β-D-glucopyranosyl (7) de-acetylated product of final compound 6 (*in situ, data to be completed*)

2-deoxy-2-amino-1, 3, 4, 6-tetra-O-triethylsilyl-β-D-glucopyranosyl (8) Glucosamine hydrochloride (7.32 g, 33.95 mmol) and DMAP (cat.) was stirred in 100 ml anhydrous pyridine for three hours at room temperature. Chlorotriethylsilane (20.47 g, 136.27 mmol) was add dropwise while the solution stirred on an ice bath at -5° C to room temperature for 16 hrs and at 40-45° C for 2 hrs. The pyridine was removed and the resulting oil was wash with 1:1 ethyl acetate and subsequently with 1:1 ethyl acetate and brine. The final organic layer was dried with Na₂SO₄ and solvent removed via rotary evaporation to give a colorless oil, which was dried overnight under vacuum, affording **8** (21.82g; 97%) as a white foam. ¹H NMR (CD₃OD): 5.39 (d, 1H), 4.83-4.80 (m, 2H), 3.89-3.47 (m, 3H), 3.09-3.01 (dd, 1H), 1.04-0.53 (m, 60H). ES1 for C₃₀H₆₉NO₅Si₄: FW 636 found *m/z* 636 [M⁺] and 522 [M + H⁺] w/loss of (C₆H₁₅).

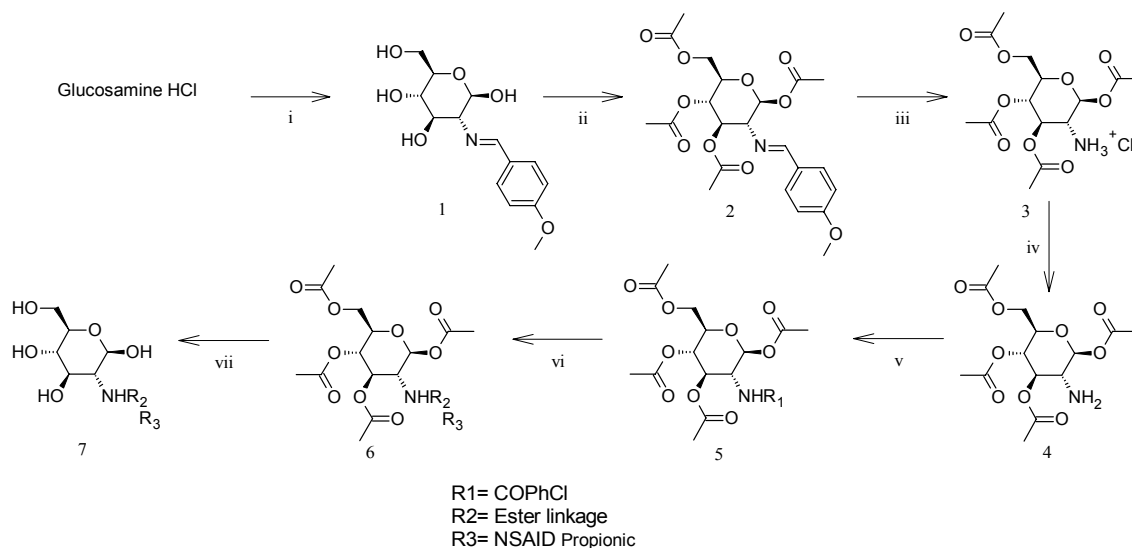
2-deoxy-2-[2-(3-benzoylphenyl)propanoic acid]amino-β-D-glucopyranosyl (10) **8** (28 g, 43.5 mmol) and DMAP (cat) was stirred in 10 ml acetonitrile on an ice bath. In a separated vessel, (2*S*)-2-(3-benzoylphenyl)propanoic acid (11 g, 43.55 mmol) in 10 ml acetonitrile was stirred on an ice bath with BuNMe₂ (8.75 ml, 120 mmol), Me₂NSO₂Cl (9.67 ml, 90 mmol) until the solution became

clear. Both solutions were mixed and stirred at 0° C to room temperature over 12 hours. The acetonitrile was removed via rotary evaporation. The resulting syrup was wash with 2 x 150 water/ethyl acetate (1:1) and the organic layer 2 x 100 ml brine. The organic layer was dried via rotary evaporation and in vacuo for 12 hours to give 2-deoxy-2-[2-(3-benzoylphenyl)propanoic acid]amino-1, 3, 4, 6-tetra-O-triethylsilyl - β -D-glucopyranosyl (**9**) (43 g, quantitative: ES1 for C₄₆H₈₁NO₇Si₄: FW 872 found m/z 857 [M + H⁺] w/loss CH₃) as a yellow syrup. **9** was directly de-protected with t-butyl ammonium fluoride/MeOH to give white precipitate that was filtered, washed with methanol and recrystallized from hot ethanol to give **10** (17.3 g, 85%). ¹H NMR (DMSO-d₆) 7.63-7.24 (m, 9H), 6.17 (s, 1H), 4.65 (s, 1H), 4.56 (s, 1H), 4.44 (s, 1H), 4.21 (s, 1H), 3.61 (s, 1H), 3.45-2.10 (m, 4H), 2.83 (d, 2H), 2.25 (d, 3H), 1.1 (s, 3H). ¹³C NMR (DMSO- d₆) 197.13, 176.39, 141.67, 137.64, 137.34, 132.54, 131.73, 129.69 (2C), 128.79, 128.47, 128.41 (2C) , 128.19, 95.20, 74.85, 72.28, 68.61, 61.90, 55.46, 45.09, 18.12 . ES1 for C₂₂H₂₅NO₇: FW 416 found m/z 416 [M + H⁺]. Mp 164° C

II. Abstract: Transdermal application

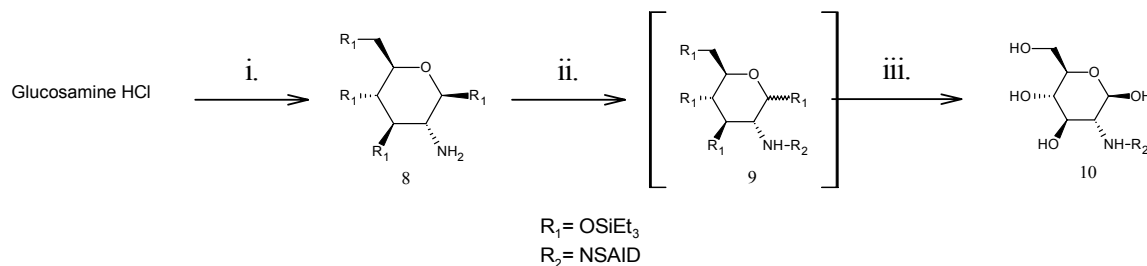
The invention describes composition and method for the application to deliver ester/s of glycosaminoglycan/s. These are biologically significant carbohydrates. They exist as free chains or constituents of proteoglycans. The composition uses an ester/s of a glycosaminoglycan/s admixed in a cream, gel, solution or ointment base consisting of fatty esters, alcohols, gel bases and water to form a transdermal and/or topical agent. The ester/s of glycosaminoglycan/s act to maintain and or repair of tissues *via* regulation of cellular events and/or physiological processes, such as cell-cell and cell-matrix interactions, and cell proliferation/differentiation.

Example 1
Scheme I: Spacer Linked Glycosaminoglycan –NSAID Mutual Prodrugs



i) 1 N NaOH, p-methoxybenzaldehyde/ 0°C - r.t. 1 hrs **ii)** pyridine (anhydrous), acetic anhydride/ r.t. 24 hrs **iii)** acetone (reflux) and 5 N HCl **iv)** triethylamine, water **v)** chlorophenylacetyl chloride, triethylamine, **vi)** NSAID, CH₂Cl₂ **vii)** methanol/ammonium (gas)

Example II
Scheme II: Directly Linked Glycosaminoglycan-NSAID Mutual Prodrugs



i) Trichloroethylsilane, pyridine (anhydrous) **ii)** NSAID, Me₂NSO₂Cl, MeCN, BuNMe₂, DMAP **iii)** TBAF, Methanol

Background

There exists a need to deliver esters of glycosaminoglycans for the repair of and maintenance of connective tissues and cartilage *via* non-invasive procedures. Currently recognized methods of delivery are by oral, subcutaneous injections and/or intramuscular/intravenous injection. The

disadvantages associated with these methods, respectively include drug loss by first pass metabolism and, invasiveness of administration, therefore a topical formulation is sought.

The invention relates to the therapeutic use of compositions containing ester/s of glycosaminoglycan/s, including chondroitin sulfates, dermatan sulfates, heparin, heparan sulfate, and keratan sulfate, but not excluding all other biologically significant proteoglycans. Examples of the esters, which are components of connective tissue and cartilage, include 1) 2-Amino-1, 3, 4, 6-acetyl- β -D-glucopyranosyl, 2) 2-Acetamido-2-deoxy- β -D-glucopyranose 1, 3, 4, 6-tetraacetate, and 3) 2-Acetamido-2-deoxy- β -D-glucopyranose as well as other esters of glycosaminoglycans formed *via* an imide, carbonyl or ester or ester linkage.

Description

The ester/s of the glycosaminoglycan/s may be applied to the affected area. The area may be affected by osteoarthritis, wrinkles, tenderness or pain. The ester may be formulated/admixed into a fatty ester, pluronic gel, lecithin, dimethyl sulfoxide or any number or combination of topical/transdermal vehicles approved by the United States Pharmacopoeia (USP) for human or veterinarian use.

Examples: Formulation admixes at any concentration containing either of the esters at concentrations ranging from 1% - 75% for 1) 2-Amino-1, 3, 4, 6-acetyl- β -D-glucopyranosyl, 2) 2-Acetamido-2-deoxy- β -D-glucopyranose 1, 3, 4, 6-tetraacetate, or 3) 2-Acetamido-2-deoxy- β -D-glucopyranose as *per*, but not confined to the following examples:

1. Isopropyl Palmitate, DMSO, Ethanol, Lecithin, Poloxamer, Ester of glycosaminoglycan

2. Isopropyl Palmitate, Water, Ethanol, Lecithin, Poloxamer, Ester of glycosaminoglycan
3. Isopropyl Palmitate, Vitamin E, Lecithin, Poloxamer, Ester of glycosaminoglycan
4. Isopropyl myristate, Poloxamer, Water, Ethanol, Lecithin, Poloxamer, Ester of glycosaminoglycan
5. Isopropyl myristate, Water, Lecithin, Poloxamer, Ester of glycosaminoglycan
6. Isopropyl Palmitate, Water, Lecithin, Poloxamer, USP approved antimicrobial agent, Ester of glycosaminoglycan
7. Vitamin E, Lecithin, Isopropyl myristate, Ester of glycosaminoglycan
8. Isopropyl myristate, Lecithin, Poloxamer, Ester of glycosaminoglycan
9. Isopropyl Palmitate, USP approved antimicrobial agent, Vitamin E, Lecithin, Hydrous or Anhydrous Lanolin, Ester of glycosaminoglycan
10. Isopropyl Palmitate, USP approved antimicrobial agent, Vitamin E, Hydrous or Anhydrous Lanolin, Ester of glycosaminoglycan
11. Isopropyl Palmitate, USP approved antimicrobial agent, Vitamin E, Lecithin, Ester of glycosaminoglycan
12. Isopropyl Palmitate, Lecithin, USP approved antimicrobial agent, Hydrous or Anhydrous Lanolin, Ester of glycosaminoglycan
13. Isopropyl myristate, Vitamin E, USP approved antimicrobial agent, Lecithin, Hydrous or Anhydrous Lanolin, Ester of glycosaminoglycan
14. Isopropyl myristate, USP approved antimicrobial agent, Vitamin E, Lecithin, Ester of glycosaminoglycan
15. Isopropyl myristate, USP approved antimicrobial agent, Vitamin E, Hydrous or Anhydrous Lanolin, Ester of glycosaminoglycan

16. Isopropyl myristate, USP approved antimicrobial agent, Lecithin, Hydrous or Anhydrous Lanolin, Ester of glycosaminoglycan
17. Isopropyl myristate, USP approved antimicrobial agent, Vitamin E, Ester of glycosaminoglycan
18. Isopropyl myristate, USP approved antimicrobial agent, Lecithin, Ester of glycosaminoglycan
19. Isopropyl myristate, USP approved antimicrobial agent, Hydrous or Anhydrous Lanolin, Ester of glycosaminoglycan
20. Isopropyl myristate, DMSO, Ethanol, Lecithin, Poloxamer, Ester of glycosaminoglycan
21. Isopropyl myristate, Vitamin E, Lecithin, Hydrous or Anhydrous Lanolin, Ester of glycosaminoglycan
22. Isopropyl myristate, DMSO, Ethanol, Vitamin E, Poloxamer, Ester of glycosaminoglycan
23. Isopropyl myristate, DMSO, Vitamin E, Poloxamer, Ester of glycosaminoglycan
24. Isopropyl myristate, DMSO, Ethanol, Vitamin E, Poloxamer, Ester of glycosaminoglycan
25. Isopropyl Palmitate, DMSO, Ethanol, Lecithin, Vitamin E, Poloxamer, Ester of glycosaminoglycan
26. Isopropyl Palmitate, Ethanol, Lecithin, Poloxamer, Ester of glycosaminoglycan
27. Isopropyl Palmitate, DMSO, Ethanol, Lecithin, Ester of glycosaminoglycan
28. Isopropyl Palmitate, DMSO, Ethanol, Poloxamer, Ester of glycosaminoglycan
29. Isopropyl Palmitate, Ethanol, Lecithin, Ester of glycosaminoglycan
30. Isopropyl Palmitate, DMSO, Ethanol, Lecithin, Vitamin E, Poloxamer, Ester of glycosaminoglycan
31. Isopropyl Palmitate, Isopropyl myristate, DMSO, Ethanol, Lecithin, Poloxamer, Ester of glycosaminoglycan

32. Isopropyl Palmitate, Isopropyl myristate, DMSO, Ethanol, Lecithin, Vitamin E, Poloxamer, Ester of glycosaminoglycan
33. Isopropyl Palmitate, Isopropyl myristate, DMSO, Ethanol, Poloxamer, Ester of glycosaminoglycan
34. Isopropyl Palmitate, Isopropyl myristate, DMSO, Ethanol, Vitamin E, Poloxamer, Ester of glycosaminoglycan
35. Isopropyl Palmitate, Isopropyl myristate, DMSO, Ethanol, Lecithin, Ester of glycosaminoglycan
36. Isopropyl Palmitate, Isopropyl myristate, DMSO, Ethanol, Lecithin, Vitamin E, Poloxamer, Ester of glycosaminoglycan
37. Isopropyl Palmitate, Isopropyl myristate, DMSO, Lecithin, Vitamin E, Poloxamer, Ester of glycosaminoglycan
38. Isopropyl Palmitate, Isopropyl myristate, DMSO, Vitamin E, Poloxamer, Ester of glycosaminoglycan
39. Isopropyl Palmitate, Isopropyl myristate, DMSO, Lecithin, Poloxamer, Ester of glycosaminoglycan
40. Isopropyl Palmitate, Isopropyl myristate, DMSO, Lecithin, Vitamin E, Ester of glycosaminoglycan
41. Isopropyl Palmitate, Isopropyl myristate, DMSO, Poloxamer, Ester of glycosaminoglycan
42. Isopropyl Palmitate, Isopropyl myristate, DMSO, Lecithin, Ester of glycosaminoglycan
43. Isopropyl Palmitate, Isopropyl myristate, DMSO, Vitamin E, Ester of glycosaminoglycan
44. Isopropyl Palmitate, Isopropyl myristate, Water, Ethanol, Lecithin, Ester of glycosaminoglycan

45. Isopropyl Palmitate, Isopropyl myristate, Water, Ethanol, Vitamin E, Poloxamer, Ester of glycosaminoglycan
46. Isopropyl Palmitate, Isopropyl myristate, Water, Ethanol, Vitamin E, Poloxamer, Hydrous or Anhydrous Lanolin, Ester of glycosaminoglycan
47. Isopropyl Palmitate, Isopropyl myristate, Water, Ethanol, Poloxamer, Hydrous or Anhydrous Lanolin, Ester of glycosaminoglycan
48. Isopropyl Palmitate, Isopropyl myristate, Water, Ethanol, Vitamin E, Hydrous or Anhydrous Lanolin, Ester of glycosaminoglycan
49. Isopropyl Palmitate, Isopropyl myristate, Water, Ethanol, Vitamin E, Lecithin, Poloxamer, Hydrous or Anhydrous Lanolin, Ester of glycosaminoglycan
50. Isopropyl Palmitate, Isopropyl myristate, Water, Ethanol, Lecithin, Hydrous or Anhydrous Lanolin, Ester of glycosaminoglycan
51. Isopropyl Palmitate, Isopropyl myristate, Water, Ethanol, Vitamin E, Hydrous or Anhydrous Lanolin, Ester of glycosaminoglycan
52. Isopropyl Palmitate, Isopropyl myristate, Water, Ethanol, Poloxamer, Hydrous or Anhydrous Lanolin, Ester of glycosaminoglycan
53. Isopropyl Palmitate, Isopropyl myristate, Water, Ethanol, Hydrous or Anhydrous Lanolin, Ester of glycosaminoglycan
54. Isopropyl Palmitate, Isopropyl myristate, Water, Ethanol, Poloxamer, Ester of glycosaminoglycan
55. Isopropyl Palmitate, Isopropyl myristate, Water, Ethanol, Ester of glycosaminoglycan
56. Isopropyl Palmitate, Isopropyl myristate, Water, USP approved antimicrobial agent, Lecithin, Vitamin E, Poloxamer, Ester of glycosaminoglycan

57. Isopropyl Palmitate, Isopropyl myristate, Water, USP approved antimicrobial agent, Vitamin E, Poloxamer, Ester of glycosaminoglycan
58. Isopropyl Palmitate, Isopropyl myristate, Water, USP approved antimicrobial agent, Lecithin, Poloxamer, Ester of glycosaminoglycan
59. Isopropyl Palmitate, Isopropyl myristate, Water, USP approved antimicrobial agent, Lecithin, Vitamin E, Ester of glycosaminoglycan
60. Isopropyl Palmitate, Isopropyl myristate, Water, USP approved antimicrobial agent, Lecithin, Ester of glycosaminoglycan
61. Isopropyl Palmitate, Isopropyl myristate, Water, USP approved antimicrobial agent, Poloxamer, Ester of glycosaminoglycan
62. Isopropyl Palmitate, Isopropyl myristate, Water, USP approved antimicrobial agent, Vitamin E, Ester of glycosaminoglycan
63. Isopropyl Palmitate, Isopropyl myristate, Water, Ethanol, Lecithin, Vitamin E, Poloxamer, Ester of glycosaminoglycan
64. Isopropyl Palmitate, Isopropyl myristate, Ethanol, Lecithin, Poloxamer, Ester of glycosaminoglycan
65. Isopropyl Palmitate, Isopropyl myristate, DMSO, Ethanol, Lecithin, Ester of glycosaminoglycan
66. Isopropyl Palmitate, Isopropyl myristate, Ethanol, Lecithin, Vitamin E, Poloxamer, Ester of glycosaminoglycan
67. Isopropyl Palmitate, Isopropyl myristate, Water, Ethanol, Lecithin, Poloxamer, Ester of glycosaminoglycan
68. Isopropyl Palmitate, Isopropyl myristate, Water, USP approved antimicrobial agent, Lecithin, Poloxamer, Ester of glycosaminoglycan

69. Isopropyl Palmitate, Isopropyl myristate, DMSO, Ethanol, Poloxamer, Ester of glycosaminoglycan
70. Isopropyl Palmitate, Isopropyl myristate, Ethanol, Lecithin, Ester of glycosaminoglycan
71. Isopropyl Palmitate, Isopropyl myristate, USP approved antimicrobial agent, Vitamin E, Lecithin, Hydrous or Anhydrous Lanolin, Ester of glycosaminoglycan
72. Isopropyl Palmitate, Isopropyl myristate, USP approved antimicrobial agent, Lecithin, Hydrous or Anhydrous Lanolin, Ester of glycosaminoglycan
73. Isopropyl Palmitate, Isopropyl myristate, USP approved antimicrobial agent, Vitamin E, Hydrous or Anhydrous Lanolin, Ester of glycosaminoglycan
74. Isopropyl Palmitate, Isopropyl myristate, USP approved antimicrobial agent, Vitamin E, Lecithin, Ester of glycosaminoglycan
75. Isopropyl Palmitate, Isopropyl myristate, USP approved antimicrobial agent, Vitamin E, Ester of glycosaminoglycan
76. Isopropyl Palmitate, Isopropyl myristate, USP approved antimicrobial agent, Lecithin, Ester of glycosaminoglycan
77. Isopropyl Palmitate, Isopropyl myristate, USP approved antimicrobial agent, Hydrous or Anhydrous Lanolin, Ester of glycosaminoglycan
78. Isopropyl Palmitate, Isopropyl myristate, USP approved antimicrobial agent, Vitamin E, Lecithin, Hydrous or Anhydrous Lanolin, Poloxamer, Ester of glycosaminoglycan
79. Isopropyl Palmitate, Isopropyl myristate, USP approved antimicrobial agent, Lecithin, Hydrous or Anhydrous Lanolin, Poloxamer, Ester of glycosaminoglycan
80. Isopropyl Palmitate, Isopropyl myristate, USP approved antimicrobial agent, Vitamin E, Hydrous or Anhydrous Lanolin, Poloxamer, Ester of glycosaminoglycan

