Topical Treatment of Blisters With Vitamin E

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Abstract

An effective method for encapsulating Vitamin E in a film was tested for the treatment of topical blisters. Vitamin E is a natural solution for skin treatment that is easily available. Alginate films are nontoxic and relatively simple to make out of a simple sodium alginate and calcium chloride combined procedure. Vitamin E is fat-soluble, making the commonly used method for making alginate films impossible based on the reliance that the film contains a water-soluble chemical solution. A hydrocolloid film for the topical treatment of blisters was created. Sodium alginate solution is mixed with methyl cellulose solution to create a solution. Vitamin E oil is added to the previous solution to produce an emulsified phase using high speed mechanical stirring. The emulsion was put in a syringe as means of filtering, and 2 microliters of the emulsion is dropped over calcium chloride in methanol on slide and an additional glass slide is put on top of the alginate and calcium chloride solution immediately after combination set to polymerize. The Vitamin E was quantified utilizing gas chromatography. The samples successfully demonstrated the encapsulation of Vitamin E and its retention for a period of two months.

Conclusion

The film, resulting from emulsion preparation, contained Vitamin E both in unaged and aged film samples. The unaged film was analyzed for Vitamin E content and found to contain 5910ppm(0.59% by weight) Vitamin E. An identical sample was tested in the GC after aging for eight weeks, and the film contained 2560ppm (0.25%) Vitamin E.

Additionally, in the aged sample, there was evidence of deterioration of the alginate-methyl cellulose emulsion in the GC analyses, as seen by the decrease in magnitude of the methyl cellulose peaks. The reduction in Vitamin E content in the film from 0.59% by weight to 0.25% by weight in eight weeks suggests that the Vitamin E has migrated from the film. No evidence of oxidized Vitamin E was evident in the GC analyses. This ability to migrate slowly with time may be useful in the topical treatment of blisters to enhance healing. While the preparation utilized 4% by weight Vitamin E, approximately 0.6% by weight was experimentally determined to be encapsulated in the aqueous film. This is 15% rate of encapsulation.

The results of the GC testing can conclude that Vitamin E was indeed encapsulated in the alginate-methylcellulose procedure. Since alginate films are water based, encapsulation of Vitamin E is difficult. The original procedure had to be altered to fit the needs of encapsulating a fat-soluble macromolecule, and thus the inclusion of methylcellulose to form an emulsion. Gas Chromatography analysis demonstrated that oil soluble Vitamin E was successfully encapsulated in an aqueous based alginate film. While there was encapsulation of Vitamin E in the film, the amount of macromolecule in the aqueous film was significantly less than the amount that was originally put into the procedure.

Running the samples through calcium chloride and alcohol wash does encapsulate the Vitamin E more effectively, but can slow down the migration rate of the macromolecule. The

migration rate is slowed because it hardens the actual film to what looks like a dried piece of spaghetti, making the film no longer a gel but a solid. Emulsions are also difficult to hold stable over time. Not all of the sample dissolved in the chloroform when performing the GC analysis, and suspensions were formed. Therefore, it is possible that more than 15% encapsulation rate of the Vitamin E occurred. In order to quantify the total amount of Vitamin E in the gel, a new analytical procedure would need to be developed specific to the procedures of this project (i.e. finding a new method of Vitamin E quantification).

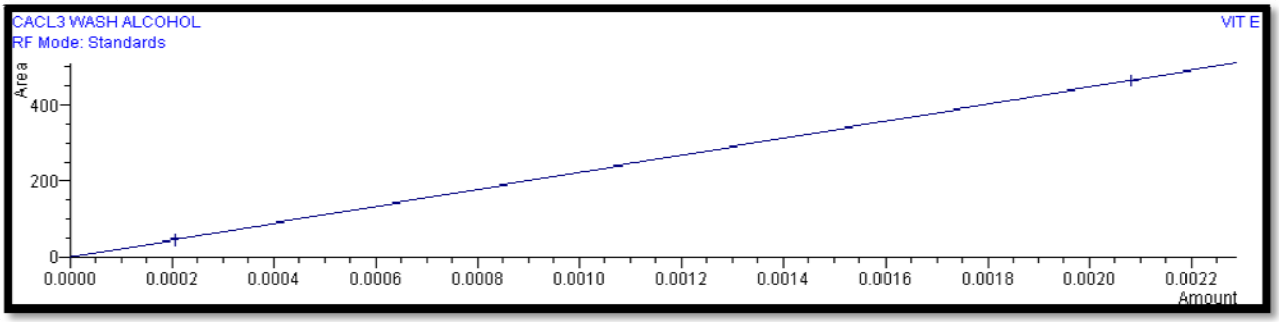
There are several avenues for continued research that could be pursued. Improving the rate of encapsulation of the Vitamin E in the alginate film is necessary for this to be commercially marketable. A 0.59% or 0.26% quantity of Vitamin E to be placed on a membrane for topical treatment of blisters would not be an effective method of treatment. Improving the quality and duration of the emulsions is also an area of improvement. There is success because the results suggest a migration rate over six months, but a faster migration rate would be desired to make this product commercially marketable. The 15% migration rate suggests that the procedure needs to be varied in order to increase the rate of encapsulation (and one would hypothesize to decrease the amount of methylcellulose or to find a weaker emulsifier). Only two samples were run through the GC analysis, thus further testing of more samples should have been conducted in order to have more data and leave less room for error to draw a more exact and duplicated conclusion.

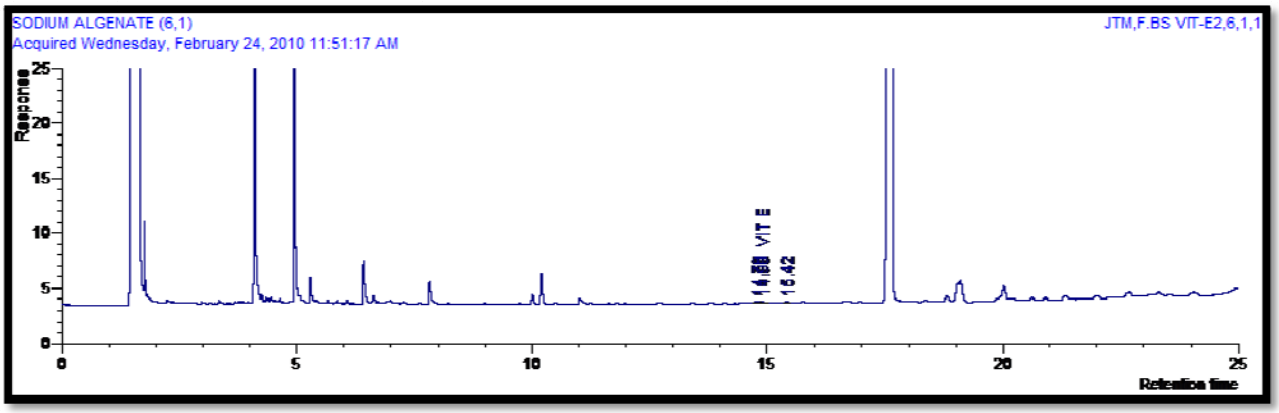
An improved analytical method specific to these studies is important for continued research. If a desired film is produced in future studies, it can be further harnessed into additional testing to determine its exact diffusion rate as suggested in the results. The films can be tested for its effective diffusion across a membrane with a similar permeability to that of human skin as to