81. Isopropyl Palmitate, Isopropyl myristate, USP approved antimicrobial agent, Vitamin E, Lecithin, Poloxamer, Ester of glycosaminoglycan
82. Isopropyl Palmitate, Isopropyl myristate, USP approved antimicrobial agent, Hydrous or Anhydrous Lanolin, Poloxamer, Ester of glycosaminoglycan
83. Isopropyl Palmitate, Isopropyl myristate, USP approved antimicrobial agent, Lecithin, Poloxamer, Ester of glycosaminoglycan
84. Isopropyl Palmitate, Isopropyl myristate, USP approved antimicrobial agent, Vitamin E, Poloxamer, Ester of glycosaminoglycan
85. Isopropyl Palmitate, Isopropyl myristate, USP approved antimicrobial agent, Poloxamer, Ester of glycosaminoglycan
86. Isopropyl Palmitate, Isopropyl myristate, Water, USP approved antimicrobial agent, Poloxamer, Hydrous or Anhydrous Lanolin, Ester of glycosaminoglycan
87. Isopropyl Palmitate, Isopropyl myristate, DMSO, Vitamin E, Lecithin, Hydrous or Anhydrous Lanolin, Poloxamer, Ester of glycosaminoglycan
88. Isopropyl Palmitate, Isopropyl myristate, DMSO, Lecithin, Hydrous or Anhydrous Lanolin, Poloxamer, Ester of glycosaminoglycan
89. Isopropyl Palmitate, Isopropyl myristate, DMSO, Vitamin E, Hydrous or Anhydrous Lanolin, Poloxamer, Ester of glycosaminoglycan
90. Isopropyl Palmitate, Isopropyl myristate, DMSO, Vitamin E, Lecithin, Hydrous or Anhydrous Lanolin, Ester of glycosaminoglycan
91. Isopropyl Palmitate, Isopropyl myristate, DMSO, Vitamin E, Hydrous or Anhydrous Lanolin, Ester of glycosaminoglycan
92. Isopropyl Palmitate, Isopropyl myristate, DMSO, Lecithin, Hydrous or Anhydrous Lanolin, Ester of glycosaminoglycan

93. Isopropyl myristate, oleic acid, ethanol, Lecithin, Hydrous or Anhydrous Lanolin, Ester of glycosaminoglycan
94. Isopropyl myristate, oleic acid, Lecithin, Hydrous or Anhydrous Lanolin, Ester of glycosaminoglycan
95. Isopropyl myristate, Lecithin, Hydrous or Anhydrous Lanolin, Ester of glycosaminoglycan
96. Isopropyl myristate, oleic acid, ethanol, DMSO, Vitamin E, Lecithin, Hydrous or Anhydrous Lanolin, Poloxamer, Ester of glycosaminoglycan
97. Isopropyl myristate, oleic acid, DMSO, Vitamin E, Lecithin, Hydrous or Anhydrous Lanolin, Poloxamer, Ester of glycosaminoglycan
98. Isopropyl myristate, oleic acid, ethanol, Hydrous or Anhydrous Lanolin, Ester of glycosaminoglycan
99. Isopropyl myristate, oleic acid, Hydrous or Anhydrous Lanolin, Ester of glycosaminoglycan
100. Isopropyl myristate, oleic acid, ethanol, Vitamin E, Poloxamer, Ester of glycosaminoglycan
101. Fatty esters, Ester of glycosaminoglycan

It will be understood that the compounds of the invention can be administered for any number of different purposes, which can include, without limitation, therapeutic and/or cosmetics purposes. It is therefore evident that many different components can be used to manufacture formulations suitable for delivery of the compound of the invention. Formulation may be prepared from various combinations of isopropyl palmitate, isopropyl myristate, a glycosaminoglycan or glycosaminoglycan ester, a stiffening agent like long chain fatty alcohols, long chain fatty alcohol esters, waxes like spermaceti, nonionic gelling/emulsifying agents like poloxamers, water and a USP approved anti-microbial agent. Certain formulation of the compound will naturally be better suited for certain purposes. For example, a preferred formulation to enhance percutaneous absorption at a

joint like the knee will most likely be different from a preferred formulation to heal/restore sun damaged skin around the eyes and/or wrinkles around the eyes caused by exposure to sun and weather. The latter use benefits from the inclusion of vitamin E to promote skin healing; however vitamin E may not provide any added benefit to a formulation intended for joint penetration, even though vitamin E will not interfere with the skin penetration process. Thus cosmetic formulations, for example, by formulation entries 102-113, below. It should be understood that, regardless of the intended use of the composition, the compositions or methods of the invention are not limited by the selection of the formulation components.

Additional suitable formulations are listed in chart below:

102. Isopropyl palmitate, isopropyl myristate, glycosaminoglycan or glycosaminoglycan ester, cetyl alcohol, poloxamer, water, antimicrobial agent
103. Isopropyl palmitate, isopropyl myristate, glycosaminoglycan or glycosaminoglycan ester, cetyl alcohol, antimicrobial agent
104. Isopropyl palmitate, isopropyl myristate, glycosaminoglycan or glycosaminoglycan ester, stearyl alcohol, poloxamer, water, antimicrobial agent
105. Isopropyl palmitate, isopropyl myristate, glycosaminoglycan or glycosaminoglycan ester, stearyl alcohol, antimicrobial agent
106. Isopropyl palmitate, isopropyl myristate, glycosaminoglycan or glycosaminoglycan ester, cetostearyl alcohol, poloxamer, water, antimicrobial agent
107. Isopropyl palmitate, isopropyl myristate, glycosaminoglycan or glycosaminoglycan ester, cetostearyl alcohol, antimicrobial agent
108. Isopropyl palmitate, isopropyl myristate, glycosaminoglycan or glycosaminoglycan ester, spermaceti (or spermaceti replacement), poloxamer, water, antimicrobial agent
109. Isopropyl palmitate, isopropyl myristate, glycosaminoglycan or glycosaminoglycan ester, spermaceti (or spermaceti replacement), antimicrobial agent
110. Isopropyl palmitate, isopropyl myristate, glycosaminoglycan or glycosaminoglycan ester, cetyl esters, poloxamer, water, antimicrobial agent
111. Isopropyl palmitate, isopropyl myristate, glycosaminoglycan or glycosaminoglycan ester, cetyl esters, antimicrobial agent
112. Isopropyl palmitate, isopropyl myristate, glycosaminoglycan or glycosaminoglycan ester, emulsifying wax, poloxamer, water, antimicrobial agent
113. Isopropyl palmitate, isopropyl myristate, glycosaminoglycan or glycosaminoglycan ester, emulsifying wax, antimicrobial agent

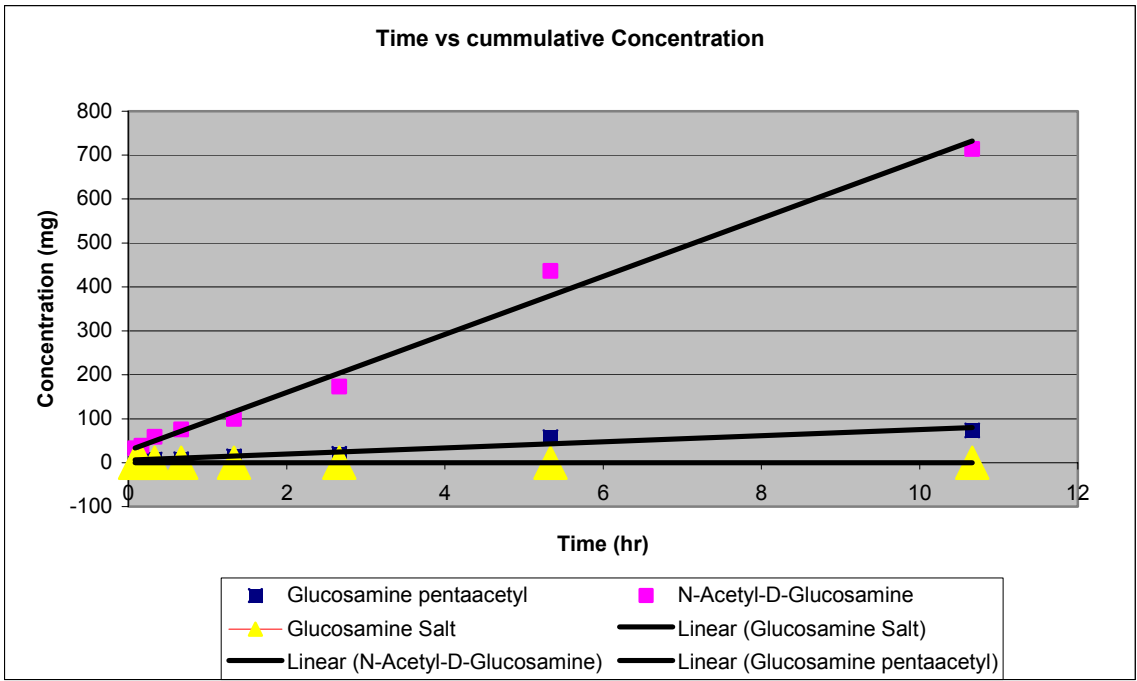
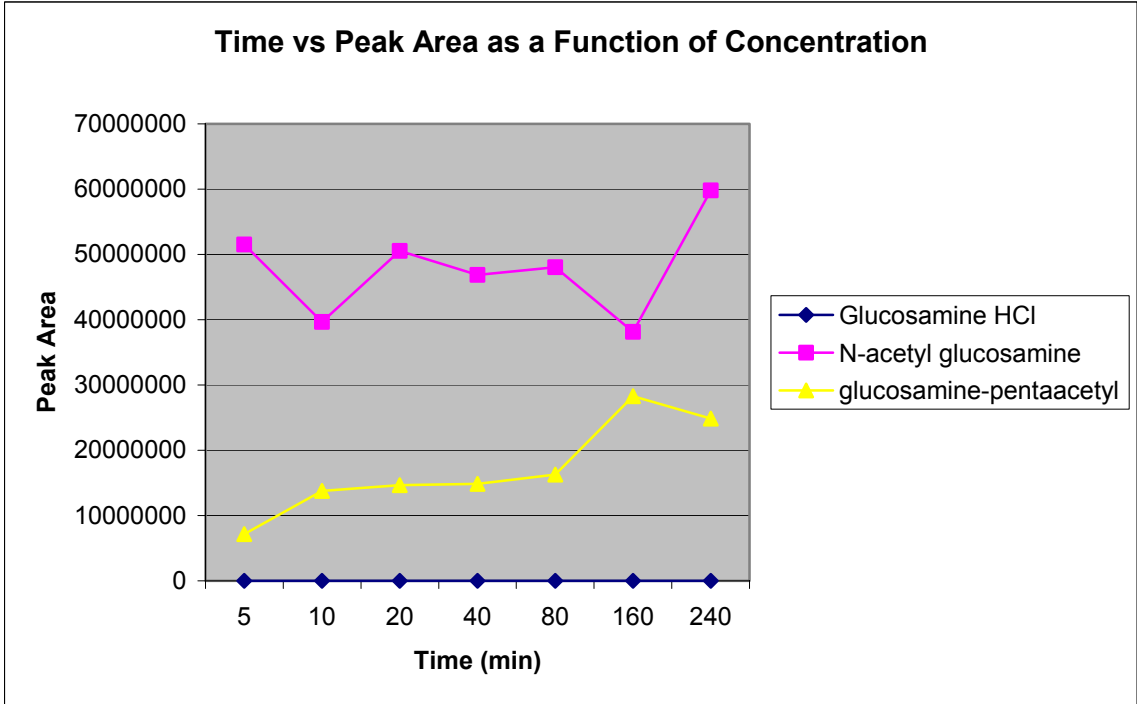
An NSAID may be incorporated into any of the aforementioned formulations

Initial Confirmatory Transport Data

Initial data shows transport our glucosamine products (esters of glycosaminoglycan). Current creams use glucosamine salts (HCl or sulfate), which are monovalent (uncharged) chemical entities that do not cross/and or penetrate the skin unless an electrical charge is applied.

Studies

In vitro transport (diffusion) was evaluated by Franz cell diffusion experiments. A model skin membrane was mounted to three Franz receptor cells filled with pH 7.4 phosphate buffer. The donor cells were clamped to the each receptor and fill with 100 mg in a 1 ml solution. Twenty micro liter (μ l) samples were taken at 5, 10, 20, 40, 80, 160 and 240 -minute intervals. Each aliquot was diluted into 1 milliliter (ml) of a buffer solution n. A ten μ l of each sample was analyzed by high performance liquid chromatography w/pulsed electrochemical detection. **Results:** There was no diffusion/transport of the model glucosamine salt. The esters of glycosaminoglycans products showed immediate and constant diffusion/transport from 5 minutes to 240 minutes as shown in the figure below. Note: Each receptor cell volume was analyzed to show an amount greater than 50% transport of esters.



APPENDIX B
ACADEMIC RESEARCH PLAN/PROPOSAL¹

¹Garner, Jr. S. T., Academic Research Plan/Proposal . Submitted to potential employers in academia

RESEARCH PLAN/PROPOSAL PREFACE

The following research plan is general overview of my immediate research objectives. The aim is to use this as a reference point towards developing a well rounded and self-supported extramurally funded interdisciplinary program in pharmaceuticals. As primary funding sources, various new investigator awards and other extramural funding will be immediately sought from the National Institutes of Health's (NIH) National Center for Complementary Medicine as well as the National Institute of Arthritis and Musculoskeletal and Skin Disease (NLAMS). Small Business Innovation Research (SBIR) and Small Business Technology Transfer (STTR) programs also offer opportunities towards outside collaborations towards extramural funding. In exploration aforementioned federal funding opportunities, the University of Georgia's President, Dr. Michael Adams' speech at the University of Georgia's College of Pharmacy's Centennial Celebration highlighted the following paraphrased statement: '...that to be successful in these times, researchers within the College of Pharmacy must become more entrepreneurial in reaching the programmatic research goals of their respective organization/s and the University...' This has especial application to my objectives in designing and achieving my research objectives towards attainment the ultimate goals.

RESEARCH PLAN

A. Specific Aims

The specific aims of this proposal are:

I. Complete Further Formulation Studies of Glycosaminoglycans as Transdermally Delivered and/or Percutaneously Absorbed Osteoarthritis Therapies

Transdermally delivered percutaneously absorbed GAGs and/or GAG components are effective in the treatment of osteoarthritis. The objective of this research is to evaluate transdermal permeability GAG components, such as glucosamine, chondroitin and n-acetylglucosamine, in order to assess the feasibility of pursuing a percutaneous formulation for local therapy to osteoarthritic joints. In

connection, a provisional patent has been filed with the US Patent and Trademark Office, (1). Oral administration of glucosamine, its salts, and NAG are affected by the liver's first-pass metabolism, (2). However, a more recent report indicates that these agents may be metabolized mostly in the gut rather than solely by the liver, (3). Few pharmacokinetic literature reports exist on the disposition of these agents in articular cartilage. Setnikar *et. al.* (4, 5) has reported on the pharmacokinetic properties of glucosamine in dogs and man. It is estimated that approximately 87% of the original glucosamine oral dose is absorbed and excreted; <13% is widely distributed in the body; and <1% reaches osteoarthritic joints. Chondroitin is known to degrade into its basic disaccharide components within the gut prior to further metabolism, (6). Although only a small fraction of glucosamine reaches the articular cartilage target site, it is reported to exhibit a high potency; and together glucosamine and chondroitin therapy demonstrate therapeutic efficacies over time, (7). Our current studies utilized NAG because it is an active metabolite of glucosamine; and as well as it is commercially available, relatively low cost and stability. It possesses the following physical and chemical characteristics making it a reasonable candidate for transdermal delivery and percutaneous absorption: a) high potency, b) reasonably lipid soluble, c) low molecular weight, d) unique biochemical pathway with active transport from blood into articular cartilage. (8)

II. Evaluate the Pharmacologic and Physicochemical Activities of NSAID-GAG mutual Prodrugs in the Treatment of Osteoarthritis

The objective along with the synthesis of NSAID-GAG mutual prodrugs has been to improve the pharmaceutical properties of drugs such as permeability, solubility and stability in relationship to a drug's pharmacological and pharmacokinetic properties. These modifications alter the physicochemical properties which may affect delivery methods i.e. route of administration in optimizing drug delivery, pharmacodynamic and pharmacokinetic properties. For example,

glucametacine is the glucosamide pro moiety and alternative to its parent drug indomethacin marketed in Europe as an effective anti-inflammatory agent in patients with chronic rheumatic conditions. It has few and usually mild side effects in comparison to its indomethacin parent moiety. Glucametacine is not promoted, nor indicated as a mutual prodrug to deliver both glucosamine and indomethacin. The working hypothesis is that the mutual prodrugs synthesized are reduced to the parent compound *in vivo* to produce the parent compounds thus providing patient with systemic pain relief and maintenance of joints leading to a reduction in GI toxicities and ADRs associated with NSAIDs and provide the benefits of GAG components in the treatment of OA.

The expected applicability of the research findings: *GAGs and GAG components are popular nutritional supplements for OA. These supplements such as glucosamine and chondroitin have shown moderate efficacy in meta-analysis and large industry-sponsored clinical trials. The National Center for Complementary and Alternative Medicine and the National Institute of Arthritis and Musculoskeletal and Skin Disease (NIAMS/NCCAM) have funded a multi-center five-arm placebo controlled study called The Glucosamine Arthritis Intervention Trial (GAIT). GAIT spans 24 weeks, enrolling 1588 subjects, at 13 centers comparing the efficacy of glucosamine sulfate, chondroitin sulfate, glucosamine with chondroitin, to placebo and compared to celecoxib for knee OA. This study will have final data released in 2005. Therefore the inevitable applicability new developments of these OA therapies will inexorably explode into new research area where little competition is currently concentrated. Thus, allowing one to become the leader in the area by situating himself in a position to provide relevant research towards an emerging market by way of producing avenues towards more efficacious GAG and/or GAG component based products for the treatment of OA and other applications whereas GAG are the basic components of skin, cartilages and tissues, thus the research opportunities within this arena immense in the potential to develop new drug delivery applications and therapies.*

B. Background and Significance

Problem

Osteoarthritis is the most common type of arthritis. It affects more than 20 million people and is accountable for more than 7 million doctor visits in the United States (9, 10). Osteoarthritis results from the breakdown and eventual loss of cartilage. Cartilage is a protein substance that supplies cushion between the bones of the joints. It is one of the most symptomatic medical problems for middle aged and older people (11). Osteoarthritis occurs more frequently as a person ages. Most men are diagnosed before the age of 45. However, after the age of 45, osteoarthritis is found more frequently in women (9). In fact, before the age of 65, over half the population has osteoarthritis. For many years, the traditional pharmacologic management of osteoarthritis has been mainly symptomatic without well-known influence on the duration of the disease and its progression (13). Even today, medical treatment is based on symptomatic treatment, and the etiology of this arthritis is not fully understood (14).

Glucosamine sulfate and chondroitin sulfate as a glycosaminoglycan (GAG) component and GAG respectively are agents marketed as nutritional supplements in the United States and considered to have beneficial effects on cartilage. These two agents are widely used for the treatment of painful osteoarthritis (OA) of the knee, hip, and hands. It is hypothesized that these act by inhibiting degradative enzymes, and up regulating cartilage and metabolism and proteoglycan matrix production (15, 16).

Solution

The use of glucosamine became popular after being featured in the book, The Arthritis Cure by Jason Theodasakis, MD, (17). Currently, glucosamine and its metabolites are not classified as

drugs but as nutraceutical/dietary supplements under United States Food and Drug Administration's Dietary Supplement Health and Education Act of 1994 (DSHEA). Oral dosage formulations of N-acetyl-D-glucosamine (NAG), and its parent compound glucosamine in salt form (sulfate, hydrochloride etc.) are commercially available nutraceuticals, and are commonly administered in conjunction with chondroitin sulfate, also a readily available nutraceutical. Glucosamine and chondroitin have been reported effective in the oral treatment of OA but have not undergone the rigorous studies needed for FDA approval as pharmaceuticals. (8, 9) The National Institutes of Health (Bethesda, MD, USA) has an ongoing multi-center study- GAIT (Glucosamine/Chondroitin Arthritis Intervention Trial) that is currently evaluating the efficacy of orally, administered glucosamine and chondroitin oral supplements, (18).

Glucosamine and chondroitin salts are charged, highly polar, aqueous soluble, and poor candidates for transdermal absorption. Currently, there are topical products containing these ingredients as salts marketed nutraceuticals for the treatment of osteoarthritis, which contains ingredients whose effects may be mistaken in the short term as being therapeutic NAG; an acetylated glucosamine metabolite is less polar and neutral appears to be a more likely candidate for transdermal delivery and percutaneous absorption.

Glucosamine, and its orally delivered salt forms, are metabolized to NAG via the hexosamine pathway; glucosamine or galactosamine, plus a uronic acid, is incorporated as a disaccharide unit into all macromolecules requiring amino sugars such as keratan sulfates, dermatan sulfates, chondroitin 4- and 6-sulfates, hyaluronates, and heparin and heparan sulfates, to produce glycosaminoglycans (GAGs). GAGs are highly negatively charged molecules, with an extended conformation, and demonstrate high viscosity and low compressibility ideal as a lubricating fluid for

anatomical joints. The majority of GAGs in the body are linked to core proteins, to form proteoglycans or mucopolysaccharides, which are basic components of skin, tissue, and cartilage. (19, 20)

GAGs offer an alternative currently widely recognized osteoarthritic therapy, mainly NSAIDs as a treatment for the root cause of OA. Whereas the common therapy of OA is NSAID that systemically treat the associated pain via the inhibition of cyclooxygenase-1 and/or -2, which has its side effects. Recently, as of September 30, 2004 the FDA recognized Merck & Company's voluntary withdrawal of its product Vioxx® (chemical name rofecoxib), a non-steroidal anti-inflammatory drug (NSAID). There were great claims made upon the development of COX-2 selective NSAIDS such as Vioxx®, owing to a reported reduction the rate of gastrointestinal (GI) ulcers and bleeding. On the other hand adverse drug reactions (ADRs) such as increased risk of colon polyps, cardiovascular events, heart attacks and stroke were seen in comparison of Vioxx® to naproxen in Merck's VIGOR (Vioxx Gastrointestinal Outcomes Research) study. The use of NSAIDs for the treatment of osteoarthritis has been proven to be beneficial. Unfortunately, there are a number of associated ADRs (21). NSAIDs cause platelet aggregation inhibition, gastric irritation, and nephrotoxicity (22). Still other side effects include: nausea, vomiting, gastrointestinal ulceration and bleeding, skin reactions, organ failure and the accelerated destruction of cartilage during osteoarthritis (23). However, one of the major side effects associated with NSAIDs is gastric irritation of which researchers are focused on preventing. Most research has occurred in the area of pharmaceuticals/pharmaceutical chemistry over recent years to develop NSAID products with fewer side effects via a formulation perspective through new synthesis.