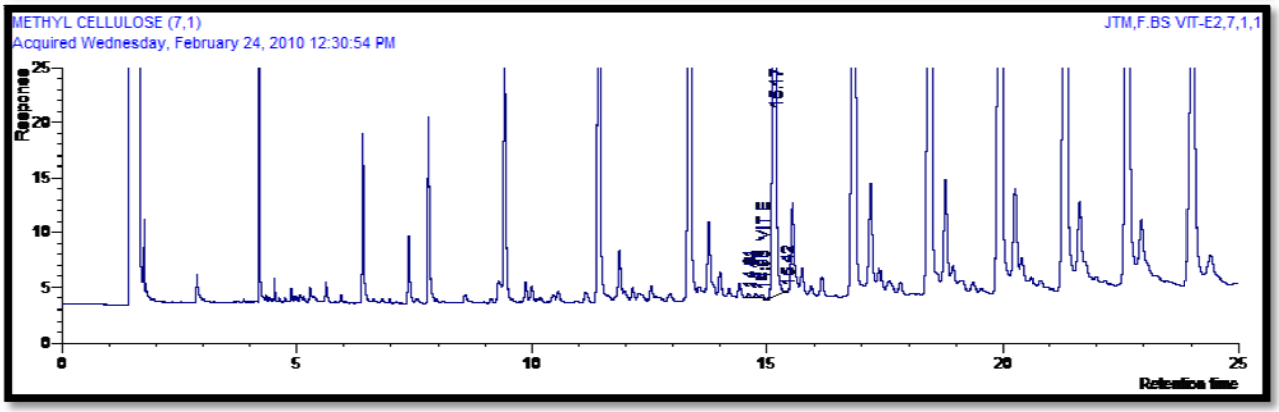
mimic the actual topical treatment. Such materials as <500Da dialysis tubing or pigskin would mimic the diffusion of the macromolecules through the membrane, and thus the amount of macromolecule to diffuse through the membrane would be quantified. The applications of the tested experimentation are numerous, and in order to continue this experimentation, more samples need to be tested as well as a refinement in the procedure and amounts of chemicals used.