C. Preliminary Studies

OA's root cause can be successfully treated via the development of transdermal/percutaneous delivery applications for components of GAGs and mutual prodrugs consisting of an NSAID and GAG component as the covalently bound parent drugs. Whereas the application development of transdermal/percutaneous delivery for the GAG component and glucosamine metabolite NAG has been developed. Current research results show that a GAG component n-acetylglucosamine can be transdermally delivered via pharmaceutically developed formulations. Furthermore, unpublished preliminary results from our laboratory show that NAG has adequate permeation from other drug delivery vehicles (1, 24). Product development leading to patenting, licensing is underway. Here lies an opportunity for rapid pharmaceutical development towards an over-the-counter (OTC) product.

On the other hand, we have produced mutual prodrugs in the pharmaceuticals arena via the synthesis of two new compounds. These compounds as models are proposed to be mutual prodrugs of GAGs, coupled to a non-steroidal anti-inflammatory drug (NSAID). Opportunities exist for collaborations to study these new chemical entities pharmacological activities in animal models towards product development as a mutual prodrug for the treatment of OA. These prodrugs are designed to deliver an NSAID without producing ADRs and a GAG for the treatment of OA. Ueda and Tani reintroduced the mutual prodrug concept in 1990 as methodology to produce a more pharmaceutically efficacious product. Their work is based on chemically bonding two drugs, usually *via* an ester linkage that is easily degraded by mammalian esterases, so that after administration each drug is released to produce its respective pharmacological action. At first, it was named a chimera prodrug, since it is composed of two parts; however mutual prodrug is a better description since both entities are active. The linking of the two drugs may impart a protective effect so that unwanted degradation, usually by stomach acids may be prevented. Also, the mutual prodrug

will usually exhibit different aqueous/lipid solubility profiles, which will aid in a drugs formulation towards its delivery.

In recent years, pharmaceuticals research has taken on more challenging projects dealing with the synthesis of more complex molecules as delivery tools for the parent compound as opposed to the production of simple salts to provide a more efficacious product during the product development phase. Some of the advances led our research in the direction of producing mutual prodrugs via non-complex synthesis in reach of a pharmaceuticals laboratories better equipped to produce physicochemical properties and finished products rather than tedious synthetic work.

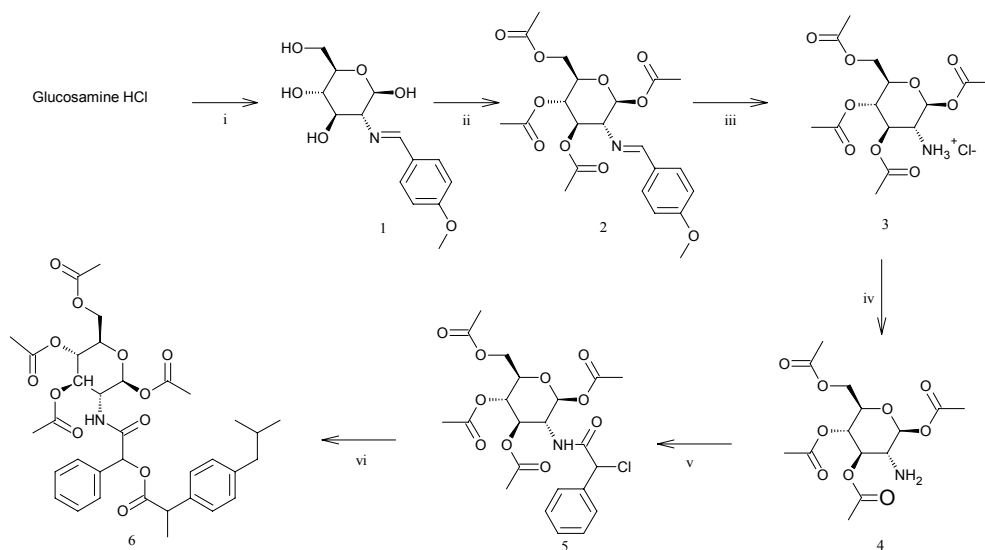
Using this mutual prodrug (MP) concept as a template, our group has developed potential osteoarthritis' therapeutics via the mutual prodrug approach. The products are two new compounds (Examples 1 and 2), which are proposed to be mutual prodrugs of glucosamine coupled to a non-steroidal anti-inflammatory drug (NSAID). Both of which are used to treat osteoarthritis Thus our research in the pharmaceuticals arena has led us to synthesize two new compounds, which are proposed to be mutual prodrugs of glucosamine, a nutraceutical used in treatment of osteoarthritis, coupled to a non-steroidal anti-inflammatory drug (NSAID). Mutual prodrugs (MP) are pharmaceutically active covalently bound drugs. MPs are best described as the conjugation of two drugs having different pharmacological activities. The concept arises from the practice of co-administering two drugs in order to enhance pharmacological activity or prevent clinical side effects. Whereas MPs are synthesized towards a pharmaceutical objective directed at improving each drug's therapeutic index in producing a chemical modified chemical entity with optimized delivery parameters and lower toxicities By which optimized delivery of the drugs is achieved as an improvement upon their pharmaceutical and or physicochemical properties.

MPs are directly linked to the prodrug concept developed by Albert and coworkers (25). MPs follow the prodrug accordingly whereas the chemical entity would be converted into the active

drug within the body through enzymatic and non-enzymatic reactions once it reached to site of action prodrugs are of the same mold as prodrugs by performing as carrier-linked prodrugs; bio-precursor prodrugs, or chemical activation prodrugs. At the site of action, the side effects of the original drug would be masked allowing the drug to work more effectively (26). MPs follow same processes/methodologies prodrugs in attempts to overcome pharmaceutical and pharmacological problems such as incomplete absorption and too rapid absorption and excretion. Some positive characteristics associated with prodrug usage include: covalent linkage, lack of intrinsic activity, lack of toxicity, and optimal kinetics of release to ensure effective drug levels.

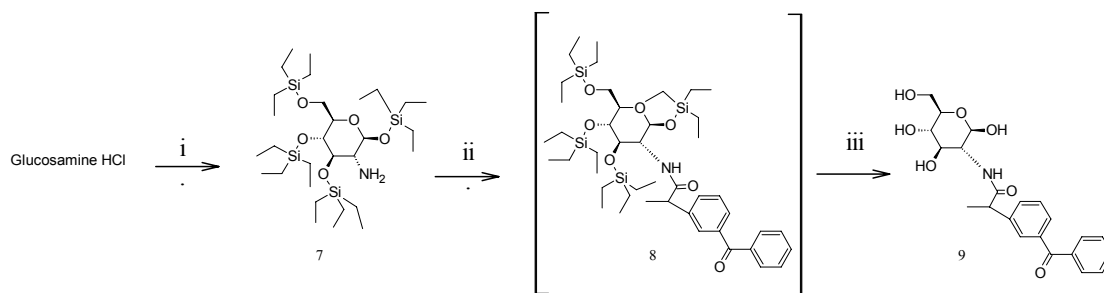
For many years patients had been dispensed mutual prodrugs as therapeutic agents before the term prodrug had been placed into research domain. Singh and Sharma (27) expressed the advances arisen from the production of Sulphasalazine to the advances in antibiotic research, which has lead to in essence mutual prodrugs of β -lactam antibiotics and their potentiating agents to produced for example ampicillin-mecillinam MP, ampicillan-sulbactam MP to form sultamicillin, Dual Action Cephalosporins as well as other MPs not typically referred to as MPs. The first mutual prodrugs were introduced as therapeutic agents before the concept of prodrug was developed.

Example 1
Scheme I: Spacer Linked Glycosaminoglycan –NSAID Mutual Prodrugs



i) 1 N NaOH, p-methoxybenzaldehyde/ 0°C - r.t. 1 hrs ii) pyridine (anhydrous), acetic anhydride/ r.t. 24 hrs iii) acetone (reflux) and 5 N HCl iv) triethylamine, water v) chlorophenylacetyl chloride (linker), triethylamine, vi) NSAID, CH₂Cl₂

Example II
Scheme II: Directly Linked Glycosaminoglycan-NSAID Mutual Prodrugs



i) Trichloroethylsilane, pyridine (anhydrous) ii) NSAID, Me₂NSO₂Cl, MeCN, BuNMe₂, DMAP iii) TBAF, Methanol

D. Research Design and Methods

Specific Aim I.

Complete Further Formulation Studies of Glycosaminoglycans as Transdermally Delivered and/or Percutaneously Absorbed Osteoarthritis Therapies

The working of this aim is that transdermally delivered percutaneously absorbed GAGs and/or GAG components are effective in the treatment of osteoarthritis.

Design and Methods

The conceptual framework of objectives to be met: 1) GAGs and their components will be obtained from commercial sources. 2). Physicochemical characterization studies will be completed towards transdermal delivery/percutaneous absorption applicability. 3) Optimized formulations will be developed.

The major milestone thus far is that analytical method development has been completed for NAG and can be utilized further utilized for these studies. Whereas NAG analysis has been carried out using high-performance anion exchange chromatography with pulsed amperometric detection (HPAE-PAD); Dionex, Sunnyvale, CA USA); on a Dionex DX-500 HPLC system consisting of a GP40 gradient pump, ED40 Electrochemical detector, AS3500 autosampler and PeakNet Chromatography Workstation, (28-31). The HPAE-PAD was equipped with CarboPac™ PA20 (3 x 150 mm), analytical anion-exchange column for the rapid, high-resolution separation of monosaccharides and disaccharides, using pulsed amperometric detection, (28-31), and a CarboPac PA20 analytical guard column (3 x 30 mm) and a carbonate trap column (25 x 15 mm). Mobile phase (A) was degassed and deionized water. The mobile phase (B) consisted of 0.02 N NaOH

prepared with deionized water and filtered with 0.45 μm filters in a solvent filtration apparatus (Waters-Millipore, Milford, MA, USA) that was degassed under vacuum. The mobile phase system was run at a gradient concentration of 16 mM NaOH at a flow rate of 0.5 ml/min. A standard calibration curve of NAG (Figure 1) was obtained with linear regression and value of $R^2=0.99$. Each sample set was run with external standards. The sample concentration values were obtained *via* the Peak Net software. These values were compared with to those obtained by calculations of the peak area and peak height observe as functions of the standard curve's linear regression equation. The instrument sensitivity was approximately 10^{-4} units.

Specific Aim II. *Evaluate the Pharmacologic and Physicochemical Activities of NSAID-GAG component, mutual Prodrugs in the Treatment of Osteoarthritis*

The working hypothesis for this aim is that the mutual prodrugs synthesize are reduced to the parent compound in vivo to produce the parent compounds to provide patient with systemic pain relief and maintenance of joints leading to a reduction in GI toxicities and ADRs associated with NSAIDS as well as provide the benefits of GAG components in the treatment of OA.

Design and Methods

The conceptual framework of general objectives to be met: 1) New chemical entities have been synthesized. 2). Physicochemical characterization studies will be completed of these two compounds along with preformulation/formulation examinations. 3) An analytical method is to be developed concurrently. 4) *In vivo* studies of the compounds in the appropriate animal models are to be completed

The major milestone thus far is that two new chemical entities with provisional patent status have been synthesized. Chemical characterization is complete. Physicochemical characterizations *in silico* and bench characterizations have led to these compounds as targets for preformulation/formulation studies as mutual prodrugs towards pre-clinical studies.

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APPENDIX C

CHALLENGES OF RECRUITING AMERICAN MINORITY GRADUATE STUDENTS: THE COACH MODEL¹

¹Capomacchia, A. C.; Garner, S. T. Challenges of Recruiting American Minority Graduate Students: The Coach Model. *Am J Pharm Educ.* 2004; 68(4): article 89. Reprinted here with permission of publisher.

ABSTRACT

Objectives. To examine the Coach Model for recruiting minority students for the pharmaceutical and biomedical sciences at the University of Georgia College of Pharmacy- The long-term goal is to establish the University of Georgia and the College of Pharmacy as a regional center for training minority scientists to solve problems concerning minority health disparities

Methods. Professional fairs, workshops, seminars, and campus visits were used to attract the most qualified students. Also vital to the recruitment effort is direct family contact, the location of supportive mentors, and funding.

Results. The 9-year project resulted in the matriculation of 22 students to graduate programs in the College of Pharmacy. Eight have graduated and 13 are scheduled to graduate within 5 years. Only one student transferred to another institution. Funds are provided by a variety of sources, including the Alfred P. Sloan Foundation, the National Institutes of Health, the Department of Pharmaceutical and Biomedical Sciences, and The University of Georgia Graduate School. All students are fully funded.

Conclusions. The project has already shown that a strong graduate program for minority students in the pharmaceutical and biomedical sciences can be executed and succeed. The project also demonstrates that faculty involvement as well as a positive instructional environment plays pivotal roles in the program's success.

INTRODUCTION

African Americans, American Indians, and Hispanic Americans are underrepresented in the biomedical sciences at the doctoral level. There is a present and immediate need for scientists,

professors, and government leaders from these minority populations in the United States. This need is recognized in the “National Institutes of Health (NIH) Strategic Research Plan to Reduce and Ultimately Eliminate Health Disparities.”¹ The Strategic Plan describes research infrastructure objectives that include minority research training and development, and minority institution support. It is reasonable to expect that the minority population participate and direct the reduction of health disparities within its own population. The shortage of minority scientists in the biomedical-related research sciences is due, in part, to a lack of students in the pipeline and a lack of programs at both historically black colleges and universities (HBCU) and predominantly white colleges and universities (PWCU) to support the development of these students.² This problem also exists in colleges of pharmacy with low numbers of minority faculty members and professional students. Numbers for faculty are found in the “2002-2003 Profile of Pharmacy Faculty”³: 8.7% black or African American; 3.9% Hispanic or Latino; 14.9% Asian or Pacific Islander; and 0.2% Native American. These numbers are not as good as they appear. The black and African American entry includes permanent residents or naturalized citizens from the Caribbean and Africa who are not minority African Americans. Furthermore, Asian faculty members are included with Hawaiians and other Pacific Islanders. The NIH considers neither Asian faculty members or students, nor non-domestic black faculty members or students to be minorities. The current report also does not consider Asian or non-domestic black students who are or have been enrolled in the graduate program at The University of Georgia (UGA), College of Pharmacy, Department of Pharmaceutical and Biomedical Sciences, as under-represented minorities.

The challenge facing educators is to locate, matriculate, educate, and graduate minority doctoral students who are committed to addressing health disparities issues. The Department of

Pharmaceutical and Biomedical Sciences in the College of Pharmacy at the University of Georgia and the UGA Graduate School have instituted an instructional program for minority biomedical scientists with the long-term objective of establishing the institution as a regional center for addressing health disparities issues at the graduate biomedical scientist level.

All 4 of the requirements necessary to achieve a successful minority graduate program, namely locating, matriculating, educating, and graduating, define the Coach Model for recruitment. An explanation of the challenges of each requirement of the Coach Model process will emphasize the qualifications that make the Model successful.

Locating and recruiting American minority students for graduate doctoral programs is an underestimated challenge. Experience dictates that success in this initial endeavor is based on the development of a trusting relationship between the recruiter and the potential student. Visits to the student's home campus as well as direct family involvement are valuable in the recruiting process. Direct family contact has proven to be a powerful factor in the student's decision. In addition, if the recruiter's campus sponsors the interview, this is especially valuable. At the expense of the recruiting institution, the student can examine the programs available and determine which are most suitable to his or her course of study. These measures nurture the development of a trusting relationship and an empathetic understanding of the student's background, and offer confidence in the student's desire to succeed.

Once the student has been located, the matriculation process begins. This involves the provision of financial aid and the selection of the appropriate graduate program and mentor to accomplish the student's objectives. This process involves extensive collaboration between a network of faculty representatives and graduate administrators at both the student's former institution and the

recruiter's institution. Again, this aspect of the matriculation process is driven by trust. The student must trust the recruiter's guidance regarding the selection of the graduate program. Most students, although bright and discerning, are usually not sufficiently experienced to make this judgment. Eventually, after successful matriculation of several candidates, the recruiter develops a reputation throughout the recruitment region for honesty and empathy in student affairs. Once this occurs, the process has evolved into a recruitment/retention program that impacts the student's educational path to graduation and future employment.

The latter phases of the Coach Model formula involve the education and graduation of the student. Research has shown that student retention is the primary problem facing minority graduate programs.³⁻⁸ Once matriculated, the student must be nurtured to success. This must become a continuum of responsibility for the recruiter, the mentor, the department, and the graduate program. After all, it is in the best interest of the department, college, and institution to select students carefully and to promote their paths to success. The best successes are a result of consistent, wise, fair, and firm mentoring. The student feels confident of the availability of sound guidance, especially early in the program. The relationship is based on trust, which forms the basis for a caring, positive environment, which is critical to the student's retention and overall success. Furthermore, if the student-mentor relationship is built on a firm foundation, it will last a lifetime.

METHODS

At the University of Georgia College of Pharmacy, the Coach Model was first employed in 1992 for the purpose of locating and matriculating domestic students. In 1992, the Department of Pharmaceutics was composed of 100% international students (Table 1). Both faculty members and students were concerned about the lack of diversity. This prompted the Department to embark on a plan to increase domestic student enrollment, with an objective to balance enrollment of domestic

to international students at a 50:50 ratio. Since the minority population in Georgia is approximately 30%, the plan was designed so that the underrepresented minority enrollment would be 30% of the domestic enrollment. In addition, students from disciplines other than pharmacy would be recruited because graduating pharmacists were not usually pursuing advanced degrees. These other disciplines included chemistry, biological sciences, physics, mathematics, and engineering. Remarkably, in 1992, this cutting-edge plan preceded the 1999 published report by the American Association of Colleges of Pharmacy, which states in Recommendation Six of the Report of the Commission on the Future of Graduate Education in the Pharmaceutical Sciences that there is a need to increase the enrollment of domestic students, including students from disciplines other than pharmacy.⁹ The Coach Model procedural plan included attending regional graduate and professional fairs, which attracted large numbers of interested students. The initial geographical area included Georgia, Alabama, South Carolina, North Carolina, Virginia, and Florida. Later Mississippi and Tennessee were added to the recruitment map. Recruiters interviewed potential graduate students from both HBCUs and PWCUs. Using the Coach Model method, personal contacts were made with as many students as possible. Detailed dialogs describing the educational opportunities and graduate programs were conducted with each student. The students provided contact information for future communication. Later the students were contacted via mail, e-mail, or telephone. The Department conducted about 25 recruiting visits each fall and was successful in enrolling students from all but 2 of the states visited. Interestingly, recruiters who were faculty members were more successful than the non-faculty recruiters. As the program grew, graduate students from the program were asked to participate in the recruiting process, especially at their alma mater, an approach that also proved successful.

RESULTS

From 1992–1998, Department of Pharmaceutics enrollment of domestic students increased from 0% to near 50%, and minority enrollment increased from 0% to approximately 20%, as shown in Table 1. The most troubling imbalance in enrollment demographics was the continued lack of minority males in the program. This was addressed by aggressive recruiting and an increase in enrollment of this group is noted in Table 2. During the 1997–1998 academic year, an administrative restructuring of the College of Pharmacy resulted in combining the Departments of Pharmaceutics, Medicinal Chemistry, and Pharmacology/Toxicology, into a single Department of Pharmaceutical and Biomedical Sciences (PBS). This was completed by the 1998–1999 academic year. Aggressive recruiting continued and currently the PBS Department has between 50 and 60 graduate students, half of whom are international students and half are domestic students, and one third of the domestic students are minority students. This is shown in Table 2 for the academic years 1998–1999 through 2002–2003.

DISCUSSION

Our goal of a 50:50 ratio has been reached. Furthermore, the low numbers of minority male students seen in Table 1 has been corrected and minority male enrollment has increased to parity that of female minority enrollment. This is the result of special efforts to recruit and matriculate male minority students during this period. Table 3 shows how student funding was managed and leveraged to maximize Department funds. From the 1994–1995 academic year to 1997–1998, the Department of Pharmaceutics solely funded the program, with the exception of funds from the NIH Minority supplement. The minority students reported in Table 3 were recruited from 12 HBCU and 10 PWCU. All were admitted under UGA Graduate School admissions standards; all have been retained and are performing as well as non-minority and international students. In the 1999–2000 academic year, the author received a grant from the Alfred P. Sloan Foundation to partially support graduate students in the Department. The grant award was based on the previously discussed Coach Model recruitment program. Owing to the grant and PBS Department

commitment to this program, funds from the Graduate School were forthcoming. From 1998–1999 to present, funding of the program came from multiple sources. Four doctoral degrees and 4 master’s degrees were granted during this period. Figure 1 shows total annual enrollment for the period 1994 to 2002. The column labeled “total/year entered” is a running sum of all students entering; while “number/year entered” are the matriculation numbers for that year. Graduation data are displayed in the same manner, and the column labeled “total/year graduated” is a running sum of all graduated students; while the column “number/year graduated” is the number of students graduating each year. The 12 minority students matriculating and graduating between 1999 and 2006 will cost the Department only 40% of the actual cost of education owing to aggressive and imaginative leveraging of funds.