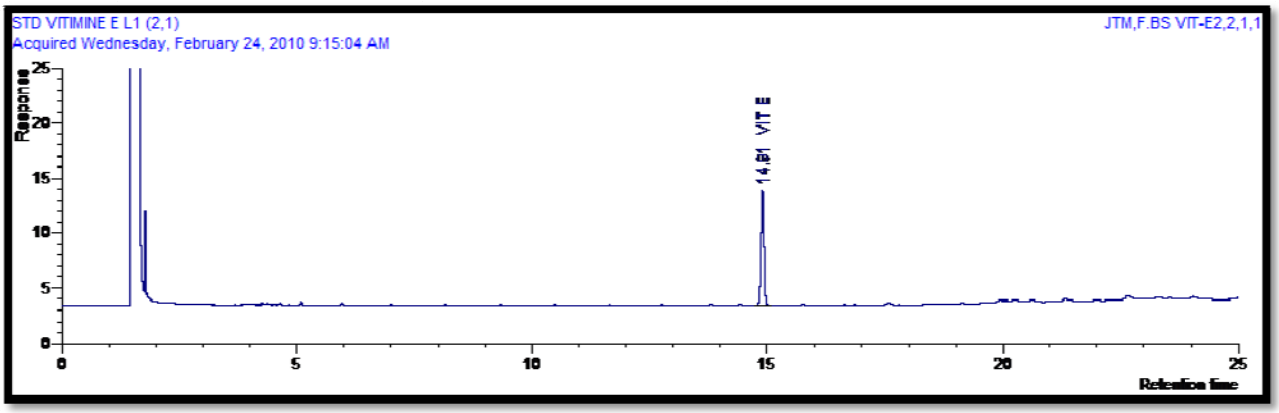
Data

The Standard Curves





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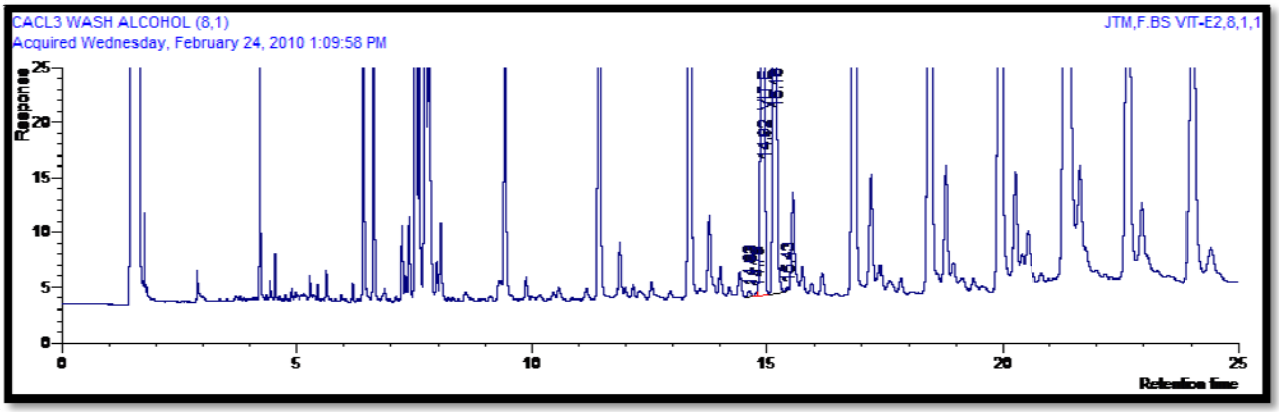
Response
14.61 VIT E
Retention time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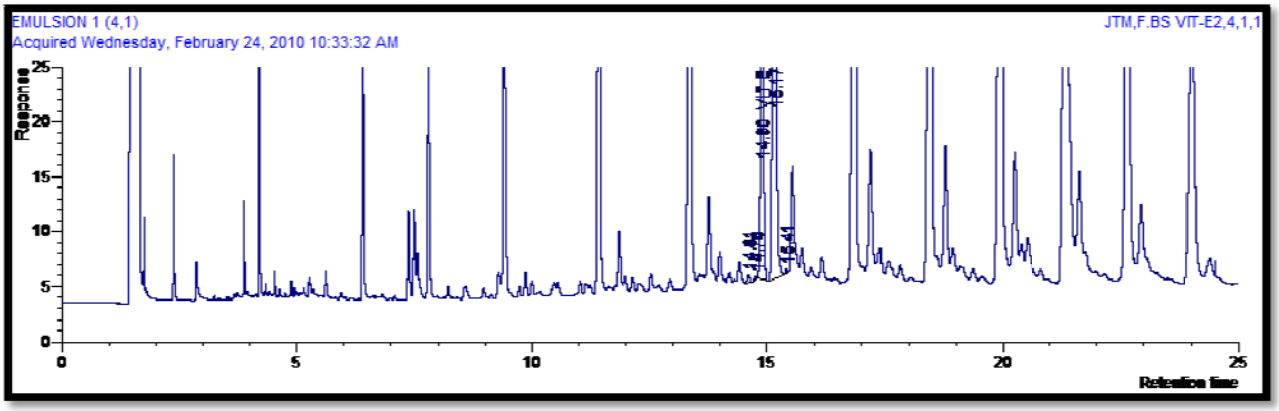
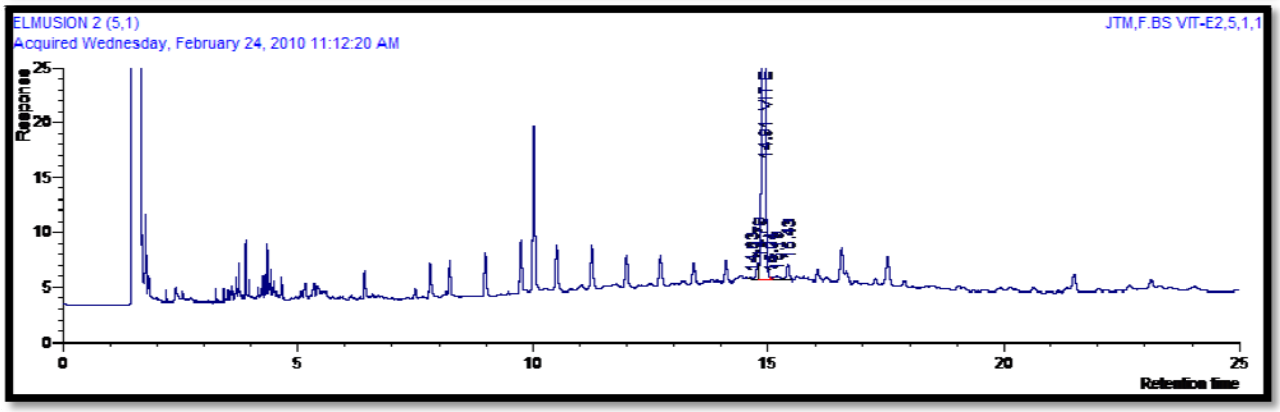
JTM,F.BS VIT-E2,8,1,1
Response
Retention time