As a result of the work presented here, a Bridges to the Doctorate grant was awarded by the National Institutes of Health/National Institute of General Medical Sciences to the author and UGA’s PBS Department in August 2002. The goal of the grant is to increase the numbers of underrepresented scientists in the biomedical sciences. The grant is designed so that a doctoral granting institution partners with an institution that only grants masters degrees, in order to seamlessly bridge the student into a doctoral graduate program. For this grant UGA partners with North Carolina A&T University, Greensboro (NCAT), and seeks to train 18 minority master’s degree students over the next 3 years to enter biomedical graduate programs at UGA. Upon entering UGA, the Graduate School funds the first 2 years for each student; the balance to be paid by the student’s respective biomedical science Department. Receipt of the grant is viewed as highly positive for attracting minority doctoral students to UGA via NCAT. It broadly expands the currently described program in PBS to include all biomedical disciplines at UGA.

CONCLUSIONS

The 4 elements of the Coach Model, locate, matriculate, educate, and graduate, are shown to be the basis for a viable recruitment program that purposefully seeks to increase the number of minority graduate students enrolled in and graduating from graduate programs at a predominantly white colleges and universities such as UGA. The crucial elements are faculty interest, Departmental and Institutional interest and support, and a positive Departmental training environment, without which the program could not succeed

ACKNOWLEDGEMENTS

The article was presented at the AACCP Annual Meeting, Minneapolis, MN, July 18–23, 2003, and presented in part at a conference, “The State of the African American Professorate: Challenges and Responsibilities in 2003,” held at Ramapo College, Mahwah, NJ, April 16–19, 2003.

The authors thank the following people for their contributions to the manuscript: SR Marcantonio, MS, RPh; Stuart Feldman, former Dean, UGA College of Pharmacy, currently Associate Director of UGA Biomedical Health Sciences Center; Douglas Boudinot, formerly Associate Dean, UGA Graduate School, currently Dean, Virginia Commonwealth University Graduate School; Gordhan L. Patel, UGA Vice President for Research, formerly Dean UGA Graduate School; Curtis Byrd, Director Recruitment and Retention, UGA Graduate School; Tony Edmonds, EEO/Civil Rights Manager, South Atlantic/Mid-south areas, USDA/ARS; and The Graduate School, UGA.

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Graduate Student Characteristics for UGA College of Pharmacy, Departments of Pharmaceutics (reorganized), and Pharmaceutical Biomedical and Sciences (PBS). PBS was formed from the Departments of Pharmaceutics, Medicinal Chemistry, and Pharmacology/Toxicology after reorganization in 1997.

Table I: Graduate Student Characteristics, Department Pharmaceutics (1992–1997), prior to College reorganization.

^a Year: f/m/t	Minority	Majority	Foreign	Total Students
1992/93:f/m/t	0/0/0	0/0/0	8/10/18	8/10/18
1993/94:f/m/t	0/0/0	3/2/5	6/7/13	9/9/18
1994/95:f/m/t	2/1/3	3/3/6	6/7/13	11/11/22
1995/96:f/m/t	3/1/4	4/3/7	5/5/10	9/12/21
1996/97:f/m/t	4/1/5	4/3/7	6/4/10	14/8/22
1997/98:f/m/t	4/1/5	3/3/6	6/5/11	13/9/22

^a f, number female students; m, number male students; t, total number students

Table II. Graduate Student Characteristics, PBS (1998-2004), after College reorganization.

^a Year: f/m/t	Minority	^b Majority	^b Foreign	Total Students
1998/99:f/m/t	4/2/6	9/11/20	15/15/30	28/28/56
1999/00:f/m/t	5/5/10	11/11/22	14/17/31	30/33/63
2000/01:f/m/t	6/5/11	12/9/21	5/17/22	23/31/54
2001/02:f/m/t	7/6/13	10/7/17	7/15/22	24/28/52
2002/03:f/m/t	7/6/13	10/7/17	8/16/24	25/29/54
2003/04:f/m/t	8/6/14	7/3/10	8/15/23	23/24/47
2004/05:f/m/t	10/4/14	7/1/8	6/11/17	23/16/39

^a f, number female students; m, number male students; t, total number students;

^b official department graduate records of majority and foreign students were lost during College reorganization; the numbers above were obtained from records maintained by the author

Table III. Minority Students in Department PBS: Matriculation, Student Funding Classification {numbers of Sloan, non-Sloan, Sloan entry, Department (PBS), UGA Graduate School sponsored (GS; and GRO), NIH, NIH-Bridges (B), Graduation Date/Degree, and Current Status

Matriculation Year	Sloan Student ^a	Sloan entry Student ^b	Non-Sloan Student ^c	Funding ^d	Graduation Date/Degree (projected) ^e	Current status
2004/05		1 1		GRO/PBS GS/B ⁱ /PBS	(2008/D) (2008/D)	Student Student
2003/4	1 1			GS-B ⁱ -Sloan GS-B ⁱ -Sloan	(2007/D) (2007/D)	Student Student
2002/3	1 1	1 1		Sloan/PBS Sloan/PBS GRO/PBS PBS	(2006/D) (2006/D) (2006/D) (2006/D)	Student Student Student Student
2001/2	1 1	1		Sloan/UW Sloan/PBS PBS	(2005/D) <u>(2005D)</u> ^f 2003/M ⁱ	Student Student Student
2000/1	1 1			Sloan/GS/PBS Sloan/GS/PBS	(2004/D) (2004/D)	Student Student
1999/0	1 1 1	1 1	1	Sloan/PBS Sloan/PBS Sloan/PBS NIH supp PBS PBS	(2003/D) (2003/D) 2004/D 2001/M ^g (2004/D) ^h 2001/M	Student Student NIH Doc.stud. Student Lee Labs
1998/9			1 1	Pharmaceutics Pharmaceutics	2002/M 2003/D	J&J BannerCaps
1997/8	none	none	None			
1996/7			1	Pharmaceutics	2000/M	Takada, Inc.
1995/6			1	Pharmaceutics	2001/D	Alkermes, Inc.
1994/5			1 1 1	Pharmaceutics Pharmaceutics NIH supp	1999/D 1999/D 1999/D	Athrogenics NIEHS FAMU

a. Sloan Foundation seeks to increase the number of minority students in graduate science programs. It provides each Sloan Student with \$30,000 for their total graduate education.

b. To access Sloan Foundation funds, Sloan estimates how many minority students on average have been enrolled in the program prior to Sloan evaluation. For PBS the baseline is one. These are Sloan entry students. PBS pays the Sloan entry student directly.

c. Non-Sloan students are those enrolled in addition to the Sloan program; they are also Sloan Scholars.

d. Sloan/PBS, matched funding; GRO/GRO (GRO are matched funds from UGA Office of Recruitment Retention and PBS); Sloan/UW matching from a University Wide TA; NIH/Chu (PBS faculty), NIH minority supplement; Sloan/GS/PBS (matching funds from Graduate School, GS).

e. Graduated student degrees in bold.

f. one student transferred to College of Education

g. one student completed MS (see h)

h. and started a Ph.D. program

i. NIH Bridges student; MS from North Carolina A&TU funded by NIGMS; GS funds first two years

j. matriculated to Howard University

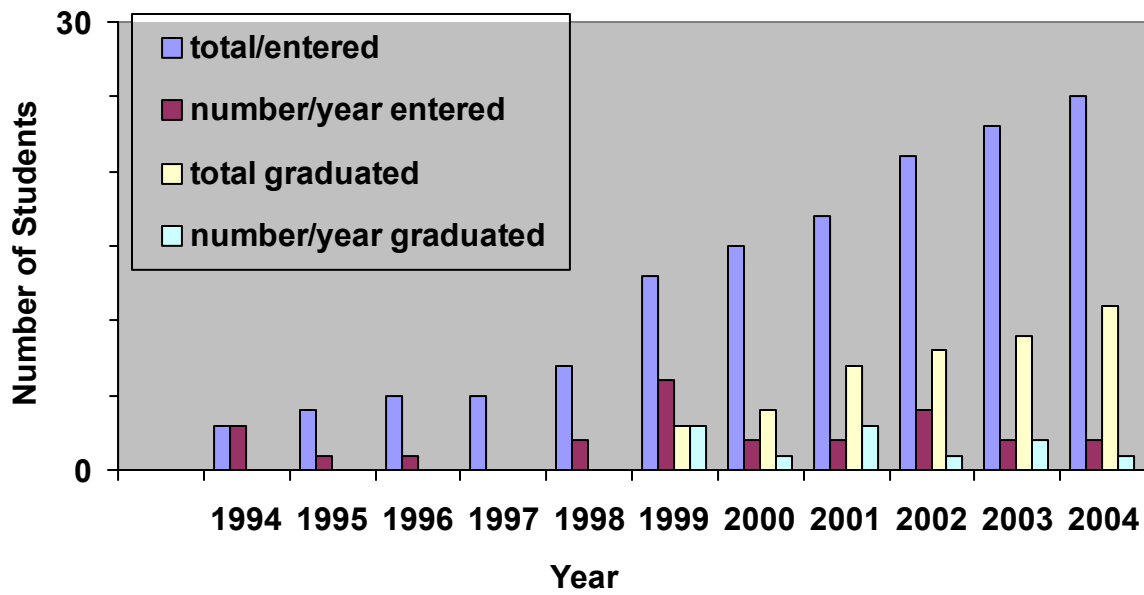


Figure 1: Annual running enrollment and graduation of minority students, 1994-2004; combined data from Departments of Pharmaceutics and Pharmaceutical and Biomedical Sciences.

Table IV. Graduate Student Characteristics, UGA/NCAT: NIH/NIGMS Bridges to the Doctorate Program MS at NCAT, Ph.D. at UGA (2003 –2005)

Year	Student Gender f/m	Ethnicity	UGA Department	Graduation Date/Degree (projected)	^a Funding
2003/04	m	African American	Physics/BMB	(2007/D)	NIH-NIGMS GS & UGA Department
2003/04	m	African American	PBS	(2007/D)	NIH – NIGMS GS & UGA Department
2003/04	f	African American	PBS	(2007/D)	NIH-NIGMS GS & UGA Department
2004/05	f	African American	PBS	(2008/D)	NIH-NIGMS GS & UGA Department

^a NIH-NIGMS, National Institutes of Health-National Institute of General Medical Science; GS, UGA Graduate School, UGA Department funds the balance of the student stipend

APPENDIX D
NATIONAL COLLEGIATE INVENTORS AND INNOVATORS ALLIANCE (NCIIA)
E-TEAM GRANT APPLICATION¹

¹Solomon T. Garner, Jr., M.S. and Dr. Charles W. Hofer, Ph.D. Submitted to the National Collegiate Inventors and Innovators Alliance (NCIIA)

The NCILA was established in 1995 with support from The Lemelson Foundation. Its mission is to foster invention, innovation, and entrepreneurship in higher education – components of the higher education curriculum that are vital to the nation’s economic future. The NCILA accomplishes its goals by supporting curricula and programs that encourage the development and the work of E-Teams – multidisciplinary teams of students, faculty, and industry mentors working together to take an idea for a technological innovation and bring it through prototype development to commercialization. The “E” stands for excellence and entrepreneurship. (This grant application was funded with \$18, 750)

Summary

ThruSkin Technologies (hereafter ThruSkin) will develop and market a patent pending new product called Rejuvalin™ that will significantly improve the lives of America’s 32 million osteoarthritis sufferers. Until recently, individuals with osteoarthritis had no effective treatments for their affliction. All they could do was to reduce the pain by use of a variety of different pain medications, most of which had serious side effects. In fact, more than 15,000 Americans die each year of overdoses of NSAIDs (non-steroid anti-inflammatory drugs), the most frequently used pain relief treatment for arthritis. A number of studies conducted over the past decade have shown that glucosamine, a natural sugar, can stop further deterioration of the arthritic joint and even help rebuild the cartilage in these joints. Consequently, consumer demand for glucosamine products has increased to over \$800 million in less than a decade. The principal limitation of these glucosamine pills is that over 80% of the glucosamine that they contain is excreted by the body, while only about 1% reaches the affected joints where the glucosamine is needed. Rejuvalin™ is a glucosamine cream that can be applied directly to affected arthritic joints. Rejuvalin™’s primary benefit is that almost 70% of the glucosamine in Rejuvalin™ penetrates the skin and reaches the arthritic joint. In short,

Rejuvalin™ is a superior treatment for osteoarthritis that offers, for the first time, the prospect of a nearly normal life for America's 32 million osteoarthritis patients.

The Purposes of This Grant

The purposes of this grant are threefold. The first is to complete the analytical studies needed to document Rejuvalin™'s effectiveness in delivering glucosamine to the affected joints and to prepare, under Good Manufacturing Practices (GMP), the bulk quantities of Rejuvalin™ required to conduct a non-invasive clinical treatment study. The second is to complete the various market research studies needed to determine the most effective ways of bringing Rejuvalin™ to the market. The third is to explore ways that Rejuvalin™ might be combined with various topically applied NSAIDs in order both to stop further joint deterioration and to reduce the pain caused by osteoarthritis.

1. Project Description (Including Its Innovative Features)

The Problem

Osteoarthritis is the most common form of arthritis. It causes degradation of the cartilage matrix resulting in inflammation, pain, and loss of movement. Osteoarthritis is a medical condition that affects more than 32 million people (about 12% of the U.S. population). Common treatments for osteoarthritis include NSAIDs, such as Ibuprofen, and prescription drugs, such as Celebrex™. However, these treatments offer no protection against further deterioration of the joint, they only reduce the pain associated with the joint deterioration.

By contrast, in peer-reviewed scientific studies, glucosamine has been consistently proven to improve joint health by lessening the effects of osteoarthritis. Glucosamine works because it is a naturally occurring building block of glucosaminoglycans that increases the production of keratan, dermatan, heparan, hyaluronic acid and chondroitin sulfates. Each of these compounds is a component of cartilage and of the synovial fluid that lubricates the joint and keeps the cartilage smooth and healthy. At the same time, glucosamine enhances chondrocyte synthesis (chondrocytes produce cartilage cells), which in turn reduces the inflammation of the membrane surrounding the synovial fluid and the joint pain. In the peer-reviewed studies that compared NSAIDs to glucosamine, NSAIDs were shown to decrease inflammation more quickly than glucosamine. However, these same studies showed that *only* glucosamine increased the production of glucosaminoglycans, thereby increasing both the synovial fluid and cartilage matrix in the arthritic joints of the individuals studied. In short, NSAIDs reduced the pain faster, but *only* glucosamine stopped further joint damage. Unfortunately, most of the glucosamine products currently on the market are pills that deliver only about 1% of their glucosamine to the affected joints. The rest of the currently available glucosamine products are topical creams, but none of them is able to get more than 3% to 5% of their glucosamine across the skin barrier.

The Solution

Rejuvalin™ uses ThruSkin's new, innovative patent pending technology to deliver significantly more glucosamine through the skin to the affected joint(s). Thus, the in vitro studies of ThruSkin's Rejuvalin™ formulation conducted to date have confirmed that more than 70% of Rejuvalin™'s glucosamine crossed the skin barrier using an approved skin model and kinetic quantitative analysis (concentration versus time). In short, ThruSkin's technology makes Rejuvalin™ a substantially more effective glucosamine treatment for osteoarthritis than any product currently on the market. Moreover, it

also appears that it may be possible to combine Rejuvalin™ with a patented, but not yet licensed, University of Georgia transdermally applied NSAID to produce a topical cream that will both stop the pain and re-build cartilage.

Rejuvalin™'s active ingredient is a Food and Drug Administration (FDA) approved dietary supplement. It is covered under the FDA Dietary Supplements Health and Education Act (DSHEA) of 1994. More specifically, in accordance with Section 413(a) of the Federal Food, Drug, and Cosmetic Act, ThruSkin will *only* need to provide the FDA with a 75-day pre-market notification of its intention of bringing Rejuvalin™ to market. Once ThruSkin submits Rejuvalin™'s product content to the FDA's Consumer Safety Officer, Division of Standards and Labeling, Office of Nutritional Products, Labeling, and Dietary Supplements, DSHEA requires that the FDA respond within 75 days, after which ThruSkin can begin selling Rejuvalin™. ThruSkin is currently completing its pre-market notification notice, which it intends to submit to the FDA by January 1, 2004. Consequently, *ThruSkin anticipates that it will be able to start selling Rejuvalin™ by mid-March 2004.*

2. Market Potential

Target Customers

ThruSkin's primary target customers for Rejuvalin™ are the 32 million American's who suffer from osteoarthritis. Secondary targets are the 38 million additional American's who have chronic joint symptoms and athletes seeking to minimize damage to their joints because of the stresses of high impact sports. The vast majority of these 70 million plus potential customers are adults, since arthritis is a disease associated with aging. Thus, 42% of all adults in the 45 to 65 age groups have arthritis, while 59%

of adults aged 65 and older have arthritis. Arthritis also affects women more than men, as 37% of U.S. female adults suffer from arthritis, while only 28% of U.S. male adults have the disease

Current Market Size and Segmentation

Currently, U.S. consumers spend well over \$2.5 billion on various products to deal with their arthritis. Over half of this total is spent for NSAIDs to relieve the pain associated with arthritis. The remainder is spent on various types of glucosamine products that offer the potential to stop the joint deterioration caused by the disease.

Overall, the U.S. market for glucosamine is both large and growing. Since 1994, total U.S. glucosamine sales have risen from about \$10 million in 1994 to over \$820 million in 1999 (Challenger, Cynthia). This represents a compounded annual growth rate in excess of 100% per year (Krause, Chemical Market Reporter). Currently, the U.S. glucosamine market is estimated to exceed \$1 billion.

The U.S. glucosamine market can be divided into two major segments – the human market and the pet market. The human market is by far the larger of the two, accounting for over 90% of all U.S. glucosamine sales, i.e., for over \$900 million in annual sales.

Future Products and Their Market Potential

ThruSkin also expects to have the opportunity to license a UGARF patented platform technology that converts solids to oils at room temperature. This technology was developed by Dr. Won Jun, a Professor in Georgia's Pharmacy School. It has already been used to develop a transdermally applied cream that increases the percentage of Ibuprofen that crosses the skin barrier from less than 3% to over 45%.

Because Ibuprofen is an NSAID, not a nutraceutical, some formal FDA testing will be required before this product can be brought to market. Consequently, ThruSkin has not placed a high priority on the near-term commercialization of this technology.

However, once ThruSkin has successfully launched Rejuvalin™, it will focus its R&D efforts on the development of a topical cream that will deliver high concentrations of both Rejuvalin™'s active ingredient and Ibuprofen through the skin to the affected joint areas. ThruSkin expects that the time needed to secure FDA approval for this product will be substantially less than that required for non-tested invasive products, since “Ibuprofens” have already been approved by the FDA and since this cream will be topically applied.

The great attractiveness of this product is that it will simultaneously reduce the pain associated with osteoarthritis, while helping to maintain and re-build the cartilage in the patients' arthritic joints. In short, it will appeal to the entire \$2.5 billion arthritis medication market.

3. E-Team Members and Their Skills

The following four individuals comprise ThruSkin's Advanced E-Team. They will carry out most of the activities covered by this grant. When ThruSkin is ready to bring Rejuvalin™ to market, ThruSkin anticipate that it need to hire a VP of Sales & Marketing and a CEO, both of whom have experience in nutraceuticals and/or pharmaceuticals.

Solomon T. Garner, Jr., Ph.D. Student, University of Georgia, Chief Technology Officer

➤ MS (Medicinal Chemistry) & BS (Chemistry)

- President, Graduate and Professional Scholars Society, a student run organization whose mission is to improve the matriculation of historically underrepresented groups at the graduate level.
- Key Skills: Medicinal Chemistry and Pharmaceutical Development

Michael B. Clark, M.D., MBA Student, University of Georgia, Chief Medical Officer

- M.D. (Doctor of Medicine) & BS (Biology)
- 4 years experience as a Flight Surgeon for the U.S. Navy
- 2 years experience as Officer in Charge, Naval Medical Clinic, Souda Bay
- Key Skills: Medical Doctor

J. Mark Moore, Ph.D., MBA Student, University of Georgia, Chief Operations Officer

- Ph.D. (Materials Science and Engineering), MS (Mechanical Engineering), & BS (Mechanical Engineering)
- 2 years experience as a Senior Process Engineer with Intel
- 5 published papers, including one on total hip replacement issues
- Key Skills: Ability to manage efforts of equipment support personnel equipment maintenance support provided by outside vendors for a quality control equipment

Tobin P. Mercer, MBA Student, University of Georgia, Chief Financial Officer

- BBA (Marketing)
- 6 years experience in sales and finance
- Key Skills: Sales and Finance

4. Project Advisors and Their Skills

Dr. Charles Hofer, Regents Professor, Terry College of Business, University of Georgia

- BS (Physics), MS (Mathematics), MBA (Marketing), DBA (Strategic Management)
- Has assisted over 12 student teams convert their plans into real world businesses
- Won 2002 Robert Foster Cherry Award for Great Teachers

Dr. Anthony Capomacchia, Associate Professor Pharmaceutical & Biomedical Sciences.

- BS (Pharmacy), Ph.D. (Chemistry),
- Advisor to the Laboratory for Pharmaceutical Development / Quality Office

Sandra B. Granade, Pharmaceutical Industry Consultant

- BS (Biology), MS (Biology)
- 25 years of pharmaceutical industry experience

5. Resources and Equipment Provided

The University of Georgia will contribute to the success of this project in several ways.

First, the University of Georgia's College of Pharmacy will provide the space and equipment (blenders, mills, wet lab equipment, and emulsifiers) needed to produce enough Rejuvalin™ to complete the documentation of Rejuvalin™'s ability to penetrate the skin. The College of Pharmacy will also provide the GMP facilities needed for the small-scale production of Rejuvalin™ for use in ThruSkin's clinical study of Rejuvalin™'s effectiveness. [Note: The latter study is not needed to

bring Rejuvalin™ to market. It will, however, allow ThruSkin to make stronger claims regarding Rejuvalin™'s effectiveness as an osteoarthritis treatment.]

Second, the University of Georgia's Complex Carbohydrate Research Complex will allow ThruSkin the use its Dionex high performance anion-exchange chromatographer (HPAEC) and technical support personnel in order to complete the analytical analysis of Rejuvalin™.

Third, all of the MBA students on ThruSkin's E-Team will receive course credit for their market research on the most effective ways of bringing Rejuvalin™ to market

Finally, ThruSkin anticipates that it will be able to secure office space in the University of Georgia's Bio-Technology Incubator sometime during the summer of 2004.

6. Project Timeline and Plan of Work

ThruSkin's Timeline for bringing Rejuvalin™ to market is as follows.

January and February 2004

- File 75-day FDA Pre-Market Notification of ThruSkin's intent to market Rejuvalin™
- Completion of Trademark Search on Rejuvalin™ Band Name.
- Completion of ThruSkin's Focus Group Market Surveys on Rejuvalin™
- Completion of Rejuvalin™ Analytical Protocol

March and April 2004

- FDA Response to 75-day Pre-Market Notification Due
- File for Trademark on Rejuvalin™ Brand Name
- Attend Nutraceutical Trade Fair
- Completion of ThruSkin's Business Plan
- Completion of ThruSkin's Web-Site

May and June 2004

- Finalization of Rejuvalin™ & Transdermal NSAID Licenses with the University of Georgia Research Foundation (UGARF)
- Presentation of ThruSkin's Plans to Potential Angel & Venture Capital Investors
- Bulk Production of Rejuvalin™ with Batch Records
- Begin Rejuvalin™ Clinical Study

July, August, and September 2004

- Finalization of ThruSkin's Startup Funding
- Setup ThruSkin Offices in University of Georgia Bio-Tech Incubator
- Finalization of Vendor Contracts for Rejuvalin™ Production
- Identification of Potential Candidates for VP of Sales Position
- Continuation of Rejuvalin™ Clinical Study

October, November, and December 2004

- Begin Sales of Rejuvalin™ through ThruSkin's Web-Site and Selected Retailers
- Completion of Rejuvalin™ Clinical Study

- Begin Development of “Info-mercials” for the National Marketing of Rejuvalin™
- Begin Development of combined Rejuvalin™/Transdermal NSAID product.

7. Project Budget

Expense Category	Done By	Amount Budgeted
1. Analytical (HPAEC) Supplies	Solomon Garner	\$3,000
2. Trademark Research	Needle & Rosenberg	750
3. Focus Group Market Research	MBA Students	3,000
4. Nutraceutical Trade Fair Expenses	Garner & MBA Students	2,500
5. “Workaround Analysis” of Patent	IP Attorney	1,000
6. Chemicals Needed for R™ Clinical Trial	Solomon Garner	8,000
7. Packaging Materials for R™ Clinical Trial	Outside Vendor	1,500
8. Miscellaneous Supplies	Various Vendors	500
Total Projected Costs		\$20,000

There are four major categories of expenses for which grant support is sought. The first is for the supplies needed to complete additional analytical studies of Rejuvalin™ to determine the specific Rejuvalin™ product formulation that maximizes the amount of glucosamine that crosses the skin barrier. Rejuvalin™ ‘s current formulation is able to get about 70% of its active ingredient across the skin barrier. While this is significantly greater than the delivery capabilities of all products currently on the market, ThruSkin is seeking funds to test some new ideas Solomon Garner has for

increasing this percentage even more. The second major expense category is for conducting several Focus Group studies to determine from a customer's perspective, the most effective ways to bring Rejuvalin™ to market. The third major expense category is the cost of sending the E-Team to a major Nutraceutical Trade Fair. This trip will allow the team to identify and evaluate the different channels that it could use to bring Rejuvalin™ to the market. The final major expense category involves the chemicals and packaging materials needed to produce enough Rejuvalin™ for a small scale clinical trial of its effectiveness once Rejuvalin™'s 75 day Notification has been approved by the FDA.

A company web site will be developed to enable direct card and 1-800 sales at an estimated cost of \$3500 + \$20/month hosting fee (from J. House media), as well as educate potential customers as to our product, company, and benefits. Features of webs site will include educational materials, registration, message board, an email link to customer service, and a sales order mechanism. The company will make its first exhibitions at the National Natural Foods Association trade show (Las Vegas) and/or the Natural Product Expo East (Washington, DC) in attempt to solicit wholesale and retail business. The average cost per visit to a trade shows has been estimated to be approximately \$3500/show.

APPENDIX E

BUSINESS PLAN FOR A TOPICAL GLUCOSAMINE PRODUCT¹

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¹Solomon T. Garner, Jr., Michael Clark, Toby Mercer, Mark Moore, Chris Miller, Heard Robinson, and Mary E. Thompson. Submitted to Dr. Charles W. Hofer's Entrepreneurship course.

BIZ 101

ON THE INSIDE: Immersing themselves in their target industry helped Michael Clark, Solomon Garner Jr., Mark Moore and Toby Mercer (l. to r.) win the SEED Business Plan contest.



Sowing the Seeds

THESE BUSINESS PLAN COMPETITION WINNERS SHOW THEY'VE GOT WHAT IT TAKES TO MAKE THEIR STARTUP IDEAS BLOOM.

BY NICHOLE L. TORRES

START A BUSINESS. START A BUSINESS. Start a business. As a reader of *Entrepreneur*, you hear that phrase often. And for the participants of the first annual 2004 SEED Business Plan Competition in Santa Barbara, California, this past February, it seemed to be the chant in the air.

SEED, short for Spirit of Entrepreneurship and Enterprise Development, is the brainchild of David Newton, founder and president of TechKnowledge Point Corp. His Santa Barbara firm helps small businesses and startups find research and business contacts to help them grow their ventures and brings academic re-

search to the business community. (TechKnowledge Point also compiled *Entrepreneur's* 2004 college rankings, which appear on page 78.) The competition is part of an overall strategy that encourages bringing together business ideas and people who have the research, capital, connections and general know-how to get a startup off the ground.

The business plan competition attracted graduate and post-graduate students from business schools across the country to present their ideas on ventures ranging from fuel-cost-management services for trucking companies to made-to-order art prints for interior designers. From rethinking old ideas to creating a brand-new niche in a growing market, these budding entrepreneurs came prepared to exceed expectations. The excitement was palpable, and, in many cases, the teams came with their businesses well beyond the planning stage, ready to enter the execution stage.

Of the 35 business plans submitted, eight were chosen to be presented at the semifinals in Santa Barbara in front of a tough group of judges who threw hard questions while making solid suggestions for the aspiring entrepreneurs. According to Newton, the plans were scored on elements such as the strength of the management team; a clearly identifiable business model; fully fleshed-out costs and cost structures; whether there was a large, viable market ready for the product or service; and the overall attractiveness to investors.

"We were pleased to find that, as opposed to a stack of 35 plans dealing with some kind of precision technical instrument or wireless telecom, the whole range was there," says Newton. From pharmaceuticals to medical devices to an adventure-travel Web services site and road-safety-testing equipment, the plans ran the gamut.

Taking home the top prize was ThruSkin Technologies, the team from the University of Georgia in Athens. Helmed by Michael Clark, 33, Solomon T. Garner Jr., 26, Toby Mercer, 28, and Mark Moore, 34, and their business plan is to create and market nutraceutical products, which include supplements from natural sources with proven health benefits.

Their first product in development is a glucosamine topical treatment to relieve the causes and symptoms of osteoarthritis. Garner, a student at the University of Georgia College of Pharmacy, had been researching the product for his studies, and he linked up with Clark, Mercer and Moore in the business school to find a way to commercialize what he'd developed in the lab.

The fact that these aspiring entrepreneurs have combined their backgrounds in medicine, pharmacology, sales and business development certainly added strength to their business plan. But what really earned ThruSkin Technologies the first-place prize of \$10,000 in seed money, an office computer system, and an automatic berth in the University of San Francisco School of Business and Management's business plan com-



INCA

Intelligent Nutraceutical Company of America

Formerly

ThruSkin Technologies

Delivering Effective Natural Treatments[™]

Business Plan



EXECUTIVE SUMMARY

Company Overview

The mission of Intelligent Nutraceutical Company of America (INCA) is to develop and market nutraceutical and select complementary nutraceutical-pharmaceutical combination products that have scientifically proven health benefits. INCA's first product is Rejuvalin™, a topically applied cream that treats osteoarthritis by delivering significantly more glucosamine directly to affected joint than any other product on the market today.

Osteoarthritis (OA): A Widespread and Growing Disease

OA is a debilitating and incurable joint disease caused by the deterioration of joint cartilage. Symptoms experienced by OA victims include localized pain, inflammation, decreased mobility, and joint deformity. In addition, OA patients must also change and carefully manage their lifestyles to prevent further joint deterioration.

Arthritis is the #1 disability in the United States today, affecting more people than cancer, diabetes, or heart disease. In 2002, over 21 million American had OA, and they spent over \$6 billion for various types of products and services to treat OA and its symptoms. According to the Arthritis Foundation, these numbers are forecast to grow to 25 million sufferers and over \$7.5 billion in annual treatment revenues by 2010.

Problems with Current Osteoarthritis Treatments

Common but ineffective treatments for OA include over-the-counter (OTC) non-steroidal anti-inflammatory drugs (NSAIDs) such as ibuprofen, and prescription drugs such as CelebrexTM. NSAIDs are frequently used to treat pain and inflammation associated with OA. However, NSAIDs treat only the symptoms of OA and do not address OA's underlying causes. Studies have shown that long-term use of these drugs to treat OA can actually cause joint deterioration to increase. In addition, NSAIDs have dangerous and life-threatening side effects associated with long-term use. For example Merck and Company withdrew VioxxTM from the market due to a study, which cited its detrimental affects. The Centers for Disease Control (CDC) reported that in 2000, over 100,000 people were hospitalized and 16,500 died due to NSAID overdoses.

Glucosamine: A Promising OA Treatment with Inadequate Delivery Methods

Glucosamine offers hope to OA sufferers, because it has been shown in a number of peer reviewed scientific studies to alleviate both the pain and inflammation symptoms associated with OA and to protect against further joint deterioration. In fact, several studies have shown that glucosamine not only promotes joint health but also is shown to reverse the effects of OA by preventing further joint decline. The National Institutes of Health (NIH) is currently conducting an ongoing multi-center study- GAIT (Glucosamine/Chondroitin Arthritis Intervention Trial) that is currently evaluating the glucosamine. The results of this study are scheduled for release in the fall of 2005. Early reports have indicated that the study is strongly confirming the benefits of glucosamine documented in earlier studies.

Due to these earlier studies and after the featuring of glucosamine in the book [The Arthritis Cure](#) by Jason Theodasakis, MD during the mid-1990s, glucosamine sales exploded. Sales of glucosamine

have been growing at an annually compounded rate in excess of 40%. Currently, U.S. glucosamine sales are estimated to be in excess of \$1.2 billion.

However, the effectiveness of glucosamine as a treatment of OA is greatly hampered by its available delivery methods. Currently, glucosamine is sold as pills, powders, drinks, creams and gels. Unfortunately, less than 1% of orally administered doses reach the joints. The form of glucosamine in current creams and gels is not able to penetrate the human skin. Consequently, while oral delivery methods may provide enough glucosamine for some individuals with mild OA, they do not provide enough for sufferers with more serious problems.

Rejuvalin™: A Significantly Improved Glucosamine Cream

Rejuvalin™ is a patent-pending glucosamine cream that has been specifically formulated to overcome delivery problems. In testing on skin models, Rejuvalin™ has been shown to deliver glucosamine across the skin's barriers. Rejuvalin's™ superior delivery capabilities' provide great hope to sufferers of osteoarthritis. Additionally, since Rejuvalin™ contains nutraceuticals and over-the-counter ingredients, intensive U.S. Food and Drug Administration (FDA) testing and approval processes will be avoided. Thus, INCA anticipates selling Rejuvalin™ by summer of 2005.

Start-up and Growth Strategies

INCA will use a focused value chain strategy concentrating its resources on the marketing and selling of Rejuvalin™ while outsourcing the production, packaging and physical distribution of its products. INCA will also outsource the effectiveness testing of its products, although it will coordinate those tests in order to ensure quality.

INCA will initially sell Rejuvalin™ through its own web site and local and regional retail outlets. One INCA has completed sufficient Rejuvalin™ testing to support strong advertising claims, it will begin nationwide distribution of the product and secure a nationally recognized spokesperson to deliver strong Rejuvalin™ ads to its target customer groups.

The Deal

INCA has recently received \$28,750 from the National Collegiate Inventors and Innovators Alliance (NCIIA) as well as the Idea to Product (I2P) competition to complete the initial testing of Rejuvalin's™ effectiveness. INCA is currently seeking \$750, 000 to launch Rejuvalin™ in summer-2005. Once Rejuvalin's™ effectiveness has been confirmed by additional studies, INCA will seek additional capital to begin marketing Rejuvalin™ on a national basis. By year five, INCA expects to generate sales in excess of \$60 million and provide its initial investors cash on cash returns significantly in excess of 10 to1.

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I. Executive Summary

Company Overview

The mission of the Intelligent Nutraceutical Company of America, LLC (INCA) is to develop and market nutraceutical products whose health benefits and efficacy have been proven scientifically through rigorous independent third party testing. INCA recognizes that there is a substantial opportunity in marketing selected nutraceutical products that can be differentiated by proprietary technology and proven to be effective through scientific testing. INCA's first product is Rejuvalin™, a transdermal cream that treats osteoarthritis by delivering significantly more glucosamine directly to affected joints than any other product on the market today.

Osteoarthritis: A Widespread and Growing Disease

Arthritis is the #1 cause of disability in the United States today, affecting more people than cancer, diabetes, or heart disease. Osteoarthritis (OA) is the most common form of Arthritis. It is a debilitating, incurable joint disease caused by the deterioration of joint cartilage. Symptoms experienced by OA victims include localized pain, inflammation, decreased mobility, and joint deformity. In addition, OA patients must also change and carefully manage their lifestyles to prevent further joint deterioration. In 2002, over 21 million Americans had OA, and they spent over \$6 billion for various types of products and services to treat their OA and its symptoms. According to the Arthritis Foundation, these numbers are forecast to grow to 25 million sufferers and over \$7.5 billion in annual treatment costs by 2020.

Problems with Current Osteoarthritis Treatments

Common but ineffective treatments for OA include over-the-counter non-steroidal anti-inflammatory drugs (NSAIDs), such as Ibuprofen, and prescription drugs, such as Celebrex™. NSAIDs are frequently used to treat the pain and inflammation associated with OA. However, NSAIDs treat only the symptoms of OA and do not address OA's underlying causes. Studies have

shown that long-term use of these drugs to treat OA can actually cause joint deterioration to increase. In addition, NSAIDs have dangerous and life-threatening side effects associated with long-term use. In 2000, over 107,000 people were hospitalized and over 16,500 died because of complications associated with NSAID use.

Glucosamine: A Promising OA Treatment

Glucosamine offers hope to OA sufferers because it has been shown in a number of scientific studies to alleviate both the pain and inflammation symptoms of OA and to protect against further joint deterioration. In fact, several studies have shown that glucosamine not only promotes joint health, in some cases, it has actually reversed the effects of OA. The National Institutes of Health (NIH) is currently conducting a long-term study of glucosamine that is scheduled to be released in mid to late 2005. Early reports have indicated that it will strongly confirm the benefits of glucosamine documented in earlier studies.

Because of these findings, total U.S. glucosamine sales have exploded since the mid 1990s, growing at an annually compounded rate in excess of 40%. Currently, annual U.S. glucosamine sales are estimated to be in excess of \$1.2 billion.

Unfortunately, the effectiveness of glucosamine as a treatment of OA is hampered by its available delivery methods. Currently, glucosamine is sold in the form of pills, powders, liquids, creams and gels. However, only about 1% of the glucosamine in pills, powders, and liquids that are consumed orally actually reaches the diseased joint and less than 2% of the glucosamine in current creams and gels is able to penetrate human skin. Consequently, while these delivery methods can be effective for those with only mild forms of OA, they are not effective for sufferers with more serious forms of the disease.

Rejuvalin™: A Significantly Improved Glucosamine Cream

Rejuvalin™ is a patent-pending glucosamine cream that has been specifically formulated to overcome glucosamine delivery problems. In testing on skin models, Rejuvalin™ has been shown to deliver over 55% of its glucosamine across the skin barrier, a delivery rate 50 times greater than that achieved by other glucosamine products. Rejuvalin™'s superior delivery capabilities provide great hope to sufferers of osteoarthritis. Also, since all Rejuvalin™'s components are nutraceuticals, Rejuvalin™ will not need to go through an FDA testing and approval process to be brought to market. Once INCA has completed testing of Rejuvalin™, the company will begin selling the product through its web site and other direct sales channels.

Start-up and Growth Strategies

INCA will use a focused value chain strategy concentrating its resources on the marketing and selling of Rejuvalin™ while outsourcing the production, packaging, and physical distribution of its products. INCA will also outsource effectiveness testing, although it will coordinate such tests in order to ensure quality. INCA's startup strategy will focus resources on low cost channels and methods in order to get to market quickly and effectively. INCA's growth strategy will include multiple promotional activities such as direct mail and email, radio advertisements, and physician awareness. As sales increase, INCA will begin to utilize more high cost promotional activities such as print media, infomercials and national spokespersons, as well as the retail sales channel.

INCA's initial product will be Rejuvalin™ cream, which is intended to treat OA. Additionally, INCA has identified several follow-on products. These include:

- Rejuvalin™ pills with enteric coating to reduce stomach indigestion
- Rejuvalin Pain Formula™ used for immediate pain relief for flareups

- Resveratrol is a natural antioxidant that has been proven to have several health benefits
- A blood pressure reducing nutraceutical discovered at the University of Georgia.

The Deal

INCA received \$18,750 from the National Collegiate Inventors & Innovators Alliance (NCIIA) and \$10,000 by winning the 2004 International “Idea-to-Product™” Competition. These funds will be used to complete the initial testing of Rejuvalin™’s delivery effectiveness. INCA is seeking \$750,000 to launch Rejuvalin™ by mid to late 2005 in return for a 15% equity position. Once INCA’s startup strategy has been effectively executed, INCA will seek additional capital to fund growth strategies. By 2009, INCA expects to be generating annual sales in excess of \$35 million and provide its initial investors cash on cash return significantly in excess of 20 to 1.

II. Osteoarthritis: A Serious, Incurable Disease

What is Osteoarthritis?

Osteoarthritis (OA) is a disease of the joints caused by degradation of articular cartilage (Exhibit 1). Cartilage is a firm but flexible substance surrounding bone that acts as a shock absorber and allows bones to travel with fluidity during physical movement. OA is characterized by the slow focal erosion of this cartilage. It is a progressive disease. In advanced stages, OA may cause the cartilage to wear away to the point where bone touches bone. The disease causes pain, inflammation, swelling, and stiffness of the effected joints. OA results from years of accumulated "wear and tear" on joints, and tends to occur in the hips, knees, and finger joints of persons above the age of 45, although some do the disease at younger ages. Nearly 100% of persons who live long enough will be affected. OA usually develops slowly over time, and can occur due to a number of factors. Genetics, obesity, lack of activity, and injury can all play a part in the onset of osteoarthritis. If left unchecked, OA sufferers may have to undergo costly and risky joint replacement surgery. The average cost of knee replacement surgery is \$32000 per occurrence, and 171,000 of these surgeries are performed in the U.S. each year¹.

The Demographics & Impact of Osteoarthritis

Arthritis is the leading cause of disability among U.S. adults.² (Exhibit 2) Currently, Osteoarthritis affects more than 21 million people in the United States. This is expected to grow to 25 million by 2020.³ Over 50% of people above age 65 and 85% of people above the age of 75 exhibit evidence of osteoarthritis.⁴ (Exhibit 3) Because of its painful symptoms and widespread nature, OA has great

1. Dept. Health and Human Svcs.

3. CDC.gov, 2001

4. www.medtap.com

5. Postgraduate Medicine, 2003

costs to U.S. society. Not only can OA be financially devastating to an individual with the high and ongoing cost of treatments, but the loss of productivity causes additional hardship. The direct cost of OA has been estimated at \$32 billion,⁵ and recent estimates of the total costs of arthritis, of which osteoarthritis is the largest component, exceed 2% of the US GDP.⁶

Traditional Osteoarthritis Treatments

Typical treatments used for the symptoms of OA include various forms of pain medication, such as acetaminophen and non-steroidal anti-inflammatory drugs (NSAIDs). Unfortunately for the OA sufferer, these pain medications do not prevent the disease from progressing, and the cartilage continues to deteriorate under this class of treatments.

Acetaminophen: First line treatment for OA usually begins with acetaminophen (Tylenol). Acetaminophen treatment is limited to control of pain and has no anti-inflammatory properties. Sufficient dosages needed to control the pain will often be 4 grams per day (12 regular strength or 8 extra strength pills). This is important because this dosage is the highest recommended dosage in *healthy* patients, and many patients with OA have other disease states that are common with aging. Acetaminophen doses of less than or equal to 4 grams a day have been shown to cause liver toxicity in patients *without* other risk factors.⁷ Moreover, acetaminophen in even small doses (650mg) has been linked to liver toxicity in patients who are heavy users of alcohol.⁸ Also, kidney failure has been noted to be 2.5 times higher in those who consume acetaminophen over long periods of time

2. Lewis, FDA.gov

6. Annals of Internal Medicine, 2000

7. Bolesta S, Haber SL. Annals of Pharmacotherapy. 2002; 36(2):331-3

8. Denison et. al. Scand J Gastroenterol 1987; 22(6):701-704

versus other individuals.⁹ Clearly, acetaminophen is not the most effective treatment for OA and poses many life threatening risks to its users.

NSAIDs: Another common treatment for the joint pain, inflammation and stiffness associated with OA is over-the-counter (OTC) non-steroidal anti-inflammatory drugs (NSAIDs). Drugs falling into this category include aspirin, ibuprofen, and naproxen.

Though OTC NSAIDs have been safely used to address the symptoms of OA in the short-term, dangerous side effects have been associated with their long-term use. Kidney failure has been noted in people who ingest NSAIDs consistently over a long period of time.¹⁰ In addition, it has been estimated that 20-30% of all hospitalizations and deaths due to gastrointestinal bleeding for people over 65 are directly linked to use of NSAIDs.¹¹ In numerical terms, there are 107,000 hospitalization and 16,500 deaths due to NSAID related toxicity in the U.S. each year¹².