Tested Results

Alginate Film Trial	Time Elapsed Before Testing	Amount of Vitamin E Present (ppm)
Emulsion 1	2 Hours	5910 (0.59% by weight)
Emulsion 2	8 Weeks	2560 (0.27% by weight)





Introduction

Blisters that are not of genetic predisposition (i.e., bullous pemphigoid[1]) occur as the result of a force being imposed on the skin and mechanically separating the skin layers. These layers of damaged skin form a pocket of trapped lymph fluid, the causation of blisters. The skin begins to shear due to an increase in temperature, additional moisture, or a lack of padding in a certain area, such as the palm of the hand or the heel of the foot (Schimelpfenig, 2008). These areas of common blister occurrence are called hot spots, usually a result of physical activity.

Prevention of blisters is much easier to perform than the actual treatment of blisters. Prevention tips include checking feet often for blisters, especially when irritation and/or pain occur. Keeping the feet dry is also a key to prevention. Moisture-filled and hot skin blisters much quicker than cool dry skin (Schimelpfenig, 2008), and it is recommended that socks are worn and changed often to allow the foot to stay dry.

Treatments for blisters vary. Most recommended treatment for blisters caused by pressure is topical in nature, and the available treatments involve a combination of an ointment and a bandage. Moisturizers such as Vaseline are recommended by podiatrists for cracked feet. Although athletes with blisters use lubricating agents (such as, Schimelpfenig, 2008) logically to prevent blisters, research has shown that it eventually increases the chance for blister development in that particular area of application. Antiperspirants are also used by athletes for

[1] Studies are being conducted for the treatment of genetically predisposed blisters using treatments such as prednisolone and doxycycline for initial treatment of bullous pemphigoid. Bullous pemphigoid has been treated orally with long-term applications of prednisolone, which can include side effects such as diabeters, osteoporosis, infections, and high blood pressure (Blister Study) (Blister Trial). Doxycycline is used for the treatment of bacterial infections and treatment of blemishes and other types of lesions (Doxycycline, 2008).

blister prevention with the logic that reduced moisture from increased sweating areas where blisters form will be reduced, thus reducing the chance of blister formation. While science has not found this technique to be effective, certain individuals and athletes feel that it is effective (Schimelpfenig, 2008). Other attempts at blister prevention include drying powders, which soaking up moisture to keep the skin dry (Schimelpfenig, 2008). Military studies have found that certain drying powders dry up moisture as intended, yet the powder clumps together and creates an abrasive surface, and one study has shown a correlation between foot powders and blister formation (Schimelpfenig, 2008).

In terms of tape and covering material available, there are many solutions. These solutions include moleskin®, paper tape, athletic tape, Hypafix®, duct tape, Elastikon®, or any medical tape that can serve as a buffer zone between the hot spot and the exterior (Schimelpfenig, 2008). While duct and athletic tape are less expensive than medical tapes, the adhesive sometimes pulls the blister off if already healing/scabbing underneath, hence causing more pain and longer recovery time. Moleskin® is a patch made from flannel cotton used for protection against future blisters or to cover an exposed blister (Schimelpfenig, 2008). Hypafix® is a breathable tape meant for use near the toes and around the heels, while paper tape (specifically Micropore™) is useful because it is thin and usually does not pull off skin when done with application and its specific use for being underneath other types of tapes.