Celebrex™: A New NSAID: Celebrex™ is a more advanced form of NSAID drug that has been developed by Pzifer to address OA. Celebrex™ is available only by prescription, and the annual cost for an uninsured person to take Celebrex™ can exceed \$1000.

9. Sandler et. al. Ann Intern Med 1991; 115(3):165-172, Perneger et. al. N Engl J Med 1994; 331(25):1675-79

10. Sandler et. al. Ann Intern Med 1991; 115(3):165-172, Perneger et. al. N Engl J Med 1994; 331(25):1675-79

11. Griffin et. al Ann Intern Med 1991;114(4):257-263

12. Singh, 1998; N Engl J Med

CelebrexTM functions as other drugs of this class to alleviate the symptoms of joint pain, stiffness, and inflammation that are associated with OA. Unlike OTC NSAIDs, CelebrexTM has been designed to block the actions of the COX-2 enzyme that is responsible for the body's pain and inflammation response, while not inhibiting the COX-1 enzyme that is involved in blood clotting, kidney function, and protection of the stomach lining.¹³ The tailored nature of CelebrexTM eliminates some of the severe side effects associated with long-term use of its OTC counterparts. However, kidney function in patients has not been maintained as well as was first thought and gastrointestinal bleeding is still caused by this drug and others like it. Though this drug is more advanced than its OTC counterparts, it still does not address the joint wear and deterioration that is the cause of OA.

VioxxTM: An Unmitigated Disaster for OA Patients: On September 30, 2004, Merck announced a full recall of its arthritis and acute pain drug VioxxTM. The company also announced that a three year study of the effects of the drug would be halted after evidence was found that VioxxTM contributed significantly to increased cardiovascular events such as heart attack and stroke. The test was intended to prove the safe efficacy of VioxxTM amid claims that it might help prevent the recurrence of certain intestinal polyps.¹⁴

BextraTM: Additionally, on November 5, 2004, Pfizer Inc. stated in an SEC filing it is in negotiations with the FDA regarding a likely requirement to add a black box warning to the label of BextraTM, its COX-2 Inhibitor due to serious a skin reaction, Stevens Johnson Syndrome, developed in a few

13. celebrex.com

14. www.vioxx.com

patients. A black box warning is the strongest warning a pharmaceutical can hold. Stevens Johnson syndrome, while mainly a skin reaction carries a 30% fatality rate.

The Current Situation for Treating Osteoarthritis

Clearly, current treatments of osteoarthritis leave much to be desired in terms of long-term effectiveness while introducing a number of health complications in the short-term. At best, sufferers of OA who use these kinds of therapy are just putting themselves into a more comfortable state while their once-healthy joints continue to erode.

III. Glucosamine: A Potential Long-term OA Treatment

What is Glucosamine?

Glucosamine (2-amino-2-deoxy-D-glucose) is an amino sugar produced in the bodies of humans and other mammals. One of the major functions of glucosamine within the body is to support joint health. In this role, it acts as a raw material for the building blocks of cartilage, tendons, and other connective tissue.

Because glucosamine is a naturally occurring substance, it is classified as a nutraceutical. This designation allows glucosamine products to be sold to the public without the need of FDA approval. Most glucosamine-based products sold today come in the form of pills or tablets taken orally, however it is also available in the form of liquids and creams.

Glucosamine's Benefits

A glucosamine-based treatment addresses not only the symptoms of OA, but also the root cause of the disease – joint deterioration. A glucosamine regimen has been demonstrated to provide pain relief and inflammation reduction that is on par with the magnitude of symptom reduction provided by ibuprofen and other NSAIDs.¹⁵ Furthermore, several studies have found that the use of supplemental glucosamine prevents further joint space narrowing in the joints of OA sufferers.¹⁶ The benefits and safety claims for glucosamine are supported by numerous clinical studies involving thousands of people nationwide.¹⁷ Exhibit 4 summarizes some recent glucosamine studies.

15. Tapadinhas et. al. *Pharmatherapeutica* 1982;3(3):157-68

16. Reginstar et. al. *Lancet* 2001; 357:251-6

17. Medline search; Glucosamine and Osteoarthritis

In addition to being a more comprehensive treatment for OA, glucosamine is safe for long-term use. Glucosamine has fewer complications than acetaminophen or NSAIDs.¹⁸ The most commonly reported side effect of oral treatment is upset stomach.¹⁹ The safety of long-term glucosamine use has been verified by studies that determined there are limited side effects to using glucosamine.²⁰ The benefits associated with taking glucosamine are normally realized within a period of four to eight weeks after treatment begins. However, these benefits will cease once the treatment is discontinued.

Some Limitations of Current Glucosamine Treatments

While, glucosamine has the potential to address both the symptoms and root cause of OA, the current methods for delivering glucosamine to a diseased joint are not effective in all cases. Only a trace amount (less than 1%) of an orally administered dose actually reaches the diseased joint²¹ (Exhibit 5). This means that glucosamine taken orally, while effective for prophylactic purposes or for treating mild forms of OA, is not able to deliver glucosamine in high enough quantities to be an effective treatment of severe OA. In cream form only about 2% of glucosamine administered topically is able to penetrate the skin barrier, a fact that was confirmed by INCA's laboratory tests that showed that virtually no glucosamine penetrated traditional skin models (Exhibit 6).

NIH Study of Glucosamine

The National Institutes of Health (NIH) is currently performing a study on the effects of glucosamine taken orally by OA patients. The results are not expected until mid to late 2005, but preliminary reports have been very positive. If the study results are strongly positive, it will have a

18. Qiu et. al. *Arzneimittelforschung* 1998; 48:469-74

19. Dietary Supplements, Melanie Cupp and Tim Tracy, eds. Humana Press 2003

20. Hungerford MW, *Foot Ankle Clin*, 2003 Jun; Vol. 8 (2), pp. 201-19

21. Setnikar et. al. *Arzneimittelforschung* 1986;36(4):729-735

dramatic effect on total US glucosamine sales by increasing the frequency that glucosamine is recommended by medical professionals.

IV. The Glucosamine Market: Large, but Underdeveloped

U.S. Retail Sales and Customer Segments

Total U.S. glucosamine sales have risen from \$10 million in 1994 to over \$820 million in 1999.²² The market is currently estimated to exceed \$1.2 billion²³. Sales of glucosamine accelerated in 1997 when renowned authority Dr. Jason Theodosakis described the benefits of glucosamine for arthritis sufferers in his book, *The Arthritis Cure*. According to Dr. Theodosakis, "Four decades of medical research around the world have shown that two nutritional supplements - glucosamine and chondroitin sulfates - can halt, reverse, and even cure osteoarthritis."²⁴ Over the last five years, glucosamine sales have been growing at a rate of 40% per year²⁵ (Exhibit 7).

The two primary customer groups who purchase glucosamine are OA sufferers and athletes, with OA sufferers making up the major portion of the total market. The allure of glucosamine stems from its ability to alleviate joint pain and to maintain joint health. Most users generally seek glucosamine as a treatment for joint pain. However, glucosamine is already being marketed and sold to athletes as a preventative treatment.

Products, Distribution Channels, and Purchase Decisions

Currently, glucosamine is marketed as an over-the-counter dietary supplement by a wide variety of companies. Major producers of glucosamine include large companies such as Rexall-Sundown and Pharmaton. Glucosamine-based products are sold by traditional retailers such as pharmacies,

22. Challenger, Cynthia. Chemical Market Reporter, Sept. 25, 2000

23. www.healthstrategy.com

24. www.drtheo.com

25. Krause, Chemical Market Reporter

grocery stores, nutritional stores, and discount retailers. Additionally, glucosamine products are sold on the internet on both manufacturer and retail web sites. The typical recommended daily oral dosage of glucosamine is 1,500 mg.

Glucosamine-based products are available to consumers in forms such as pills, caplets, liquids, powders, drinks, and creams, and marketed under more than 100 different brands. Consumers take specific recommendations from their doctor and other respected medical authorities as to the type and brand of glucosamine to purchase. Though recommendations from medical professions can drive the initial purchase of a particular brand, customers will continue to use only the products that are effective. Once consumers find a brand that works, they tend to remain loyal to that brand.

Because of the widespread and debilitating nature of OA, sufferers are highly networked. One implication of this is that information on new treatments and products is disseminated quickly and is relatively easy to obtain. The success of glucosamine is due in part to the network of information available to the general public through the internet; articles in journals, magazines and newspapers; and information received from doctors.

The Market Potential of a More Effective Glucosamine Product

The current glucosamine market is very large, but it is also significantly below its potential. Considering that 21 million Americans suffer from OA and could potentially benefit from glucosamine treatment, total annual sales of glucosamine to this market segment could exceed \$6 billion. A more effective delivery method of glucosamine therapy, combined with positive results from the NIH study will play an important part in increasing the sales of glucosamine (Exhibit 8).

V. Rejuvalin™: An Improved Glucosamine Cream

What is Rejuvalin™?

Rejuvalin™ is a patent-pending topical cream that is applied directly to the osteoarthritic joint. Rejuvalin™ is designed to penetrate through all three layers of the skin and deliver glucosamine directly to the joint cartilage. Users will simply rub Rejuvalin™ on the affected joint area once a day, and Rejuvalin™'s glucosamine will penetrate through the skin and reach the targeted cartilage. As with other glucosamine treatments, customers will have to continue to use Rejuvalin™ to continue to experience its benefits.

Rejuvalin's™ Benefits

Rejuvalin™ reaches the affected cartilage using INCA's patent-pending technology. Exhibit 9 shows that Rejuvalin™ immediately and continuously penetrates a scientifically accepted skin mode delivering as much as 50 times more glucosamine to the joint.

In addition, since Rejuvalin™ bypasses the gastrointestinal (GI) tract, it will not cause any GI issues such as stomach upset or diarrhea, which are common side effects reported for glucosamine-based oral supplements, especially when taken in quantities large enough to treat moderate to severe osteoarthritis.

Exhibit 10 compares Rejuvalin™ with current pain management treatments and glucosamine supplements. Rejuvalin™ is superior to both classes of products because it addresses the root cause of OA and is delivered more effectively with minimal side effects.

Product Pricing

Rejuvalin™ cream will be offered in 3 oz. tubes. The cream will be marketed to patients who have OA in a limited number of joints. It will deliver a substantially larger amount of glucosamine to the target area. This proprietary product will be INCA's flagship offering.

INCA anticipates introducing Rejuvalin™ cream at an initial retail price of \$24.99 per 3 oz. tube which provides enough product for one month of treatment. This price is commensurate with the cost of a month's supply of other high-quality glucosamine products, but will deliver much better value because of increased effectiveness.

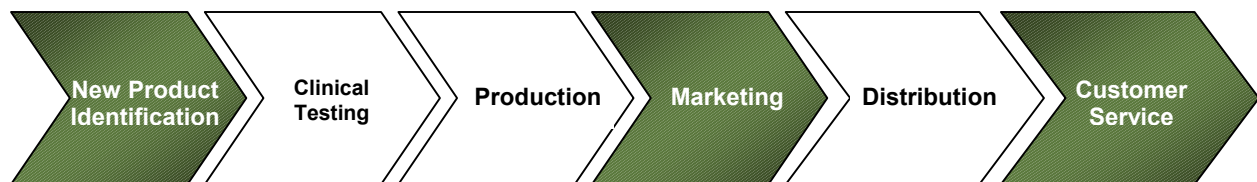
VI. INCA's Goals & Strategy

INCA's Mission & Vision

INCA will develop, test, and market innovative, superior-quality nutraceutical products that are scientifically proven to be effective. INCA's vision is to become a leading international developer & marketer of highly effective nutraceutical products.

INCA's Focused Value Chain Strategy

INCA's primary focus will be on new product identification, marketing and customer service. The majority of INCA's resources will be devoted to marketing and advertising to generate consumer demand for Rejuvalin™. INCA's new product efforts will be directed toward the identification and licensing of effective nutraceutical products and toward limited clinical testing of products ready for market. Raw materials procurement, product production, and packaging will be outsourced to experienced contract manufacturing firms. INCA will also contract with a fulfillment company to distribute Rejuvalin™ to customers who have ordered it, including retail outlets. Outsourcing the manufacturing and distribution portions of the business to established leaders in the area will enable INCA's management team to focus entirely on developing a market for Rejuvalin™.



Start-up Strategy

During the first year of operations, INCA will focus company resources on effective skimming of the market for Rejuvalin™. More specifically, INCA will generate initial revenues by distributing Rejuvalin™ through direct marketing channels such as internet, mail order, and home shopping channels. To generate awareness, INCA will use internet advertising, as well as an awareness

campaign for medical professionals. In addition, negotiations are currently underway to secure Dr. Jason Theodosakis as one of INCA's key partners. An endorsement from Dr. Theodosakis would carry weight with hundreds of thousands of OA sufferers and dramatically boost sales of Rejuvalin™.

Growth Strategy

After establishing profitability, INCA will begin to increase market penetration of Rejuvalin™. This will include marketing and advertising directly to potential customers the roll out of Rejuvalin™ into retail stores. INCA will initially sell product through health food stores, which have not sticking fees, and gradually expand into chain drug and grocery stores. INCA will also begin to build a sales force to market and sell Rejuvalin™ nationwide with particular emphasis given to selling Rejuvalin™'s benefits to medical professionals once the results of the NIH study are announced to the profession.

VII. Patents, Licenses & FDA Notification

Rejuvalin™'s Patent

A provisional patent on Rejuvalin™ was filed by the University of Georgia Research Foundation (UGARF) in early April 2004, and a final application will be filed in April 2005. Key patent claims include: (1) the unique form of glucosamine compound Rejuvalin™ uses to treat osteoarthritis, (2) the transdermal application of this compound and its embodiments and formulations, (3) the combination use of this compound and its transport agents with transdermal NSAIDS. Over the past 20 years, over 95% of the patents filed by UGARF have lead to issued patents.

INCA's Workaround Analysis

F. R. Denton, J.D., Ph.D. (Biochemistry), a patent attorney with over 10 years experience as a work-around specialist at Motorola has been contracted to do a complete work-around analysis on UGARF's provisional patent application in preparation for the final patent application.

INCA's License

INCA is in the process of negotiating an exclusive world-wide license on the transdermal penetration method used to formulate Rejuvalin™. Terms of this contract are expected to include the cost to UGARF of getting the patent (\$15,000 - \$30,000), the payment of all international patent filing fees, a royalty equal to 2% of net sales and 2% equity ownership in the company. However, no royalties would be paid, nor would the equity position vest until the patent issues.

FDA Notification

Because Rejuvalin™ is comprised of nutraceutical compounds, the regular product formulation is not regulated by the FDA. INCA will be able to manufacture and market Rejuvalin™ without having to secure FDA approval. INCA plans to send an "intent to market" letter to the FDA as

soon as the final patent application is filed. INCA will then be legally able to begin distribution of Rejuvalin™ 75 days later. All patent and regulatory hurdles should be cleared by July 1, 2005.

Product Testing

INCA also plans to perform clinical testing on Rejuvalin™ so that it can make stronger claims about the product's effectiveness in its marketing and advertising materials. Since these tests will not have to meet Federal Drug Administration (FDA) requirements, their costs will be kept low. However, the tests will be conducted in such a way as to meet the standards that are acceptable to the scientific and medical community in order to support strong advertising claims. Experienced counsel will be consulted to ensure that any advertising claims that are made are in compliance with what is acceptable to the FDA.

VIII. INCA's Marketing Strategy

Target Customers

INCA's primary customer segment for Rejuvalin™ is 45+ year olds who have been diagnosed with OA. There are over 21 million Americans currently suffering from OA, and the vast majority of them are 45 years of age or older. INCA's secondary target for Rejuvalin™ are athletes such as runners, tennis players, weight lifters, and football players who are seeking a preventative treatment for cartilage break down and joint pain relief.

Rejuvalin's™ Major Customer Benefits

Rejuvalin™ will be positioned as a safer and more effective treatment to OA and joint damage than existing products. It has differential and competitive advantages over current pain management techniques and other glucosamine-based supplements. Rejuvalin™ delivers a more effective (higher) dose directly to the source of OA, treating the root cause of osteoarthritis. It also avoids the gastrointestinal upset that is the common side effect of glucosamine-based supplements taken orally.

Marketing Strategy

INCA plans to launch Rejuvalin™ by the fall of 2005. INCA will use multiple sales channels such as direct to the consumer, home shopping channels and retail establishments. The Startup Strategy focuses INCA on low cost channels and methods in order to get to market quickly and effectively. The Growth Strategy will include two phases. In Phase I, INCA will use multiple promotional activities including direct mail and email, radio advertisements, and physician awareness. INCA will test many of these promotional activities regionally so the best methods for reaching our targeted customer base can be quickly determined. Phase II will include more high cost promotional activities such as print media, infomercials and national spokespersons, as well as the retail sales channel. An overall marketing budget is included in Exhibit 11.

Startup Strategy

The first sales channel to be used will be direct sales from the company website. INCA's website will feature information about Rejuvalin™, osteoarthritis, marketing messages encouraging product purchase, a credit card purchase feature, an automatic repeat purchase feature, a database to keep track of customer purchases, a message board, and links to national arthritis organizations. The estimated cost for a high-quality site created by J. House Media (Athens, GA) is \$3500 + a \$20 a month hosting fee. Coincident with the roll-out of INCA's website, INCA will begin taking direct orders from customers who wish to purchase Rejuvalin™ by telephone, fax or mail. The fulfillment of these orders will be contracted to a fulfillment center, Innotrac.

Additionally, INCA will register as a vendor on home shopping channels such as QVC and Home Shopping Network to sell Rejuvalin™ through the television. Home shopping networks primary audience includes females aged 40+ with an average income of \$63,000. Products are sold to QVC at wholesale cost, and there is no fee for the airtime. This will minimize expenses while increasing product awareness.

Upon completion of the clinical trials, residents from Medical College of Georgia will pursue publications in medical journals highlighting the result of the trials. INCA will highlight and reference these publications in advertisements in order to strengthen the advertising claims. This will provide the medical community the information they need to recommend Rejuvalin™ to their patients.

Once these tests are complete, Dr. Theodosakis, as one of INCA's key partners, will reference and link INCA's website to DrTheo.com under the Recommended Brands section.

Efforts will also be made to partner with affiliate websites such as the Arthritis Foundation (headquartered in Atlanta, GA), WebMD, and on-line sellers of nutraceutical products such as Drugstore.com, MotherNature.com and Vitacost.com. For all sales generated through these affiliate websites INCA will pay a fee of 18% in year 1, 10% in year 2, and 5% in year 3 for each product purchased. In addition, INCA will send targeted emails to health discussion boards, and banner ads on websites servicing our key customer demographic such as the AARP and Arthritis Foundation websites.

Growth Strategy - Phase I

Once the initial launch is complete, INCA will move into Phase I of the Growth Strategy. This first phase will include low cost marketing methods, as well as regional testing of some high cost methods.

Direct Mail and Email: INCA will work with Acxiom to procure mailing lists and email addresses of individuals within our target market. Information on Rejuvalin™ and coupons will be included in these direct mail pieces. The coupons and other promotional incentives will include reference codes so the effectiveness of each promotional activity can be tracked. Additionally, for \$900, the AARP will blast an email with information on Rejuvalin™ to 25,000 AARP subscribers. In addition to direct mailings, sponsorship of a popular national radio show such as Paul Harvey or Rush Limbaugh could also radically increase Rejuvalin™ awareness.

Physician Education: INCA will seek to educate the medical community on the benefits and clinical results of Rejuvalin™ through direct mail and medical conferences such as the Florida Geriatrics Society Conference and the Gerontological Society of America – 58th Annual Meeting.

Growth Strategy - Phase II

Phase II will include more high cost promotional activities such as infomercials, print media and national spokespersons, as well as the retail sales channel.

Infomercials: An infomercial that precisely and effectively conveys the benefits of Rejuvalin™ to our target customers will be developed with some of the capital raised in the second round of financing. This infomercial will feature INCA's national spokesperson, discuss product benefits, provide testimonials from satisfied Rejuvalin™ users and give viewers and opportunity to purchase Rejuvalin™. Care will be taken to run the infomercial on channels and at times that maximize the impact and value that INCA will receive.

Print Media: INCA will advertise in magazines and newspapers targeted to INCA's target market. Some print advertising venues that are promising include magazines such as Arthritis Today, Natural Health, Alternative Medicine, Total Health, and Yoga Journal. The cost of advertising in some of these magazines has been included in Exhibit 12.

National Spokesperson: A national spokesperson will be signed to promote product credibility and awareness to our potential customer base. The spokesperson's image and endorsement will appear in print advertising. We are considering partnering with a high profile athlete such as John Smoltz or Joe Montana. This partnership would create a pull strategy for Rejuvalin™, allowing INCA to benefit from the instant familiarity that a high profile athlete would bring. With a high profile spokesperson INCA will be able to accelerate the national expansion while utilizing more mass direct advertising such as radio. In exchange for their endorsement and promotion, INCA will

negotiate a spokesperson fee. We will look to have agreements with a spokesperson (s) before the national launch of Rejuvalin™.

Retail Sales: Once significant demand is generated for Rejuvalin™ efforts will be directed toward securing shelf space for Rejuvalin™ at natural health, nutrition and drug retailers. Strong preference will be given to retailers who require no or low cash payments to secure such space. Expected mark-ups for these retailers will be 50%. Some retail establishments include Earth Fare, GNC, CVS, Eckerd Drugs and Walgreens.