There are a few combination treatments that have both medication and covering, usually in the form of a bandage. A few examples of these are Spenco Second Skin®, Spenco Blister Pads®, Blist-O-ban®, and Band-Aid Blister Block and Blister Relief. Second Skin® is a hydrogel (a natural or synthetic water-insoluble polymer effective for absorption) used for

treatment of open blisters, claiming to be effective to protect the blister from friction and pressure (Schimelpfenig, 2008). Second Skin Pads® involves the use of a hydrocolloid pad.

Chemical cauterization is also a treatment method, specifically Mercurochrome or benzoin; however, the treatment is seen as controversial because of the pain experienced by the patient (Schimelpfenig, 2008). The value in the treatment is the leftover tough skin that remains after the treatment. Debatable scientific treatment is the pain and anguish expressed by the patient who gets to have an open cauterized wound complete after trimming away the skin surrounding the blister.

Vitamin E

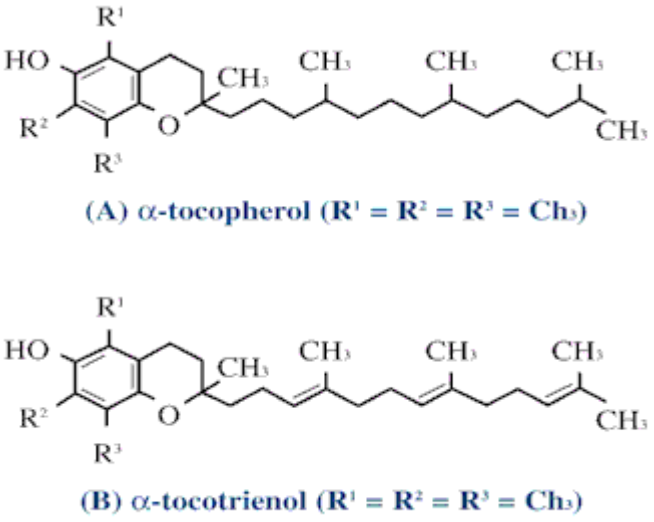


Figure 1: The chemical structures of tocopherols and tocotrienols (Kwiat, 2009)

Vitamin belongs to a family of eight molecules with chromanol rings and carbon chains (Best, 2004). Topical Vitamin E is a natural option for skin treatment. It is composed of eight different tocotrienols and tocopherols (Brown, 1952), side chains containing three double bonds and saturated side chains respectively.

Tocotrienols are well absorbed by the skin and used in Vitamin E cream, as found in high concentrations of palm oil (Best, 2004). Tocopherols make up about ninety percent of animal tissue, and are well assimilated in digestion; tocotrienols are not viably digestible and not a part of animal tissue. The various alpha, beta, delta, and gamma forms of the tocopherols are

currently being researched for the possible treatment of cancer, specifically in laboratory kidney rats, and coronary heart disease (Best, 2004). Approximately fifteen milligrams per day of Vitamin E is an effective form of preventing the most potent form of breast cancer because of the gamma-tocopherol chain in Vitamin E (Best, 2004). Synthesizing Vitamin E is only from plants, and it is therefore part of the daily animal diet to consume Vitamin E. Gamma-tocopherols are predominantly part of the diet because of vegetable oil and nuts, but the liver prefers alpha-tocopherols in th body for cholesterol. Gamma-tocopherols are more concentrated on tissues compared to the alpha-tocopherols located in the bloodstream (Best, 2004). While most research in the field of Vitamin E is conducted in the tocopherol area, the tocotrienols help with aging effects, wound treatment, and scar healers.