Tradeshows: Retailers will also be targeted at national trade shows. Four of the primary trade shows that INCA will attend include the National Natural Foods Association (NNFA) trade show (Las Vegas), the Natural Product Expo East (Washington, DC), the Natural Products Expo West (Anaheim, CA), and the National Association of Chain Drug Stores (NACDS) Marketplace. The NNFA trade show caters to the natural foods and supplement market with major retailers and buyers of supplements attending the show. The cost per booth is \$25-\$33 per square feet. 67% of the attendees, which include buyers from grocery, health food drug stores, placed orders in 2002 (nnfa.org). The Natural Product Expo East caters to the same audience and boasts 20,000 attendees were at the 2003 trade show. Of the retail buyers that attended this show, 73% bought one or more products (expoeast.com). The costs to attend these trade shows are estimated to be \$3500 per show.

IX. INCA's Manufacturing & Distribution

INCA will outsource the sourcing, manufacturing, packaging, and distribution of Rejuvalin™.

Raw Material Sourcing

Active and inactive ingredients will be purchased from Ferro-Pfanstiehl Laboratories. The company is a high purity chemical manufacturer in the field of health and beauty. They will supply active ingredients at a cost of \$125/kg and are able to provide a certificate of analysis to verify the ingredients. Pfanstiehl is able to supply the active ingredients in any quantity needed in the foreseeable future, but INCA has compiled a list of other manufacturers who are also capable of providing these materials in order to hedge against the risk of relying on a single supplier.

Manufacturing

INCA is negotiating with Sigma Aldrich regarding the formulation and packaging of Rejuvalin™. Sigma is a high volume producer who operates in a secure and regulated environment, and is able to scale up to any foreseeable level of production.

To effectively execute its startup strategy, INCA is also currently negotiating with a number of smaller producers who are capable of doing small volume production runs. Once selected, this manufacturer will produce unmarked Rejuvalin™ and placebo tubes for clinical trials. This startup manufacturer will also supply initial Rejuvalin™ inventory. Low cost production strategies will be used until demand reaches an appropriate level to scale up production.

Rejuvalin™ will be packaged in 3 oz plastic pump tubes with high-quality, visually appealing labeling specifying the ingredients used, some limited product claims, directions for product use, and the company's web site. Each 3 oz tube is designed to last for one month and is targeted to contain 6 g of active ingredient.

Based on unit sales of approximately 10,000 in the first year, initial estimates of production costs result in a variable cost of \$3.74 per unit. This is composed of a cost for the ingredients, labor, manufacturing, and materials.

Distribution

INCA will outsource distribution of Rejuvalin™ to Innotrac, an experienced contract fulfillment firm. Innotrac will accept and fill individual orders for Rejuvalin™ placed by internet, telephone and fax. The company will also distribute Rejuvalin™ to retailers who stock the product.

Distribution cost is estimated at \$5.50 per order. This cost includes storage of inventory, order processing, and handling. The customer will bear the cost of shipping the product.

X. INCA's Product Development

Product Acquisition Strategy

INCA will sustain its long-term growth rate through the acquisition and development of additional new nutraceutical products whose effectiveness is confirmed through scientific testing. INCA will budget funds to identify and license third-party product R&D. In particular, INCA will seek a strategic alliance with the University of Georgia College of Pharmacy. INCA will assist the college's product development efforts by funding research that leads to highly marketable products. The College of Pharmacy is an ideal third-party strategic alliance partner because it has a proven track record in developing nutraceutical and OTC pharmaceutical products with high market potential.

Future Products

At the current time, INCA has identified four potential follow on products that it will seek to bring to market after Rejuvalin™ is successfully launched. They are: Rejuvalin™ pills, Rejuvalin™ Pain Formula, Resveratrol, and Hyperease™.

Rejuvalin™ Pills

Once INCA has reached breakeven and secured a number of loyal customers, it will begin development of an improved glucosamine pill. Rejuvalin™ pills will be enteric coated, which will make it possible to increase the dosage of glucosamine while reducing gastrointestinal side effects. These pills will also be marketed to customers who normally take glucosamine for treatment or prophylactic purposes. Rejuvalin™ pills can be used jointly with Rejuvalin™ cream to provide a complete treatment option that meets individual customer needs.

Rejuvalin™ Pain Formula

INCA intends to seek an exclusive license for the ternary melt technology that facilitates the transport NSAIDs through human skin. A patent has already been issued for this technology. INCA will use this technology in combination with Rejuvalin™'s technology to produce Rejuvalin™

Pain Formula. Because it involves an NSAID, INCA will need to secure FDA approval to sell this product. INCA plans to fund these FDA tests from the revenues generated by the sale of Rejuvalin™.

Hypertension-ease

The first non-glucosamine product for which INCA may seek a license is a nutraceutical that is currently being tested for its ability to reduce blood pressure. If the results of this testing verify product efficacy, and the product license is secured, INCA's customer base could be greatly expanded by adding this product.

Resveratrol

Finally, INCA is exploring the possibility of marketing resveratrol tablets. Resveratrol has been documented to have antioxidant health benefits, especially in the cardiovascular system. It has been shown to be the source of most of the health benefits associated with daily wine consumption. There is no proprietary position associated with the production of Resveratrol available to INCA. However, INCA has a unique sourcing opportunity that will serve as a competitive deterrent and protect market share.

XI. INCA's Management & Key Advisors

INCA's management team possesses all the skills needed to successfully implement its business model through the conclusion of the product testing phase. Upon successful completion of these tests, INCA will hire a Chief Executive Officer to lead the company forward. This individual will assume leadership of the company after RejuvalinTM has been proven effective in order to orchestrate the product launch. The ideal candidate will have experience in the upper management of a nutraceutical or pharmaceutical company. Preference will be given to individuals who have experience with the launch of a product similar to RejuvalinTM. Ideally, the individual will have substantial industry contacts which INCA can leverage.

XII. INCA's Financials

Capital Structure

INCA has recently raised nearly \$30,000 from a National Collegiate Inventors & Innovators Alliance (NCIIA) grant and by winning the 2004 International "Idea-to-Product™" Competition. INCA will use these funds to complete initial testing of Rejuvalin™'s delivery effectiveness. Once INCA's testing has established Rejuvalin™'s effectiveness, the company will seek \$750,000 to commence operations. The company is anticipating offering an equity position of 15% ownership stake in the company for this investment. This capital will be used to finalize manufacturing arrangements, to market Rejuvalin™, and to fund working capital. The investment will fund INCA's operations for approximately twelve months with no revenue. INCA anticipates a second round of funding in 2007 to fund increased growth.

Pro Forma Financials

INCA will be able to begin operations with low fixed costs due to the outsourcing of manufacturing and distribution. INCA forecasts initial sales of \$250 thousand in 2005 which will increase to over \$60 million in 2010. Losses will total \$303 in 2005, however the company will reach break-even by summer of 2006. By 2010, INCA will expect profits of \$16.5 million.

	2005	2006	2007	2008	2009	2010
Unit Sales	10	65	370	720	1,600	2,900
Revenues	\$250	\$1,599	\$8,537	\$16,194	\$35,519	\$61,643
COGS	\$37	\$241	\$1,374	\$2,949	\$7,577	\$13,555
Royalties	\$5	\$32	\$171	\$324	\$710	\$1,233
Gross Profit	\$208	\$1,327	\$6,992	\$12,921	\$27,233	\$46,855

Marketing & Distribution Expense	\$179	\$563	\$1,937	\$3,998	\$7,627	\$13,717
Product Testing Expense	\$50	\$200	\$500	\$1,500	\$3,000	\$4,000
G&A Expense	\$22	\$46	\$63	\$66	\$98	\$101
Salary Expense	\$159	\$377	\$527	\$702	\$852	\$1,183
Net Income after Tax	(\$205)	\$80	\$2,369	\$3,977	\$9,374	\$16,688

*all figures in thousands

Exhibits 13-16 contain INCA's detailed financial projections.

Exit Strategy and Investor Returns

INCA expects exit through a sale of the company toward the end of year 5 to provide liquidity for its investors. Based on nutraceutical industry EBITDA multiplier of 7.5, the market capitalization of INCA is estimated to be \$117 million by the end of year 2009. This will result in a return of \$17.5 million to our investors, resulting in a cash-on-cash return of over 20 to 1. Considering the low risks and high rewards that are present in this opportunity, INCA represents a tremendous investment value.

XIII. Key Risks & Contingency Plans

INCA's long-term success could be influenced by several key risks including: (1) Lower than expected sales (2) Development of an equally effective glucosamine cream (3) FDA regulation of Nutraceuticals and (4) Demand for Rejuvalin™ exceeds expectations.

1. Lower than Expected Sales

Lower than expected sales of Rejuvalin™ are highly unlikely. INCA has constructed a forecast based on very conservative sales projections of less than 1% penetration of the target market over five years. In addition, INCA expects to finalize an endorsement agreement with Dr. Theodosakis upon completion of product testing, and believes that this powerful endorsement will generate demand far in excess of sales projections. Even if demand is lower than expected, INCA will be able to operate on an appropriate scale to profitably capitalize on the level of product demand.

2. Development of an Equally Effective Glucosamine Cream

There is no evidence that any competitors are attempting to develop any glucosamine creams that would be an effective competitors to Rejuvalin™. Nonetheless, it is possible that such a development could occur. Such an event would not be devastating to INCA for several reasons. The current U.S. glucosamine market is huge and growing in terms of dollar sales and the topical glucosamine market, though a subset of the overall market, remains large and unsatisfied. Increased competition in this market will not significantly hurt INCA because of the fragmented nature of the competition. In fact, INCA expects to benefit from any competitor's marketing efforts to convince consumers of the efficacy of topical glucosamine treatments.

3. The FDA Begins Regulation of Nutraceuticals

It is highly unlikely that the FDA will attempt to regulate either glucosamine or other nutraceuticals. However, if such regulations were put in place, they would significantly increase the cost of doing

business in the nutraceutical industry. This would negatively affect most nutraceutical firms, as they would have to turn their resources from marketing to product testing. However, INCA would be in an extremely advantageous position, since it will already have completed limited testing the effectiveness of all of its products. Thus FDA regulation is more likely to help than to hurt INCA because of the damaging effect it will have on INCA's competitors.

4. The Demand for Rejuvalin™ Exceeds Expectations

INCA is anxiously awaiting the results of the NIH study of glucosamine effects because a positive report could have a very powerful and positive effect on sales of Rejuvalin™. In fact, if the report strongly supports claims made about the effectiveness of glucosamine, it is possible that demand for Rejuvalin™ could vastly exceed INCA's current projections. Should such an event occur, INCA will respond by quickly ramping up production through its suppliers, manufacturers and distributors, all of whom have been selected because of their ability to scale upward should demand explode. In addition, INCA has also identified a number of other suppliers who are capable of manufacturing Rejuvalin™ and are available on relatively short notice should the need arise.

XIV. Future Growth & Development

INCA has two major avenues for growing the sales of Rejuvalin™. These prospects give INCA the potential to multiply initial revenues from sales of Rejuvalin™ many times over. These prospects are: Increased market penetration of Rejuvalin™ and Marketing of Rejuvalin™ internationally.

Increased Market Penetration

INCA's first strategy for growth will be to increase the market penetration of Rejuvalin™ by marketing it for additional uses. The first of these is prophylactic treatment. Many customers who are not sufferers of OA, but who are at risk of developing the disease later in life currently use glucosamine as a preventative treatment. This group represents a potential target for Rejuvalin™ because Rejuvalin™ is more highly effective at delivering glucosamine to the user's at-risk joints. INCA will expand advertising and promotion to target this customer segment. As a second part of the increased market penetration strategy, INCA will begin targeting athletes as primary customers. Athletes typically suffer from increased stress and strain on joints and may benefit from Rejuvalin™'s ability to deliver heightened glucosamine dosages to damaged joints. Sport versions of INCA's products will be developed and promoted to this market segment. These products will be packaged in containers that are distinct from those used for the regular product line. Advertising channels will also be expanded to venues that better reach this new segment (e.g. ESPN, sports magazines, etc.). In total, this segment is as large as the osteoarthritis segment, and although the need is not as strong as with osteoarthritis users, this market could nearly double sales of Rejuvalin™.

International Expansion

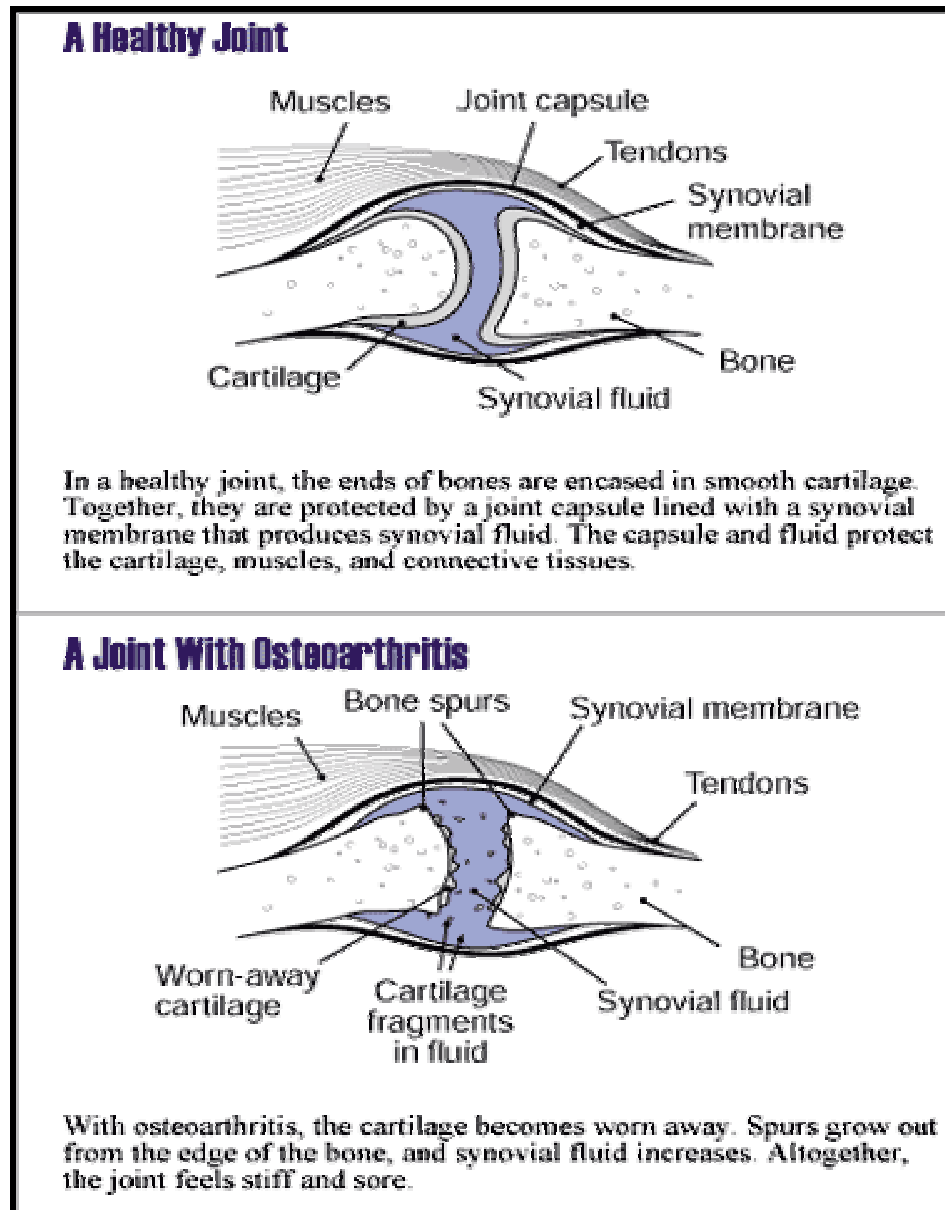
International expansion represents an opportunity for INCA to multiply its scale of operations by a factor of 3 or more. Though glucosamine is becoming a popular treatment in the U.S., it has been widely accepted in Europe and Australia for a long period.

Thus, glucosamine is currently the leading treatment for arthritis in Spain, Portugal and Italy. INCA intends to extend sales efforts into Canada by year 3 and will begin marketing Rejuvalin™ beyond North America by the sixth year of operation.

The first target for international expansion will be the European Union. There are currently 378 million residents in EU nations with an estimated 28 million OA sufferers, a market 35% larger than the US market. This implies a potential glucosamine market of \$8.5 billion. Like Canada and the US, Europe enjoys high standards of living. Initial focus will be to target Spain and Italy, which have 40 million and 58 million people and potential markets of \$896 million and \$1.3 billion, respectively. Both of these countries already have strong consumer bases for glucosamine products. After successful introduction in these two countries, INCA will introduce Rejuvalin™ to the remainder of the European Union.

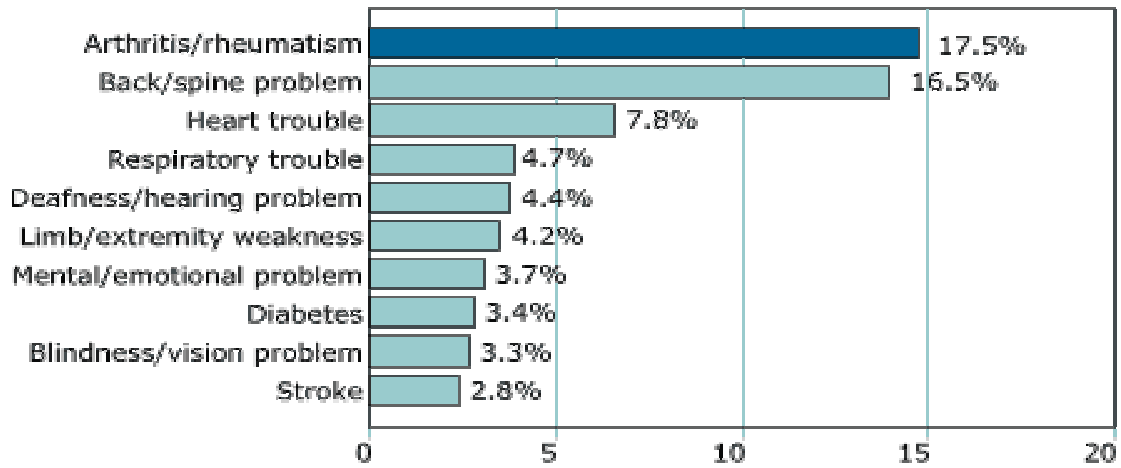
Asia and Australia represent a third possibility for international expansion, and INCA will expand its operations into these markets as well. Initial product launch will begin in Australia and Japan and then be expanded to other countries such as Singapore, Taiwan, and South Korea. In order to protect our intellectual property, we will seek international patents to protect our intellectual property in these arenas. Finally, INCA may desire to license the technology in these countries should a significantly positive opportunity arise.

Exhibit 1
Illustration of an Osteoarthritic Joint



Source: National Institutes of Health
<http://www.niams.nih.gov/hi/topics/arthritis/oahandout.htm>

Exhibit 2
Arthritis is the #1 Disability in the U.S.



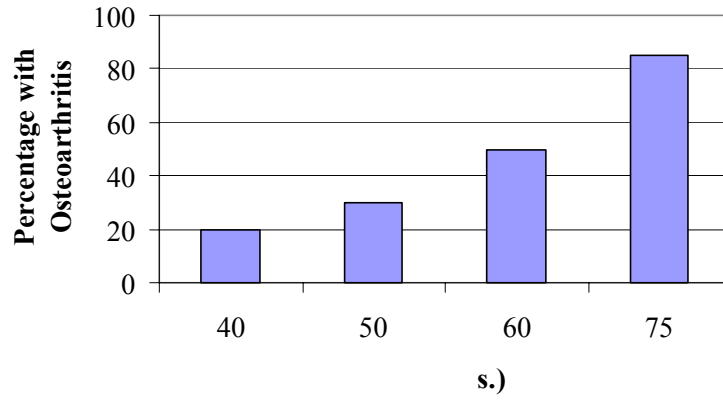
Percent of 41.2 million persons with a disability

Source: **Centers for Disease Control and Prevention**

www.cdc.gov/nccdphp/arthritis/index.htm

Exhibit 3
Osteoarthritis is a Disease of Aging

Demographics of U.S. OA sufferers



Source: Sack, KE. "What therapies for this disease of many causes?"
Post Graduate Medicine, 114(5), Nov 2003.

Exhibit 4
Summary of Recent Glucosamine Studies

Authors	Primary Variable	Number of Patients	Duration of Study	Conclusions
Pavelka et. al. Arthritis Rheum 2000; 9(suppl):S384	Oral Glucosamine sulfate (1500 mg/day) vs. placebo	202	3 years	-Glucosamine prevented further deterioration of knee joint -Symptom relief was significantly better in glucosamine group
Reginster et. al. Lancet 2001; 357:251-6	Oral Glucosamine sulfate (1500 mg/day) vs. placebo	212	3 years	-Glucosamine reduced the amount of further knee joint deterioration experienced -Symptom relief was significantly better in the glucosamine group
Vega et. al Menopause 2004:11:13-143	Oral Crystalline Glucosamine Sulfate (1500 mg/day) vs. placebo	414	3 years	-Glucosamine reduced the amount of further knee joint deterioration experienced -Pain and function were improved for group taking glucosamine over group taking placebo

Exhibit 5 Oral and Transdermal Glucosamine Delivery

OA Sufferer ingests glucosamine supplement orally.