The focus of the project at hand is the use of the tocotrienols and the natural applications for topical treatment of blisters. Because the skin has a permeability with a molecular weight of less than five hundred Daltons (Da) (Brown, Traynor, Martin, Akomeah, 2008), the application of the Vitamin E on the topical wound must be smaller than five hundred Da. By topically applying a supplement, the skin has shown to improve and rebuild through these active methods. In this particular experimentation, the Vitamin E would be applied to the blister in the form of a topical treatment because the blister is not of genetic predisposition.

Alginate

Alginate gel is a popular wound dressing that is used in the medical field. These alginate dressings are under the greater family of hydrocolloids, all gel-forming agents (i.e. carboxymethylcellulose) for wound products (Thomas, 1997). The combination of a hydrocolloid and an adhesive with a sheet of some waterproof foam or film usually consisting of

polyurethane forms a wound dressing (Thomas, 1997). The type of hydrocolloid used will depend on the purpose and topical use of the dressing. The formation of alginate gel can be made into films. Extra thin alginate films have shown to be an effective technique for protoplast cultures through a study for which a procedure for making alginate films was used. Extra thin alginate films result in the higher plating efficiency as well as better observation of the colonies through a microscope (Pati, Sharma, Ahuja 2005). While the protoplast culture formation does not aid the current research topic, the procedure for the film was of use. Fifty micro liters of calcium chloride was formed and dropped on clean autoclaved glass then combined with a fifty micro liters sodium alginate; a cover glass immediately was applied on top of the bottom glass (Pati, Sharma, Ahuja, 2005). While this method is efficient for the production of alginate film, vitamins have the characteristic of being fat-soluble, thus making this water soluble method inefficient for the formation of a Vitamin E gel.

Alginate films are macromolecular proteins released on a target area for a certain period of time, and both beads and films control the release of the desired substance. Hermes, Narayani, 2002 demonstrated Bovine serum Albumin as a protein to be in encapsulation in the gel and compared with the amount of loading in the gel/film as the control. The Bovine serum Albumin concluded to be effective for long time release with a twenty percent cross-linked concentration. The ten percent was effective for less than twenty days compared to the twenty percent's one month. While the beads were more uniform and faster in their release rate, there was less drug concentration in those particular beads (Hermes, Narayani, 2002). The experiment demonstrated that alginate beads and films are effective for the transfer of macromolecular agents, with beads more suitable for oral purposes compared to the film's transdermal or medical possibilities.

Sodium alginate has many applications in medical and chemistries. While it is an ideal solvent for spectrophotometric and chromatographic work (Sodium Alginate, 2008), one of its most primary applications is its use in the food industry. It is a gelling agent as well as an emulsifier, stabilizing food and being used in applications such as dietary food and reducing serum cholesterol (Sodium Alginate, 2008). It is also a natural biodegradable and biocompatible compound that has been proven a suitable entrapment for water (Hermes, Narayani, 2002). Sodium alginate is also used in the chemical industry as a thickener and stabilizer, and has recently been applied to the synthesis of protein sequencing and DNA synthesis (Sodium Alginate, 2008). The application in this particular experiment for sodium alginate is for the formation of alginate gel for wound application.

Figure 2: Image of Sodium Alginate (Lianyungang Changhaol Seaweed Auxiliaries, 2009)

Dialysis Tubing

Dialysis tubing is a semi permeable membrane that will serve as the substitution for skin in the experiment. The tubing has a permeability of less than five hundred Da [the permeability of human skin (Brown, Traynor, Martin, Akomeah, 2008)], and the Vitamin E films will be placed on the tubing for testing its permeability. Also for testing is the procedure to make