OA Sufferer applies currently available glucosamine cream to affected area

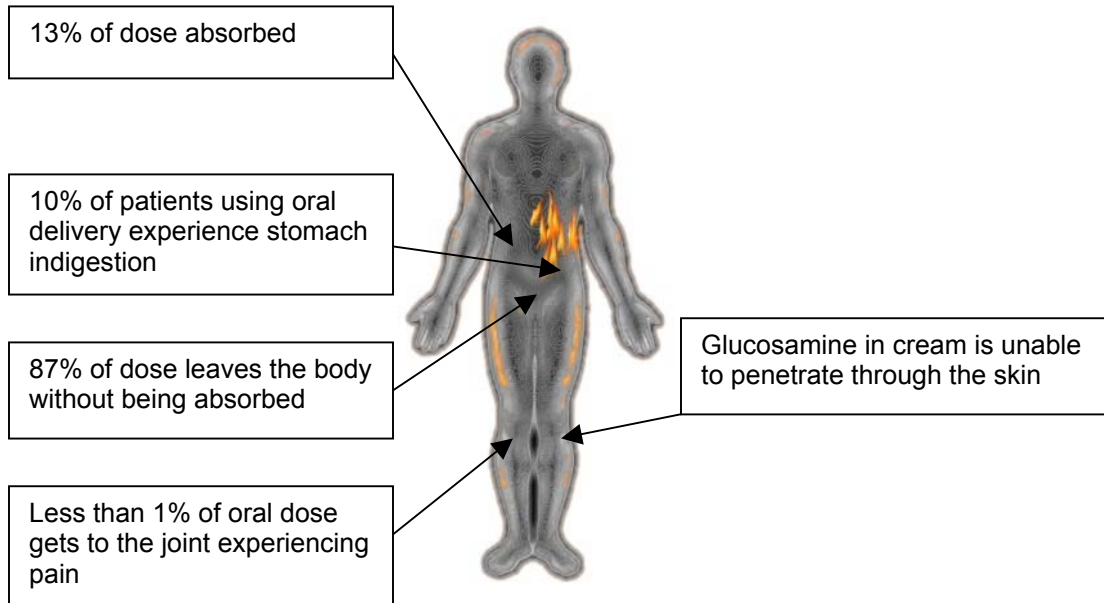
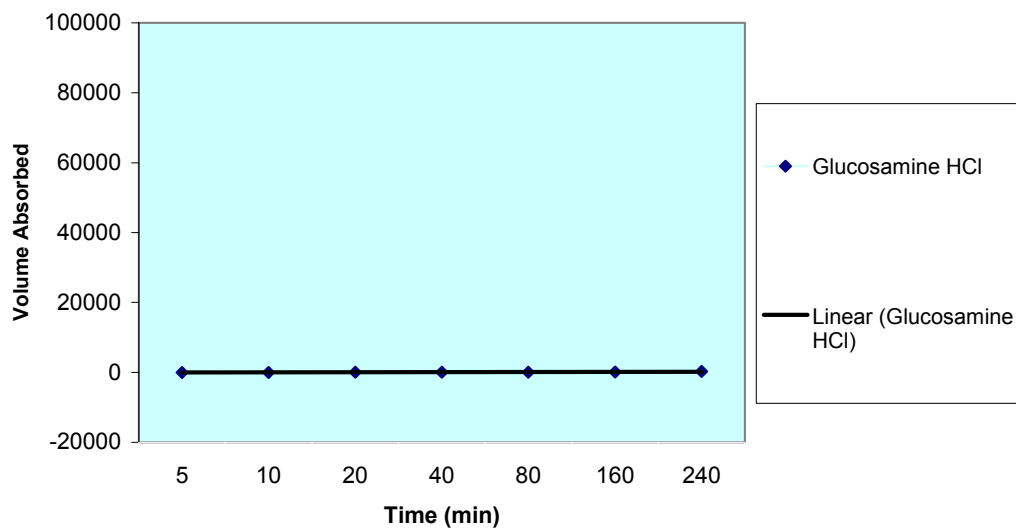


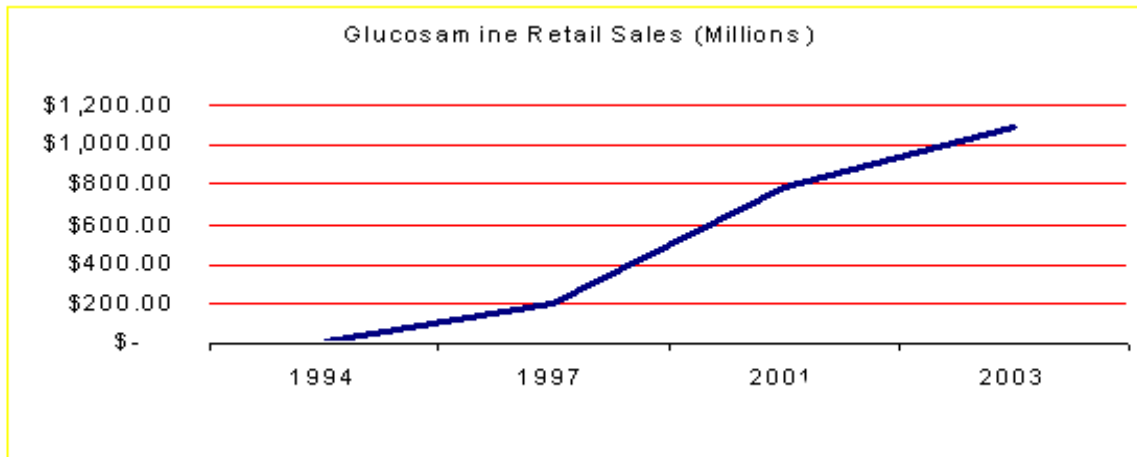
Exhibit 6 Traditional Glucosamine Creams Transported Across the Skin Over Time

Time vs Peak Area as a Function of Concentration



Source: Experiment, UGA Research Foundation, March 2004

Exhibit 7
U.S. Retail Glucosamine Sales



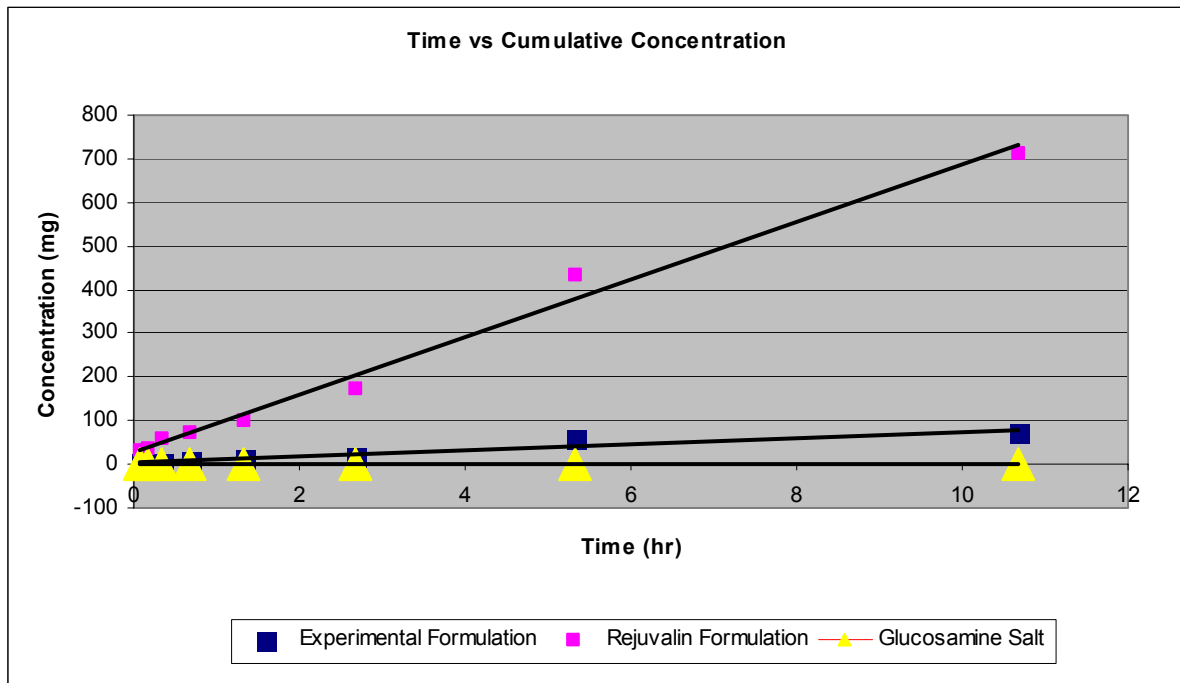
Source: "Specialty supplements are the bright spot in US dietary supplement market." Chemical Market Reporter, Vol. 264 (1). July 14,2003

Exhibit 8
Potential U.S. Market for Glucosamine

U.S. Population	290,000,000
<hr/>	
Americans with Osteoarthritis	21,000,000
Average glucosamine cost per year (\$25* x 12)	\$300
<hr/>	
Potential U.S. Glucosamine Market	\$6.3 billion
<hr/>	

*Average Monthly Cost of Osteo Bi-flex, Flex-a-min, & Cosamin DS
Source: Prices based on surveys of CVS and Eckerd retail stores in Athens, GA, 2004

Exhibit 9 Skin Penetration of Two Rejuvalin™ Formulations



Source: Experiment, UGA Research Foundation, March 2004

Exhibit 10
Rejuvalin™: A Better Solution

Benefits Products	Safe Delivery	Effective Delivery	Pain Relief	Mild OA Joint Recovery	Severe OA Joint Recovery
NSAIDS e.g. Ibuprofen	X	✓	✓	X	X
Celebrex™	X	✓	✓	X	X
Other Products w Glucosamine	✓	X	After 4 Weeks	Yes for some	X
Rejuvalin™	✓	✓	After 4 Weeks	✓	✓

**Exhibit 11
Marketing Budget**

	<u>2005</u>	<u>2006</u>	<u>2007</u>	<u>2008</u>	<u>2009</u>	<u>2010</u>
Avertising Expense						
Print Media	-	80,000	120,000	825,000	1,200,000	2,300,000
Radio	35,000	60,000	85,000	120,000	280,000	600,000
TV Commercials	-	-	-	225,000	575,000	1,350,000
Infomercials	-	-	400,000-	500,000	725,000	1,225,000
Direct Mailings	60,000	120,000	100,000	80,000	80,000	80,000
Other	15,000	28,000	56,000	120,000	240,000	320,000
Total	110,000	288,000	765,000	1,870,000	3,100,000	5,875,000
Promotion Expense						
Promotional Materials	15,000	30,000	50,000	100,000	155,000	315,000
Travel Expense	5,000	7,000	9,000	10,000	15,000	30,000
Other	-	-	-	10,000	15,000	20,000
Total	20,000	37,000	55,000	120,000	185,000	365,000
Other Marketing Exp.						
Website Commissions	4,500	16,000	42,500	-	-	-
Total	4,500	16,000	42,500	-	-	-
Total Planned Expenditures	134,500	341,000	862,500	1,990,000	3,285,000	6,240,000

Exhibit 12
Potential Regional and National Advertising Exhibit 14
Potential Regional and National Advertising

Media	Reach	Price
Direct Email to AARP Subscribers	25,000	\$900
AARP Banner Ad	22,000,000	\$25,000 3 months
Arthritis Today	650,000	\$28,152 (1 Color Page) \$19,665 (1BW Page)
Alternative Medicine	105,000	\$4,725 (1 Color Page) \$2,642 (1 BW Page)
Total Health	700,000*	\$2,835 (1 Color Page) \$2,415 (1 BW Page)
QVC	86,000,000	No fee: % of sales

Sources: www.aarp.com, www.arthritistoday.com, www.alternativemedicine.com,
www.totalhealthmagazine.com, www.qvc.com)

Exhibit 13
INCA Pro-forma Income Statement
Fiscal Year Ended 12/31/05 - 12/31/10
All figures in thousands

	2005	2006	2007	2008	2009	2010
Revenues						
RejuvalinTM Cream	\$250	\$1,499	\$8,163	\$13,495	\$23,824	\$41,650
RejuvalinTM Pills	\$0	\$100	\$373	\$1,079	\$2,599	\$4,998
RejuvalinTM Pain Formula	\$0	\$0	\$0	\$1,619	\$9,097	\$14,995
Total	\$250	\$1,599	\$8,537	\$16,194	\$35,519	\$61,643
COGS						
RejuvalinTM Cream	\$37	\$224	\$1,309	\$2,244	\$4,114	\$7,480
RejuvalinTM Pills	\$0	\$16	\$65	\$195	\$488	\$975
RejuvalinTM Pain Formula	\$0	\$0	\$0	\$510	\$2,975	\$5,100
Royalties to UGARF	\$5	\$32	\$171	\$324	\$710	\$1,233
Total	\$42	\$273	\$1,545	\$3,273	\$8,287	\$14,788
Gross Profit	\$208	\$1,327	\$6,992	\$12,921	\$27,233	\$46,855
Operating Expenses						
Distribution Expense	\$30	\$192	\$1,024	\$1,943	\$4,262	\$7,397
Advertising Expense	\$150	\$371	\$913	\$2,055	\$3,365	\$6,320
Product Testing Expense	\$50	\$200	\$500	\$1,500	\$3,000	\$4,000
Office Expense	\$22	\$46	\$63	\$66	\$98	\$101
Salary Expense						
Executive & Administrative	\$65	\$169	\$169	\$221	\$234	\$299
Operations	\$29	\$65	\$85	\$78	\$85	\$91
Marketing	\$33	\$72	\$195	\$319	\$442	\$689
R&D	\$33	\$72	\$78	\$85	\$91	\$104
Total	\$159	\$377	\$527	\$702	\$852	\$1,183
Dep. & Amort. Expense	\$2	\$7	\$17	\$27	\$33	\$41
Total	\$413	\$1,193	\$3,044	\$6,293	\$11,610	\$19,042
EBIT	-\$205	\$134	\$3,948	\$6,628	\$15,623	\$27,813
Interest Expense	\$0	\$0	\$0	\$0	\$0	\$0
EBT	-\$205	\$134	\$3,948	\$6,628	\$15,623	\$27,813
Income Tax	\$0	\$53	\$1,579	\$2,651	\$6,249	\$11,125
Net Income After Tax	-\$205	\$80	\$2,369	\$3,977	\$9,374	\$16,688

- All figures in thousands except unit price and unit COGS
- Price is assumed at \$24.99/unit for Cream, \$19.99 for Pills & \$29.99 for Pain Formula
- Revenues are calculated assuming all products are sold through retail channels with 50% markup
- Distribution cost is assumed to be 12% of sales
- Revenue calculations assume 0% retail distribution in year 1, ramping up to 50% in year 6
- COGS is assumed as detailed in the operations section
- Licensing expense assumed to be 2% of revenues

- Patent expense is capitalized
- Assets are amortized / depreciated over 15 years

Exhibit 14
INCA Pro-forma Statement of Cash Flows
Fiscal Year Ended 12/31/05 – 12/31/10
All Figures in thousands

	2004	2005	2006	2007	2008	2009	2010
Cash at beginning of Year	0	\$29	\$439	\$304	\$4,056	\$6,387	\$11,924
Financing Cash Flows							
Paid in capital	\$29	\$ 750	\$0	\$ 3,000	\$0	\$0	\$0
Operating Cash Flows							
Sales	\$0	\$250	\$1,599	\$8,537	\$16,194	\$35,519	\$61,643
COGS	\$0	\$37	\$241	\$1,374	\$2,949	\$7,577	\$13,555
Royalty Payments	\$0	\$5	\$32	\$171	\$324	\$710	\$1,233
Operating Expenses	\$0	\$411	\$1,186	\$3,026	\$6,266	\$11,577	\$19,001
Taxes	\$0	\$0	\$53	\$1,579	\$2,651	\$6,249	\$11,125
Net cash from Operations	\$0	(\$203)	\$87	\$2,386	\$4,003	\$9,407	\$16,728
Investment Cash Flows							
Change in A/R	\$0	\$112	\$145	\$767	\$919	\$2,319	\$3,135
Change in Inventories	\$0	\$20	\$182	\$652	\$766	\$1,933	\$2,612
Change in A/P	\$0	\$25	\$185	(\$56)	\$173	\$501	\$650
Investment in PPE	\$0	\$5	\$5	\$10	\$10	\$20	\$50
Patent Expense	\$0	\$25	\$75	\$150	\$150	\$100	\$100
	\$0	(\$137)	(\$222)	(\$1,635)	(\$1,672)	(\$3,870)	(\$5,247)
Net cash at end of Year	\$29	\$439	\$304	\$4,056	\$6,387	\$11,924	\$23,405

- A/R are assumed to be 44 days
- Inv buffer is 50% of expected sales
- A/P are assumed to be 37 days payable

Exhibit 15
INCA Pro-forma Balance Sheet
Fiscal Year Ended 12/31/05 – 12/31/10
All figures in thousands

	2005	2006	2007	2008	2009	2010
Assets						
Cash	\$439	\$304	\$4,056	\$6,387	\$11,924	\$23,405
A/R	\$112	\$257	\$1,024	\$1,943	\$4,262	\$7,397
Inventories	\$20	\$202	\$854	\$1,619	\$3,552	\$6,164
Total Current Assets	\$571	\$763	\$5,934	\$9,950	\$19,738	\$36,966
Fixed Assets	\$28	\$101	\$243	\$377	\$463	\$573
Total Assets	\$599	\$864	\$6,177	\$10,326	\$20,201	\$37,539
Liabilities						
A/P	\$25	\$210	\$154	\$327	\$829	\$1,479
Total Current Liabilities	\$25	\$210	\$154	\$327	\$829	\$1,479
Long-Term Debt	\$0	\$0	\$0	\$0	\$0	\$0
Total Liabilities	\$25	\$210	\$154	\$327	\$829	\$1,479
Owner's Equity						
Paid in Capital	\$779	\$779	\$3,779	\$3,779	\$3,779	\$3,779
Retained Earnings	-\$205	-\$125	\$2,244	\$6,220	\$15,594	\$32,281
Total OE	\$574	\$654	\$6,022	\$9,999	\$19,373	\$36,060
Total Liabilities and Owners Equity	\$599	\$864	\$6,177	\$10,326	\$20,201	\$37,539

- A/R are assumed to be 44 days
- Inv buffer is 50% of expected sales
- A/P are assumed to be 37 days payable
- Fixed assets include capitalized patent expense
- Assets are depreciated / amortized over 15 years

Exhibit 16
INCA Pro-forma Monthly Statement of Cash Flows
Fiscal Year Ended 12/31/05 – 12/31/06
All figures in thousands

	Jun-05	Jul-05	Aug-05	Sep-05	Oct-05	Nov-05	Dec-05	Year Total	Jan-06	Feb-06	Mar-06	Apr-06	May-06	Jun-06	Jul-06	Aug-06	Sep-06	Oct-06	Nov-06	Dec-06	Year total
Cash at beg. of period	\$29	\$690	\$630	\$573	\$522	\$480	\$450	\$29	\$432	\$390	\$281	\$326	\$308	\$297	\$279	\$281	\$290	\$292	\$314	\$341	\$432
Financing Cash Flow																					
Paid in capital	\$750	\$0	\$0	\$0	\$0	\$0	\$0	\$750	\$0	\$0	\$0	\$0	\$0	\$0	\$0	\$0	\$0	\$0	\$0	\$0	\$0
Operating Cash Flow																					
Sales	\$0	\$7	\$17	\$30	\$47	\$65	\$82	\$250	\$88	\$96	\$104	\$112	\$120	\$128	\$136	\$144	\$152	\$160	\$176	\$184	\$1,599
COGS	\$0	\$1	\$3	\$4	\$7	\$10	\$17	\$42	\$13	\$14	\$16	\$17	\$18	\$19	\$20	\$22	\$23	\$24	\$26	\$60	\$273
Royalty Payments	\$0	\$0	\$0	\$1	\$1	\$1	\$2	\$5	\$2	\$2	\$2	\$2	\$2	\$3	\$3	\$3	\$3	\$3	\$4	\$4	\$32
Operating Expenses	\$59	\$59	\$59	\$59	\$59	\$59	\$59	\$413	\$99	\$99	\$99	\$99	\$99	\$99	\$99	\$99	\$99	\$99	\$99	\$99	\$1,193
Taxes	\$0	\$0	\$0	\$0	\$0	\$0	\$0	\$0	\$0	\$0	\$13	\$0	\$0	\$13	\$0	\$0	\$13	\$0	\$0	\$13	\$53
Net Income	(\$59)	(\$53)	(\$44)	(\$34)	(\$20)	(\$5)	\$5	(\$210)	(\$26)	(\$20)	(\$27)	(\$7)	\$0	(\$7)	\$13	\$20	\$13	\$33	\$47	\$8	\$48
Net Op. Cash Flow	(\$59)	(\$53)	(\$44)	(\$34)	(\$20)	(\$5)	\$5	(\$210)	(\$26)	(\$20)	(\$27)	(\$7)	\$0	(\$7)	\$13	\$20	\$13	\$33	\$47	\$8	\$48
Investment Cash Flow																					
Change in A/R	\$0	\$7	\$13	\$17	\$23	\$26	\$26	\$112	\$14	\$85	(\$66)	\$12	\$12	\$12	\$12	\$12	\$12	\$12	\$20	\$12	\$145
Change in Inv.	\$2	\$2	\$3	\$4	\$4	\$11	(\$6)	\$20	\$2	\$2	\$2	\$2	\$2	\$2	\$2	\$2	\$2	\$4	\$50	\$112	\$182
Change in A/P	\$2	\$3	\$3	\$5	\$5	\$12	(\$4)	\$25	\$0	(\$2)	\$6	\$2	\$2	\$2	\$2	\$2	\$2	\$4	\$50	\$113	\$185
Investment in PPE	\$5	\$0	\$0	\$0	\$0	\$0	\$0	\$5	\$3	\$0	\$0	\$0	\$0	\$0	\$3	\$0	\$0	\$0	\$0	\$0	\$5
Patent Expense	\$25	\$0	\$0	\$0	\$0	\$0	\$0	\$25	\$38	\$0	\$0	\$0	\$0	\$0	\$38	\$0	\$0	\$0	\$0	\$0	\$75
Net Inv. Cash Flow	(\$30)	(\$7)	(\$13)	(\$16)	(\$22)	(\$25)	(\$23)	(\$137)	(\$15)	(\$89)	\$71	(\$11)	(\$11)	(\$11)	(\$11)	(\$11)	(\$11)	(\$11)	(\$19)	(\$11)	(\$142)
Net cash, period end	\$690	\$630	\$573	\$522	\$480	\$450	\$432	\$432	\$390	\$281	\$326	\$308	\$297	\$279	\$281	\$290	\$292	\$314	\$341	\$338	\$338