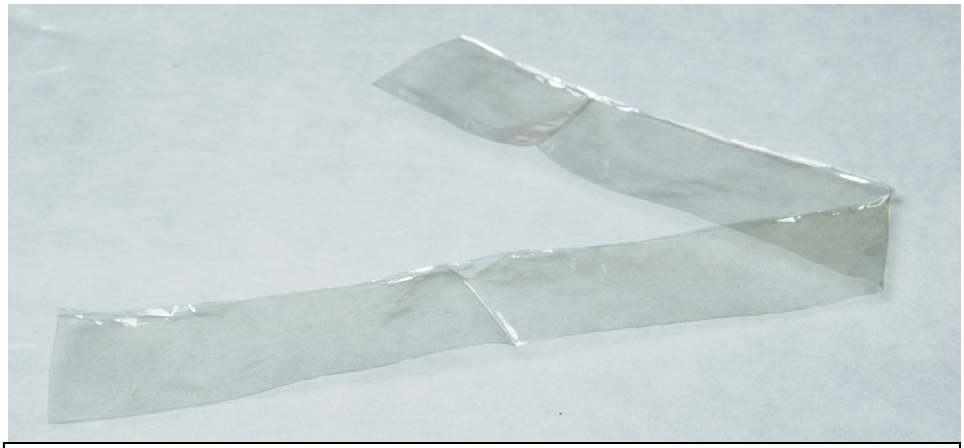


Figure 3: Image of dialysis tubing (Athabasca University- Center for Science, n.a.)

the alginate films, given that Vitamin E is an emulsion and therefore not applicable to the previous procedure for water-soluble sodium alginate films. The procedure involves filtering the Vitamin E through a series of alcohols and filters to change the size of the oil droplets of the Vitamin E to form a smooth and emulsion of oil.

The purpose of the testing is to create an alginate film made of Vitamin E for the topical treatment of blisters. This film will be tested using dialysis tubing for its effectiveness and permeability on the skin. An HPLC procedure will be used to quantify the amount of Vitamin E in the films.

Materials

Chemicals/ Consumables	*Supplies*	*Equipment*
• Sodium Alginate • Methyl Cellulose • Vitamin E • Calcium Chloride • Methanol	• Syringe • High speed mechanical magnetic stirring • Glass slides • <500 Da Dialysis Tubing	• Vacuum • HPLC with UV Detection

Procedure

4% (by weight) sodium alginate solution was mixed under high sheer mixing with 12% methyl cellulose solution to form an emulsion. 4%Vitamin E oil was added to produce an emulsified phase using high speed mechanical mixing. High sheer mixing apparatuses were used specifically to induce emulsification of oil into an aqueous phase, resulting in emulsion-containing oil droplets in the 1-3 micrometer range. The emulsion was filtered through a capillary syringe, passing through a 2.5% calcium chloride in methanol solution. Calcium ions were exchanged with sodium metal of the alginate, resulting in shape-retaining calcium-alginate beadlets that encapsulate Vitamin E. The alcohol was removed by evaporation. Films of the encapsulated Vitamin E emulsion were prepared on a glass slide. An additional glass slide was put on top of the film to increase surface area while forming a thin film of the emulsion containing Vitamin E oil droplets in the 1-3 micrometer range.

In order to quantify the Vitamin E in the films, two hundred milligrams of each sample was weighed into a 25 ml volumetric and diluted to the mark with HPLC grade chloroform. They were sonicated and vortexed for about 30 minutes. They were also heated slightly while in the sonicator. None of the samples completely dissolved, but suspensions were formed. They were then filtered through a 0.2 micron filter and run on the GC (Agilent 6890 with Flame ionization detector). 0.1 and 1.0 % w/w solutions of vitamin E were run as standards. The capsule content was used for the standard, considered 100% pure for the purpose of this study.

Samples of calcium chloride, sodium alginate, and methyl cellulose were tested using flame ionization gas chromatography in addition to the tested films. The Vitamin E could then be identified based on where the peaks were located in relation to the normal standard of where the standard for Vitamin E is located. The standard for Vitamin E was identified by running a sample of Vitamin E through the GC to determine the peak's level, and the amount of Vitamin E in the actual film was determined by finding the area under the curve. The area was calculated by taking the weight of the sample multiplied by the area of the standard.

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